INNOVATIVE APPROACHES IN THE MANAGEMENT OF BONE AND JOINT INFECTION

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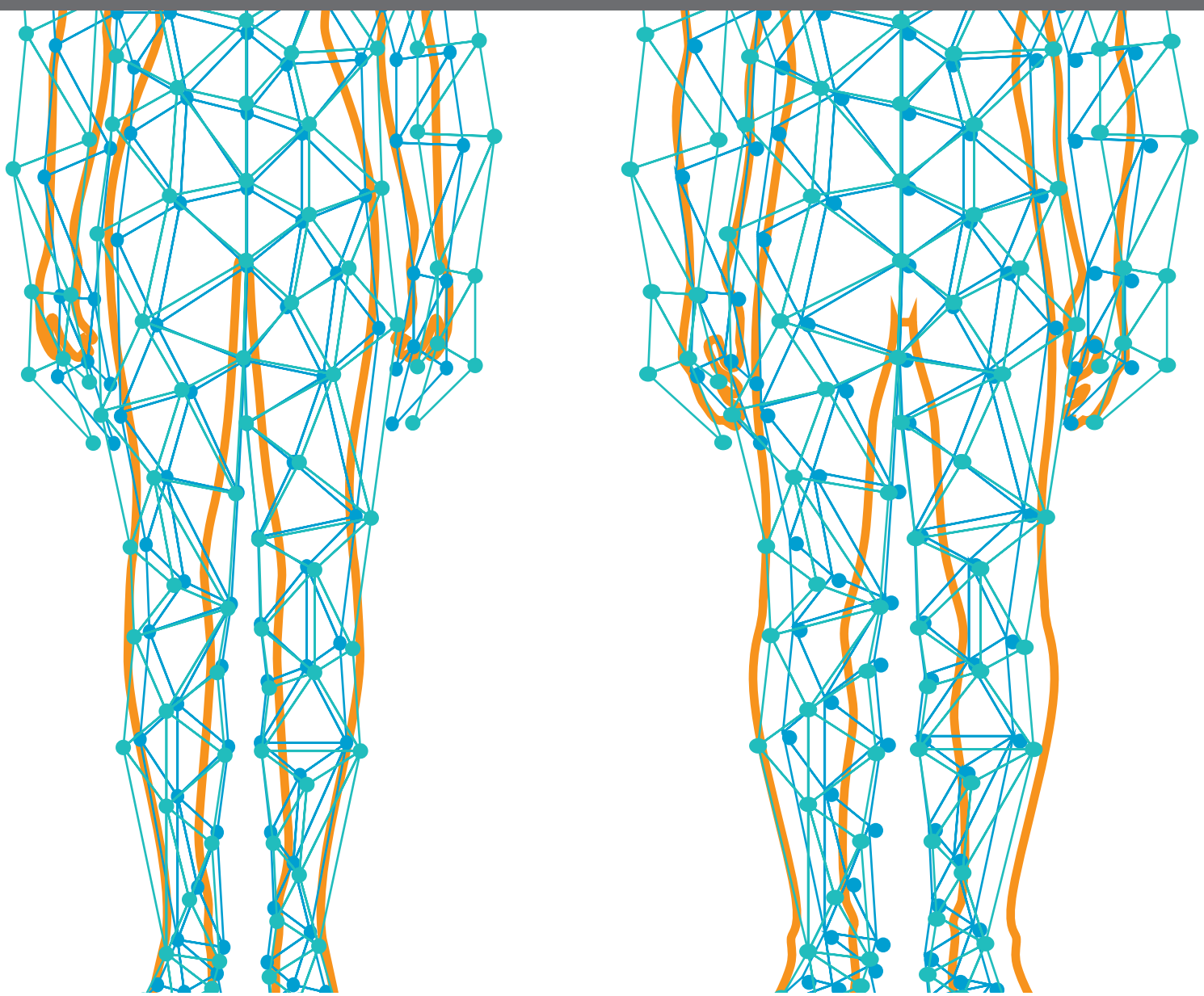
PUBLISHED IN: Frontiers in Medicine and Frontiers in Surgery



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ISSN 1664-8714
ISBN 978-2-88971-783-5
DOI 10.3389/978-2-88971-783-5

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INNOVATIVE APPROACHES IN THE MANAGEMENT OF BONE AND JOINT INFECTION

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Citation: Ferry, T., Lustig, S., Laurent, F., Soriano, A., eds. (2021). Innovative Approaches in The Management of Bone and Joint Infection. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-783-5

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frontiers in Medicine

EDITORIAL
published: 18 November 2021
doi: 10.3389/fmed.2021.789092

Editorial: Innovative Approaches in the Management of Bone and Joint Infection

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Keywords: bone and joint infection, prosthetic-joint infection, phage therapy, bacteriophage, lysin, OSCAT, personalized medicine, suppressive antimicrobial therapy

Editorial on the Research Topic

Innovative Approaches in the Management of Bone and Joint Infection

Bone and joint infections (BJI) are one of the most difficult-to-treat bacterial infectious diseases. Its management is complex, and requires a multidisciplinary approach, from the diagnosis to the medico-surgical strategy. This Research Topic brings together several breakthrough papers in microbiological diagnosis, personalized common or new therapies, and prevention of superinfection (**Figure 1**).

At present, diagnosis is easy for the acute forms, while most of the chronic infections remain undiagnosed or are discovered too late, leading to catastrophic clinical situations. Pham et al. reported a case where genomic analysis was decisive for the best therapeutic strategy, in a patient who experienced two episodes of *Streptococcus dysgalactiae subsp. Equisimilis* prosthetic-joint infection (PJI), that were finally unrelated. To improve the diagnosis of low-grade PJIs, Kolenda et al. showed that the treatment of an explanted prosthesis with dithiothreitol (a chemical agent that disrupts biofilm) used in a particular device could improve the microbiological diagnosis, by shortening the duration of cultures, or by identifying additional pathogens. This could provide an alternative to sonication, which is not so easy to implement and requires significant technical time.

To treat BJI, different medico-surgical strategies are proposed, depending on the type of BJI. However, the rate of success remains very disappointing, especially for patients with implant-associated BJI. In such patients, finding a way to cure the infection without adverse body reactions such as organ failure, or loss of function, remains a challenge, and deciding the treatment strategy for a particular patient with a specific form of BJI, is mainly based on experience, rather than precision medicine and the patient's clinical health data. Wouthuyzen-Bakker et al. reviewed the importance of risk scores for debridement, antibiotics, and retention of the implant (DAIR) in patients with PJI, and discussed the potential of implementing machine learning (artificial intelligence) in identifying patients who are at the highest risk for failure of DAIR will be addressed. The ultimate goal is to maximally tailor and individualize treatment strategies and to avoid treatment generalization, as also proposed by Baldan and Sendi. In this way, outpatient parenteral antimicrobial therapy (OPAT) is a potential option in particular patients with BJI, as described in the paper by Ferry et al. Moreover, Goutelle et al. demonstrated in a case series that

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *04 October 2021*
Accepted: *11 October 2021*
Published: *18 November 2021*

Citation:
Ferry T, Lustig S, Laurent F and Soriano A (2021) Editorial: Innovative Approaches in the Management of Bone and Joint Infection. Front. Med. 8:789092. doi: 10.3389/fmed.2021.789092

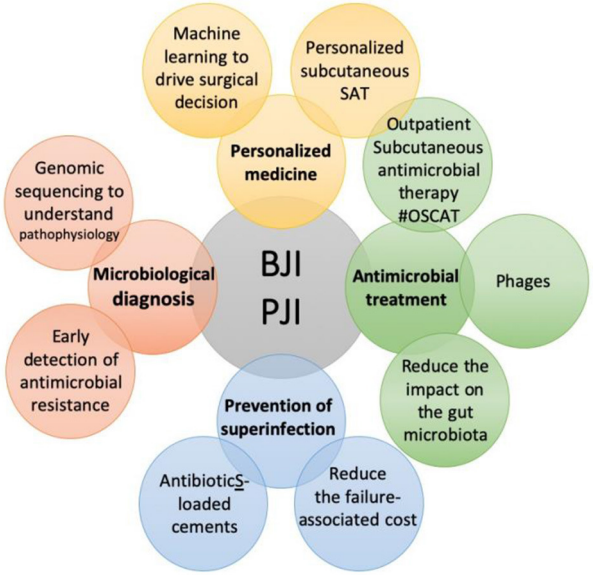

FIGURE 1 | The specific themes covered in the Research Topic "Innovative Approaches In The Management Of Bone and Joint Infection".

pharmacokinetic/pharmacodynamic dosage individualization of suppressive beta-lactam therapy administered by subcutaneous route only three times in a week (without need for a central catheter) was feasible, safe, and beneficial for particular complex patients with PJI.

Chauvelot et al. present new important data about the pathophysiology and treatment of Corynebacterium BJI, an often-neglected etiological agent of post-traumatic and/or post-operative BJI. This infection requires complex and collaborative medical-surgical management as it is associated with a poor prognosis, which is mostly driven by the initial surgical debridement. Furthermore, if biofilm formation did not appear as a pivotal physiopathological mechanism of Corynebacterium in BJI, bone cell invasion via the cellular β1 integrin allows the formation of an intracellular reservoir that leads to chronic infection.

The most important contributions to this Research Topic relate to non-common anti-infective agents, such as bacteriophages or lysins, that are new ways to specifically target the pathogen. They have antibiofilm activities and Ferry et al. report emergent approaches to keep the function in patients with chronic PJI for whom phages were administered during open debridement, during arthroscopy, or within a hydrogel in a patient with knee megaprosthesis infection. Ferry et al. also reported the use of exebacase (CF-301), a lysin that targets staphylococci species, as salvage therapy in elderly patients with relapsing multidrug-resistant *S. epidermidis* prosthetic knee infection. These approaches probably improve the efficacy of suppressive antibiotics and prevent major loss of function, and clinical trials are now needed. These breakthrough papers have been viewed and are already largely cited, and will help to promote the creation of nation-wide phage therapy centers dedicated to implant-associated infections and other difficult-to-treat infections, especially if a multidrug-resistant bacteria are involved (1).

Finally, a better knowledge of the costs, risks, and consequences of the management of patients with chronic PJI is crucial. In the paper by Bourbotte-Salmon et al., patients with chronic total knee arthroplasty infection, requiring revision using rotating hinge implant, had good functional outcomes but experienced a high rate of septic failure, mostly due to bacterial superinfection. These patients need optimal antimicrobial systemic prophylaxis and innovative approaches to reduce the rate of superinfection. The cost of superinfection was evaluated in the paper by Serrier et al. This study revealed that chronic PJI requiring a 2-stage revision is costly, with significant costs arising from the reimplantation procedure (about 15 k€). However, following reimplantation, the rate of subsequent new infection remained high, and the cost of reimplantation following a new infection is considerable, reaching 50 k€ per patient. These first cost estimates of managing chronic PJI with 2-stage exchange in France underline the economic interest of preventing new infections. A one-stage approach for patients with chronic PJI, including patients with fistula, as described by Marmor et al., could be an option for reducing the cost attributed to superinfection attributable to the 2-stage approach, as the infection control rate at 2 years was 95.3% in patients treated in this monocentric study. However, generalization of one-stage procedure in this context could be associated with bacterial persistence, which needs to be prevented (2). In patients for whom cemented prosthesis is needed for septic revision is required, the use of commercially available bone cement, used for prosthesis fixation and loaded with gentamicin plus vancomycin or clindamycin, can help in preventing superinfection. Indeed the paper published by Cara et al. compared the prophylactic anti-biofilm activity of various commercial cements on different staphylococcal strains that are resistant to antibiotics, and found that cement eluting a combination of antibiotics had a significantly better ability to inhibit biofilm formation than the cement eluting only gentamicin. Finally, Benech et al. evaluated the potential adverse effect on the gut microbiota of systemic antibiotics used to treat patients with BJI. Systemic antibiotics significantly altered the gut microbiota diversity and composition, with a rapid but partial recovery observed at 2 weeks after antibiotic withdrawal. Antibiotic duration or the use of fluoroquinolones did not seem to affect this resilience. In this paper, the acquisition of multidrug-resistant bacteria carriage in the gut remained one of the most challenging side effects of long-term exposure to antibiotics. Innovative microbe-based therapies could be a promising tool to address these issues.

Taken together, each of the articles published in this Research Topic has contributes to improving the diagnosis, management, and outcomes of BJI. Promoting translational research in expert centers such as CRIOAc (3), the compilation of such studies in this Research Topic, aims to share experiences in multidisciplinary societies and congresses such as the European

Bone and Joint Infection Society. These types of initiatives are crucial to innovate and considerably reduce the burden of BJI, which remains a neglected infectious disease in many industrialized countries.

AUTHOR CONTRIBUTIONS

TF initiated the Research Topic and wrote the draft of the manuscript. All authors approved the submission.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
published: 21 February 2020
doi: 10.3389/fmed.2020.00053

Second Periprosthetic Joint Infection Caused by *Streptococcus dysgalactiae*: How Genomic Sequencing Can Help Defining the Best Therapeutic Strategy

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Specialty section:
This article was submitted to Infectious Diseases – Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *11 December 2019*
Accepted: *04 February 2020*
Published: *21 February 2020*

Citation:
Pham TT, Lazarevic V, Gaia N, Girard M, Cherkaoui A, Suva D and Schrenzel J (2020) Second Periprosthetic Joint Infection Caused by Streptococcus dysgalactiae: How Genomic Sequencing Can Help Defining the Best Therapeutic Strategy. Front. Med. 7:53. doi: 10.3389/fmed.2020.00053

Primary and revision arthroplasties are increasing worldwide, as are periprosthetic joint infections (PJI). The management of PJI requires surgery, the strategy of which is dictated by the acute or chronic nature of the infection, with an exchange of the implant in the event of a chronic PJI or in the case of recurrence with the same pathogen. We report the case of a 63-year-old man with two episodes of *Streptococcus dysgalactiae* subsp. *equisimilis* PJI within 9 months. Based on clinical suspicion of an haematogenous PJI, the patient was treated by DAIR (debridement, antibiotics, implant retention), while genomic sequencing revealed two different strains, confirming our hypothesis that no additional surgery was needed. Hence, we report a case where genomic analysis was decisive for the decision of the best therapeutic strategy.

Keywords: periprosthetic joint infection, PJI, *Streptococcus dysgalactiae*, next-generation sequencing, NGS

INTRODUCTION

There is an increasing trend for primary and revision arthroplasties in the United States (US) (1) and in Europe (2, 3). The incidence of periprosthetic joint infections (PJI) ranges from 0.5 to 2.0% (4). Twenty-five to thirty-eight percent of knee replacements are due to infections and have been associated with a significant morbidity and a 5-fold increase in mortality at 1 year (4, 5). The management of peri-prosthetic joint infections is challenging and therefore requires a multidisciplinary approach, typically including infectious diseases specialists, microbiologists, and orthopedic surgeons to decide which of the following options may be the best in each case: 1 or 2-stage exchange, DAIR (Debridement, Antibiotic, and Implant Retention), ablation of the prosthesis (Girdlestone procedure for example), arthrodesis or amputation.

Herein, we report two episodes of a prosthetic joint infection due to *Streptococcus dysgalactiae* subsp. *equisimilis* within a 9 month-interval. Genomic sequencing of the two strains showed that the 2 strains were genetically unrelated. The patient was treated by DAIR for the second PJI, hypothesizing an hematogenous infection rather than a recurrence or a chronic infection, and therefore additional surgical intervention was avoided.

CASE REPORT

We report the case of a 63-year-old man, known for a right Charcot foot, lymphedema associated with venous insufficiency of the right lower extremity and bilateral knee arthroplasties (right knee 6 months prior without complications, left knee 4 years prior). Two days prior to admission, the patient presented with redness of the right knee, pain and increased swelling, fever, and shivering. The patient did not report any trauma before this episode.

He consulted at a private clinic where a blood test showed a C-reactive protein (CRP) at 50.9 mg/L [0–10 mg/L]. An arthrocentesis was performed which revealed 156,660 leucocytes/μL (36% neutrophils), 70,000 erythrocytes/μL, and direct examination showed the presence of gram-positive cocci. A treatment with cefuroxime administered intravenously (IV) was initiated. The next day, the patient's condition deteriorated with hypotension not responding to IV fluid administration. Antibiotic treatment was changed to amoxicillin-clavulanate 2.2 g IV and the patient was transferred to our hospital.

On arrival, the patient was in septic shock and blood tests revealed a CRP at 426.5 mg/L [0–10 mg/L], and an acute kidney failure with creatinine at 355 μmol/L [62–106 μmol/L] (eGFR 15 mL/min/1.73 m^2). He was promptly taken to the operating room (OR) where a large amount of pus was collected around the prosthesis and a DAIR procedure with exchange of moving parts was performed considering an acute PJI. Cultures of the purulent material obtained in the OR became positive for *S. dysgalactiae* subsp. *equisimilis* and antibiotic therapy was initially changed to ceftriaxone 2 g/day IV and subsequently to penicillin G 4 MioU six times daily, after excluding an infective endocarditis. A second and third look were performed, because of steady increase in CRP on post-operative day (POD) 4: the prosthesis was ablated with the placement of a spacer with decision of a 2-stage exchange. However, abscesses of the right foot were formed requiring a new surgical intervention on POD14 and the treatment was empirically changed to amoxicillin-clavulanate 1,000/200 mg four times daily. Operative samples showed *S. epidermidis* and vancomycin 1 g IV twice daily (bid) was introduced. Clinical response was documented, and antibiotic treatment was subsequently narrowed down to fusidic acid 500 mg orally (PO) three times daily with rifampicin 600 mg PO once daily. He received a total of 6 weeks of antibiotics after the prosthesis was removed, and eventual reimplantation on POD90. Cultures obtained during this surgery were all negative.

Six months after reimplantation, the patient presented with fever, shivering and right knee pain. Upon presentation, he was again in septic shock and on clinical examination he had a warm, red, and swollen right knee with a clear cellulitis extending to the leg. Blood tests showed a leukocyte count of 16,400 cells/μL [4,000–11,000 cells/μL], CRP 310 mg/L [0–10 mg/L]. Arthrocentesis revealed opaque fluid, with 120,338 leukocytes/μL (98% neutrophils), 193,306 erythrocytes/μL, and direct examination showed gram-positive cocci. The patient was quickly taken to the OR where a DAIR was performed and treatment with cefazoline 2 g three times daily IV and vancomycin 1 g twice daily IV was initiated post-operatively. Blood cultures obtained on admission and perioperative cultures became positive for *S. dysgalactiae* subsp. *equisimilis*. Although a chronic, insufficiently treated infection was suspected, in view of the cellulitis with lymphedema and chronic vein insufficiency, the origin of this second episode was considered to be the cellulitis with *S. dysgalactiae* bacteremia and secondary seeding. A DAIR was therefore a valid option, particularly after considering the recent implantation of a revision implant.

In order to confirm the source of this infection (relapse or new infection), we performed a genomic analysis of the strains GE-044 and GE-045 (**Table 1**) recovered from the periprosthetic purulent aspirate of the first and second episode, respectively. Genomic sequences were generated in the same sequencing run on an Illumina iSeq 100 benchtop system with 2 × 151 cycles. Comparison of sequence contigs revealed that the two strains had an average sequence identity (ANI) (7) of only 98.81%, while each strain showed higher, >99% ANI to several *S. dysgalactiae* subsp. *equisimilis* isolates available in the NCBI database (https://www.ncbi.nlm.nih.gov/assembly). The seven MLST alleles of strains GE-44 (*gki*_3, *gtr*_3, *murI*_2, *mutS*_8, *recP*_9, *xpt*_6, *atoB*_6) and GE-45 (*gki*_3, *gtr*_2, *murI*_4, *mutS*_2, *recP*_20, *xpt*_1, *atoB*_3) perfectly matched (100% alignment and 100% identity) those of sequence types ST-20 and ST-55, as revealed using the BIGSdb software at the *S. dysgalactiae* MLST website (https://pubmlst.org/sdysgalctiae/) (8). *gki*_3 was the only allele common to both isolates. A single ST-55 strain previously reported in the PubMLST database was collected in Europe (Portugal). Eleven ST-20 type strains already present in the PubMLST database originated from four continents (Asia, Australia, Europe, North

TABLE 1 | *S. dysgalactiae* strains isolated in this study.

Strain	Isolation date (month-year)	Total length (nt); number of contigs >500 nt[a]	Sequencing depth	Best ANI hit; strain; GenBank assembly accession	Sequence type
GE-044	03-2018	2,067,268; 74	52 ×	99.687; *S. dysgalactiae* subsp. *equisimilis* UT_4242_AB; GCA_001682815.1	ST-20
GE-045	12-2018	2,023,504; 75	53 ×	99.957; *S. dysgalactiae* subsp. *equisimilis* T642; GCA_002094215.1	ST-55

[a] *Sequencing data were assembled using SPAdes (6).*

America). The results of our genomic analysis suggest a new infection rather than relapse.

Antibiotic treatment was transitioned to penicillin G 4 MioU every 4 h IV on day 1 after surgery for 2 weeks with good response and hospital discharge by day 15 on clindamycin 900 mg three times daily PO for 4 additional weeks, for a total of 6 weeks of antibiotic treatment post-surgical intervention. Subsequently, the patient was put under suppressive antibiotic therapy with penicillin V 0.5 MioU twice daily considering the high risk of recurrence and a second severe streptococcal infection within 6 months. At 1 year post-surgical procedure, the patient had no further relapse.

DISCUSSION

The management of peri-prosthetic joint infections remains challenging and requires consideration of several parameters. In the context of chronic infections, it is preferable to exchange the prosthesis (one- or two-stage approach), because of the presence of a mature biofilm, that may be almost impossible to sterilize without changing the material (9). On the other hand, in the setting of acute infection or when the patient's condition does not allow it (e.g., comorbidities, age, etc.), a DAIR may be preferable (10, 11).

The time between arthroplasty and infection should also be considered, historically divided into early (within the first 3 months), delayed (between 3 and 12 months) and late (>12 months) post-operative infections. For instance, early post-operative infections are usually due to more virulent bacteria, such as *Staphylococcus aureus* and gram-negative bacilli. During the delayed post-operative period, infections are mainly due to less virulent bacterial pathogens, including *Cutibacterium acnes* and *Staphylococcus epidermidis*, among others. Finally, during the late post-operative period infections may be due to low virulent pathogens, but also due to direct inoculation and/or hematogenous seeding in the setting of a concomitant transient or sustained bacteremia (12).

In this case report, despite the isolation of the same type of organism in two episodes of PJI within a period of 9 months, the second episode represented a new infection, as suggested by: (i) the clinical presentation compatible with cellulitis of the leg, favored by chronic lymphedema and venous insufficiency which are known risk factors (13), with bacteremia and consequently a hematogenous PJI and (ii) a genomic analysis, which revealed that the two isolates collected at a 9 month interval were genetically distant. Notably, streptococci are the second most frequent cause of PJI of hematogenous origin after *S. aureus* (14). Therefore, because of the hematogenous origin, a management by DAIR with exchange of moving parts instead of complete exchange of prosthesis was considered, thus a new surgery could be avoided. Next-generation sequencing (NGS) provides better resolution for strain differentiation than other molecular methods. Starting from a cultured isolate, or directly from a clinical specimen, genomic sequence of a pathogen may be obtained and analyzed in <30 h (15) when NGS is implemented as a routine procedure. While direct NGS of a clinical specimen greatly reduces the overall turnaround time (by bypassing the need to culture bacteria), the completeness of the genomic sequence obtained for a given pathogen depends not only on the sequencing depth but also on the efficacy of human DNA removal, bacterial load and the nature of infection (monomicrobial vs. polymicrobial or monoclonal vs. polyclonal). In the present research study, the reagent cost of NGS for genomic testing of two samples was ∼700 CHF. This figure does not take into account labor costs and infrastructure (NGS and computational) investment. Implementing of NGS in routine diagnostics may substantially reduce the cost per sample by multiplexing more samples in the same sequencing run; labor costs for data analysis and interpretation will depend on the computational resources, the level of automation of bioinformatics processes, the availability of curated databases and the nature of each individual clinical case.

Suppressive antibiotic therapy was administered in this patient presenting with a second *S. dysgalactiae* infection with the necessity of hospitalization in the intensive care unit. According to Thomas et al., penicillin as a suppressive treatment is effective in preventing recurrent cellulitis, although the protective effect gradually decreases once treatment is stopped (16). Furthermore, streptococcal PJIs treated with DAIR have a high recurrence rate, with 42.1% treatment failure (17). This high rate of failure has led some experts to suggest suppressive treatment for at least 1 year for streptococcal infections. Additional data suggest that suppressive antibiotic therapy may be associated with better outcomes in streptococcal PJI (93 vs. 57%, $p = 0.002$, median follow up of 13 months, range 0.5–111 months) (18). Although long-term follow-up data are not available for this patient, he remained without recurrent episodes for at least 1 year on low-dose penicillin secondary suppressive prophylaxis treatment.

CONCLUSION

Identification of the same organism in recurrent PJIs, found in 31% of cases (19) is commonly considered as indicative of either chronic or relapsed infections. Our case report, based on NGS data analysis, illustrates the fact that recurrent infections due to same bacterial pathogens could represent a new infection, which could have significant implications in the management of those patients. A multidisciplinary approach, including infectious disease, microbiology, and orthopedic surgery specialists, to take into account the case as a whole and determine the best management strategy is required. Genomic sequencing, by the virtue of precisely determining genetic relatedness of sequentially collected clinical isolates from the same patient, can occasionally be the determinant factor for choosing the best approach. In fact, this case report is the first to demonstrate a clear contribution that genomic sequencing can make to the strategy of peri-prosthetic joint infections management.

DATA AVAILABILITY STATEMENT

Assembly contig fasta files of stains GE-044 and GE-045 were uploaded to the *S. dysgalactiae* PubMLST BIGSdb database (https://pubmlst.org/sdysgalctiae/) (8).

ETHICS STATEMENT

According to hospital protocol, no formal ethics approval was required. The patient agreed and provided written informed consent for publication of this case report.

AUTHOR CONTRIBUTIONS

TP, VL, DS, and JS analyzed and interpreted patient data. NG, MG, and AC performed the experiments. NG and VL analyzed the genomics data. TP and JS wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

CASE REPORT
published: 31 July 2020
doi: 10.3389/fmed.2020.00342

The Potential Innovative Use of Bacteriophages Within the DAC® Hydrogel to Treat Patients With Knee Megaprosthesis Infection Requiring "Debridement Antibiotics and Implant Retention" and Soft Tissue Coverage as Salvage Therapy

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OPEN ACCESS

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Specialty section:
This article was submitted to Infectious Diseases-Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *08 April 2020*
Accepted: *09 June 2020*
Published: *31 July 2020*

Citation:
Ferry T, Batailler C, Petitjean C, Chateau J, Fevre C, Forestier E, Brosset S, Leboucher G, Kolenda C, Laurent F and Lustig S (2020) The Potential Innovative Use of Bacteriophages Within the DAC® Hydrogel to Treat Patients With Knee Megaprosthesis Infection Requiring "Debridement Antibiotics and Implant Retention" and Soft Tissue Coverage as Salvage Therapy. Front. Med. 7:342. doi: 10.3389/fmed.2020.00342

Infection is the most dramatic complication in patients with knee megaprosthesis. Its management is more complex in comparison with patients with primary arthroplasty, with a high risk of relapse. Lytic bacteriophages are considered to have a high potential in patients with prosthetic joint infection as it has been demonstrated that they have a synergistic anti-biofilm activity with antibiotics. The Defensive Antibacterial Coating (DAC®) hydrogel is a hydrogel available in the market that has been designed to prevent the adherence of bacteria on a prosthetic joint and to have the ability to transport and release anti-bacterial substances such as antibiotics. We report here the case of a patient with a catastrophic relapsing *Staphylococcus aureus* knee megaprosthesis infection without prosthesis loosening. We firstly perform phage susceptibility testing of the patient's strain to select an active cocktail, under the supervision of the French health authority. Then, we performed, as salvage therapy, a debridement and implant retention procedure with application of a selected cocktail of bacteriophages that was prepared extemporaneously within the DAC® hydrogel. A free flap for soft tissue coverage was required and empirical antibiotic treatment was started immediately after the surgery. Unfortunately, at 5 days after the surgery, while the local aspect of the surgical site was favorable, the patient developed myocardial infarction which required emergency stenting and dual antiplatelet therapy that were rapidly associated with

bleeding at the surgical site, leading to a new prosthesis exposition. As a consequence, a transfemoral amputation was finally performed several months later. We also evaluated *in vitro* the impact of DAC® hydrogel on bacteriophage activity and showed that the selected phages were released very rapidly from the DAC® hydrogel, and then their titers were stable for at least 6 h. This case demonstrated the feasibility of the use of bacteriophages within a hydrogel to treat patients for knee megaprosthesis infection during a debridement procedure. The implementation requires identification of the pathogen before the debridement in order to perform phage susceptibility testing of the patient's strain and to identify a hospital pharmacist who will accept to do the preparation and to take the responsibility of the magistral preparation.

Keywords: prosthetic joint infection, bacteriophage, phage therapy, hydrogel, megaprosthesis

INTRODUCTION

Knee megaprosthesis is used for patients with bone cancer or trauma that requires distal femur resection (1). Infection, which occurs in 3–40% in such patients, is one of the most terrible complications (2, 3). Its management is more complex in comparison with patients with primary arthroplasty as: (i) the "Debridement Antibiotics and Implant Retention" (DAIR) procedure is potentially associated with a higher rate of failure and (ii) one- or two-stage exchange is associated with higher morbidity and loss of function, especially if there is no loosening of the implants. DAIR is usually contraindicated in patients with chronic prosthetic joint infection (PJI) or in patients with PJI with prosthesis exposition.

Lytic bacteriophages rapidly kill *in vitro* specifically the targeted bacteria and self-replicate in an exponential and self-sustained reaction (4). They are considered to have a high potential in patients with PJI as it has been demonstrated that they have a synergistic anti-biofilm activity with antibiotics (5). In a few patients with relapsing chronic PJI for whom explantation was not possible, we previously performed DAIR and used a selected cocktail of bacteriophages that was injected into the joint as compassionate therapy, with a good clinical response (6). This approach is not a simple option for patients with infected knee megaprosthesis, especially in the case of prosthesis exposition that require soft tissue coverage. Indeed in this critical clinical situation, the surface of the infected joint is large, and there is no anatomical joint to contain the phages administered during DAIR surgery.

The Defensive Antibacterial Coating (DAC®) hydrogel (Novagenit, Mezzolombardo, Italy) is a hydrogel composed of two bioresorbable polymers (hyaluronic acid and poly-lactic acid) and that has been designed to prevent the adherence of bacteria (that are usually attracted by the hydrophobic surface of the implant) and to have the ability to transport and release anti-bacterial substances such as antibiotics. In a prospective observational multicenter study in patients for whom primary arthroplasty or prosthesis revision was performed, the use of the DAC® hydrogel was associated with a significant reduction of the rate of post-operative infection (7). In patients with PJI, two studies with a limited number of patients revealed that the use of the DAC® hydrogel during a one- or two-stage exchange may provide better infection control (8, 9).

We report here the case of a patient with a catastrophic relapsing *Staphylococcus aureus* knee megaprosthesis infection. The patient presented with prosthesis exposition, fistula, and purulent discharge, but without prosthesis loosening. We performed, as salvage therapy, a DAIR with the application of a selected cocktail of lytic bacteriophages within the DAC® hydrogel (as magistral preparation) after susceptibility testing of the phages against the patient's strain, and we finally performed a free flap for soft tissue coverage. We also evaluated the impact of DAC® hydrogel on bacteriophage activity.

CASE DESCRIPTION

A 49-year-old man had a past history of trauma in 2012 with right scapula fracture, sternoclavicular luxation complicated by brachial plexus palsy, and open left distal femoral fracture. A knee megaprosthesis was used for reconstruction in 2013. As the patient developed skin and knee extensor necrosis, patellectomy and gastrocnemius skin and soft tissue flap were performed. In 2015, a multidrug-resistant *Staphylococcus epidermidis* PJI was diagnosed, and a two-stage exchange of the megaprosthesis was performed. Unfortunately, in 2016, a purulent discharge appeared. As methicillin-susceptible *S. aureus* (only resistant to penicillin) grew in culture from the discharge sample, but also from the puncture of an abscess in close contact with the prosthesis, clindamycin was prescribed as suppressive therapy. A new change of the megaprosthesis was considered to be not feasible, and the patient refused transfemoral amputation due to the terrible functional consequences as he still had the right brachial plexus palsy, making it impossible to walk with crutches. Finally, the patient developed two fistula (**Figure 1A**), with purulent discharge and prosthesis exposition (**Figures 1B,C**), without prosthesis loosening on X-ray (**Figure 1D**). We proposed, as salvage therapy, to perform a DAIR with local application of a selected cocktail of lytic bacteriophages under the supervision of the French National

Agency for Medicines and Health Products Safety (ANSM) and in collaboration with the hospital pharmacist. Indeed phage therapy is not yet approved by the European Medicines Agency but "compassionate" use is however possible in France, under the supervision of ANSM, if the patient's status matches with article 37 of the Declaration of Helsinki, i.e., if proven interventions do not exist or if other known interventions have been ineffective (10). The final mix of bacteriophages has to be performed extemporaneously, under the responsibility of the hospital pharmacist, as this preparation becomes a "compounded" drug product, also called "magistral" preparation in Europe. In this particular case, the application of the mix of bacteriophages in a liquid formulation was complex as the infection was not limited to the joint but also concerned a large part of the femoral compartment of the megaprosthesis. Moreover, as the patient also had a large skin and soft tissue defect, with previous local flap, we planned to perform a free deep inferior epigastric perforator (DIEP) flap (i.e., taking skin and soft tissue from the abdomen to cover the megaprosthesis) (11). Considering all these elements, a carrier such as a gel was essential for phage application to keep the phages at the implant surface during the skin and soft tissue coverage. We proposed to use the DAC® hydrogel, which is available in the market and usable for patients with PJI.

MATERIALS AND METHODS

Phages

The two phages, PP1493 and PP1815 [both *Caudovirales* (tailed bacteriophages), *Herelleviridae* family], administered to the patient were selected from the Pherecydes phage bank. These bacteriophages, which were still in a development process, were not yet approved as drugs. Although the manufacturers followed the same processes as those established by the Good Manufacturing Practice (GMP) guidelines, they were produced in a research and development (R&D) laboratory (not GMP). The ANSM carefully reviewed the quality control tests applied to these batches, in collaboration with the hospital pharmacist and before the salvage therapy.

Phagogram

The efficiency of these bacteriophages against the patient's strain was tested using the plaque assay to calculate the efficiency of plating (EOP) score and looking at the impact of the phages on the bacterial growth kinetics, hereafter referred to as kinetics assay. Plaque assay was based on the visualization of bacterial lysis when serial 10-fold dilutions of phages were spotted on solid medium containing either the patient's strain or the reference strain (spot plaque assay). When plaque-forming units (PFU) were observed, the EOP score was calculated by dividing the phage titer on the patient's strain by the phage titer on its reference strain showing the highest titer. The closer to 1 the score is, the more efficient the phage is. For the kinetic assay, the patient's strain was inoculated in a 96-well plate at a starting concentration of 1×10^6 colony-forming units/ml with or without phages. The activity of each phage was tested individually at three different concentrations to obtain theoretical multiplicities of infection (MOI, ratio of phage/bacteria) equal to 1, 10, and 100 phages per bacteria and classified as low, intermediate, or high MOI. Bacterial growth was monitored over time by measuring the OD_{600nm}.

Impact of DAC® Hydrogel on Bacteriophage Activity

The suspension of phages was prepared by diluting 1 ml of each phage in 4 ml of water for injection (WFI). Then, 5 ml was added to the DAC® powder. Once homogenous, the hydrogel containing the phages was incubated for 10 min at room temperature. Once it turned solid, it was transferred into 10 ml of Dulbecco's phosphate-buffered saline (DPBS) and incubated at 37°C for 6 h. The phage titers were controlled in the 5-ml dilution (before powder addition) as well as in the DPBS at T_0, $T_{0.5h}$, T_{1h}, T_{2h}, T_{4h}, and T_{6h}.

RESULTS

Phagogram

The EOP assay revealed that phage PP1493 was active and very efficient on the patient's strain with visualization of PFU (EOP score of 6.4×10^{-1}). The PP1815 phage was also active, with a partial bacterial lysis of the lawn where the phages were spotted. However, no PFU was observed. The minimum concentration of the spotted phages leading to the spot partial lysis was 5.09×10^5 PFU/ml. In the kinetic assay, we observed a complete inhibition of the bacterial growth with PP1493 whatever the phage concentration was. For PP1815, the highest phage dose (1×10^9 PFU/ml, corresponding to "high" MOI) also led to a total control of the bacterial growth, while the intermediate phage dose (1×10^8 PFU/ml, corresponding to "intermediate" MOI) led to a partial control of the bacterial population, and the lowest phage dose (1×10^7 PFU/ml, corresponding to "low" MOI) had no effect (**Figure 2A**). Even if PP1815 seemed to be less active than PP1493, we concluded that both of them were active against this *S. aureus* strain and have to be mixed to avoid the acquisition of phage resistance.

Impact of DAC® Hydrogel on Bacteriophage Activity

PP1493 and PP1815 were diluted within WFI, which is recommended by the DAC® hydrogel supplier. Before the DAC® powder addition, PP1493 and PP1815 were at 8.0×10^9 and 7.3×10^9 PFU/ml, respectively. In the DPBS, upon transfer, the phage titers were 1.7×10^8 and 1.3×10^8 PFU/ml, respectively. Between $T_{0.5h}$ and T_{6h}, the titers ranged between 3.1×10^8 and 9.3×10^8 PFU/ml for PP1493 and between 1.6×10^9 and 2.2×10^9 PFU/ml for PP1815, respectively (**Figure 2**). These results indicated that PP1493 and PP1815 were released very rapidly from the DAC® hydrogel, and then their titers were stable for at least 6 h (**Figure 2B**).

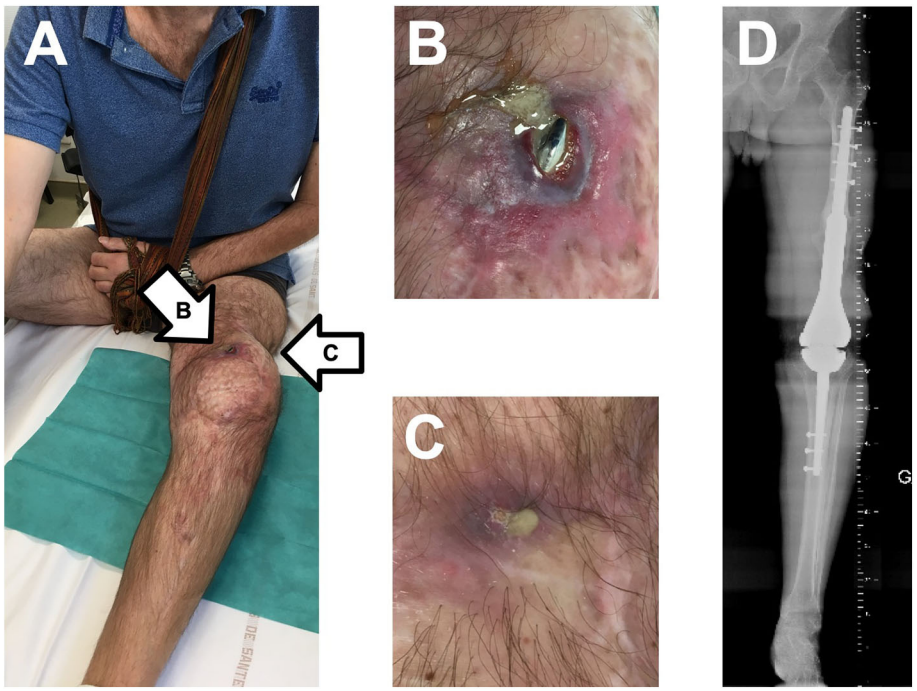

FIGURE 1 | Clinical and X-ray status of the patient at baseline, with two fistulas regarding the femoral part of the megaprosthesis **(A)**, with purulent discharge and prosthesis exposition **(B,C)**, and without prosthesis loosening on X-ray **(D)**.

DIAGNOSTIC ASSESSMENT, THERAPEUTIC INTERVENTION, FOLLOW-UP, AND OUTCOMES

We planned the therapeutic intervention under the supervision of the ANSM, and the patient signed a written consent. Two vials containing 1 ml of 10^{10} PFU/ml suspension of each bacteriophage in DPBS were received by our hospital pharmacist. Reconstitution of the DAC® hydrogel was performed according to the manufacturer's instructions. The prefilled syringe, containing 300 mg of sterile DAC® powder, was filled extemporaneously at the pharmacy, under sterile conditions, with a solution of 5 ml sterile water for injection, and 1 ml of each bacteriophage (10^{10} PFU/ml) was added instead of adding antibiotics. We performed open DAIR, which revealed, as expected, large suppuration in close contact to the femoral part of the prosthesis and into the joint. Several samples were taken for bacterial culture. Synovectomy and excision of infected tissue were performed, followed by a large irrigation with saline using a pulse lavage system. After the DAIR (**Figure 3A**), we applied the magistral phage preparation within the DAC® gel on the megaprosthesis surface (**Figures 3B,C**). Finally, the skin and soft tissue coverage with the DIEP free flap was performed (**Figure 3D**). Intravenous empirical antibiotic treatment with daptomycin (850 mg, one injection/day) and tigecycline (100 mg as initial dose, followed by 50 mg injected every 12 h) was started immediately after the surgery, pending the microbiological results, as the patient previously experienced a multidrug-resistant *S. epidermidis* infection. *S. aureus* grew in all microbiological peroperative samples, with the same antibiogram than that obtained before the surgery, except for a subpopulation of *S. aureus* that acquired erythromycin and clindamycin resistance. Unfortunately, at 5 days after the surgery, while the local aspect of the surgical site was favorable, the patient developed chest pain in relation with myocardial infarction. A coronarography was performed and revealed underlying atherosclerosis which, up to now, has been asymptomatic. An emergency stenting with dual antiplatelet therapy with salicylic acid and ticagrelor (a P2Y12 receptor antagonist) was required and prescribed. Bleeding at the surgical site rapidly occurred. At 25 days after the surgery, the free flap was perfectly integrated (without any sign of necrosis), but the bleeding persisted though the scar and led to a new prosthesis exposition. A local debridement, performed 1 month after the phage administration, revealed a hematoma under the free flap that communicated outside along the prosthesis exposition infection. A bacterial culture of the hematoma revealed superinfection with *Pseudomonas aeruginosa*, *Achromobacter* spp., and *Proteus mirabilis* in culture. No *S. aureus* grew in culture. Daptomycin was continued and tigecycline was replaced with ceftazidime, ciprofloxacin, and rifampin. As a discharge persisted, a new debridement was performed at 1 month later, without any bacteria in culture. Unfortunately, a new prosthesis exposition occurred and the patient decided to completely stop the antimicrobial therapy. At 1 year after the phage administration, a transfemoral amputation was finally performed, whereas the prosthesis exposition persisted, with a discharge. During amputation, the surgical samples revealed

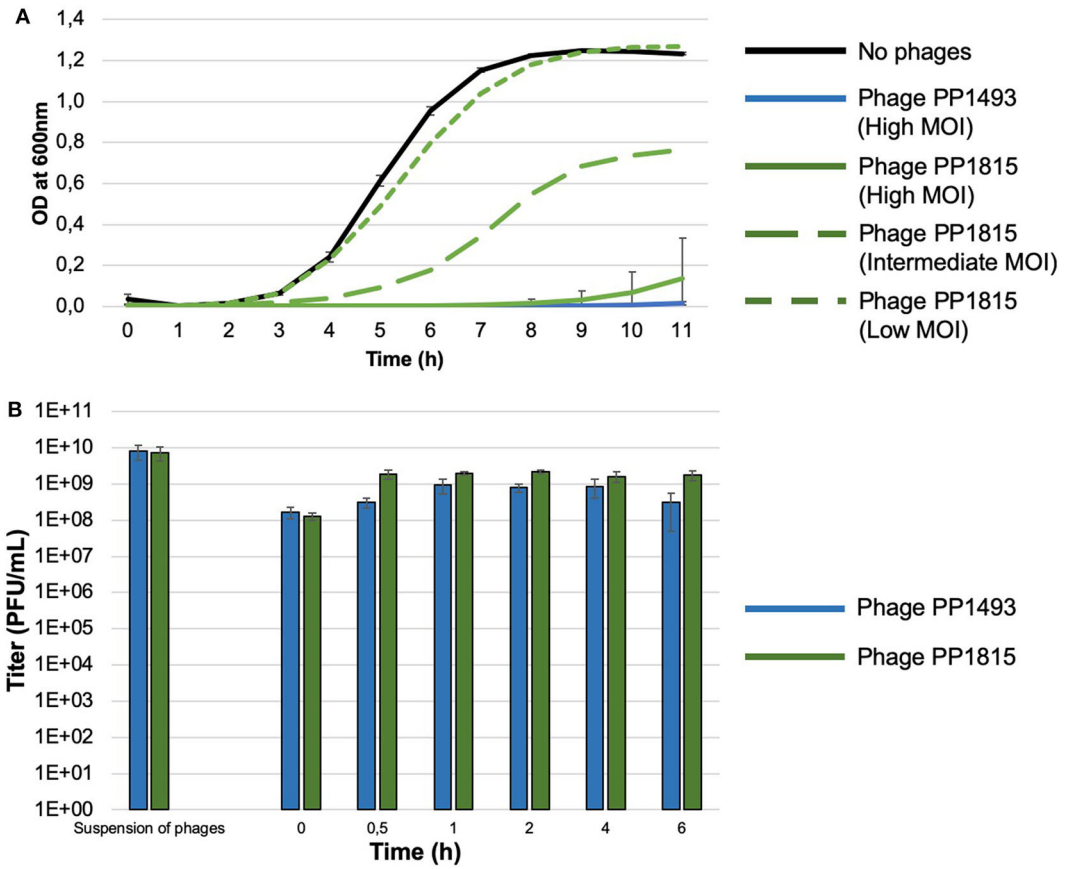

FIGURE 2 | (A) Kinetic assay of phages PP1493 and PP1815 on a patient's strain at different multiplicities of infection (MOI). The X-axis represents the time and the Y-axis indicates the OD at 600 nm. The patient's strain growth without phage is represented with a full black line and with PP1493 with a blue line, no bacterial growth was observed whatever the MOI (only the high MOI is represented); with PP1815 (green lines), the bacterial growth was MOI dependent, with inhibition of the bacterial growth only at high MOI. **(B)** Impact of DAC® hydrogel on bacteriophage activity. The X-axis represents the time in hours and the Y-axis indicates the titer in PFU/ml. PP1493 is shown in blue, and PP1815 is shown in green.

a polymicrobial infection with anaerobic flora, *Streptococcus anginosus*, *Finegoldia magna*, *P. mirabilis*, and *S. aureus* in culture (only resistant to penicillin and erythromycin; this latter strain was not genetically related to the first isolate as it belonged to the clonal complex 398, whereas the first strain belonged to the clonal complex 30). A pathology analysis of the bone did not reveal infiltration by inflammatory cells.

DISCUSSION

The risk of infection after the implantation of megaprosthesis is particularly high, especially due to the accumulation of several risk factors such as iterative past surgeries, extended incision, duration of surgery, implant's surface size, and chemotherapy or radiotherapy in oncologic patients. A conservative approach is a huge challenge in patients with infection of knee megaprosthesis without loosening. Indeed its management is considerably more complex in comparison with the management of primary prosthesis. The DAIR procedure followed with the administration of systemic antibiotics is a therapy generally offered to patients with acute or late acute PJI. Unfortunately, DAIR frequently fails in patients with knee megaprosthesis infection, at least in part due to the persistence of the pathogen on the implant's surface. The use of adjuvant agents that could have local anti-biofilm activity during the DAIR procedure seems to be of importance to control the infection in such patients and could facilitate the success of a potential subsequent suppressive antibiotic treatment (1–3, 12–20).

Bacteriophages are candidates to be used locally in patients with PJI to target the biofilm. Several past and recent data demonstrated that bacteriophages have an antibiofilm activity (21–23). The antibiofilm activities of the two phages used to treat the present case were also evaluated *in vitro* in a publication from our group. The activity of these two bacteriophages against the biofilm-embedded *S. aureus* was dose dependent. In addition, synergistic effects were observed when the bacteriophages were combined with antibiotics used at the lowest concentrations (5).

The administration of bacteriophages in patients with knee megaprosthesis is also conditioned by the use of an adequate

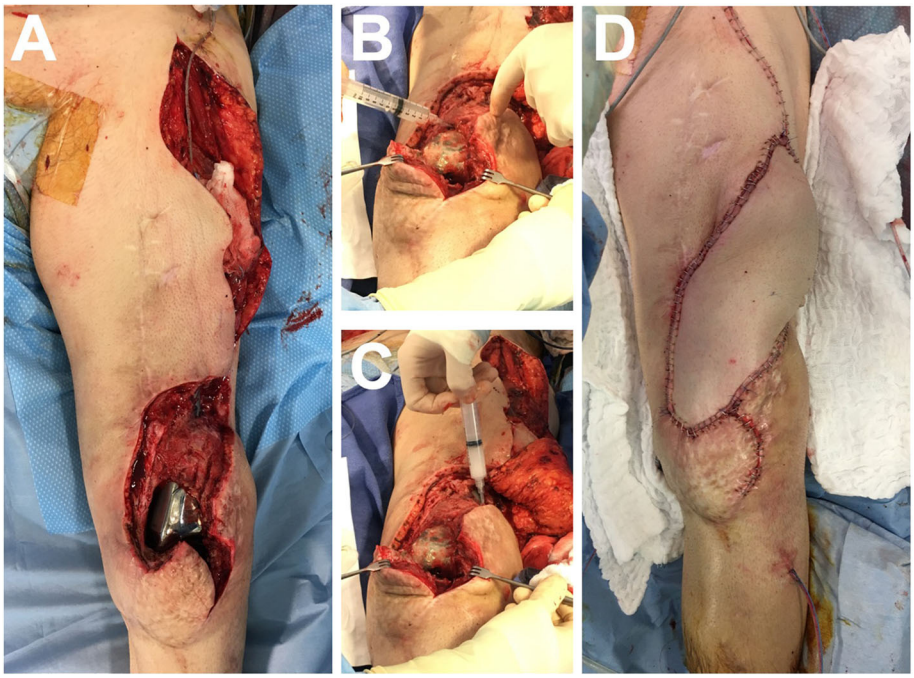

FIGURE 3 | Peroperative pictures after the "Debridement Antibiotics and Implant Retention" **(A)**, during the application on the megaprosthesis surface of the magistral preparation containing the phages within the DAC® hydrogel **(B,C)**, and skin and soft tissue coverage with the deep inferior epigastric perforator free flap **(D)**.

dosage form that could cover the implant surface and deliver the phages locally. The gel formulation could perfectly fulfill these conditions, but it is important to demonstrate the stability of the phages within the gel and to evaluate its capacity to release phages. The DAC® gel is of interest as it is a CE-marked medical device approved to act as a physical barrier against bacterial colonization of the implant surface. Moreover, this hydrogel could be mixed with a bioactive agent, such as antibiotics, that could complement the gel's primary function. The manufacturer notifies that including a bioactive agent has to be taken at the surgeon's discretion, in the best interest of the patient under treatment. Finally, this gel has been used in several previous studies (7–9).

We demonstrated the *in vitro* activity of the phages on the patient's strain. Although PP1815 was only active at high MOI, according to the kinetic assay, it has been estimated that, thanks to the debridement, the adequate ratio of phage/bacteria could be achieved during the surgery. We also showed that phages can be released from the DAC® hydrogel and that it has no major impact on PP1493 and PP1815 activity. The association of DAC® hydrogel and bacteriophages seemed compatible; thus, we used purified selected phages into the DAC® hydrogel to treat this patient.

Unfortunately, post-operative myocardial infarction (that was not considered as a phage-related serious adverse event as the patient had previous asymptomatic atherosclerosis lesions) led to the formation of a hematoma under the free flap with important bleeding and prosthesis exposition, with secondary infection, and finally with the performance of an amputation. We do not think about a putative interaction between the antiplatelet drugs and the phages administered locally as the antiplatelet treatment was prescribed days after the phage administration and as bleeding is quite common with these drugs if they are prescribed after a surgery. During the amputation at 1 year after the phage administration, whereas the patient stopped all antibiotics for several months, *S. aureus* was again detected in culture but belonged to another clonal complex. It would be a new contamination of the exposed prosthesis, with a different *S. aureus* strain.

Concerning the safety of local phage administration within the gel, the patient developed myocardial infarction, with underlying atherosclerosis that was not known before surgery. We then observed the occurrence of hematoma that led to new prosthesis exposition, in relation with the prescribed antiplatelet treatment. These "adverse events" were not considered to be in relation with the phage administration.

This case demonstrated the practical feasibility of the use of bacteriophages within a hydrogel to treat patients for knee megaprosthesis infection during a DAIR procedure. The implementation requires identifying the pathogen before the DAIR, performing phage susceptibility testing of the patient's strain on the supervision of ANSM, and identifying a hospital pharmacist who will accept to do the preparation and to take the responsibility of the magistral preparation.

PERSPECTIVE

This is a potentially innovative approach to target the biofilm in patients with megaprosthesis knee infection. However, a

prospective study including patients with such infection is complex to set up as there are some heterogeneity between the type of megaprosthesis, the clinical presentation of the infection, and the type of pathogen involved. An animal model of PJI demonstrating the microbiological and the clinical response to this therapeutic approach could be the next step.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethic committee of Hospices Civils de Lyon. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TF managed the patient, directly interacted with the French Health authority and the companies to propose the study plan and to obtain the bacteriophages and the gel, and wrote the manuscript. CP and CF performed the preclinical stability study. GL prepared the phages. CB, JC, and SL performed the surgery. FL and CK performed the microbiological diagnosis. EF also participated in patient care. All authors participated in the literature review and the improvement of the manuscript.

FUNDING

This study was based on the care of patients managed in our regional reference center. No dedicated funding was required. The patients were treated under the supervision of the French Health ministry. Pherecydes Pharma provided the bacteriophages, and Adler Ortho provided the DAC hydrogel.

ACKNOWLEDGMENTS

Lyon Bone and Joint Study Group (list of collaborators): Coordinator: Tristan Ferry; Infectious Diseases Specialists—Tristan Ferry, Florent Valour, Thomas Perpoint, Patrick Miailhes, Florence Ader, Sandrine Roux, Agathe Becker, Claire Triffault-Fillit, Anne Conrad, Cécile Pouderoux, Nicolas Benech, Pierre Chauvelot, Marielle Perry, Fatiha Daoud, Johanna Lippman, Evelyne Braun, and Christian Chidiac; Surgeons—Elvire Servien, Cécile Batailler, Stanislas Gunst, Axel Schmidt, Matthieu Malatray, Elliot Sappey-Marinier, Michel-Henry Fessy, Anthony Viste, Jean-Luc Besse, Philippe Chaudier, Lucie Louboutin, Quentin Ode, Adrien Van Haecke, Marcelle Mercier, Vincent Belgaid, Arnaud Walch, Sébastien Martres, Franck Trouillet, Cédric Barrey, Ali Mojallal, Sophie Brosset, Camille Hanriat, Hélène Person, Nicolas Sigaux, Philippe Céruse, and Carine Fuchsmann; Anesthesiologists—Frédéric Aubrun, Mikhail Dziadzko, and Caroline Macabéo; Microbiologists—Frederic Laurent, Laetitia Beraud, Tiphaine Roussel-Gaillard, Céline Dupieux, Camille Kolenda, Jérôme Josse; Pathologist; Marie Brevet, and Alexis Trecourt; Imaging—Fabien Craighero, Loic Boussel, Jean-Baptiste Pialat, and Isabelle Morelec; PK/PD Specialists—Michel Tod, Marie-Claude Gagnieu, and Sylvain Goutelle; and Clinical Research Assistant and Database Manager—Eugénie Mabrut.

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Conflict of Interest: CP and CF are employed by the commercial company Pherecydes Pharma.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 17 September 2020
doi: 10.3389/fmed.2020.00527

Synergistic Effects of Pulsed Lavage and Antimicrobial Therapy Against *Staphylococcus aureus* Biofilms in an *in-vitro* Model

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: 15 January 2020
Accepted: 27 July 2020
Published: 17 September 2020

Citation:
Poilvache H, Ruiz-Sorribas A, Sakoulas G, Rodriguez-Villalobos H, Cornu O and Van Bambeke F (2020) Synergistic Effects of Pulsed Lavage and Antimicrobial Therapy Against Staphylococcus aureus Biofilms in an in-vitro Model. Front. Med. 7:527. doi: 10.3389/fmed.2020.00527

Background: Prosthetic joint infections (PJI) are difficult to treat complications of joint arthroplasty. Debridement with implant retention is a common treatment strategy and frequently involves the use of pulsed lavage (PL). However, PL effects on biofilms and antibiotic activity have been scarcely studied *in-vitro*. We report the effects of PL, vancomycin or flucloxacillin used independently or in combination against *Staphylococcus aureus* biofilms.

Methods: Biofilms of 3 methicillin-susceptible (MSSA) and of 3 methicillin-resistant (MRSA) *S. aureus* were grown on Ti6Al4V coupons in TGN (TSB + 1%glucose + 2%NaCl). After 24 h, PL was applied to half of the samples (50 mL saline from 5 cm). Samples were either reincubated for 24 h in TGN or TGN + flucloxacillin or vancomycin. Analyses included CFUs counts, biomass assays or fluorescence microscopy.

Results: PL transiently reduced bacterial counts by 3–4 Log_{10} CFU/coupon, but bacterial regrowth to baseline levels was seen after 24 h. At 20 mg/L, flucloxacillin reduced both the CFU counts (3 Log_{10} CFU/coupon) and biomass (−70%) in one MSSA only, while vancomycin had no effects against MRSA. PL combined with a 24 h reincubation with vancomycin or flucloxacillin at 20 mg/L was synergistic (−5 to 6.5 Log_{10} CFU/coupon; 81–100% biomass reduction). Fluorescence microscopy confirmed that PL removed most of the biofilm and that subsequent antibiotic treatment partially killed bacteria.

Conclusions: While PL only transiently reduces the bacterial load and antibiotics at clinically relevant concentrations show no or limited activity on biofilms, their combination is synergistic against MRSA and MSSA biofilms. These results highlight the need for thorough PL before antibiotic administration in PJI.

Keywords: biofilm, MRSA, MSSA, pulsed lavage, vancomycin, flucloxacillin, prosthetic joint infection

INTRODUCTION

Prosthetic Joint Infections (PJI), defined as infections involving joint replacement implants and the surrounding articular tissues, are devastating complications, affecting 0.5 to 2% of patients benefiting from hip or knee replacement (1, 2) and are among the most common causes of arthroplasty failures (3, 4).

These infections result from either a peri-operative contamination of the joint, generating acute (less than 4 weeks from the index surgery) or late infections, or an hematogenous seeding of bacteria to the joint following a bacteremia (2). The prevalence of the causative micro-organisms varies depending on the origin and interval from the index surgery, with *Staphylococcus aureus* being the most frequently isolated in cases of acute PJI, whereas coagulase-negative staphylococci and *Streptococci* spp. predominate late infections and hematogenous infections, respectively (5–8).

Infections by *S. aureus* are characterized by the rapid adhesion of bacterial cells to the implant surface, followed by the development of a self-produced extracellular matrix composed of poly-*N*-acetylglucosamine, extracellular DNA, proteins, and lipids, forming complex communities known as biofilms (6). The development of the biofilm induces phenotypic changes of the bacteria, which, combined with the isolating effects of the matrix, make bacteria tolerant to antibiotics at up to 1000x the minimal inhibitory concentration (MIC) observed in a planktonic state (9). This explains the limited success of antimicrobial therapy and the necessity for surgical strategies aiming to disrupt or remove the biofilm (2).

The Debridement, Antibiotics and Implant Retention (DAIR) strategy is often recommended for the treatment of acute PJI due to lower morbidity and costs than staged implant replacement. The surgical procedure consists in the open debridement of the infected joint, with the excision of necrotic tissues and a synovectomy, replacement of bearing surfaces if possible, followed by a thorough lavage of the joint space usually performed with a pulsed-lavage device which projects normal saline intermittently at pressures between 30 and 350 kPa (10, 11). However, DAIR presents a relatively high failure rate (16–57.4%), with a worse prognosis for patients infected with *S. aureus* (12–16). These failures may be partly explained by an inadequate removal of biofilms during the debridement surgery and their tolerance to antibiotics. However, only a few studies have looked into the effects of irrigation performed using standard pulsed lavage devices against *S. aureus* biofilms grown on metallic substrates, and none of these investigated the effects of its combination with antibiotics at clinically relevant dosages for systemic administration (17–20). The purpose of this study was to describe the effects of pulsed lavage and clinically relevant antibiotics used at recommended concentrations for systemic use, (i) in combination or (ii) independently, on the amount of cultivable cells, the biomass, and the microscopic aspects of MRSA and methicillin-susceptible *S. aureus* (MSSA) biofilms on titanium alloy coupons.

TABLE 1 | MIC (mg/L) values for the tested strains[a].

Strains		Oxacillin[b]	Flucloxacillin		Vancomycin	
		CA-MHB	CA-MHB	TGN	CA-MHB	TGN
MSSA	ATCC 25923	0.25	0.13	0.06	1	8
	578	0.25	0.25	0.13	2	8
	611	0.25	0.25	0.06	1	8
MRSA	ATCC 33591	>64	>64	>64	1	4
	749	>64	>64	>64	1	8
	676	>64	64	64	1	8

[a] *CLSI breakpoints values (in CA-MHB; mg/L): Flucloxacillin: N/A; Oxacillin: S≤2, R≥4; Vancomycin: S≤2, R≥16.*

[b] *used to check the MRSA character of the strain, according to CLSI guidelines (21).*

MATERIALS AND METHODS

Bacterial Strains

The laboratory strains ATCC 25923 and ATCC 33591 were used as references for MSSA and MRSA biofilms, respectively. Two MSSA clinical isolates (strains 578 and 611) and two MRSA clinical isolates (strains 676 and 749), collected from orthopedic device-related infections cases were also studied.

Antibiotics

Oxacillin (powder potency: 81.5%) was obtained as a microbiological standard from Sigma-Aldrich (Sigma-Aldrich Corp., Saint-Louis, MO, USA). Vancomycin (Vancomycin Mylan, powder potency: 97.5%, Mylan Inc, Canonsburg, PA, USA) and flucloxacillin (Floxapen, powder potency: 91.9%, Actavis Group, Hafnarfjördur, Iceland) were used as a powder for injection approved for human use in Belgium.

Susceptibility Testing

MICs were determined by broth microdilution in cation-adjusted Mueller-Hinton broth (CA-MHB, Sigma-Aldrich Corp., Saint-Louis, MO, USA) as per the Clinical & Laboratory Standards Institute protocol (21), and in Tryptic soy broth (VWR Chemicals, Leuven, Belgium) supplemented with 1% glucose (Sigma-Aldrich Co., Saint-Louis, MO, USA) and 2% NaCl (VWR Chemicals, Leuven, Belgium) (TGN) (**Table 1**).

Biofilm Culture

Biofilms were grown on titanium alloy Ti6Al4V coupons (Biosurface Inc., Bozeman, MT, USA) in order to mimic implant surface characteristics. These coupons are unpolished cylinders measuring 12.7 mm in diameter and 3.175 mm in height. The initial inoculum was prepared from bacteria grown overnight on Tryptic Soy Agar (VWR, Leuven, Belgium) (TSA), suspended in Phosphate Buffer Saline (PBS), adjusted to an optical density at 620 nm of 0.5 (CECIL 2021 spectrophotometer, CECIL, United-Kingdom) and diluted 1:100 in TGN, reaching a bacterial density of ∼6.5 $\log_{10}$ CFU/mL. Sterile coupons were incubated for 24 h at 37°C in 12 wells plates containing 2mL of bacterial suspension in TGN per well, under a continuous orbital shaking of 50 rpm in order to induce shear stress. Biofilms reached maturity after 24 h (i.e., no meaningful change in biomass or

bacterial counts was observed when prolonging the incubation for 48 h, **Supplementary Data Figure 1**) and used for testing the treatments.

Biofilm Treatments

Irrigation

Half of the biofilm samples (referred to as irrigation samples) were irrigated with 50 mL of sterile saline (Baxter International Inc, Deerfield, IL, USA) from 5 cm, using Interpulse battery-powered irrigation devices (Stryker Co., Kalamazoo, MI, USA). The Interpulse were fitted with soft tissue tips, delivering the sterile saline at a flow rate of 700 ml/min and a pressure of 68.95–82.74 kPa or 10–12 PSI (manufacturer's data). The samples were then rinsed twice in sterile PBS before allocation to one of the subgroups. The other half of the samples (referred to as control samples) were rinsed twice in sterile PBS before being allocated to one of the subgroups.

Antibiotic Treatments

Control and irrigation coupons were allocated to one of the subgroups: immediate analysis (T0); 24 h reincubation in TGN (T24h—TGN); 24 h reincubation in TGN containing antibiotic at MIC (T24h–MIC); 24 h reincubation in TGN containing therapeutic concentration of antibiotic (T24h–ThC) according to the flowchart shown in **Figure 1**. Reincubations were done at 37°C, under a continuous rotating movement at 50 rpm. As antibiotics, flucloxacillin was used for MSSA biofilms, considering as therapeutic concentration 20 mg/L, an estimate of the serum concentration 3 h after injection when administered 2 g IV four times daily, as inferred from pharmacokinetic data (22). MRSA biofilms were reincubated with vancomycin at a therapeutic concentration of 20 mg/L, corresponding to target trough serum concentration for bone and joint infections (23).

Biofilm Analysis

CFU Counts

Coupons were individually placed in 15 mL conical tubes (Greiner Bio-One International GmbH, Kremsmünster, Austria) containing 2 mL of sterile PBS. The tubes were vortexed for 30 s, sonicated for 5 min (Branson 5510 Ultrasonic bath, Emerson Electric, Saint-Louis, MO, USA) and vortexed 30 s again. Aliquots of the supernatant were serially diluted, plated on TSA and incubated for 24 h at 37°C. CFU counts were performed using an automated method [image acquisition using Gel Doc XR+ and image processing using Quantity One (BioRad, Hercules, CA, USA)].

Biomass Quantification

After drying overnight at 60°C, coupons were stained with 1 mL of 1% crystal violet (Sigma-Aldrich Corp., Saint-Louis, MO, USA). After eliminating the excess of dye by rinsing the samples with deionized water, biofilm-bound crystal violet was resolubilized in 1 mL of a 66% acetic acid (Merck KGaA, Darmstadt, Germany). The coupons were then removed from the solution and the absorbance was read at 570 nm using a Spectramax M3 spectrophotometer (Molecular Devices, San Jose, CA, USA).

Fluorescence Microscopy

Samples were stained using the FilmTracer LIVE/DEAD biofilm viability kit (ThermoFisher, Waltham, MA, USA) following the manufacturer's instructions. DAKO fluorescence mounting medium (Agilent, Santa Clara, CA, USA) was added after staining and a coverslip was placed. Images were acquired as Z-stacks using an AxioImager.Z1 microscope fitted with an ApoTome1 attachment (Zeiss, Oberkochen, Germany) at a 20x magnification using the structured light illumination technique. Image post-processing was performed using FIJI (24, 25). Images were reconstructed using Maximum Intensity Projection (MIP) and were further post-processed by increasing the brightness of each channel separately to the maximum value.

Statistical Analysis

CFU counts were transformed to logarithmic values before statistical analysis. Biomass values were normalized as the percentage of positive controls after subtracting the average value of negative controls (coupons incubated in sterile TGN). Statistical analysis was performed using the mean of each repetition ($n = 4$, with $n = 3$ per replicate) using GraphPad 7.01 (GraphPad Software, San Diego, CA, USA). Means were compared using 2-way ANOVA, followed by Holm-Sidàk *post-hoc* test. Differences were considered statistically significant when $p < 0.05$. Synergy was defined as a significant interaction factor (26).

RESULTS

Antimicrobial Susceptibility

Minimal inhibitory concentrations (MIC) for the MSSA and MRSA strains are shown in **Table 1**. MSSA strains exhibited low MICs to flucloxacillin in either media, with MICs in TGN one to two dilutions lower than in CA-MHB. MRSA strains were resistant to flucloxacillin in both media. All strains were susceptible to vancomycin in CA-MHB, but their MIC was 2 to 3 dilutions higher when tested in TGN.

Antimicrobial Activity in Biofilms

Bacterial counts (CFU) are shown in **Figure 2A** for MRSA strains and in **Figure 3A** for MSSA strains. The CFU counts of control coupons of all strains did not change between T0 and T24, indicating biofilm maturity at T0. Incubation of control MRSA coupons with vancomycin at either MIC or 20 mg/L concentrations did not result in reductions in CFU counts when compared to the T0 control samples. A similar observation was made for MSSA biofilms exposed to flucloxacillin at MIC. However, exposure to flucloxacillin at 20 mg/L resulted in a statistically significant decrease of the CFU counts in strains ATCC 25923 (-2.98$\log_{10}$) and 611 ($-1.49\log_{10}$). The results of biomass assays are shown in **Figure 2B** for MRSA strains and **Figure 3B** for MSSA strains. As we observed with CFU counts, no statistically significant differences in biomass were observed between controls at T0 and T24, except for strain 578 (+29.6%, $p < 0.001$). Twenty-four hours exposure to vancomycin at MIC did not reduce biomass in control coupons of all MRSA strains. Exposure to a concentration

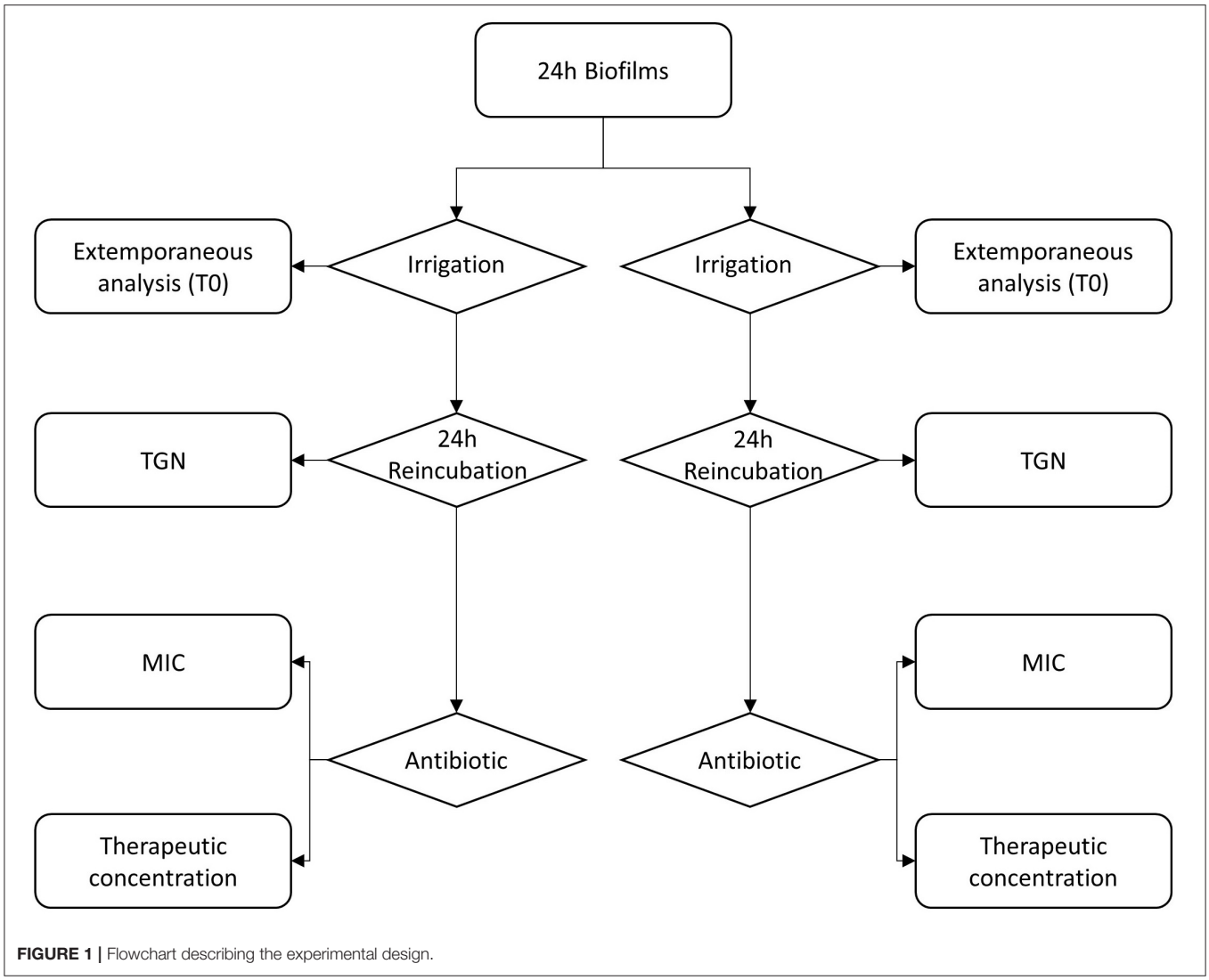

FIGURE 1 | Flowchart describing the experimental design.

of 20 mg/L did not affect the biomass, except for strain 676 (−27.5%, $p = 0.03$). The biomass of control MSSA samples was not modified in a statistically significant manner after a 24 h incubation with flucloxacillin at MIC. The diminutions of biomass of ATCC 25923 samples observed after a 24 h exposure to flucloxacillin at MIC and 20 mg/L did not reach statistical significance (-30.3%, $p = 0.79$ and −69.9%, $p = 0.05$ respectively). The incubation with a 20 mg/L concentration of flucloxacillin caused a significant biomass reduction for biofilms of strain 611 (−24.1%, $p = 0.04$). Fluorescence microscopy maximum intensity projection images of the Z-stacks at 20x magnification are shown in **Figure 2C** for strain ATCC 33591 and in **Figure 3C** for strain ATCC 25923. ATCC 33591 control biofilms uniformly covered the surface of the coupons. Live (green) cells were the most prevalent, but a small proportion of dead (red) cells was observed. Re-incubated controls were comparable without or with vancomycin. ATCC 25923 control biofilms appeared to have a looser aspect than those of ATCC 33591. The proportion of dead cells remained stable after re-incubation without flucloxacillin but appeared to increase when the samples were reincubated with flucloxacillin at MIC or 20 mg/L.

Antimicrobial Effects of Pulsed Lavage With and Without Sequential Antibiotics

The use of pulsed lavage significantly reduced the CFU counts in all MRSA and MSSA strains by 2.74 to 4.04 $\log_{10}$ when compared to T0 controls (**Figures 2A**, **3A**). The remaining bacterial load was found to be sufficient to promote the regrowth of bacteria within the biofilms to baseline (T0) levels after 24 h. The addition of vancomycin at MIC inhibited the regrowth of the biofilms of strains ATCC 33591 and 749 and reduced the CFU counts of strain 676 ($-1.29\log_{10}$). Flucloxacillin at MIC inhibited the regrowth of strains 578 and 611 and reduced the CFU counts of strain ATCC 25923 ($-2.11\log_{10}$). Sequential treatment with pulsed lavage and then 24 h exposure to either

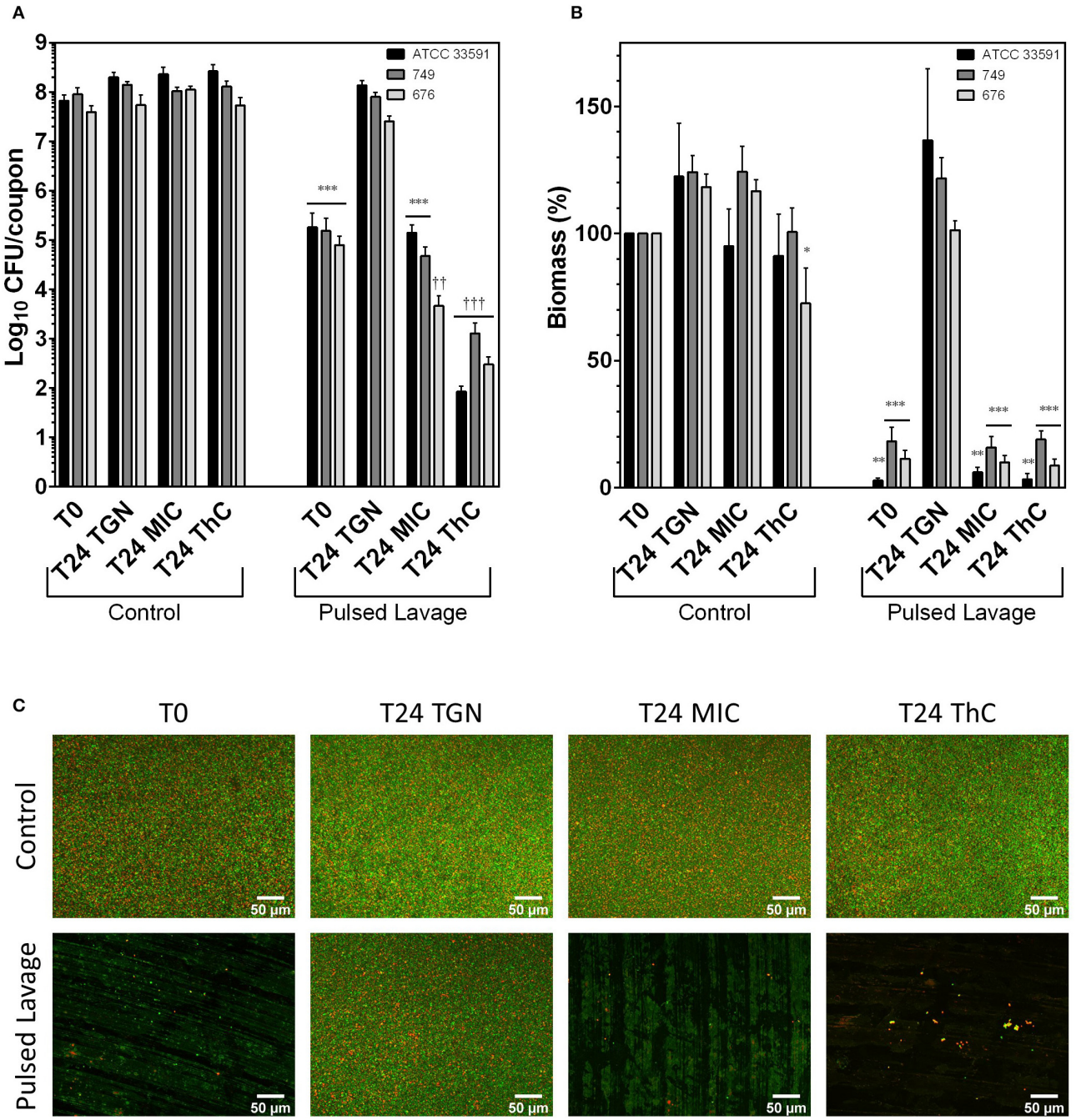

FIGURE 2 | Effects of irrigation and vancomycin on MRSA biofilms grown for 24 h in TGN. **(A)** CFU counts; **(B)** biomass expressed in percentage of T0 controls; **(C)** MIP of Z-stack acquired at 20x magnification following Live (green fluorescence, Syto 9) /Dead (red fluorescence, Propidium Iodide) staining. Scale bar: 50 µm. Control: control groups; Pulsed Lavage; groups treated with pulsed lavage; T0: samples analyzed after 24 h of growth; T24 TGN: T0 samples analyzed after 24 h of reincubation in TGN; T24 MIC: samples analyzed after 24 h of reincubation in TGN with vancomycin at MIC; T24 ThC: samples analyzed after 24 h of reincubation in TGN with vancomycin at 20 mg/L (therapeutic concentration). Data expressed as means of experiments and SEM. N experiments ≥ 3. Statistical analysis: two-way ANOVA followed by Holm-Sidàk *post-hoc* test. Comparisons to T0 control samples: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Comparisons to T0 irrigation samples: $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$.

vancomycin (MRSA strains) or flucloxacillin (MSSA strains) at a 20 mg/L concentration reduced the CFU counts in all strains by 1.90 to 2.54$\log_{10}$ when compared to coupons analyzed after pulsed lavage alone. The two-way ANOVA revealed a highly significant ($p < 0.001$) interaction parameters for all strains when considering the exposure to pulsed lavage and the reincubation of

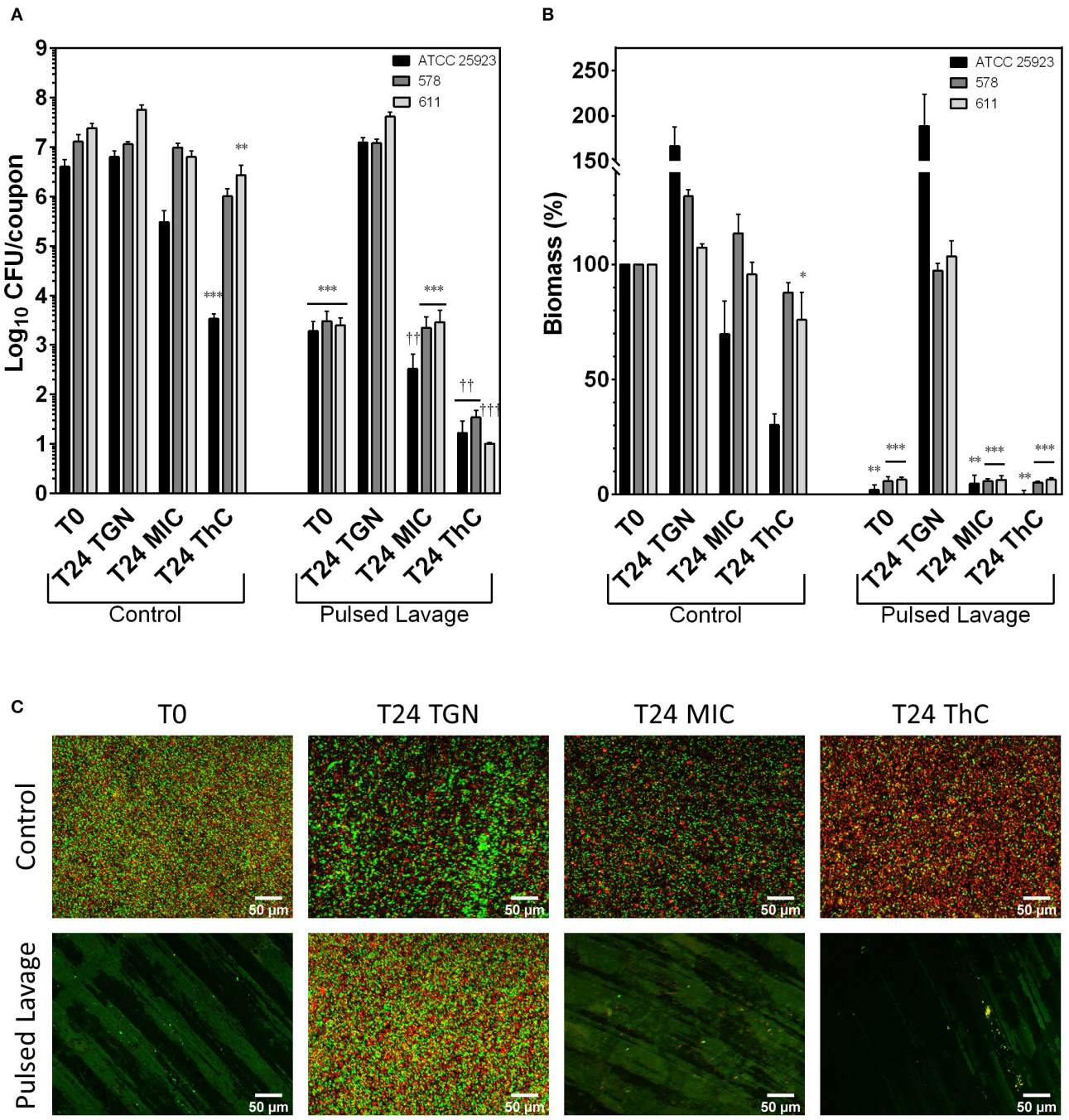

FIGURE 3 | Effects of irrigation and flucloxacillin on MSSA biofilms grown for 24 h in TGN. **(A)** CFU counts; **(B)** biomass normalized as a percentage of T0 controls; **(C)** MIP of Z-stack acquired at 20x magnification following Live (green fluorescence, Syto 9) /Dead (red fluorescence, Propidium Iodide) staining. Scale bar: 50 µm. Control: control groups; Pulsed Lavage; groups treated with pulsed lavage; T0: samples analyzed after 24 h of growth; T24 TGN: T0 samples analyzed after 24 h of reincubation in TGN; T24 MIC: samples analyzed after 24 h of reincubation in TGN with flucloxacillin at MIC; T24 ThC: samples analyzed after 24 h of reincubation in TGN with flucloxacillin at 20 mg/L (therapeutic concentration). Data expressed as means of experiments and SEM. N experiments ≥ 3. Statistical analysis: two-way ANOVA followed by Holm-Sidàk *post-hoc* test. Comparisons to T0 control samples: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Comparisons to T0 irrigation samples: $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$.

the samples as factors (**Supplementary Data Tables 1, 2**). These significant interaction parameters indicate a synergy of pulsed lavage and antibiotic therapy on CFU counts.

Pulsed lavage significantly reduced the biomass in all strains of MRSA and MSSA by 81.7% to 98% (**Figures 2B**, **3B**). As was observed for CFU counts, the remaining

bacteria restored the biofilms to control levels after a 24 h reincubation in medium. The successive exposure to pulsed lavage and vancomycin or flucloxacillin at either MIC or 20 mg/L inhibited the restoration of the biofilms to control levels. No subsequent reduction in biomass following exposure to antibiotics was observed. Comparably to CFU counts, the two-way ANOVA analysis showed a highly significant ($p = 0.005$ to $p < 0.001$) interaction parameter for all strains when considering the exposure to pulsed lavage and the reincubation of the samples as factors (**Supplementary Data Tables 3, 4**). Likewise, these results point toward a synergy of pulsed lavage and antibiotic therapy on biomass.

Microscopy was used to evaluate pulsed lavage samples (**Figures 2C**, **3C**). Pulsed lavage removed most of the cells for both strains, leaving small clusters on the surface of the coupons. The remaining cells appeared to be mostly viable. As was observed for CFU counts and biomass measurements, the incubation of samples treated with pulsed lavage without antibiotics resulted in a complete restoration of the biofilms, with images similar to those of the controls (**Figures 2C**, **3C**). The incubation with vancomycin or flucloxacillin at MIC or 20 mg/L did not alter the density of cell clusters of the samples. The proportion of dead cells seemed to increase when the samples were exposed to a therapeutic concentration of antibiotics when compared to controls.

DISCUSSION

Our results show a synergistic effect of sequential pulsed-lavage and antimicrobial therapy with either vancomycin or flucloxacillin at clinically relevant concentrations against *Staphylococcus aureus* biofilms grown on titanium coupons. This combination of pulsed lavage with antibiotics at concentrations compatible with a parenteral administration to simulate PJI has not been previously reported. Knecht et al. published on the combination of pulsed lavage and incubation with tobramycin- and vancomycin-loaded calcium sulfate beads, showing a strong synergy (20). However, the antibiotic concentrations eluted in the culture medium were not determined, limiting the extrapolation of the results. Wolcott et al. (27) studied the combination of pulsed lavage and gentamicin against *S. aureus* biofilms in a chronic wound model. A synergy of the two treatments was observed, but the concentration of gentamicin was far above the human C_{max} after the administration of a conventional dose, limiting the extrapolation of the results to a clinical setting. Collectively, prior data and the data herein underline the importance of surgical irrigation of infected wound and implant surfaces prior to the administration of adequate antibiotic doses to observe a strong synergy and achieve maximal sustainable reduction in bacterial inoculum.

The independent use of pulsed lavage against *S. aureus* biofilms appeared to remove most of the biofilm cells and biomass in our experiments. This contrasts with previous studies that reported a 1 to 2 $\log_{10}$ reduction in cell numbers following pulsed lavage (17, 18, 20). This discrepancy between our results and previous studies may be due to differences in strains, material surfaces or culture conditions. However, the conditions used here likely closer simulate clinical conditions, with a comparatively short distance between the nozzle and the coupons, and a volume of fluid to sample surface ratio of 40 mL/cm². Moreover, we studied a larger variety of strains, limiting the confounding factor of strain-dependent effects.

Despite a substantial reduction in CFU counts, we noted that residual bacteria on the coupons after pulsed lavage were sufficient to restore a biofilm after a 24 h incubation, consistent with what was previously shown by other authors (18).

We observed that vancomycin at its recommended serum trough concentration had no effect on reducing MRSA bacterial inocula via CFU or biomass if the biofilms were not first disrupted by pulsed lavage, consistent with previous data that vancomycin activity within biofilms is poor. Several authors have described that vastly supratherapeutic concentrations of vancomycin were required to observe a significant reduction in CFU counts (28) or in the metabolic activity (23, 29) of MRSA biofilms. The low penetration of vancomycin in *S. aureus* biofilms may explain these observations (30).

In contrast, a limited strain-dependent effect of flucloxacillin was observed against MSSA biofilms in the absence of pulse lavage. Flucloxacillin is a narrow spectrum β-lactam antibiotic, directed against *Staphylococci* and *Streptococci*, which is recommended in combination with rifampicin for MSSA and methicillin-susceptible *S. epidermidis* prosthetic joint infections, alongside nafcillin and oxacillin (31–33). Only a few conflicting studies have been published about the *in vitro* effect of flucloxacillin against *S. aureus* biofilms, pointing toward a variable, strain-dependent effect (34–37), analogous to our observations.

Our study presents several limitations. First, biofilms were grown only on Ti6Al4V as a substrate. This decision was based on the previous observation by Urish et al. (17) that the differences between metallic substrates are tenuous when considering the effect of pulsed lavage. Second, we limited the growth period of the biofilms before treatment to 24 h. While the biofilms were mature from a microbiological perspective, it could be argued that older biofilms would develop a more complex structure that could change the effect of the treatments we used. Third, we used antibiotics at set concentrations. While vancomycin is often administered in a continuous infusion, flucloxacillin is usually administered on a 2 g, 4 times per day regimen and important variations in serum concentrations are observed over time between 2 administrations. We decided to use, in addition to the MIC, a concentration equivalent to the one observed 3 h after administration of a 2 g dose in order to mitigate this limitation. However, as this concentration remained constantly above the MIC for 24 h, the observed effects of flucloxacillin may be overestimated.

CONCLUSION

A synergy of pulsed lavage and vancomycin or flucloxacillin was observed against *S. aureus* biofilms grown on titanium alloy coupons. This effect was never reported when considering clinically relevant antibiotic concentrations. These results confirm the need for thorough irrigation of the metallic surfaces of implants during DAIR procedures to facilitate the subsequent action of antibiotics.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

HP, AR-S, OC, and FVB contributed to the conception and design of the study. GS and HR-V provided clinical strains for the study. HP carried out experiments and analyzed data. HP and FVB wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work was supported by grants from the Fonds pour la Recherche dans l'Industrie et l'Agriculture (FRIA; Fonds de la Recherche Scientifique (FRS-FNRS) (grant J.012.19) and the Orthenzy project (Win2Wal program of the Region Wallonne). HP received successive Ph.D. fellowships from the Fondation Saint-Luc (Cliniques Universitaires Saint Luc, Université catholique de Louvain) and the FRIA (grant 5211220F). FVB is Research Director from the Belgian FRS-FNRS.

ACKNOWLEDGMENTS

The authors thank Mrs Caroline Bouzin from the IREC imagery platform and Mr Alix Mangin from the Laboratoire de Pharmacologie Cellulaire et Moléculaire for their technical advice and support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00527/full#supplementary-material

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Conflict of Interest: The Interpulse pulsed-lavage devices used for the experiments were gifted by Stryker Co., Kalamazoo, MI, USA.

The authors declare that the research was conducted in the absence of any other commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 16 October 2020
doi: 10.3389/fmed.2020.540929

One-Stage Exchange Arthroplasty for Fistulizing Periprosthetic Joint Infection of the Hip: An Effective Strategy

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Background: Prosthetic hip infection (PHI) is a disastrous scenario after an arthroplasty. International guidelines contraindicate one-stage exchange arthroplasty for fistulizing chronic prosthetic hip infection (FCPHI), nevertheless few surgical teams, mostly from Europe, support one stage procedure for this indication.

Questions/Purposes: Analysis of infection recurrence and implant failure of a series of FCPHIs treated with one stage arthroplasty.

Patients and Methods: Sixty-six FCPHIs treated with one-stage exchange arthroplasty were prospectively followed up at least 2 years. Clinical, radiological and bacteriological signs suggestive of reinfection were sought, as well as implant failures and PHI related deaths.

Results: Thirty-four females and thirty-two males with median age of 69.5 years [61–77] and BMI of 26 kg/m² [22-31] were included. Fistulae were productive in 50 patients (76%). *Staphylococcus* was responsible for 45% of PHI and 21% were polymicrobial. Twenty-nine patients (44%) received preoperative antibiotic therapy. After a median 60-month follow-up [35–82], 3 patients (4.5%) presented reinfection (two new infections, one relapse) and 3 patients experienced implant failure (1 femoral fracture, 1 stem breakage, 1 recurrent dislocation). One death was related to PHI. After a minimum of 2 years, the infection control rate was of 95.3% (±0.02).

Conclusion: One-stage exchange arthroplasty for FCPHIs showed a good infection control rate similar to that of non-fistulizing PHI. Systematic preoperative microbiological documentation with joint aspiration and, in some specific cases, the use of preoperative antibiotic therapy are among the optimizations accounting for the success of the one-stage arthroplasty. In light of these results, and those of other studies, international recommendations could evolve.

Level of Evidence: Descriptive therapeutic prospective cohort study. Level of evidence: IV.

Keywords: hip, infection, joint, prostheses, one stage exchange arthroplasty, fistula

OPEN ACCESS

Edited by:
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Hospices Civils de Lyon, France

Reviewed by:
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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *06 March 2020*
Accepted: *08 September 2020*
Published: *16 October 2020*

Citation:
Marmor S, Kerroumi Y, Meyssonnier V, Lhotellier L, Mouton A, Graff W and Zeller V (2020) One-Stage Exchange Arthroplasty for Fistulizing Periprosthetic Joint Infection of the Hip: An Effective Strategy. Front. Med. 7:540929. doi: 10.3389/fmed.2020.540929

BACKGROUND

The treatment of chronic periprosthetic hip infection (PHI) is still a controversial issue. There are currently two conventional surgical treatment procedures. The two-stage exchange arthroplasty is the most common treatment worldwide; nevertheless, a one-stage exchange procedure is gaining more and more ground (1–4). This technique is encouraged by satisfactory results of infection control rate in selected patients, at a minimum follow-up of 2 years [Wroblewski et al. (5), 91%; Loty et al. (6), 91%; Raut et al. (7), 86%; Winkler et al. (8), 92%; Klouche et al. (9), 100%; Hansen et al. (10), 70%; Choi et al. (11), 82%; Zeller et al. (12), 96%].

Other obvious benefits of one-stage surgery are the reduction in cost-burden, operating time, anesthetic risk, and complications inherent in multiple hospitalizations and surgeries.

The choice between those two strategies is guided by bacteria nature and its antibiotic susceptibility, PHI prior treatment, bone quality, patient's underlying conditions, and soft tissue inflammatory state, which indicates when severe, two-stage arthroplasty according to some authors (13–18).

Studies on fistulizing chronic periprosthetic hip infections (FCPHIs), treated with one-stage arthroplasty, are scarce and report only a few cases of FCPHIs with satisfactory infection control (5, 12, 19, 20). To our knowledge, only one prospective study described specifically the results of a series of 57 PHIs with productive fistulae, reporting a rate of 86% of reinfection-free survival after a mean follow-up of 7 years (7). Although no studies have compared one- and two-stage arthroplasty in FCPHI treatment, expert panels and international recommendations favor the two-stage strategy, arguing the likelihood of an assumed higher risk of reinfection with one-stage surgery in this indication (15, 17, 21–23).

Therefore, we asked (1) what is the reinfection-free survival rate after one-stage arthroplasty revision for patients with FCPHIs at a 2-year follow-up? (2) What is the implant failure-free survivorship for the same patients at the same follow-up?

MATERIALS

Study Population

Patients included were sampled from a cohort of 541 PHIs between 2003 and 2014. Three hundred and seventy-three were managed with one-stage exchange arthroplasty, 97 with two-stage surgery, 30 with debridement, antibiotics, and implant retention, and 41 with other strategies (resection, delayed reimplantation). In this cohort, the presence of a fistula was never a contraindication to performing one-stage arthroplasty. Until 2008, two-stage strategy indications were either major bone defects or unknown PHI-causative germ. Afterward, we performed a one-stage exchange arthroplasty to almost all PHIs.

Abbreviations: PHI, periprosthetic hip infection; FCPHI, fistulizing chronic prosthetic hip infection; Sd, standard deviation; CRP, C-Reactive Protein; ASA score, American Society of Anesthesiologist score.

We included in this single-center, prospective cohort study patients over 18 years of age undergoing one-stage exchange arthroplasty for FCPHI in our referral center of osteoarticular infection.

Endpoints

The primary endpoint of the study was the occurrence of prosthetic hip reinfection. Reinfection corresponds to a recurrence of the prostheses infection, which could be either a relapse with the same bacteria or a new prosthetic infection due to a different one. The secondary endpoint is the occurrence of implant failure. It may be a loosening, dislocation, or any other mechanical event occurring in the patient's prostheses, without any clinical, biological, or radiological sign suggesting a PHI. In addition, the cultures of preoperative joint aspiration fluid and intraoperative samples must be sterile in case of revision.

Methods

All patients were treated and followed at least 2 years after surgery. They were reviewed at the end of the antibiotic therapy period (3 months) and then, at 1 year, 2 years post-operatively, then every 2 years. Phone interviews were conducted to gather the latest news from patients who were unable to attend follow-up visits.

At each visit, we sought clinical (pain, fever, local inflammation), radiological (appearance of periosteal bone apposition/radiolucent line, geodes...), and biological [increase in C-reactive protein (CRP)] and polymorphonuclear neutrophil count signs suggestive of reinfection or implant failure. Deaths were monitored as well. In the absence of clinical, biological, and/or radiological signs of infection, PHI was considered healed after 2 years of follow-up (24).

Ethics Statement

All participants were informed and gave their consent before the start of this study, which was approved by the Local Ethics Committee.

Statistics

Qualitative variables were described according to frequency. Quantitative variables were assessed for normality. They were described by their mean and standard deviation (Sd) if they met a normal distribution, otherwise by their median and interquartile range. They were compared from baseline to 24th month using the non-parametric Wilcoxon signed-rank test. The reinfection-free survival of implant failure and PHI-related mortality was analyzed using Kaplan–Meier's method and expressed as a rate with its Sd. Log-rank (Mantel–Cox) test was used to compare the survival distributions of the two groups. A $p < 0.05$ was considered significant. All statistical tests were performed with SPSS.20 software.

Diagnosis and Therapeutic Strategy

PHI diagnosis was based on the presence of one or more fistulae, which is a major criterion for periprosthetic joint infection diagnosis (24, 25), and confirmed by the results of microbiological cultures of preoperative joint aspiration

and/or intraoperative samples. For infection recurrence, the diagnosis was established through the same workup as the initial diagnosis.

The pathogen was considered causative of PHI when it was isolated from ≥2 different intraoperative specimen samples or joint fluid aspirates. The diagnosis and surgical strategy for all patients were validated during the weekly multidisciplinary consultation meeting, involving at least one orthopedic surgeon, one infectiologist, and one microbiologist.

At least 2 weeks after discontinuing any ongoing antibiotic therapy, preoperative aspiration of the joint fluid was done in the Department of Radiology under fluoroscopic guidance and strict sterile conditions. In addition, two joint washing-aspirations with the saline solution were performed. Specimens were intended for the determination of differential white blood cell counts and microbial identification.

Joint aspiration was completed with media contrast injection to view the fistula pathway via arthrography.

One-stage exchange arthroplasty was the surgical technique adopted in this series. It involved the excision of the old scar and the fistula pathway through the former incision or a new one to permit a double approach.

After thorough debridement, the old prosthesis was removed. In some cases, trochanterotomy and/or femorotomy were carried out to facilitate the endofemoral cement excision, implant extraction, and joint exposure.

Debridement consisted of an extensive and circumferential synovectomy. All macroscopically infected or suspect tissues were excised. Osteosynthesis hardware and cement were removed. During the surgical excision procedure, at least five intraoperative specimens were sampled from synovial, acetabular, and femoral sites. Specimens were immediately transported to the laboratory of microbiology, then diluted and crushed. Afterward, the final suspension was aliquoted and cultured. When necessary, non-antibiotic-impregnated bone allograft was performed to fill the bone loss. Finally, the new prosthesis was implanted after one saline washing. Most of the time, the implant was cementless, and when cemented, no antibiotics were added. All patients had drain suction during 3–5 days post-operatively.

Antibiotics susceptibility testing was performed for all isolated germs, according to the recommendations of the French Society of Microbiology (26).

Polymicrobial infection included different genera. The presence of different staphylococcal species defined mixed staphylococcal PHIs. The antibiotic therapy was initially guided by the results of the culture of the preoperative joint aspirate and subsequently adapted to the microbiological results of the intraoperative samples.

PHI was classified according to Tsukayama's classification (27); two PHI groups were considered post-operatively acquired, i.e., without signs of hematogenous spread. Early-post-operative infection was defined as surgical site pain, redness with or without drainage, associated or not with fever, occurring within 30 days after joint arthroplasty. Late-chronic infection was defined as progressive pain, joint dysfunction with or without a fistula, occurring ≥1 month after joint arthroplasty.

A hematogenous infection was defined as occurring after a symptom-free interval of ≥1-month post-surgery, with sudden onset of pain, joint dysfunction with or without fever, and/or chills, a virulent bacterium compatible with hematogenous dissemination (*Staphylococcus aureus*, *Streptococcus*, *Enterobacteriaceae*…), or identification of a portal of entry.

All patients received post-operative antibiotic therapy, which was launched intraoperatively with at least one intravenous (IV) antibiotic through a central venous catheter. Continuous infusions administered vancomycin, cefazolin, ceftazidim, piperacillin-tazobactam, and clindamycin. The monitoring of antibiotic serum levels was performed for all IV antibiotics. Fusidic acid, minocycline, levofloxacin, and linezolid were administered by oral regimen (28–31).

When the result of preoperative joint fluid culture identified monomicrobial infection with *S. aureus*, *Streptococcus* sp., *Enterobacteria*, or *Pseudomonas aeruginosa*, preoperative antibiotic therapy was initiated.

The duration of post-operative IV antibiotic therapy was 4–6 weeks, relayed by an oral regimen for a total duration of 12 weeks, in accordance with French and international recommendations (21, 32).

At the beginning of this cohort study, all patients received 6 weeks of IV antibiotics and 6 weeks of an oral regimen. From 2008, we decided to decrease the duration of the IV phase to 4 weeks if PHI was due to an organism deemed susceptible, such as methicillin-susceptible *Staphylococcus* and/or anaerobes from the skin flora.

RESULTS

Baseline Characteristics

Sixty-six FCPHIs occurred in 66 patients (34 females and 32 males) with a median age of 69.5 years [61–77] and body mass index of 26 kg/m^2 [22–31]. Osteoarthritis was the indication for the index implantation of a hip prosthesis in 46 cases (70%), followed by fractures in 15 cases (23%). Forty-one patients (62%) had cardiovascular history, 15 (23%) had diabetes, 8 (12%) dyslipidemia, 6 (9%) thromboembolism disorder, 5 (8%) hepatitis, 7 (11%) cancer, 3 (5%) renal failure, and 3 (5%) had inflammatory rheumatism. The American Society of Anesthesiologists (33) score was grade I in 3 (5%) patients, II in 46 (70%), III in 16 (24%), and IV in 1 (1%). Twenty-two patients (33%) experienced prior medical–surgical treatment failure of their PHI in other hospitals (19 debridement, antibiotics, and implant retention, 2 one-stage exchange arthroplasties, and 1 two-stage exchange arthroplasties). Nineteen (29%) underwent prior failed antibiotic therapy without surgery.

Infection Description

According to Tsukayama classification, the initial infection mechanism was for 16 (24%) PHIs as early post-operative (<1 month), 30 (45%) as late post-operative (>1 month), 12 (18%) as hematogenous, and 8 (12%) as undetermined (27).

At the time of PHI treatment in our department, all patients have a chronic infection with symptoms duration >30 days. The

median symptoms duration of this series was 241 days (100–530) before one-stage surgery.

Sixty-one patients (92%) had a single fistula, 4 patients (6%) had two fistulae, and 1 had three. On baseline visit, fistulae were productive in 50 patients (76%).

Staphylococcus was the most frequent-isolated bacteria, responsible for 30 (45%) PHIs, of which 15 (23%) were due to methicillin-resistant strains, whereas 14 (21%) PHIs were polymicrobial (**Table 1**).

Initial Workup

Radiographs showed in 14 patients (21%) both acetabular and femoral loosening, in 11 (17%), an acetabular, and in the other 11 (17%), a femoral loosening. Median CRP was 27 mg/l (11–56), and the median leukocyte count was 7,580/mm^3 (6,475–8,800).

Preoperative joint aspiration was performed in all patients, arthrography in 54 patients (82%), showing in 41 cases (62%) a communicating pathway between the fistula and joint space (**Figure 1**). The culture of joint fluid aspirate was positive in 63 cases (95%). It yielded the same bacteria as the intraoperative samples culture in 48 cases (73%). Among the three negative joint fluid cultures, two had positive and one negative intraoperative culture. The latter was operated for an abscess before the exchange arthroplasty. The intraoperative samples yielded *Streptococcus agalactiae*, considered PHI-causative bacteria. Sonication has not been performed for the three negative joint aspiration cultures because our lab was not equipped with a sonication device at that time.

TABLE 1 | Infecting organisms in their frequency.

Germes	Cases	%
Staphylococcus/[MR*]	30/[15]	45/[23]
Staphylococcus aureus/[MR]	16/[6]	24/[9]
Staphylococcus epidermidis/[MR]	11/[8]	17/[12]
Staphylococcus CN**/[MR]	3/[1]	5/[2]
Polymicrobial	14	21
Mixed staphylococcus species	4	6
Mixed bacteria	10	15
Streptococcus	4	6
GNB***	5	8
Escherichia coli	2	
Pseudomonas aeruginosa	1	
Serratia marcescens	1	
Prevotella nigrescens	1	
Propionibacterium sp.	4	6
Corynebacterium sp.	3	5
Enterococcus faecalis	2	3
Negative culture	1	2
Other****	3	5

*Methicillin-resistant.
**Coagulase-negative.
***Gram-negative bacillus.
****Finegoldia magma, mycobacterium tuberculosis, peptostreptococcus micros.

Antibiotic Therapy

Twenty-nine patients (44%) received preoperative antibiotic therapy with a median duration of 4 days (2–9). In all other cases, antibiotic therapy began intraoperatively after bacteriological samples had been taken from the surgical site.

The median duration of total antibiotic therapy was 84 days (83–90), of which 42 days (30–43) were IV and 42 days (41–55) were oral.

One Stage Surgery Procedure

One-stage exchange arthroplasty was performed via posterior approach in 50 patients (76%), combined with a double approach to excise a distinct fistula pathway in 13 patients (20%) and via direct anterior approach in 3 patients (5%). A femorotomy or trochanterotomy was necessary in 30 cases (45%). Reimplantations were mostly cementless (45 cases or 68%); the others were cemented without antibiotic-loaded cement. Eleven patients (17%) received an acetabular bone graft to fill bone defects (four graded as Paprosky type 2A, 1 as 2B, 4 as 3A, and 2 as 3B). Among them, three also had a femoral allograft (one graded as Paprosky type 1, 1 as 3A, and 1 as 3B) (34, 35).

Outcomes

The median follow-up was of 60 months (35–82) with an Sd of 31.3. Sixty-five (98%) patients were seen at 24th month post-operatively, and one was called by phone to collect follow-up data of this visit. No patient was lost to follow up.

The functional score for Postel Merle d'Aubigné (36) rose from 12 (9–15) (95% CI 10.8–13.2) preoperatively to 17 (14–18) (95% CI 14.7–16.3) at 2 years post-operatively with a median

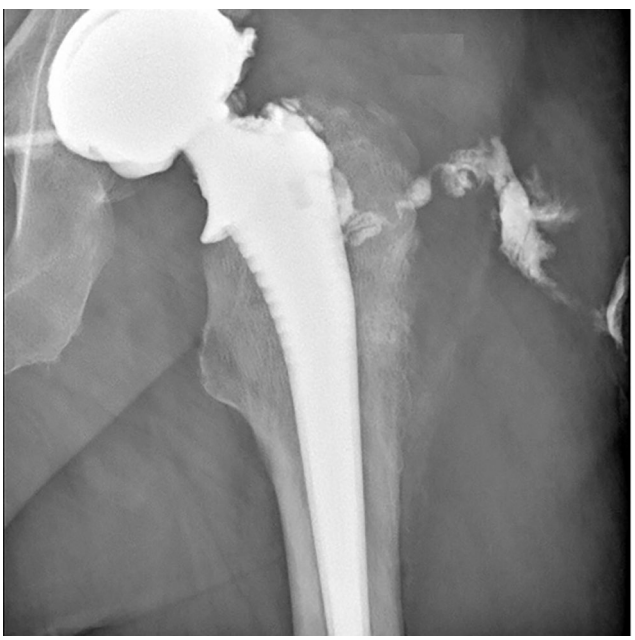

FIGURE 1 | Arthrography showing the fistula pathway.

difference of 3.5 (1–6) (95% CI 2.5–4.4). The three-item scores showed a significant improvement in pain ($p < 0.0001$), mobility ($p < 0.0001$), and function ($p < 0.0001$).

TABLE 2 | Details of the three PHI reinfections.

Initial germ	Polymicrobial**	Mixed *Staphylococcus* species***	MRSE
One stage surgery with femorotomy	Yes	No	Yes
Bone graft	No	No	No
Reinfection type	New infection	New infection	Relapse
Germ of reinfection	Polymicrobial*	*Enterococcus faecalis*	MRSE
Age (years)	77	79	65
Medical history	Prostate and colon cancer, HBP, pulmonary embolism	HBP, AF under anticoagulant, depression	Diabetes, HBP, gout, systemic scleroderma
BMI (cm/kg²)	31	38	31
ASA	2	3	2
Number of previous procedures	1	0	0
Delay for reinfection (months)	1	10	21
Reinfection treatment	2 stage	PSAT	1 stage
Vital status	PHI-unrelated death	PHI-unrelated death	Alive

**Methicillin-resistant Staphylococcus epidermidis, Klebsiella pneumonia, and Staphylococcus kloosi.*
***Methicillin-resistant Staphylococcus aureus and Enterococcus faecalis.*
****Methicillin-sensitive Staphylococcus aureus and methicillin Staphylococcus epidermidis.*
MRSE, Methicillin-resistant Staphylococcus epidermidis; PHI, Prosthetic hip infection; HBP, High Blood Pressure; AF, Atrial fibrillation; PSAT, prolonged suppressive antibiotic therapy.

Three patients (4.5%) had reinfections: We observed one relapse due to methicillin-resistant *Staphylococcus epidermidis* in a patient who initially had a late post-operative PHI. Two patients developed a new infection after their initially classified early post-operative PHIs. Their characteristics, treatments, and vital status are summarized in **Table 2**. Among the patients who underwent preoperative antibiotic therapy, one patient had PHI relapse. Three implant failures occurred in three patients: one case of stem breakage, which required replacement of the femoral stem, one case of recurrent dislocation (four episodes) treated by femoral stem replacement after three failed reductions, and one case of femoral fracture treated by osteosynthesis.

Nineteen patients died during the observation period, including three females within 2 years after surgery: a 72-year-old patient with several comorbidities (high blood pressure, dyslipidemia, pulmonary embolism, superficial venous insufficiency, chronic ethylism, peripheral arterial obstructive disease, and smoking) died a month and a half after the operation after a lung cancer diagnosed during the preoperative assessment of her PHI. A 77-year-old patient with no medical history died 5 months post-operatively from a pulmonary embolism. The third death, the only one considered related to PHI, occurred at 8 months post-operatively in a 79-year-old patient with numerous comorbidities (cardiac insufficiency, peripheral arterial obstructive disease, high blood pressure, atrial fibrillation, diabetes, renal failure, and progressive cancer). She had post-operative multiple organ failure and died from sepsis due to *Escherichia coli*, not similar to her PHI-causative germ.

The other deaths occurred after 2 years post-operatively and were unrelated to prosthetic infection. Two event-free PHIs in patients who passed away because of a PHI-unrelated cause, <2 years after surgery, were excluded from survivorship analysis.

The survival analysis, according to Kaplan–Meier, showed a cumulative reinfection-free survival rate of 95.3% (±0.02) (**Figure 2A**) and implant failure-free rate of 96.9% (±0.02) at 2 years (**Figure 2B**). The PHI-related mortality rate was 1.6% (±0.01) throughout the follow-up. The log-rank test showed cumulative reinfection-free survival rates of 100% for patients

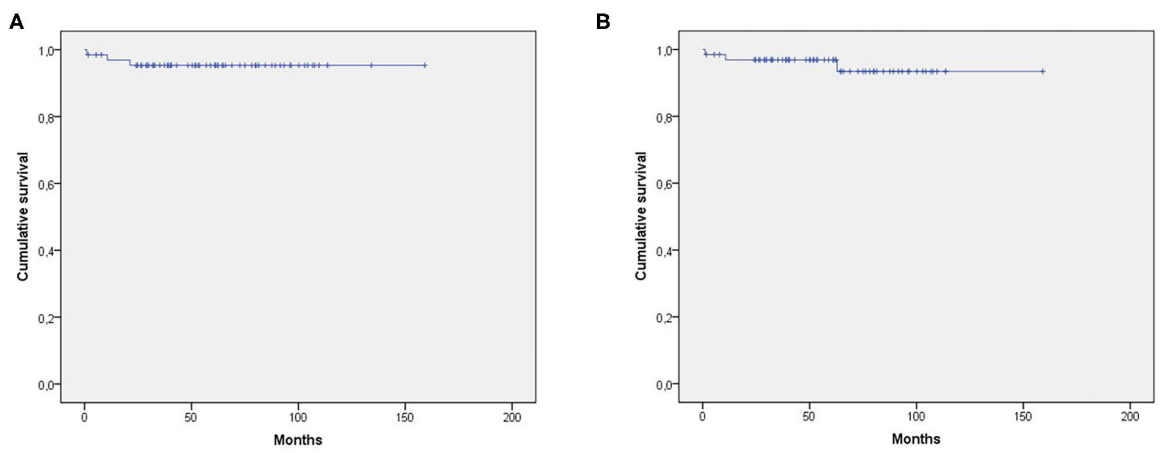

FIGURE 2 | (A) Cumulative survival free of reinfection. **(B)** Cumulative implant failure-free survival.

who had polymicrobial PHI and 94% (±0.03) for those with monomicrobial one ($p = 0.347$).

DISCUSSION

Since 2003, Zimmerli and his team have consistently proposed two-stage exchange arthroplasty as the surgical treatment of choice for FCPHIs (13, 37). The American recommendations also indicate the same strategy and contraindicate a one-stage procedure in the treatment of prosthetic hip infection with fistula (21, 22).

This choice is justified on the one hand by a risk (deemed high) of failure, due to the mediocre quality of the soft tissues, raising the risk of wound healing complications and, on the other hand, the risk of contamination of the preoperative samples through the fistula, which can prevent the identification of the PHI-causative bacteria.

We reported a series of 66 chronic FCPHI cases treated with one-stage exchange arthroplasty with very satisfactory outcomes. We observed one related death, one relapse, and two new infections, which correspond to a cumulative recurrence rate of 4.7% (±0.02). This rate is not higher than that observed in the literature, in patients treated with one-stage arthroplasty for PHI, without fistula (38).

The outcomes of our study are good and of the same order as those reported by Raut et al. (7), the exclusive series of FCPHIs published in 1994 with an infection control rate of 86%. Other studies in the literature reported series of PHIs with a small proportion of FCPHIs treated with one-stage arthroplasty, achieving success rates comparable to ours [Wrobleski (5), 92%; Hope et al. (19), 85.7%; and Rudelli et al. (20), 93%]. These data are supported by the outcomes of a systematic review of 44 studies, which compared the risk of reinfection between the two revision strategies using pooled individual participant data. Statistical analysis showed that one-stage arthroplasty might be as effective as two-stage in treating PHIs. Surprisingly, the one-stage group had higher CRP levels and a higher proportion of patients with abscess, sinus, draining wound, or fistula, a clinical presentation that often favors the 2-step surgery (39). The authors underlined that the one-stage strategy is an appropriate treatment for a patient with characteristics that had previously been thought to be inappropriate for one stage, such as those with sinus tracts. In addition, a recent study showed that two-stage prosthesis exchange arthroplasty only enables 80% of patients to be reimplanted at the second step (40).

One of the characteristics of our series is the high frequency of polymicrobism, observed in 14 cases (21%), which is higher than in Raut's series (7%) (7), but the same as in Rudelli's one (22%) (20). The presence of a fistula, with a pathway communicating between the joint and the external environment, could lead to superinfection through the fistula of an initially monomicrobial infection. The other reason could be the important frequency of the initially classified acute post-operative PHIs in which polymicrobial PHIs are frequently observed (41).

In this series, no fistula fluid samples were taken into account because we believe that the commensal flora of the skin is likely to be sampled and could skew a microbiological interpretation. For that reason, only joint aspirate was performed preoperatively as well as numerous intraoperative samples to distinguish contaminating from infecting germs.

Kaplan–Meier analysis did not show any difference in reinfection-free survival between polymicrobial and monomicrobial FCPHIs in this series (14 vs. 51)[1]. The reinfection-free success rates were 100% for polymicrobial PHIs and 94% (±0.03) for monomicrobial PHIs at a 2-year follow-up (log-rank, $p = 0.347$).

Few data in the literature are available on polymicrobial prosthetic joint infections. They are limited, divergent, and mostly concern prosthetic joint infections treated with two-stage exchange arthroplasty (42–45). Data on polymicrobial PHIs with fistula are rare and do not bring details to compare with our outcomes (7, 20).

Another feature of our study is the administration of preoperative antibiotic therapy to select patients (44%). This procedure was only used if the bacteriological results of the preoperative joint aspiration culture were consistent. Preoperative antibiotic therapy was initially used to avoid post-operative severe sepsis or septic shock. It also decreased local inflammation and facilitated the quality of surgical excision. To note, antibiotic treatment in PHI management is recommended in recent Spanish guidelines in patients undergoing one-stage exchange arthroplasty, 3–5 days before surgery if the etiological diagnosis has already been made, especially if it is caused by *S. aureus* and gram-negative bacteria (46). Nineteen out of 29 patients (66%) of this series underwent 1- to 5-day preoperative antimicrobial therapy and 10 (34%) more than 5 days.

When used, cement was never antibiotic-loaded in our practice, and prostheses were mostly cementless. Overall, the literature still lacks an appropriately sized randomized clinical trial to better support the use of antibiotic-loaded cement, which still remains a matter of debate (47–49).

Optimization of microbiological diagnosis and medical–surgical treatment (one-stage arthroplasty and extended IV and oral post-operative antibiotics) can account for the success of the one-stage exchange arthroplasty, including in FCPHIs.

The limitations of our study are the small size of the series, as well as its observational, monocentric, and non-comparative type. However, there are no randomized controlled studies assessing one-stage vs. two-stage surgery in the treatment of PHIs, either with or without fistula.

CONCLUSION

One-stage exchange arthroplasty strategy for FCPHIs shows a good success rate similar to that of non-fistulizing PHIs. Systematic preoperative microbiological documentation with joint aspiration and, in some specific cases, the use of preoperative antibiotic therapy are among the optimizations accounting for the success of this strategy. In light of our results, we believe that the presence of a fistula is not, in itself, a contraindication to performing a one-stage exchange arthroplasty for PHIs.

[1] Preoperative and intraoperative specimens culture was sterile for one patient.

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available because the local regulatory requires to keep confidential subject's data. Hence only researchers working on this article have access to data but not the general public. Requests to access the datasets should be directed to ykerroumi@hopital-dcss.org.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de protection des personnes Ile de France VI. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SM: article writing. YK: data capture, literature bibliography, statistical analysis, and article writing. VM, LL, AM, and WG: article reviewing. VZ: article reviewing and correction. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank Ms. Valérie Delorme for her assistance in data capture.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a shared department or other, though no other collaboration, with one of the authors, SM, at the time of review.

frontiers in Medicine

ORIGINAL RESEARCH
published: 26 October 2020
doi: 10.3389/fmed.2020.513242

Pseudomonas aeruginosa Implant-Associated Bone and Joint Infections: Experience in a Regional Reference Center in France

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: 19 November 2019
Accepted: 22 September 2020
Published: 26 October 2020

Citation:
Cerioli M, Batailler C, Conrad A, Roux S, Perpoint T, Becker A, Triffault-Fillit C, Lustig S, Fessy M-H, Laurent F, Valour F, Chidiac C and Ferry T (2020) Pseudomonas aeruginosa Implant-Associated Bone and Joint Infections: Experience in a Regional Reference Center in France. Front. Med. 7:513242. doi: 10.3389/fmed.2020.513242

Background: *P. aeruginosa* implant-associated bone and joint infections (BJI) is considered to be one of the most difficult to treat BJI. The data focusing specifically on this pathogen are sparse, and it seems difficult to extrapolate the results obtained with *Enterobacteriaceae*.

Methods: We performed a retrospective observation study of all *P. aeruginosa* implant-associated BJI diagnosed at our institution from 2011 to 2018. We defined failure as any type of relapse, including persistence of the same *P. aeruginosa*, superinfection by another organism(s) or any other cause of relapse such as the need for a subsequent surgery. Nonparametric statistical methods were used to compare the study groups and Kaplan-Meier curves and multivariate Cox analysis and were used to detect determinants associated with treatment failure.

Results: A total of 90 patients (62% men, median age 60 years IQR 47–72) including 30 (33%) prosthetic-joint infections and 60 (66%) other implant-associated BJIs were studied. Most of them were acute (62%). During the prolonged follow-up, (median 20 months; IQR 9–37), 23 patients (26%) experienced treatment failure. Optimal surgical treatment (DAIR for acute forms, explantation, 1-stage or 2-stage exchange for others) was significantly associated with a higher success rate in the univariate analysis ($p = 0.003$). Sixty-four (71%) patients received effective initial treatment against *P. aeruginosa* administered and 81 of them (90%) did for at least 3 weeks: both these parameters correlated with a higher success rate. In the multivariate Cox-analysis optimal surgical treatment, IV effective treatment of at least 3 weeks and treatment with ciprofloxacin for at least 3 months proved to be independently associated to a better outcome in patients with *P. aeruginosa* implant-associated BJI.

Conclusion: *P. aeruginosa* implant-associated BJI is one of the most difficult-to-treat BJI, with a strong impact on the prognosis of the surgical strategy. An effective initial IV antibiotic treatment for at least 3 weeks seems to be required, followed by oral ciprofloxacin for a total duration of 3 months.

Keywords: pseudomonas, osteomyelitis, ciprofloxacin, implant-associated bone infections (IABI), bone and joint infection

INTRODUCTION

Implant-associated bone and joint infection (BJI) is an uncommon, but dreadful complication of arthroplasties and orthopedic trauma. Despite technological and medical effort in preventing such conditions, the amount of implant-associated infections is growing because of the increasing number of implant devices (1, 2). According to Zimmerli et al., the infection rate during the first 2 years varies according to the site and it is <1% in hip and shoulder prostheses, <2% in knee prostheses, and <9% in elbow prostheses (3). With regard to internal fixation devices, about 5–10% becomes infected, with a significant disproportion between the major rate of infection after internal fixation of grade 3 open fractures (which may exceed 30%) against the 0.5–2% rate of infection after internal fixation of closed wounds (1).

The most frequently isolated microorganisms in implant-associated BJI are Gram-positive cocci, with *Staphylococcus aureus* being the most recurrent cause, while Gram-negative bacteria (GNB) are responsible for 10–23% of all episodes, causing most often acute and polymicrobial infections (1, 3–6). Even if GNB cause a minor- yet, substantial- proportion of all implant-associated BJI, they draw the attention of the medical community in light of the fact that the treatment is rather complicated and they show a less optimal outcome with longer hospitalizations -and higher costs- due to their peculiar virulence, their growing resistance to antibiotics and the comorbidities of the patients they usually infect, generally immunocompromised ones (6–9). *P. aeruginosa* is a particular GNB, commonly considered as non-fermenting bacterium, that causes 5 to 20% of the GNB infections, and recent data revealed that 14% of patients with open fracture suffered from *P. aeruginosa* infection (10, 11). *P. aeruginosa* is considered as one of the most difficult-to-treat GNB, as a result of its growing rate of multidrug-resistant strains and its ability to develop particular virulence and persistence mechanisms, such as biofilm formation and production of small colony variants (12).

Treatment strategies for staphylococcal implant-associated BJI are somewhat standardized, with a clear percentage of success, since they represent a significant cause of infection, which makes them easier to sample and study (1, 13, 14). On the contrary, our path to mastering Gram-negative implant-associated infections has been paved with scarce published experience, mostly retrospective studies, which showed inconsistent data concerning surgical and antimicrobial treatment (6–8, 15–18). Currently, guidelines for antibiotic treatment of GNB implant-associated infections recommend beta-lactams and ciprofloxacin (1, 13). This has also been supported by a large multicentre study which deals with acute GNB infections treated with debridement antibiotics and implant retention (DAIR), reporting a 79% success rate in ciprofloxacin-susceptible GNB PJI (19). In this very same setting, *P. aeruginosa* caused up to 20% of the GNB infections (19). Of note, none of these studies focused specifically on *P. aeruginosa* infections.

To our knowledge, along the years there have been just a few publications with *in vitro* studies supporting the role of fluoroquinolones against *P. aeruginosa* (20–22). However, some antimicrobial combinations, such as cefepime-ciprofloxacin and ceftazidime-ciprofloxacin, have been reported as successful options in the treatment of *P. aeruginosa* bone and joint infections (7, 16). Moreover, ciprofloxacin has been connected to a better treatment outcome when administered in case of susceptible GNB (15, 17, 23) and also of *P. aeruginosa* (19, 24).

The aims of the present study are to review our experience with the treatment of acute, delayed or chronic implant-associated *P. aeruginosa* BJI, and to analyze the impact of optimal surgical treatment, effective antimicrobial IV therapy and ciprofloxacin use on the prognosis.

MATERIALS AND METHODS

Study Design and Population

We performed a retrospective study at the Croix Rousse hospital (Hospices Civils de Lyon, France), that is the national French reference center for osteoarticular infections of the South-East region (CRIOAc Lyon; http://www.crioac-lyon.fr). We included all patients, independently of time on follow-up, with *P. aeruginosa* implant-associated infection managed in our institution between January 2011 and June 2018. All cases present in this cohort were discussed and handled thanks to the cooperation of our multidisciplinary group. Data were obtained from the electronic and written medical records, collected into a Microsoft Access Database. This study is subject to declaration with the local Commission for Data Protection and Liberties under the n°18-176 and is registered on ClinicalTrial under the n°NCT03624855.

Definitions

Implant-associated infection caused by *P. aeruginosa* was diagnosed according to the definition of organ/space surgical site infection proposed by the CDC (25) and also fulfilled the IDSA definition for patients with PJI and the new definition proposed by Metsemakers et al. for patients with internal fixation associated infections (13, 26). We identified as hematogenous acute BJIs those cases in which the patient had a normal joint function after the implantation, but experienced a sudden onset of symptoms more than 3 months after the index surgery, as previously reported by Wouthuyzen-Bakker et al. (27). At least one positive sample with *P. aeruginosa* in culture from deep perioperative samples was required.

Implant-associated infections in this study were defined as "early" if they occurred within 1 month from the date of implantation, "delayed" if they occurred between 1 and 3 months from the date of implantation and "chronic" if the onset of symptoms was >3 months from the date of implantation.

Treatment failure was defined as any type of relapse of implant-associated infection including persistence (new surgery with *P. aeruginosa* in culture), superinfection [isolation of another organism(s)] or any other cause of relapse such as the need for a subsequent surgery. Treatment was considered successful if the infection was in remission at the end of the course of antibiotics and during the entire usual follow-up in our institution. In case of need, suppressive therapy was undertaken

for the treating physician to prolong the antibiotic treatment indefinitely in patients at high risk of persistence and relapse.

Optimal surgical treatment was evaluated according to the type of surgery and the timing of the infection. In case of acute infections, we defined "DAIR" as an optimal choice of intervention if performed within 1 month following the date of implantation and for patients with hematogenous infection. If the *P. aeruginosa* implant-associated BJI was itself a superinfection on an implant previously infected by another microorganism, and if the current episode of the infection was asymptomatic and discovered accidentally on systematic bone biopsies (i.e., without clinical signs of infection), we reckoned the surgical treatment as optimal independently from the timing. While if the superinfection was accompanied by the onset of new clinical symptoms or by the worsening of the patient conditions, we assessed as optimal only the surgical treatment which was undertaken within 1 month from the previous surgery.

Effective initial antibiotic treatment against *P. aeruginosa* was defined by the use of an active IV beta-lactam drug, based on drug-susceptibility on the antibiogram.

According to the classification of the Common Terminology Criteria for Adverse Events (CTCAE), serious adverse events (SAE) were defined as CTCAE grade 3–5 (28). All SAE were reviewed by a pharmacist and were attributed (or not) to the antibiotic on *P. aeruginosa*.

Statistical Analysis

Descriptive statistics were used to estimate the frequencies of the study variables, described as effective (%) for dichotomous values and medians [interquartile range (IQR)] for continuous values. For the percentage calculation of each variable, the number of missing values was excluded from the denominator. Nonparametric statistical methods were used to compare the study groups (chi-square test, Fisher's exact test, or Mann-Whitney U test, as appropriate). Univariate Cox analysis and Kaplan-Meier curves (using the log-rank test) were used to determine determinants associated with treatment failure. Multivariate Cox analysis that includes significant determinants identified in the univariate analysis was performed, by adopting a ratio of 10 events per independent variable to avoid overfitting (maximum of three variables in the present study, selection based on the univariate analysis). A p-value of <0.05 was considered significant. Statistical analyses were performed using SPPS Statistics Base 17.0 (Softonic International, San Francisco, CA, USA).

RESULTS

Among the 1,638 implant-associated BJI occurring over the 7-year study period, 90 patients (5.5%) from the beginning of 2011 to end of 2017 were infected by *Pseudomonas aeruginosa* (including 30 with a PJI) according to our definition and were included. Basic demographic information can be found in **Table 1**.

Twenty-five patients experienced 28 adverse events during a course of treatment with antibiotics that were active on *Pseudomonas aeruginosa* (13 were SAE, 16 caused the interruption of the effective treatment, while six of them occurred in the ending phase of treatment and shortened merely the course of antibiotic hopefully without nicking the quality of the medical therapy).

TABLE 1 | Characteristics of the 90 patients with *P. aeruginosa* implant-associated BJI according to the outcome.

Characteristics	Whole population ($n = 90$)	Failure ($n = 23$)	Remission ($n = 67$)	p[a]
Age in years (median, IQR)	60 (47–72)	61 (43–74)	59 (47–72)	0.90
Male sex (*n*, %)	56 (62)	17 (74)	39 (58)	0.18
BMI ≥ 30 (*n*, %)	24 (28)	6 (29)	18 (29)	1
Active smoking (*n*, %)	29 (35)	10 (44)	19 (32)	0.34
Score ASA > 2 (*n*, %)	30 (34)	8 (35)	22 (33)	0.90
Score Charlson > 4 (*n*, %)	24 (27)	7 (30)	17 (25)	0.64
Previous infection at the same site (*n*, %)	19 (21)	6 (26)	13 (19)	0.50
Prosthesis (*n*, %)	30 (33)	7 (30)	23 (34)	0.73
Age of implant in days (median, IQR)	47 (21.7–247.5)	40 (21–222)	63 (26–798)	0.29
Type of infection (*n*, %)				
Acute	56 (62)	14 (61)	42 (63)	0.98
Sub-acute	8 (9)	2 (9)	6 (9)	
Chronic	26 (29)	7 (30)	19 (28)	
Polymicrobial infection (*n*, %)	66 (73)	18 (78)	48 (71)	0.54
BJI due to *P. aeruginosa* ciprofloxacin-resistant (*n*, %)	11 (12)	9 (39)	2 (3)	<0.001
Optimal surgical treatment[b] (*n*, %)	54 (64)	9 (39)	45 (72)	0.004
Effective initial IV treatment[c] (*n*, %)	64 (71)	12 (52)	52 (77)	0.020
Treatment with ciprofloxacin[d] (*n*, %)	79 (88)	13 (57)	66 (99)	<0.001

IQR, interquartile range.

[a] The p-value was determined by using chi-square or Fisher's exact test for categorical variables, Mann-Whitney U test for continuous variables.

[b] After exclusion of the five patients who finally received suppressive antimicrobial therapy.

[c] Such as piperacilline, piperacilline-tazobactam, ceftazidime, cefepime, imipenem-cilastatin, ceftolozane-tazobactam, ceftazidime-avibactam, based on the susceptibility on the antibiogram.

[d] After exclusion of the two patients that received ciprofloxacin as suppressive therapy.

Sites of infection were: knee (16), spine (15), hip (15), tibia (8), jaw (7), skull (6), ankle (5), femur (4), elbow (2), shoulder (2), foot (2), calcaneum (2), others (2), pubis (1), sacroiliac bone (1), humerus (1), patella (1), heel (1).

Fifty-eight (64%) patients were considered to have optimal surgical treatment, including 21 DAIR for an acute infection, 2 had incomplete implant removal, 31 complete implant removal for a chronic infection, one complete ablation followed by amputation and one DAIR followed by amputation. Among the thirty-two (36%) patients who do not meet these criteria, 20 had DAIR for a delayed or chronic infection, 1 had an incomplete implant removal, 13 had a complete implant removal. During a prolonged follow-up [median follow-up of 20 months (IQR, 9–37)]; 24 patients without failure were followed at least 2 years, 23 patients experienced a treatment failure: seven patients

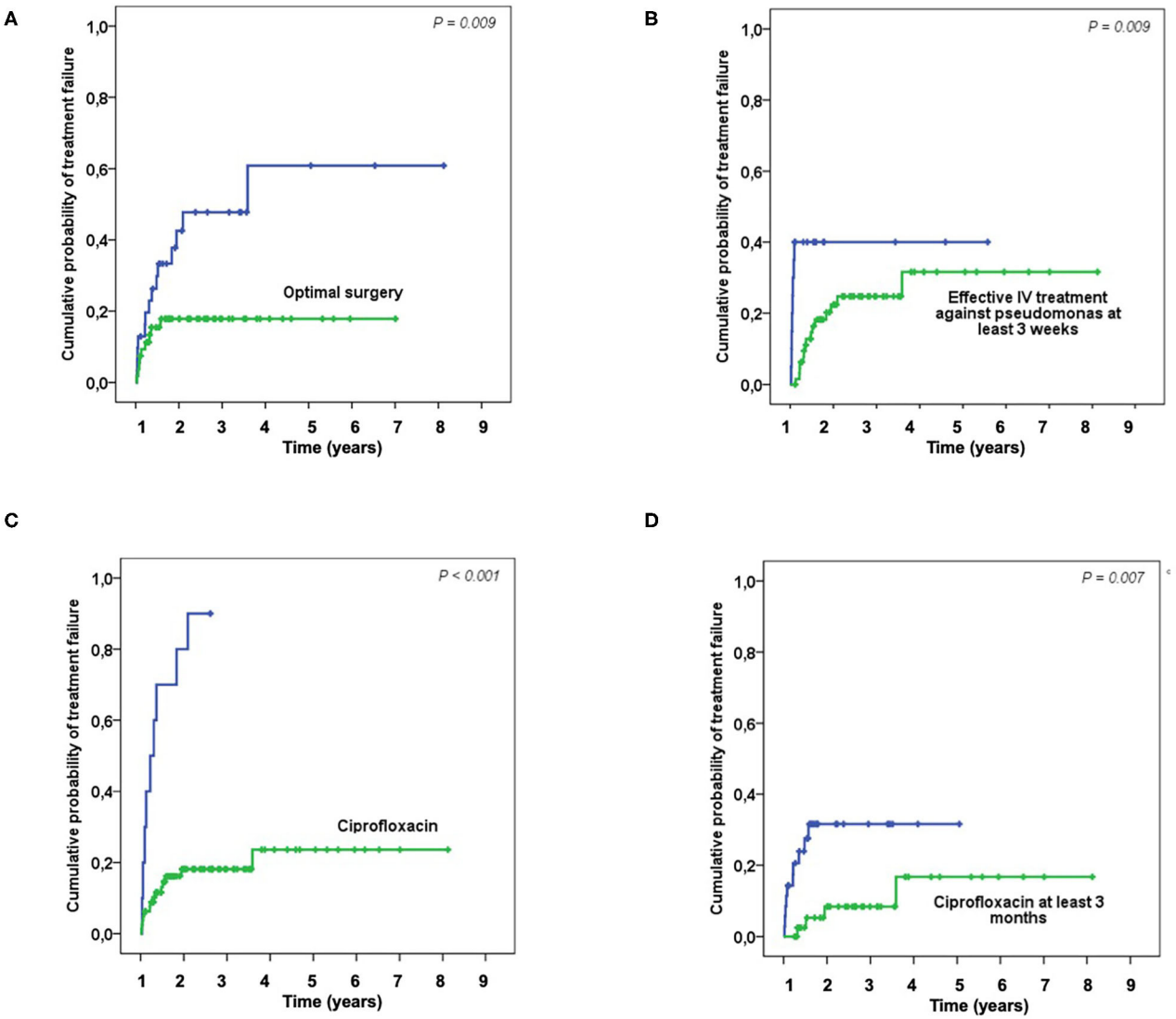

FIGURE 1 | Kaplan-Meier curves showing the probability of treatment failure depending on the surgical and medical treatments: Optimal surgery **(A)**; Effective IV treatment against pseudomonas at least 3 weeks **(B)**; Treatment with ciprofloxacin **(C)**; Treatment with ciprofloxacin at least 3 months **(D)**.

experience a persistence of *P. aeruginosa* after treatment, while 16 had a superinfection caused by another organism(s). Of note, 40 patients were lost to follow-up during the first 2-years, but these patients were not excluded in the final analysis. Optimal surgical treatment was significantly associated with a higher success rate in the univariate analysis ($p = 0.003$) and in the Kaplan-Meyer survival curve (log-rank test, p=0.009) (**Table 1**; **Figure 1A**). As long as it concerns the antimicrobial treatment, sixty-four (71%) patients received effective initial treatment against *P. aeruginosa* administered by IV, while 26 (29%) did not. Two patients with MDR *P. aeruginosa* (17%) received ceftolozane/tazobactam or ceftazidime/avibactam. Not receiving an effective initial IV drug exposed the patient to an early failure (blue line in **Figure 1B**) and when we considered an IV treatment of at least 3 weeks, which was undertaken by 90 (81%) patients, we found that it correlates with a higher success rate both in the univariate analysis ($p = 0.020$) and according to the Kaplan-Meyer curve (log-rank test, $p = 0.009$) (**Table 1**, **Figure 1B**). Eleven (12%) patients had an infection due to a *P. aeruginosa* resistant to ciprofloxacin and this impacted as well ($p < 0.001$). In the end, we evaluated the effectiveness of the treatment with ciprofloxacin. Seventy-nine (88%) patients received a course of therapy with ciprofloxacin and we found this as significantly associated with a higher success rate in the univariate analysis ($p < 0.001$) (**Table 1**; **Figure 1C**). Moreover, we observed a higher risk of failure if patients received <3 months of ciprofloxacin (log-rank test, $p = 0.007$) (**Figure 1D**). In the multivariate Cox analyses, we included in the final model three variables which finally depict the optimal pattern of treatment: optimal surgical treatment, IV effective treatment of at least 3 weeks and treatment with ciprofloxacin for at least 3 months (**Table 2**).

TABLE 2 | Multivariate Cox analysis that includes significant determinants for failure identified in the univariate analysis.

	HR	95% CI	*p*
Optimal surgical treatment*	0.32	0.11–0.98	0.045
IV effective treatment of at least 3 weeks*	0.15	0.004–0.054	0.003
Ciprofloxacin for at least 3 months*	0.23	0.07–0.75	0.015

HR, Hazard ratio; 95% CI, 95% confidence interval.
**After exclusion of the five patients who finally received suppressive antimicrobial therapy.*

DISCUSSION

We have presented a case series of 90 implant-associated BJI caused by *P. aeruginosa* at our structure, managed at our institution during 2011–2017, which accounted for the 3% of all BJI in this 7-year experience. To our knowledge, this is the largest and only study about implant-associated infections due to *P. aeruginosa* along with the one of Shah et al., which was only focused on PJIs (29).

Our data show that these infections are mostly acute and often polymicrobial, possibly due to the high comorbidity index of the patients involved and the opportunistic nature of a *P. aeruginosa* infection (5, 6, 29). After a long-term follow-up, the remission rate of patients with a *P. aeruginosa* implant-associated BJI was 74% (67 out of 90), which is consistent with the results of Rodriguez-Pardo et al. on a smaller sample of *P. aeruginosa* cases ($n = 43$) included in a larger Gram-negative PJI study (19).

Surgical treatment is the cornerstone of Implant-associated infections and all of our patients underwent surgical procedures. Choosing the correct operation for the case among the number of options (DAIR, 1-stage or 2-stage exchange, palliative treatment) is much more subtle than what it looks like, and the decision should follow as possible the current guidelines. It must be a multidisciplinary, meticulous process, and it must take into account the patient status and integrate its functional prognosis in case of implant removal (13, 30). Lora-Tamayo et al. reported 33 patients with *P. aeruginosa* infected PJI and reached an overall success rate of 81% by treating early post-surgical and hematogenous infections with stable devices and good soft tissue conditions with DAIR, while they opted for an implant removal for the chronic cases (31). Ascione et al. described 11 cases of *P. aeruginosa* PJI, as part of a broader study, treated with DAIR (80% overall success rate) or 2-stage exchange for late infections (85% overall success rate) (32). Once more, Veltman et al. presented a study on 12 early post-operative *P. aeruginosa* PJI treated with DAIR, reporting a success rate of 66% (24). These data are rather promising and in accordance with guidelines instructions, yet in contrast with the biggest *P. aeruginosa* PJI study (102 episodes in 91 patients), which pointed out a 5-year cumulative incidence of failure of 50% when treating PS PJI and an especially worse outcome for those treated with DAIR (2 year cumulative survival free rate of 26%) (29). However, this study took into account infections occurred over a long period, therefore their optimal management was clearly limited by the lack of an established protocol, as proved by the fact that most patients who underwent DAIR had chronic infections (29). Among our patients the average duration of IV treatment was of 79 days [median 63 days, IQR (44–96)], while the average duration of the oral treatment with ciprofloxacin was of 111 days (median 79 days: IQR, 29–99). A recent study on 242 GNB PJIs, among which the 20% was caused by *P. aeruginosa*, DAIR was successful in 68% of cases, with an increase to 79% in ciprofloxacin-susceptible GNB PJI treated with ciprofloxacin (19). By judging the adequateness of all our patients' surgical treatment according to the current guidelines (13, 30), we found that optimal surgical treatment was significantly associated with a higher success rate, as previously reported in a study with *S. aureus* PJI (33).

Effective initial antibiotic betalactam treatment against *P. aeruginosa* proved to be a factor correlated with a better outcome ($p = 0.020$) in accordance to the guidelines and previous experiences (7, 13, 16, 34). Even if such antibiotics are recommended as initial therapy, their optimal duration is unclear. In patients with fluoroquinolone-susceptible *Enterobacteriaceae*, it is largely admitted that the duration of IV treatment could be shortened to 2 weeks (20, 23, 35). In the study of Rodriguez-Pardo et al., *P. aeruginosa* cases were treated for a median of 60 days, with a combination of antibiotics in half of them, mainly an antipseudomonal beta-lactam plus ciprofloxacin. The median duration of the intravenous therapy (i.e., of the beta-lactam) was 18 days (19). As *P. aeruginosa* is considered to be a more difficult-to-treat bacterium in comparison with *Enterobacteriaceae,* as it has been speculated by some authors (8, 18), it is difficult to translate the results obtained with these latter bacteria exclusively, or with a minority of *P. aeruginosa*.

The treatment with ciprofloxacin was a factor significantly associated with a better outcome in our study. In the study of Shah et al., that included patients with *Pseudomonas* PJI in a period of time during which ciprofloxacin was not widely used during initial therapy (only nine out of the 102 received ciprofloxacin), the rate of success was particularly low (26%) in patients treated with DAIR (29).

This finding is in line with what has already been suggested by Martinez-Pastor et al. (23), who examined GNB BJI and fluoroquinolones in general. As already proposed by Rodriguez-Pardo et al., this finding supports the idea that the success of treatment depends on the susceptibility to this antibiotic and its use rather than on the causative microorganism (19). In this study, 28 of the 43 *P. aeruginosa* cases received ciprofloxacin, for a median of 43 days. The overall success rate was 79% (33 of 42 cases), which increased to 88% (29 of 33) when only patients with ciprofloxacin were considered (19).

According to the literature, ciprofloxacin proved itself to be effective given its qualities (namely oral availability, diffusion into the bone, activity against biofilms) (20, 36). Concerning the optimal duration of the fluoroquinolone treatment in GNB BJI, it is probably ranged from 6 weeks−3 months, as we also found by checking the median duration of treatment in other studies

(19, 24). In patients with fully susceptible *Enterobacteriaceae* native BJI, 6 weeks of treatment seem to be adequate. In patients with implant-associated BJI, a treatment course of 3 months has to be discussed, especially if *P. aeruginosa* is involved, as we found that such a duration was associated with a better outcome.

Of note, infection with a ciprofloxacin-resistant *P. aeruginosa* has a huge impact on the outcome: it has already been spotted as a risk factor advocating for implant removal even in acute infections (23), among the 11 patients infected with a ciprofloxacin-resistant *P. aeruginosa* in our study, nine experienced a failure. For this reason, fluoroquinolones should be avoided as empirical and initial therapy, yet they must be given only once having reduced the bacterial load, after a course of intravenous beta-lactam (15). There is no standard treatment for MDR GNB infection and *P. aeruginosa* is peculiarly challenging to treat, with scarce therapeutic options, that generally recur to combination of a new generation beta-lactam such as ceftolozane/tazobactam (37) or ceftazidime-avibactam (38) with colistin, which are inherently associated with high risk of toxic effects, while some *in vitro* and animal studies suggest a potential activity of the rifampin-colistin combination (39, 40).

Our work is an observational retrospective study that presents all the limitations implied by the inherent nature of this kind of study design. However, in the face of implant-associated infections, surgical and clinical management cannot be randomized; thus, observation studies are the best quality information we will ever have in this scenario. Secondly, it is crucial to focus accurately on patients with *P. aeruginosa* implant-associated BJI, as conclusions obtained with *Enterobacteriaceae* are not completely transposable. Finally, as *P. aeruginosa* implant-associated BJI is a potentially severe disease and as our center is a reference center for the management of BJI, we particularly try our best to follow these patient population. Even if the rate of lost to follow-up after 2 years was not negligible, very few data are lacking in our medical records, leading to interpretable results obtained from this study.

CONCLUSIONS

P. aeruginosa implant-associated BJI is one of the most difficult-to-treat BJIs, with a strong impact on the prognosis of the surgical strategy. An effective initial IV antibiotic treatment for at least 3 weeks seems to be required, followed by oral ciprofloxacin for a total duration of 3 months.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospices Civils de Lyon Ethic Committee. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MC wrote the first version of the manuscript. MC and TF performed the literature review. CB, AC, SR, TP, AB, CT-F, SL, M-HF, FL, FV, CC, and TF participated to the patient care. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

Lyon Bone and Joint Infection Study Group: TF, Coordinator; TF, FV, TP, Patrick Miailhes, Florence Ader, SR, AB, CT-F, AC, Cécile Pouderoux, Marie-Elodie Langlois, Marielle Perry, Fatiha Daoud, Johanna Lippman, Evelyne Braun, CC, Infectious Diseases Specialists; SL, Elvire Servien, CB, Romain Gaillard, Stanislas Gunst, Julien Roger, Charles Fiquet, MH-F, Anthony Viste, Philippe Chaudier, Jean Luc Besse, Lucie Louboutin, Gaël Gaudin, Tanguy Ledru, Adrien Van Haecke, Quentin Ode, Marcelle Mercier, Florie Alech-Tournier, Sébastien Martres, Franck Trouillet, Cédric Barrey, Emmanuel Jouanneau, Timothée Jacquesson, Ali Mojallal, Fabien Boucher, Hristo Shipkov, Joseph, Chateau, Sophie Brosset, Philippe Céruse, Carine Fuchsmann, Arnaud Gleizal, Surgeons; Frédéric Aubrun, Mikhail Dziadzko, Caroline Macabéo, Anesthesiologists; FL, Laetitia Beraud, Céline Dupieux, Camille Kolenda, Jérôme Josse, Microbiologists; Fabien Craighero, Loic Boussel, Jean-Baptiste Pialat, Imaging; Isabelle Morelec, Marc Janier, Francesco Giammarile, Nuclear Medicine; Michel Tod, Marie-Claude Gagnieu, Sylvain Goutelle, PK/PD specialists; Eugénie Mabrut, Clinical research assistant and database manager.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

PERSPECTIVE
published: 10 November 2020
doi: 10.3389/fmed.2020.580671

Precision Medicine in the Diagnosis and Management of Orthopedic Biofilm Infections

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Orthopedic biofilm infections are difficult to treat and require a multidisciplinary approach to diagnostics and management. Recent advances in the field include methods to disrupt biofilm, sequencing tools, and antibiotic susceptibility tests for bacteria residing in biofilm. The observation of interclonal differences in biofilm properties of the causative microorganisms, together with considerations of comorbidities and polypharmacy in a growing aging population, calls for a personalized approach to treat these infections. In this article, we highlight aspects of precision medicine that may open new perspectives in the diagnosis and management of orthopedic biofilm infections.

Keywords: biofilms, osteomyelitis, implant-related infection, minimal biofilm eradication concentration, next-generation sequencing, metagenomic sequencing

OPEN ACCESS

Edited by:
Alex Soriano,
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Reviewed by:
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Marjan Wouthuyzen-Bakker,
University Medical Center Groningen, Netherlands

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *06 July 2020*
Accepted: *22 October 2020*
Published: *10 November 2020*

Citation:
Baldan R and Sendi P (2020) Precision Medicine in the Diagnosis and Management of Orthopedic Biofilm Infections. Front. Med. 7:580671. doi: 10.3389/fmed.2020.580671

INTRODUCTION

Biofilms generally form on a nonliving surface. In bone and joint infections, microorganisms adhere either to dead bone (sequesters) or to implants. Hence, orthopedic biofilm infections include chronic osteomyelitis and implant-associated infections. They represent a serious threat for the patient and a substantial burden on the global health care industry. More than a million knee and hip arthroplasties are performed every year in the United States (1). Projections for arthroplasties in the United States show—in comparison to 2000 to 2014—an increase by 75, 129, and 284% (hips) and by 110, 182, and 401% (knees) in 2025, 2030, and 2040, respectively (2). Periprosthetic joint infections (PJIs) occur in 0.3–1.7% of patients after total replacement of the hip, in 0.5–2% after total replacement of the knee, and in 2–9% after total replacement of the ankle (3). The incidence of surgical site infection following open reduction and internal fixation of a fracture of the extremities is 1–5% (4). In addition, the implant can be infected via the hematogenous route as long as it remains in the body (5, 6). These arguments underscore the importance of the endeavor to constantly advance research on bone and joint infections.

Biofilm development plays a pivotal role in implant-associated infections and allows microorganisms to survive in an environment protected by antimicrobial agents and the host immune system (7). Successful management of orthopedic biofilm infections eradicates the infection while preserving a pain-free functional musculoskeletal apparatus (with or without implant). This is best achieved by combining an appropriate surgical procedure with antimicrobial treatment and by considering the characteristics of each individual patient.

An optimal and individualized approach considers several factors, including stability of the implant, causative pathogen and type of infection, patient's concomitant comorbidities, and

surgical procedure limitations. The concept of "precision medicine" is the new frontier of the modern health care industry and it combines multiple fields of expertise (e.g., genetics, genomics, big data analytics, and population health) (8). It recognizes individual variability in genes, environment, and lifestyle for each person. Precision medicine aims to replace the classic "one-size-fits-all" therapeutic strategy and to achieve "the right drug, with the right dose at the right time to the right patient" (9). By identifying patients most likely to benefit from a specific treatment, clinical outcomes can be improved and side effects and costs reduced. To achieve this, precision medicine must rely on accurate diagnostic tools to effectively maximize benefits and reduce risks to patients.

In orthopedic biofilm infections, precision medicine applies to biofilm properties of the causative microorganisms. Identifying factors associated with so-called low or high biofilm production can influence treatment strategies. The ability of bacteria to adhere to nonliving surfaces and the antibiotic susceptibility patterns of biofilm—together with host and surgical factors—may aid decision making regarding implant removal. In this article, we examine diagnostic advances in the identification of causative microorganisms and antimicrobial susceptibility testing of biofilm bacteria, putting into perspective how these methods can help individualize the management of bone and joint infections.

ADVANCES IN THE DIAGNOSIS OF ORTHOPEDIC BIOFILM INFECTIONS

Culture of bone or peri-implant tissue samples is the gold standard for identifying the organism causing the infection. Low-frequency ultrasound (i.e., sonication) is a useful tool for the clinical microbiologist in the diagnosis of biofilm-associated infections. Culture of sonication fluid increases the sensitivity and microbiological yield by disrupting the bacterial biofilm and is less affected by prior antimicrobial treatment than are prosthetic tissue samples (10, 11).

Molecular Diagnostic Tests

Molecular diagnostic tests directly applicable to clinical samples have rapidly developed in recent years, improving the sensitivity of PJI diagnosis. They can be applied to both tissue and sonication samples. **Table 1** provides an overview of different molecular techniques evaluated for the diagnosis of PJI, and displays their corresponding sensitivity and specificity. Reported sensitivities of PCR assays applied to DNA extracted from sonication fluid range from 70 to 96% (12–15). Broad-range PCR assays can identify organisms present in the panel, but will miss atypical, rare, and nontargeted pathogens (34). Assays targeting the universal 16S rRNA gene followed by sequencing allowed researchers to partially overcome this limit. Although, the different regions that can be chosen as the target and the limited resolution among closely related species still represent a barrier. This obstacle can be tackled by next-generation sequencing (NGS)-based assays. The term NGS, also known as massive parallel sequencing, refers to non-Sanger-based high throughput DNA sequencing technologies, that allow millions of small or large (depending on the use of short or long read-based technologies) DNA fragments to be sequenced and deciphered simultaneously and independently (35). NGS can be applied using either a targeted or untargeted approach. Targeted NGS focuses on selected portions of genome, while untargeted NGS adopts an unbiased, hypothesis-free approach to detect any portion of genome. In clinical microbiology, after its introduction, NGS was initially applied to cultured isolates to obtain the complete DNA sequence of their genome at a single time (whole genome sequencing, WGS). More recently, the method was applied directly to clinical samples, allowing the identification of pathogens and prediction of antimicrobial resistance (36–42). When applied directly to clinical samples using an untargeted approach, NGS allows the comprehensive characterization of all nucleic acids (microbial and human) present in the sample. This approach is called untargeted metagenomic NGS (mNGS) (43). This technique can significantly reduce the turnaround time to diagnosis compared with culture methods and can detect pathogens not identified by conventional methods (38–41).

The utility of NGS/mNGS in providing a clinically useful diagnosis was first demonstrated in infections of the central nervous system (38, 44). It has also been successfully applied to orthopedic infections to determine their etiology. Street et al. showed that mNGS on sonication fluid had 88% species-level sensitivity (28). The meta-analysis published by Li et al. showed that sequencing assays to diagnose PJI, including NGS-based assays, had favorable diagnostic accuracy, with a pooled sensitivity of 0.81 and a specificity of 0.94 (33). Thoendel et al., using mNGS on sonicate fluid, were able to identify new potential pathogens in 44% of culture-negative PJIs (26). Tarabichi et al. found that NGS was able to identify an organism in almost 90% of PJI cases compared with 61% for culture and to detect a potential pathogen in 80% of culture-negative PJIs. The samples included synovial fluid, deep-tissue specimens, and swabs from medullary canals. In 88% of samples, the results were concordant with culture results, and in 9 of 11 culture-negative PJIs, the authors detected three or more organisms (24). Wang et al. evaluated the efficacy, safety, accuracy, and reliability of mNGS for identifying pathogens in culture-negative PJIs. They found that antibiotic-related complications, duration of intravenous antibiotic treatment, and antibiotics costs in the mNGS-based treatment group were lower than in the empiric treatment group and yielded a favorable outcome in less time. Outcome was defined as control of the infection and absence of recurrence during follow-up (45). Compared to PCR-based assays, mNGS is a relatively young technique with plenty of potential that will inevitably improve, as demonstrated by four recent studies where its sensitivity to diagnose PJI was above 90% in one of them and 95% in three of them, respectively (29–32) (**Table 1**). A same-day NGS diagnostic result may significantly increase precision and efficiency while reducing the cost of PJI care. Long-read Nanopore technology, allowing real-time sequencing and analysis, seems promising to achieve same-day PJI diagnosis. Wang at al. conducted a preliminary assessment of this technology (46). The authors were able to identify

TABLE 1 | Overview of sensitivity and specificity of different molecular techniques applied to the diagnosis of PJI.

Molecular method	Clinical sample type tested	No. of samples tested	Sensitivity	Specificity	References
Multiplex panel PCR	Sonication fluid	37	78%	na	(12)
Multiplex panel PCR	Sonication fluid	24	96%	100%	(13)
Multiplex panel PCR	Sonication fluid	144	77.1%	97.9%	(14)
Multiplex panel PCR	Periprosthetic tissue, sonication fluid	64	15.6%, 68.8%	96.8%, 100%	(15)
16S rRNA - Sanger	Sonication fluid	69	86%	93%	(16)[c]
16S rRNA – NGS (pyrosequencing)	Sonication fluid	92	90%	88%	(17)[c]
16s rRNA - Sanger	Sonication fluid	366	70%	98%	(18)[c]
16S rRNA - Sanger	Periprosthetic tissue and synovial fluid	122	68% (82%[b])	98% (96%[b])	(19)[c]
16S rRNA - Sanger	Biofilm[a]	157	80% (100%[b])	94.5% (98%[b])	(20)[c]
16S rRNA - Sanger	Periprosthetic tissue	67	75%	94%	(21)[c]
16S rRNA - Sanger	Periprosthetic tissue	264	73% (70%[b])	95.5% (96%[b])	(22)[c]
16S rRNA – NGS	Sonication fluid	101	(86%[b])	(98%[b])	(23)[c]
16S rRNA – NGS	Synovial fluid, deep tissue specimens and swabs	65	89%	73%	(24)[c]
16S rRNA – NGS	Synovial fluid	86	(87%[b])	(82%[b])	(25)[c]
mNGS	Sonication fluid	408	(71%[b])[d]	(96%[b])	(26)[c]
mNGS	Synovial fluid	168	(67%[b])[e]	(93%[b])	(27)[c]
mNGS	Sonication fluid	97	88%	88%	(28)
16S rRNA – Sanger, mNGS	Synovial fluid	63	82%, 96%	94%	(29)
mNGS	Synovial fluid	25	92%	92%	(30)
mNGS	Periprosthetic tissue	44	95.5%	91%	(31)
mNGS	Synovial fluid	49	96%	95%	(32)

na, not available.

[a] *Biofilm was defined as scratched samples from the surface of implants.*

[b] *The numbers in brackets represent calculations made by other authors in the context of a systematic review and meta-analysis (33).*

[c] *The study results were included in the systematic review and meta-analysis of Li et al. (33).*

[d] *In 44% of culture-negative PJI, a microorganism was identified.*

[e] *In 16% of culture-negative PJI, a microorganism was identified.*

the causative microorganisms of PJI within approximately 12 hours after sample collection. Furthermore, the detection of corresponding antimicrobial resistance determinants was faster compared to short-read based mNGS (46). NGS performed on cultured microorganisms isolated from a patient experiencing two episodes of PJI within a 9-month period was crucial to understand that the second episode was a new infection with the same bacterial species (47). Taken together, these studies indicate that molecular diagnostic tests in diagnosing and managing osteoarticular infection are helpful—and potentially superior to conventional culture methods—when applied in the appropriate context.

Are We Ready for Metagenomics in Routine Diagnostics?

Multiple factors promote the implementation of NGS-based tests in routine clinical service. These include decreasing costs of NGS technology, cost savings from the replacement of other diagnostic tests, amount of information provided in a single test (prediction of virulence and drug resistance, outbreak analysis, difficult/unculturable species detection), and availability of portable rapid sequencing technology offering real-time data analysis, i.e., Nanopore Technologies (48, 49).

Because of the high sensitivity of NGS, findings must be interpreted in the clinical context, as detection of DNA is not sufficient to conclude that an identified microorganism is the cause of the infection. On the other hand, sensitivity is critically affected by the background level generated by human DNA present in the sample. Implementation of NGS-based tests in clinical settings requires standardized protocols and validation of each step, from DNA extraction and library preparation to bioinformatics analysis and validation and interpretation of sequencing results. The US Food and Drug Administration (FDA) has published general guidelines for the validation of infectious diseases with NGS-based diagnostic tests; mNGS tests have been meanwhile successfully validated by several groups in Clinical Laboratory Improvement Amendments-certified clinical laboratories (50–55). The vast number of detectable species makes it necessary to continuously monitor and independently confirm uncommon or unexpected results. Currently, for PJIs,

mNGS is recommended only when patients have an inconclusive diagnosis despite the contribution of a multidisciplinary expert team, or in order to further investigate culture-negative PJIs (33, 56, 57).

DIFFERENCES IN BIOFILM PRODUCTION

Biofilm formation is influenced by strain-specific properties. It is an interaction between environmental factors, surface structure, bacterial growth phase, and genetic determinants. Several methods have been published for the visualization or quantification of biofilm, including crystal violet staining and absorbance measurement, growth on polymethylmethacrylate beads followed by washing and sonication, scanning electron microscopy, and others. The physical and chemical surface properties in these assays may not necessarily reflect those in human infections, but they are responsible for the amount and structure of the biofilm (58). Bacteria can adhere to various metals (59) and biofilm formation may be different than on nonmetal material. However, irrespective of the method applied, several studies have demonstrated differences in biofilm production of the same species within a sample collection (60, 61). Post et al. demonstrated that, in a collection of isolates obtained from orthopedic implant-related infections, PJI isolates were more frequently strong biofilm formers than were isolates from fracture fixation-device infections (60).

The differences in biofilm production between various clones within the same species are important for personalized medicine; the transfer of these findings into clinical practice, however, is more challenging. The relation of biofilm production *in vitro* to infection manifestations in humans has not yet been established. For individual patients, it is important to know whether their infection is caused by a low or a high biofilm producer. Of note, these terms are schematic expressions without precise definition. Additional questions include whether or not there is an antimicrobial agent that is active against the causative microorganism, as well as which antibiotic concentrations are needed, and for how long, to cure the infection (62). Notably, the site of infection is in an extravascular compartment. Current strategies to tackle these questions include molecular tests for biofilm properties (section Genes Involved in Biofilm Formation and section Antimicrobial Susceptibility Testing of Biofilm Bacteria).

Genes Involved in Biofilm Formation

The accessory gene regulator (*agr*) system in *Staphylococcus aureus* controls the expression of MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) and regulates the quorum-sensing system along with the P2 and P3 promoters (63). *Agr* is required for *S. aureus* emigration from implant-associated biofilm (64). Post et al. observed statistically significant differences between orthopedic implant-related and non-implant-related infection isolates for the *sdrE*, *can*, *clfA*, and *bbp* genes (60). Thus, it is conceivable that molecular analysis may be helpful in categorizing bacterial clones into low and high biofilm producers.

Beyond the genes associated with biofilm production, bacterial toxins may interact with biofilm production, staphylococcal toxins and their impact on bone and joint infections, and factors associated with the aggregation of *S. aureus* in synovial fluid. Biofilm and exotoxin interactions have been recently reviewed elsewhere (65) and are beyond the scope of this article.

In group B streptococcus, the two-component system CovR/S regulates the expression of surface adhesion proteins (e.g., BsaB/FbsC), and CovR/S mutants show increased adherence to host cells and biofilm formation (66–68). Patras et al. (69) identified the biofilm regulatory protein A (BrpA) in group B streptococci. The carbon catabolite protein A (CcpA) is involved in the regulation of biofilm formation in oral streptococci (70). In addition, pili—long filamentous structures growing from the bacterial surface—have been associated with biofilm formation (71, 72). Genes encoding for pilus components and their development (i.e., backbone and accessory proteins, sortase enzymes) are clustered in genomic pathogenicity islands named PI-1,−2a, and−2b (73). Among these, PI-2a has been associated with the strongest biofilm-forming capacity (72).

While quantitative proteomics of strong and weak biofilm formers reveal important regulators of biofilm formers [e.g., *Enterococcus faecalis* in (74)], there is yet not a direct association of genetic elements and clinical failure. Numerous factors in the host-pathogen interaction and treatment concepts of biofilm-related infections contribute to clinical failure (75).

The constantly growing list of research findings illustrates the high potential for detecting biofilm properties via NGS in routine diagnostics. Thanks to technological advances, the entire pathogen's genome can be characterized in near real time with a sequence coverage sufficient to detect minor genetic variants, critical for directing clinical care decisions (48, 76, 77). This also allows clinical scientists to obtain a pathogen's detailed profile in terms of clone type and presence of genetic determinants associated with biofilm production.

Antimicrobial Susceptibility Testing of Biofilm Bacteria

The minimal inhibitory concentrations (MICs) of antibiotics are routinely determined by using planktonic bacteria and do not match the concentrations that are effective in preventing, inhibiting, reducing, or eradicating biofilm bacteria (78). Different biofilm susceptibility endpoint parameters have been proposed to guide the treatment of biofilm-associated infections. These include minimal biofilm eradicating concentration (MBEC), minimal biofilm inhibitory concentration (MBIC), biofilm bactericidal concentration, and biofilm prevention concentration.

The MBEC of an antibiotic agent is the concentration of antibiotic needed to kill all viable bacteria within an established biofilm, including persister cells. MBEC determination is not offered in clinical microbiology diagnostic laboratories, it is not standardized or validated to be performed routinely, and

antibiotic exposure time is not reported. Methods for MBEC determination have technical difficulties, leading to considerable variability in results (79, 80). Furthermore, biofilm age is an important factor; thus, MBEC depends on the point of readout (e.g., 24 vs. 120 hours) (81). Host factors such as plasma and heme increase tolerance to antibiotics. The same aged biofilm in normal media vs. media with human plasma has been shown to have up to a 100-fold difference in tolerance (82). This again illustrates the importance of considering differences between *in vivo* and *in vitro* when interpreting MBEC results. For Gram-positive organisms, MBECs of beta-lactams and glycopeptides are typically several 100 to 1000 times higher than the corresponding MIC of planktonic bacteria (62). The MBEC:MIC ratio for aminoglycosides and rifampin is typically lower than are those for beta-lactams. However, *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. may display high-level gentamicin resistance (62, 83).

For MBECs to provide a clinically useful result, a standardized assay is needed, in particular when considering antibiotic therapy as part of personalized medicine (84). Consequently, interest in antimicrobial susceptibility testing in biofilms is ongoing, and several methods have been implemented in the last few years (78).

Many published results derive from the MBEC device (formerly called the Calgary device, Innovotech Inc., Edmonton, Alberta, Canada) (85). The method is challenging, as it requires a specific protocol for every species and a antimicrobial agent [supplementary material in (85)]. Recently, a promising steam-based method has been developed by Tasse et al. (86), showing similar results to those of the MBEC device. This easy-to-handle and easy-to-implement method points toward interinstitutional comparability of MBEC results. However, there is a lack of data and direct link between MBEC results and clinical outcome, and hence, there is no scientific reasoning to favor one of the methods.

The challenge reoccurs when transferring MBEC results to antibiotic dosing in patients and treatment concept. It may seem logical that high MBEC results are associated with high biofilm production, failure to achieve the required antibiotic concentration at the site of infection, and hence, clinical failure. Conversely, low MBEC results may be associated with low biofilm production, success in achieving the required antibiotic concentration at the site of infection, and considerable chance of clinical cure. Unfortunately, there is no such simple linearity because many technical, material, and environmental factors influence the microbiological result, as outlined earlier. Nonetheless, a similar concept was recently applied on a case basis in a context other than orthopedic infections, namely, in a patient with a cardiac device-related infection and conduit valve prosthesis endocarditis caused by nutritionally variant streptococci (87). Biofilm production was investigated on three different materials (bone cement, glass, plastic), and several different culture media. Biofilm eradication concentrations were examined with two different methods. All tests uniformly demonstrated that the causative microorganism did not produce biofilm. The MBEC results were similar to MIC results. Conservative treatment without device removal proved to be successful. Although the example is only a single case, it raises (at least) three thoughts. Firstly, these concepts require fruitful collaboration of many disciplines in a joint team effort. Secondly, the number of required investigations for a single case is still too high for immediate transfer to routine clinical practice. And thirdly, depending on the complexity and specific circumstances of a clinical case requiring treatment decision making, the hypothesized linearity of low MBEC results and low biofilm production may be more tempting to believe than the presumed association of high MBEC results and high biofilm production.

PERSPECTIVE: PRECISION MEDICINE TO AID DECISION MAKING IN ORTHOPEDIC BIOFILM INFECTIONS

The concept of considering clone-level (rather than only species-level) bacterial properties in the medical decision-making process represents a promising perspective. The more invasive the surgical procedure for removing a device, the higher the complication rate. If we are able to identify microorganisms with poor biofilm production by using reliable methods prior to the planned surgical intervention, patients could potentially benefit from conservative treatment. Conversely, unsuccessful attempts at implant retention could be avoided in the presence of microorganisms with strong biofilm production. Lack of clonal analysis beyond species identification for predicting outcome in established treatment concepts reflects a knowledge gap, and findings supporting this approach will affect further research beyond the field of septic surgery. Implementation of such a concept will have cost-saving effects, considering the duration of hospitalization expenditures associated with avoidable surgical infections and the complications associated with these interventions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

RB drafted and co-wrote the manuscript and performed the literature review. PS revised the manuscript, supervised the work, and co-wrote the manuscript. Both authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

Barbara Every, ELS, BioMedical Editor, St. Albert, AB, Canada, provided English language editing.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
published: 16 November 2020
doi: 10.3389/fmed.2020.570572

Phage Therapy as Adjuvant to Conservative Surgery and Antibiotics to Salvage Patients With Relapsing *S. aureus* Prosthetic Knee Infection

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OPEN ACCESS

Edited by:
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Reviewed by:
Jingmin Gu, Jilin University, China
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Specialty section:
This article was submitted to Infectious Diseases – Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *08 June 2020*
Accepted: *28 September 2020*
Published: *16 November 2020*

Citation:
Ferry T, Kolenda C, Batailler C, Gustave C-A, Lustig S, Malatray M, Fevre C, Josse J, Petitjean C, Chidiac C, Leboucher G and Laurent F (2020) Phage Therapy as Adjuvant to Conservative Surgery and Antibiotics to Salvage Patients With Relapsing S. aureus Prosthetic Knee Infection. Front. Med. 7:570572. doi: 10.3389/fmed.2020.570572

Objectives: To report the management of three consecutive patients with relapsing *Staphylococcus aureus* prosthetic knee infection (PKI) for whom explantation was not feasible who received a phage therapy during a "Debridement Antibiotics and Implant Retention" (DAIR) procedure followed by suppressive antimicrobial therapy.

Methods: Each case was discussed individually in our reference center and with the French National Agency (ANSM). The lytic activity of three phages targeting *S. aureus*, which was produced with a controlled and reproducible process, was assessed before surgery (phagogram). A hospital pharmacist extemporaneously assembled the phage cocktail (1 ml of 1×10^{10} PFU/ml for each phage) as "magistral" preparation (final dilution 1×10^{9} PFU/ml), which was administered by the surgeon directly into the joint, after the DAIR procedure and joint closure (PhagoDAIR procedure).

Results: Three elderly patients were treated with the PhagoDAIR procedure. Phagograms revealed a high susceptibility to at least two of the three phages. During surgery, all patients had poor local conditions including pus in contact to the implant. After a prolonged follow-up, mild discharge of synovial fluid persisted in two patients, for whom a subsequent DAIR was performed showing only mild synovial inflammation without bacterial persistence or super-infection. The outcome was finally favorable with a significant and impressive clinical improvement of the function.

Conclusions: The PhagoDAIR procedure has the potential to be used as salvage for patients with relapsing *S. aureus* PKI, in combination with suppressive antibiotics to avoid considerable loss of function. This report provides preliminary data supporting the setup of a prospective multicentric clinical trial.

Keywords: bacteriophages, phage therapy, prosthetic-joint infection, *S. aureus*, phagotherapy

INTRODUCTION

Prosthetic-joint infection (PJI) is the most dramatic complication after joint arthroplasty. *Staphylococcus aureus* is frequently involved in patients with relapsing PJI as this bacterium is a strong biofilm producer, which facilitates its persistence on the implant surface (1). In patients with chronic PJI, the recommended strategy is prosthesis exchange to mechanically eradicate the biofilm (1–4). However, prosthesis explantation is sometimes not feasible, especially for the knee location in elderly patients with multiple comorbidities at risk of dramatic loss of function, reduction of the bone stock, fracture, or death. Debridement Antibiotics and Implant Retention (DAIR) could be used for such patients but the risk of relapse is particularly high due to the bacterial persistence in biofilm on the implant surface, even if suppressive antibiotic treatment (SAT) is usually proposed for these patients (1–4). In this context, the use of new adjuvant therapies that locally target the bacterial biofilm is of great interest as it may increase the success rate of SAT.

Lytic bacteriophages are viruses that specifically target bacteria (5). They are considered to have a high potential in patients with PJI, as it has been demonstrated that they have a synergistic anti-biofilm activity with antibiotics (6). In a patient with relapsing chronic PJI, we already performed DAIR and used bacteriophages that were injected into the joint with a good clinical response (7). Since then, three other consecutive patients included in the Lyon BJI cohort study (NCT02817711) and presenting a *S. aureus* relapsing prosthesis knee infection (PKI)

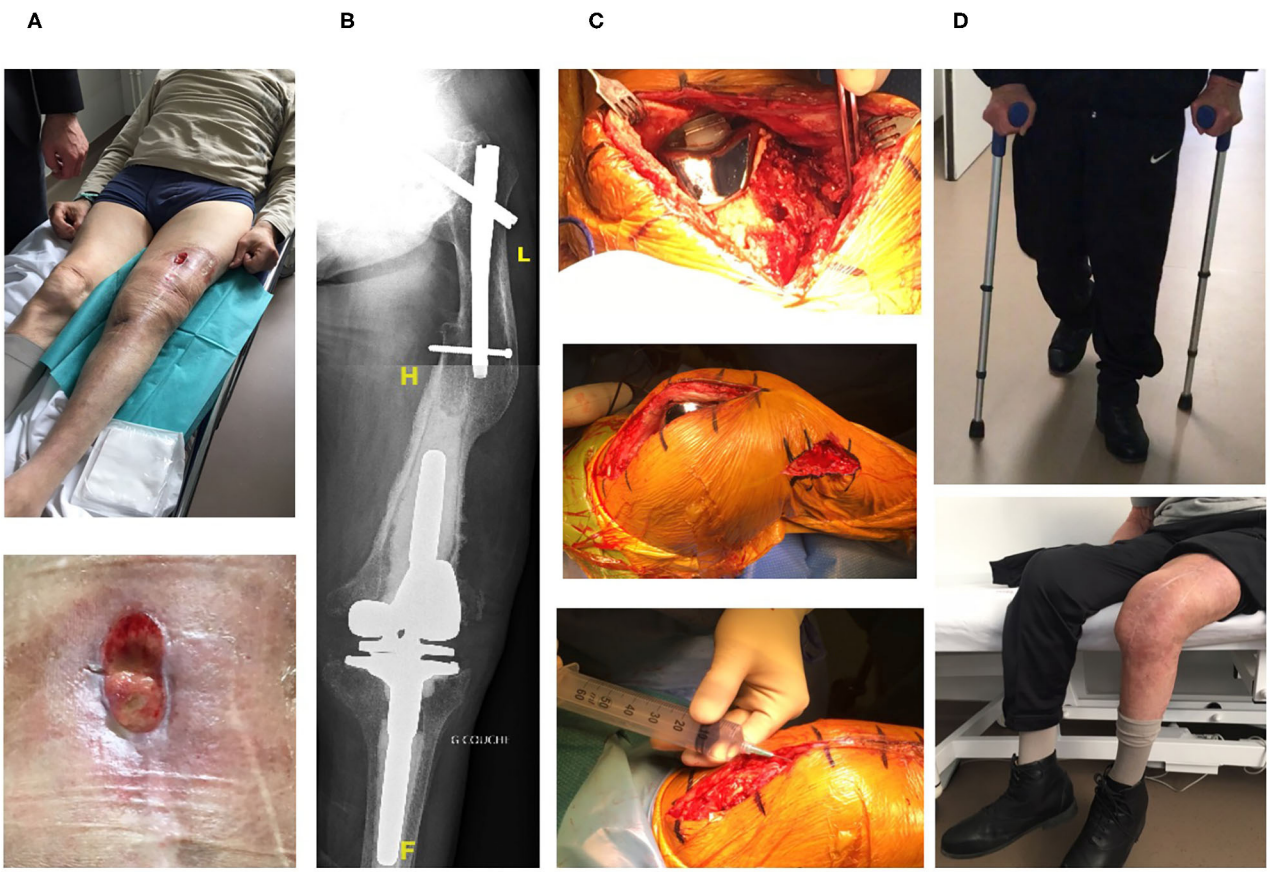


FIGURE 1 | The first patient is an 80-year-old man with past history of Parkinson disease, cardiac arrhythmia, hypertension, right hip prosthetic joint, and left hip fracture treated with osteosynthesis. A left knee prosthetic joint arthroplasty was performed in 2004. In 2014, the patient developed signs of septic arthritis of the left knee due to a methicillin-susceptible *S. aureus*. A one-stage exchange was performed with reimplantation of a hinged knee prosthesis with long cemented stem. The patient received intravenous daptomycin and rifampin orally, followed by cotrimoxazole and clindamycin for a total duration of 3 months. Unfortunately, a relapse occurred in April 2015 with persistence of the *S. aureus*, and pristinamycin (a streptogramin A+B antibiotic available in France) was prescribed as suppressive antimicrobial therapy. Despite pristinamycin treatment, the patient developed signs of left septic arthritis with severe pain, bedridden and large anterior fistula with purulent discharge **(A)**. X-ray revealed a complex orthopedic situation with radiological signs of prosthesis loosening of the femoral stem **(B)**. Open DAIR was performed showing poor soft tissue condition with pus in contact to the implant and the personalized cocktail of phages was injected in joint just after closure **(C)**. The patient improved quickly, but at 3 months, a mild discharge of synoviual fluid persisted (**Figure 5**). A new DAIR was performed, revealing a drastic improvement of the local conditions, with only mild signs of non-specific synovitis. Multiple samples were performed for bacterial culture, but no recurrency/superinfection was diagnosed (cultures remained sterile, specific *S. aureus* PCR was negative). Cefalexin was then prescribed as suppressive therapy, and the outcome was favorable after 2 years and half of follow-up with no sign of infection, a negative C-reactive protein, and a pain-free walking [**(D)**, **Supplementary Video 1**].

in therapeutic dead-end (for whom revision was not feasible) benefited from DAIR with local administration of a cocktail of bacteriophages followed by SAT and were proposed in the present report.

METHODS

In accordance with the local ethics committee, each case was discussed individually during multidisciplinary meetings in our regional reference center (8), and then with the French National Agency for Medicines and Health Products Safety (ANSM) to validate that no other options could be proposed without excessive risk of loss of function or death. Each patient signed a written consent. Phages PP1493, PP1815, and PP1957 (from the Pherecydes Pharma library), targeting *S. aureus*, were produced with a controlled and reproducible process in an appropriate environment under the supervision of ANSM. These phages are strictly lytic natural phages isolated from environmental sources and selected for the complementarity of their host spectrum on a clinical reference panel of *S. aureus* (not shown). They belong to the *Silviavirus* and *Rosenblumvirus* genus (ICTV 2018). A "phagogram" was performed using two complementary techniques (spot plaque assay, kinetic assay) to assess the lytic activity of the bacteriophages on clinical strains collected from joint puncture performed before surgery (7). The DAIR procedure was performed during open surgery, as previously described (9). A hospital pharmacist extemporaneously assembled the cocktail of the three phages (1 ml of 1×10^{10} PFU/ml for each phage) as "magistral" preparation (final dilution 1×10^{9} PFU/ml), and each cocktail was administered by the surgeon directly into the joint, after the DAIR procedure and joint closure (PhagoDAIR procedure).

RESULTS

Three consecutive elderly patients were treated with the PhagoDAIR procedure. All of them presented treatment failure despite a one-stage exchange followed by prolonged SAT (patient

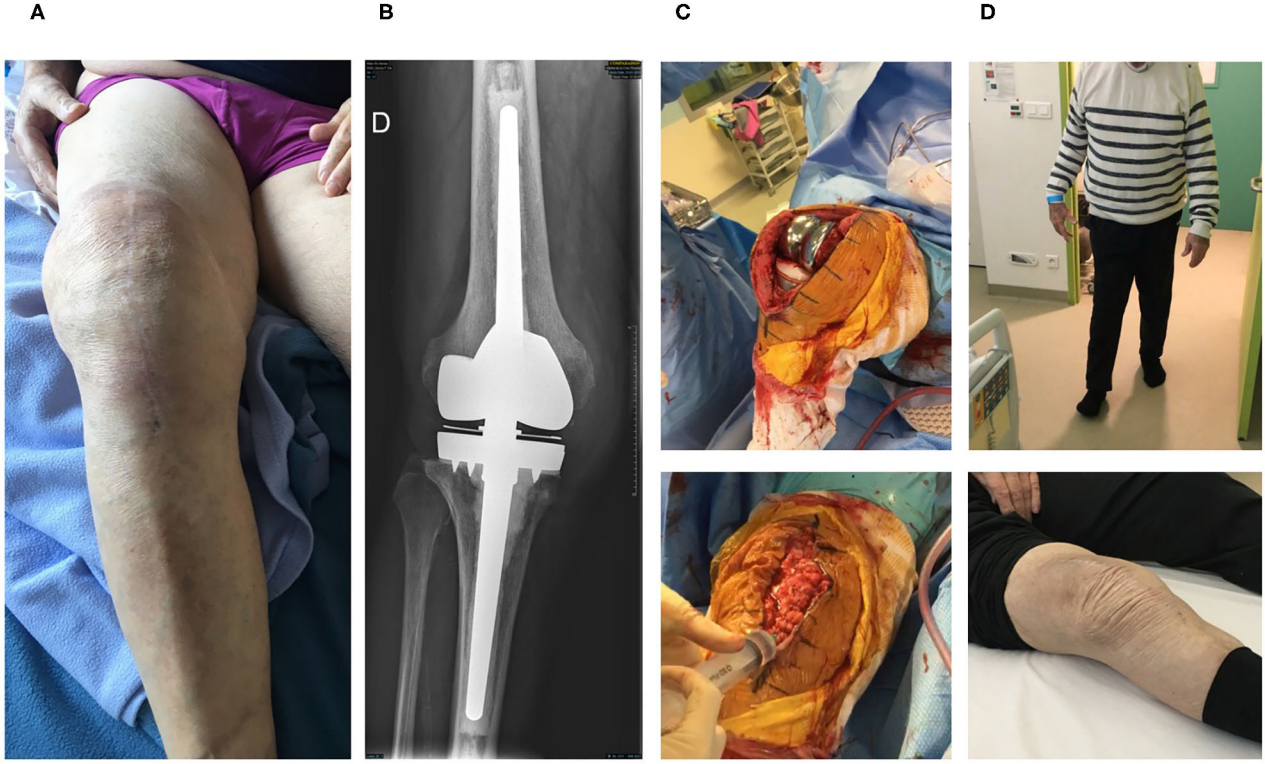


FIGURE 2 | The second patient is an 84-year-old man with past history of dyslipidemia and right prosthetic-knee arthroplasty in 2006. A two-stage exchange was performed in 2007 for a *S. epidermidis* PKI. As the patient kept a painful knee, a one-stage exchange was performed in 2016, with implantation of a hinged cemented knee prosthesis with long stem. Cultures remained sterile. In 2019, the patient developed clinical signs of acute septic arthritis with fever. A DAIR without polyethylene exchange was performed and methicillin-susceptible *S. aureus* grew in perioperative samples and blood cultures. Endocarditis was excluded. The patient was treated using intravenous cefazolin and rifampin orally. Cefazolin was switched to ofloxacin 3 weeks after the DAIR and was combined with rifampin. Under this treatment, new signs of septic arthritis occurred 6 weeks after the DAIR, with local erythema, pain, and large joint effusion **(A)**. A joint puncture was performed, but no pathogen was isolated in cultures, and the failure was attributed to *S. aureus*. X-ray showed no loosening of the prosthesis **(B)**. Open DAIR was performed showing significant local inflammation and pus into the joint. The personalized cocktail of phages was administered after joint closure **(C)**. The patient improved quickly, doxycycline was then prescribed as suppressive therapy, and the outcome was favorable at 7 months with no signs of infection, a negative C-reactive protein and pain-free walking [**(D)**, **Supplementary Video 2**].

1, **Figure 1**) or a previous DAIR followed by adequate antibiotics (patients 2 and 3; **Figures 2**, **3**, respectively). All patients had knee prosthesis with long stem (revision prosthesis), without loosening, for whom polyethylene exchange was not feasible (**Figures 1**–**3B**). Phagograms revealed a high susceptibility to at least two of the three phages at high MOI (**Figure 4**). Phages with partial lytic activity, or phages only active at high MOI, were still preserved in the final cocktail, to prevent the acquisition of phage resistance under treatment, as these phenomena has been previously observed in a previous case report (10). Patient 3 was infected with two genetically different strains (*agr* typing), showing different phage susceptibility (**Figures 4C,D**). During surgery, all patients had poor local conditions including pus in contact to the implant (**Figures 1**–**3C**). Polyethylene exchange was technically not feasible, and soft tissue flap was required for one of them (**Figure 3C**). After the PhagoDAIR procedure, patients were treated with antibiotics in combination during 6 to 12 weeks, followed by SAT, according to the IDSA guidelines (**Table 1**) (2). After a follow-up of 7, 11, and 30 months, respectively, the outcome was favorable with a significant and

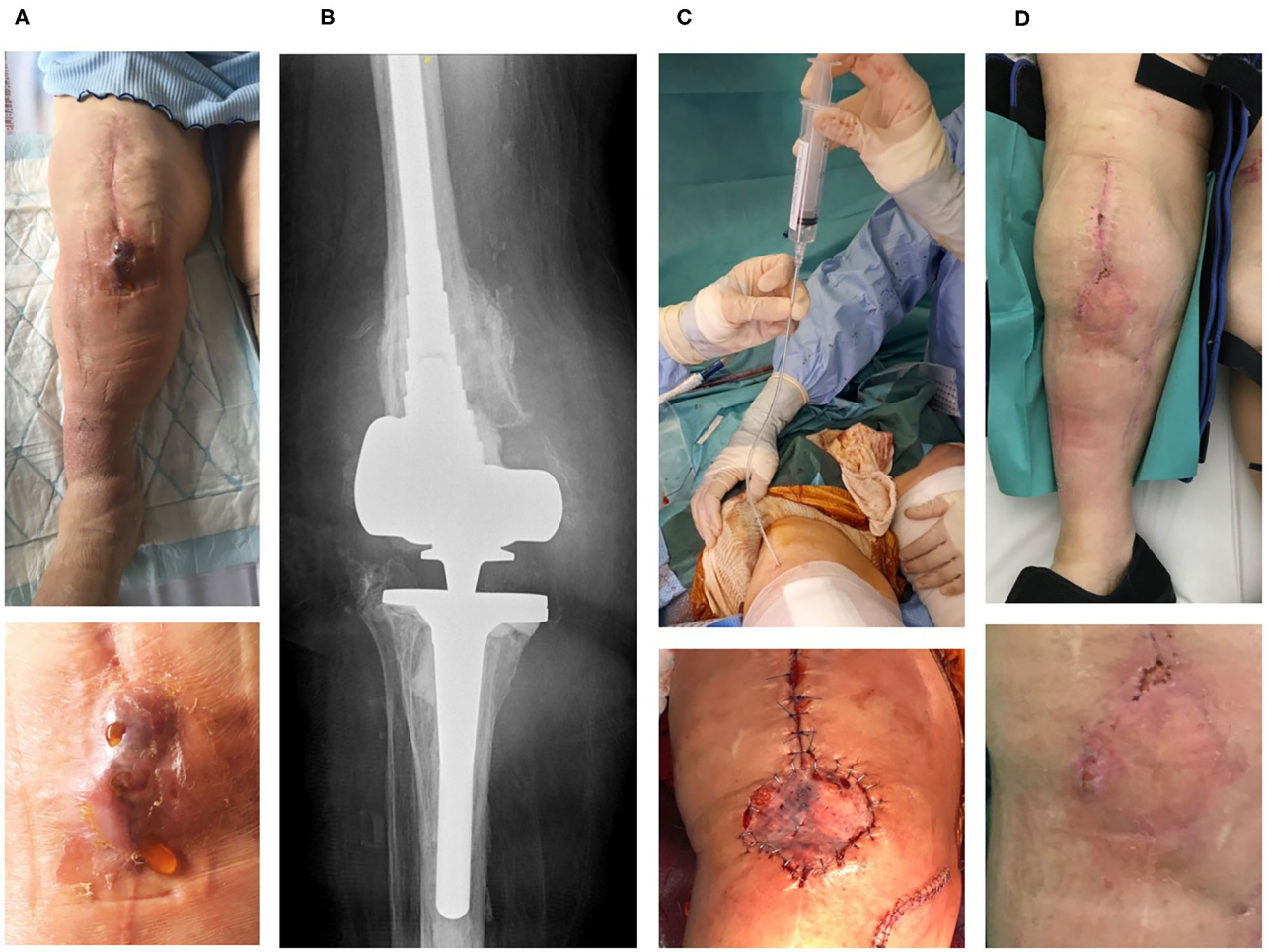
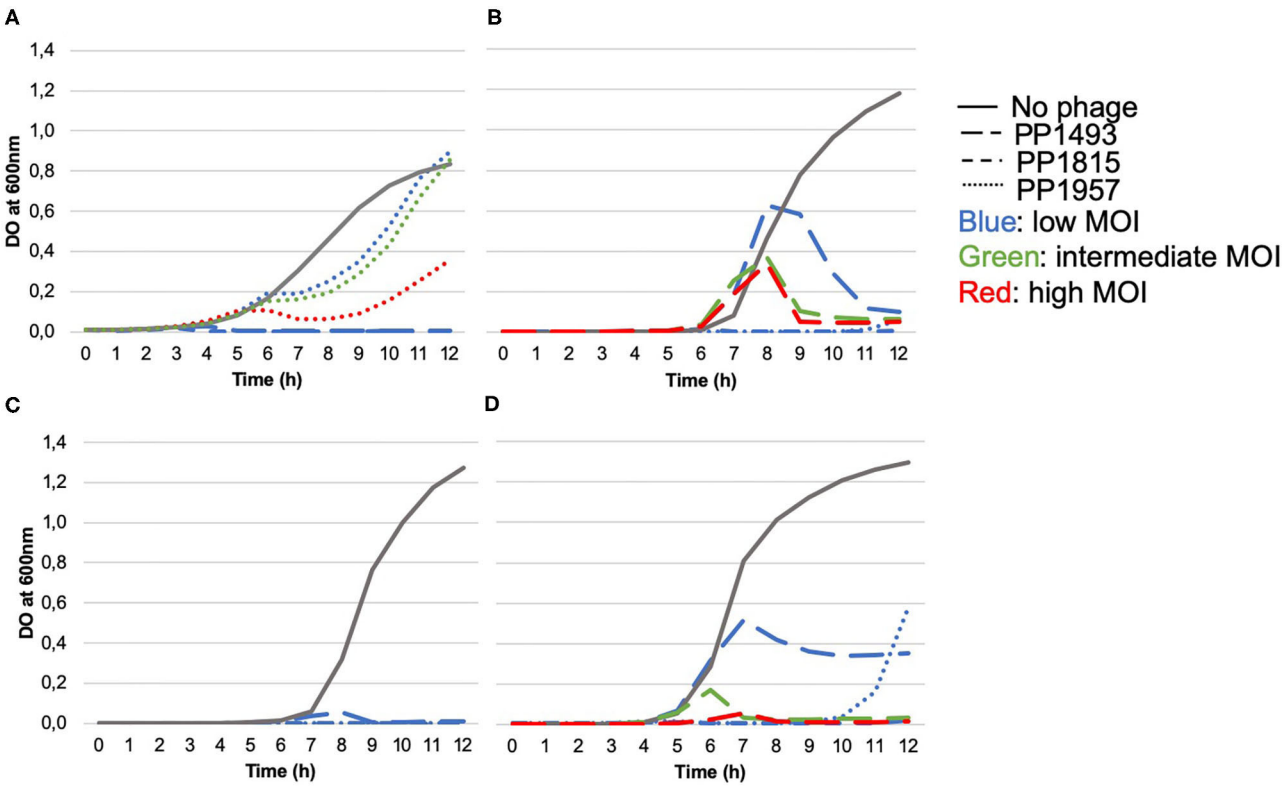


FIGURE 3 | The third patient is an 83-year-old woman with past history of hypertension and lymphoedema. Right knee arthroplasty was performed in 2000. As prosthesis loosening occurred, a one-stage exchange was performed in 2018 with implantation of a hinged cemented knee prosthesis with long stem. In April 2019, a fistula occurred close to the tibial tuberosity, and in May 2019, the patient developed signs of PKI with fever. Open DAIR without polyethylene exchange was performed and methicillin-susceptible *S. aureus* grew in blood cultures, and in perioperative samples (with different phenotypes on agar for these latter, but with the same antibiogram. Determination of *agr* type by PCR showed that one strain belong to *agr* type I, and the other one to *agr* type II). Endocarditis was excluded. The patient received intravenous cloxacillin and rifampin orally. Unfortunately, the outcome was not favorable with occurrence of a large fistula with *Bourgeon charnu* **(A)**. A joint puncture was performed, but no pathogen was isolated in cultures, and the failure was attributed to *S. aureus*. X-ray did not reveal prosthesis loosening **(B)**. Open DAIR was performed showing catastrophic local condition with pus into the joint, and soft-tissue coverage with local flap was required. The personalized cocktail of phages was administered after joint closure, using a tube placed directly into the joint to preserve the flap **(C)**. The patient improved quickly, but a mild discharge of synovial fluid persisted after 4 months **(D)**. A new DAIR was performed, but no superinfection was diagnosed (cultures remained sterile, specific *S. aureus* PCR was negative). Doxycycline was then prescribed as suppressive therapy. At 11 months, a pain-free walking was observed, but the patient had persisting mild intermittent discharge of synovial fluid associated with a fistula and a C-reactive protein ≈20 mg/L (**Figure 6**, **Supplementary Video 3**), without any superinfection at the joint puncture performed at the end of the follow-up (cultures still sterile, and PCR still negative).



E

	Panel A	Panel B	Panel C	Panel D
PP1493	$1.4x10^{-1}$	1.7	$3.3x10^{-1}$	Too small
PP1815	$4.2x10^{-1}$	1.1	1.0	Too small
PP1957	$2.9x10^{-4}$	$5.6x10^{-1}$	1.2	0 (No PFU)

FIGURE 4 | Phagograms performed using the kinetic assay **(A–D)** and the spot plaque assay **(E)**. For the kinetic assay, the bacterium was incubated with or without bacteriophages, tested individually at three different initial concentrations to obtain low, intermediate, and high multiplicity of infection (MOI, ratio of phages/bacteria). **(A)** Corresponds to bacterial growth kinetic (Optical Density at 600 nm) obtained for the strain isolated on patient 1; **(B)** from patient 2; and **(C,D)** from the two different strains isolated from patient 3. Except for PP1957 on strain A (that only delayed bacterial growth whatever the MOI), all phages were able to inhibit bacterial growth at high MOI **(A–D)**. Of note: (i) a slight delayed inhibition of the bacterial growth of strain B was observed with phage PP1493; (ii) only low MOI are represented concerning the phage activity on the strain C (blue lines), as intermediate and high MOI totally inhibited the bacterial growth; (iii) a partial growth inhibition of strain D (one of the two strains infecting patient 3) was observed with the phage PP1493 at low MOI, and a late growth of strain D appeared in presence of PP1957 at low MOI. The plaque test assay relies on the determination of the efficiency of plating score (EOP), calculated dividing the phage titer on the patient's strain by the phage titer on the reference strain (highly susceptible strain, used for phage amplification). The closer to 1 is the score is, the more efficient the phage is and likely active at low dose. Panel E showed the EOP scores of each strain and revealed that PP1493, PP1815, and PP1957 were active and very efficient on strains A, B, C from patients 1, 2, and 3 since EOP scores ranged from 1.4×10^{-1} to 1.7 with the exception of PP1957 strain A, which showed a low efficiency, consistently with kinetic assay results. However, for strain D (second strain from patient 3), PFU formed with PP1493 and PP1815 could be observed but they were too small to be enumerated confidently. The minimum concentration of spotted phages leading to PFU was 5.5×10^5 and 3.9×10^4 PFU/ml, respectively. Partial lysis without PFU was observed for PP1957 (result concordant with the kinetic assay as only a late growth at the lowest dose was observed).

impressive clinical improvement of the function for all patients (**Figures 1–3D**; **Supplementary Videos 1–3**). A mild discharge of synovial fluid persisted in two patients (patient 1, **Figure 5**), for whom a new DAIR was performed showing only mild synovial inflammation without bacterial persistence or super-infection. For these patients, a new phage administration was not performed. At the end of the follow-up, total disappearance of signs of infection was noticed except for one patient (patient 3, who was infected with two different *S. aureus* strains with different phage susceptibility) for whom a fistula with a mild intermittent synovial fluid discharge persisted despite the iterative DAIR (**Figure 6**).

DISCUSSION

We report here the impressive positive outcome of patients with relapsing *S. aureus* PKI treated with the PhagoDAIR procedure. This innovative procedure has been set up in our center for salvage therapy in patients with complex PJI after individual multidisciplinary and ethical discussions under supervision of

TABLE 1 | Details about the prosthetic knee infection history of the three patients treated with the PhagoDAIR procedure.

Patient ID	Age (sex)	Putative mechanism of inoculation	Time since prosthesis implantation (months)	Duration of clinical symptoms before the PhagoDAIR procedure (days)	Delay from the previous surgery performed for the current infection to the PhagoDAIR procedure (days)	Antimicrobial resistance	Successive primary antimicrobial therapies after the PhagoDAIR procedure (duration in days)	Successive SAT after the primary antimicrobial therapy(ies) until the last follow-up (duration in days)
Patient 1	80 (male)	Perioperative	40	976	One-stage exchange (1,371)	Penicillin G	Daptomycin–cloxacillin (4)* Levofloxacin–rifampin (123)	Doxycycline (45)*** Cephalexin (739)
Patient 2	84 (male)	Hematogenous	35	82	Open DAIR without PE exchange (78)	Erythromycin	Daptomycin–levofloxacin (14)** Ofloxacin–doxycycline (72)	Doxycycline (189)
Patient 3	83 (female)	Perioperative	11	122	Open DAIR without PE exchange (98)	Penicillin G	Daptomycin–cefepime–rifampin (14)** Levofloxacin–rifampin (111)	Doxycycline (200)

SAT, suppressive antimicrobial therapy; DAIR, debridement antibiotics and implant retention; PE, polyethylene.
**This regimen was switched to oral antibiotics due to loss of the central line.*
***This regimen was switched to oral antibiotics at the reception of the final culture results.*
****This regimen was switched to cephalexin due to oral ulceration attributed to doxycycline.*

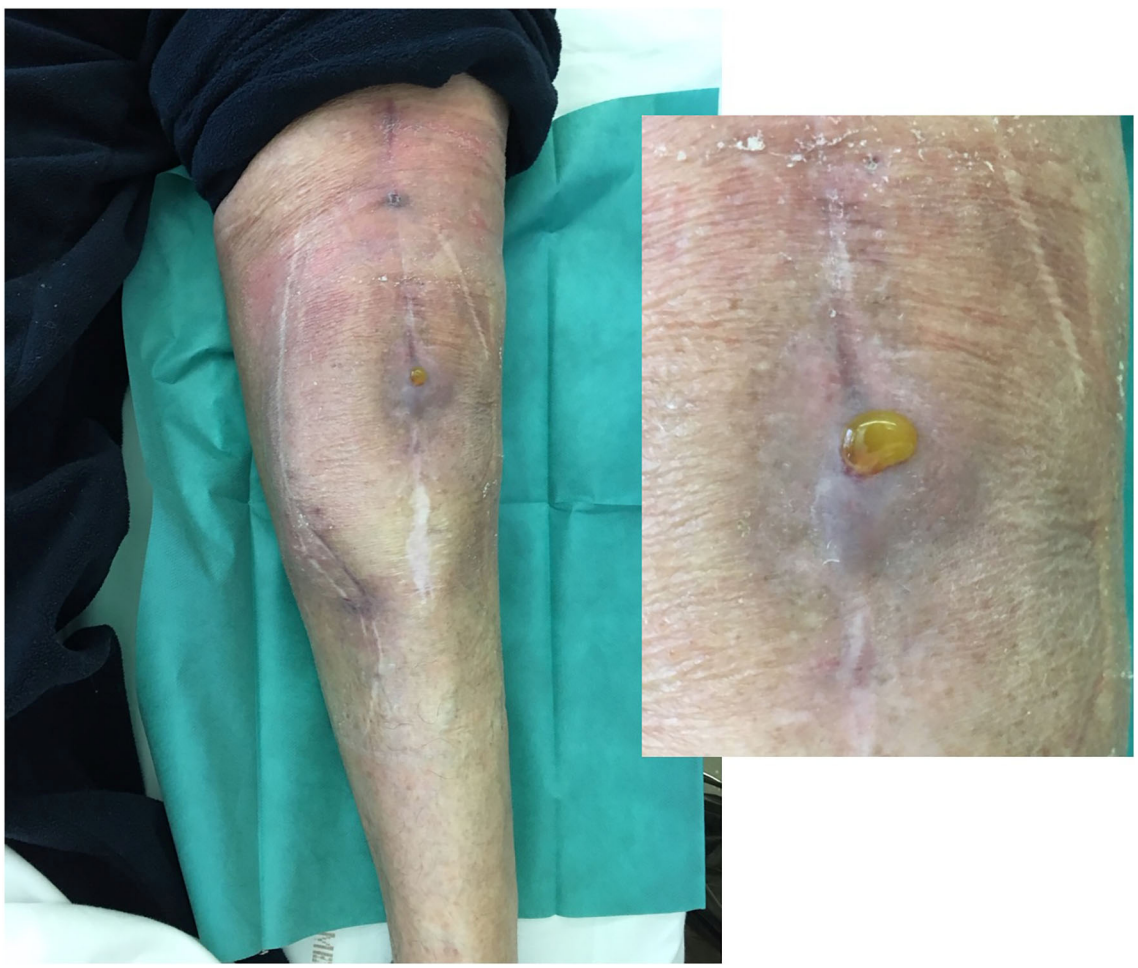


FIGURE 5 | Local status of patient 1 showing, 3 months after the phagoDAIR procedure, the significant improvement of inflammatory signs of infection, with persistence of mild discharge of synovial fluid through the scar, for which a new DAIR was performed to exclude a superinfection. After the new DAIR, the outcome was favorable.

ANSM. We previously published a case report using these phages, as salvage treatment, during a DAIR procedure in a patient with *S. aureus*, but also plurimicrobial, prosthetic hip infection. As we observed a positive outcome, we considered this approach as a possible opportunity to treat other patients with dead-end clinical situation (7). Of note, the three patients treated here

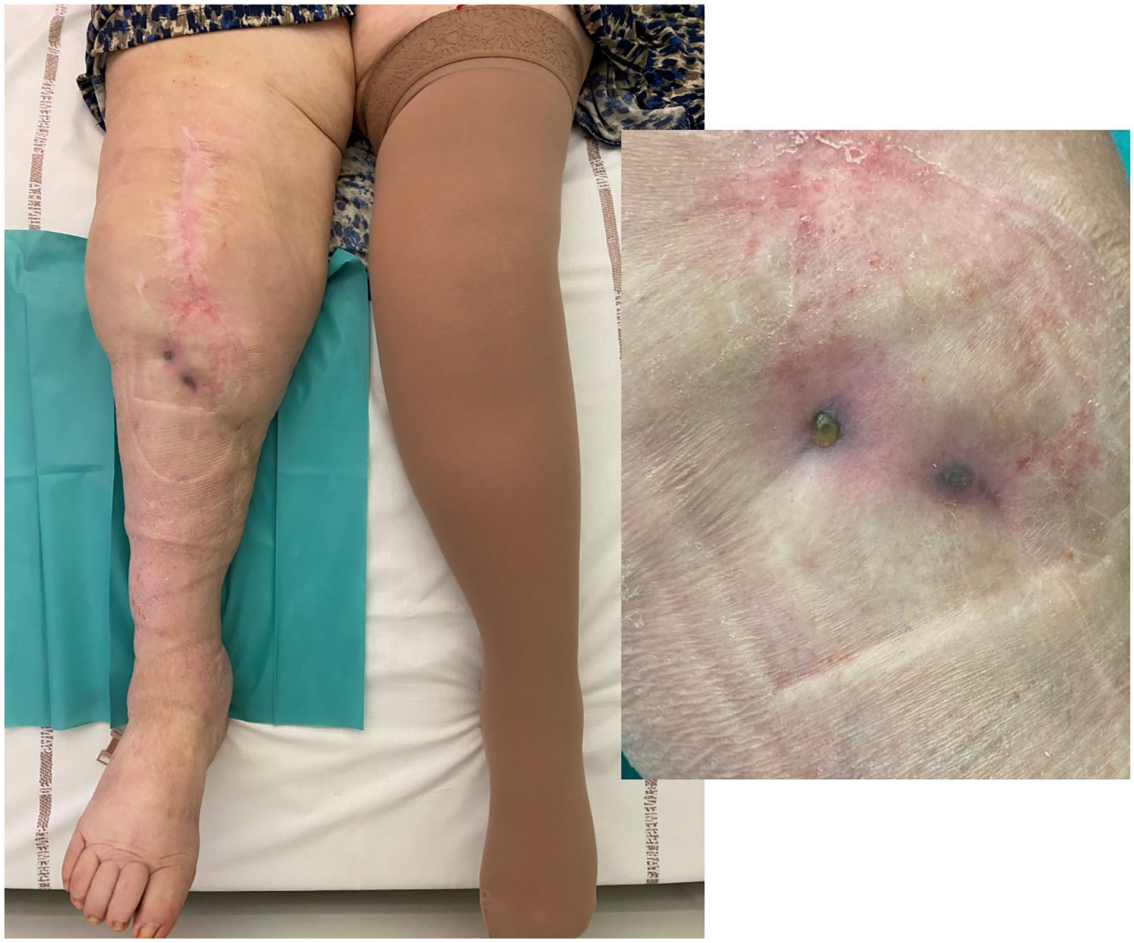


FIGURE 6 | Local status of patient 3 (infected with two different *S. aureus* strains and for whom a soft-tissue coverage was required) showing 8 months after the phagoDAIR procedure a significant improvement of inflammatory signs of infection, but with fistula and discharge that persisted despite the phagoDAIR procedure.

experienced a previous treatment failure despite a one-stage exchange followed by prolonged SAT or despite a DAIR followed by adequate antibiotics. All of them had knee prosthesis with long stem (revision prosthesis), without loosening. For patient 1, the previous medico-surgical treatment was optimal, with a one-stage exchange followed by adequate antimicrobial antibiotics during 3 months. However, pristinamycin (a streptogramin A+B antibiotic available in France) was directly prescribed 1 year later, at the diagnosis of relapse, without any subsequent surgery. For patient 2, DAIR and adequate antibiotics were prescribed for a hematogenous infection, but polyethylene was not exchanged during DAIR, and the relapse occurred during rifampin–ofloxacin treatment. Concerning patient 3, a DAIR procedure and adequate antibiotics potentially followed by SAT were proposed, even if the infection was chronic. This later patient also experience a failure under antimicrobial therapy that included rifampin.

Globally, in patients with PJI, targeting the biofilm is a potential key determinant. In patients with chronic infection, if getting rid of the biofilm by prosthesis exchange is not feasible, DAIR followed by SAT is usually proposed, but the success rate remains low (1–4). By using phage therapy as adjunctive therapy, the aim is to act locally on bacteria embedded in biofilm stuck on the implant surface into the joint cavity, as demonstrated recently in an animal model (11). The anti-*S. aureus* phages used to treat our patients demonstrated dose-dependent anti-biofilm activity *in vitro*. In addition, in the same study, synergistic effects were reported when phages were combined with antibiotics used at concentrations below MICs (6).

This report has several major limitations: (i) the non-comparative design, (ii) the small number of patients, (iii) the use of phage therapy as adjuvant to surgery and antibiotics that leads to question about the intrinsic capacity of the phage therapy to improve the outcome, and (iv) the subsequent DAIR performed in two patients during the follow-up. However, the clinical history of the three patients was homogeneous, with a dead-end situation. As they presented relapsing *S. aureus* PKI after previous standard of care treatments, the expected success rate of iterative DAIR procedure followed by SAT was close to zero. First of all, *S. aureus per se* is considered as

the most virulent pathogen in PJI and is an independent risk for DAIR failure (9). Secondly, a previous one-stage or DAIR procedure was performed, unsuccessfully, and a subsequent DAIR is an independent risk factor for failure (12). Finally, it was technically not feasible to replace the polyethylene in these patients, which has been associated with failure in several studies (2–4, 12). Concerning the DAIR performed in two patients after the PhagoDAIR procedure, the indication was based on the persistence of a mild discharge of synovial fluid, whereas all patients had already improved significantly, and only non-specific mild synovitis, without positive cultures, was found. For one of these patients, the outcome was finally favorable. Put together, these different points suggest that the PhagoDAIR procedure highly participated into the clinical improvement in the patients reported here.

CONCLUSION

The PhagoDAIR procedure has the potential to be used as salvage for patients with relapsing *S. aureus* PKI, in combination with suppressive antibiotics to avoid considerable loss of function. This report provides preliminary data supporting the setup of a prospective multicentric clinical trial.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospices Civils De Lyon Ethic Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

TF managed all the patients, directly interacted with the French Health authority, and wrote the manuscript. CB, SL, and MM participated to the surgical management. CK, C-AG, CF, JJ, and CP performed bacteriological experiments. GL performed the magistral preparation. All authors participated in the literature review and the improvement of the manuscript.

ACKNOWLEDGMENTS

We acknowledge Marine Trapani and Mathieu Medina from Pherecydes Pharma who participated in the performance of the phagograms. Drs. Laurence Legout, Julien Saison, and Florence Suy who have referred the patients to the CRIOAc Lyon. Drs. Antoine Schneider, Tarik Ait Si Selmi and Charles Fiquet.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.570572/full#supplementary-material

Supplementary Video 1 | Video showing patient 1 walking without any pain 1 year after the PhagoDAIR procedure.

Supplementary Video 2 | Video showing patient 2 walking without any pain 7 months after the PhagoDAIR procedure.

Supplementary Video 3 | Video showing patient 2 walking without any pain 11 months after the PhagoDAIR procedure.

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Conflict of Interest: CF and CP are employed by the company Pherecydes Pharma.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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PERSPECTIVE
published: 23 December 2020
doi: 10.3389/fmed.2020.585658

Outpatient Subcutaneous Antimicrobial Therapy (OSCAT) as a Measure to Improve the Quality and Efficiency of Healthcare Delivery for Patients With Serious Bacterial Infections

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Edited by:
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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *21 July 2020*
Accepted: *24 November 2020*
Published: *23 December 2020*

Citation:
Ferry T, Lodise TP, Gallagher JC, Forestier E, Goutelle S, Tam VH, Mohr JF III and Roubaud-Baudron C (2020) Outpatient Subcutaneous Antimicrobial Therapy (OSCAT) as a Measure to Improve the Quality and Efficiency of Healthcare Delivery for Patients With Serious Bacterial Infections. Front. Med. 7:585658. doi: 10.3389/fmed.2020.585658

Since the 1970s, outpatient parenteral antimicrobial therapy (OPAT) has been a viable option for patients who require intravenous antibiotics when hospitalization is not warranted. While the benefits of OPAT as a measure to improve the efficiency of healthcare delivery (i.e., reduced hospital days) and patient satisfaction are well-documented, OPAT is associated with a number of challenges, including line complications and reliance on daily healthcare interactions in some cases at home or in a clinic. To minimize the continued need for intensive healthcare services in the outpatient setting, there is trend toward patients self-administering antibiotics at home without the presence of healthcare workers, after adequate training. In most cases, patients administer the antibiotics through an established intravenous catheter. While this OPAT practice is becoming more accepted as a standard of care, the potential for line complications still exists. Outpatient subcutaneous antimicrobial therapy (OSCAT) has become an increasingly accepted alternative route of administration of antibiotics to IV by French infectious diseases physicians and geriatricians; however, currently, no antibiotics are approved to be administered subcutaneously. Antibiotics with longer half-lives that are completely absorbed and have a favorable local tolerability profile are ideal candidates for OSCAT and have the potential to maximize the quality

and efficiency of parenteral antibiotic delivery in the outpatient setting. The increasing development of wearable, on-body subcutaneous delivery systems make OSCAT even more viable as they increase patient independence while avoiding line complications and potentially removing the need for direct healthcare professional observation.

Keywords: OPAT, OSCAT, BJI, healthcare system, antibiotics, catheter-related complications, subcutaneous antibiotic

INTRODUCTION

Outpatient parenteral antimicrobial therapy (OPAT) is defined by the Infectious Disease Society of America (IDSA) as the administration of parenteral antimicrobial therapy in at least two doses on different days without intervening hospitalization. Dedicated guidelines for the prescription and management of OPAT have been published and updated in 2018 (1). OPAT is particularly relevant for the treatment of serious infections in patients who require long-term antibiotic therapy, especially when oral agents are not feasible, practical, or indicated, such as in bone and joint infections (BJI). However, OPAT has some drawbacks including the potential need for daily healthcare practitioner assessments due to the significant rate of catheter-related complications that can arise. To minimize the continued need for intensive healthcare services in the outpatient setting, there is a trend toward appropriate patients self-administering antibiotics at their own home, independent of healthcare workers. In most cases, patients administer antibiotics through an established intravenous (IV) catheter. However, the potential for IV catheter complications still exists with this practice, and there has been growing interest toward outpatient subcutaneous antimicrobial therapy (OSCAT) whereby the reliance on IV catheters can be eliminated. Herein, we (1) describe the limitation of current IV administration OPAT practices, (2) review available published data on SC administration of antibiotics in the outpatient setting, including PK data, (3) discuss the characteristics of parenteral antibiotics best suited for SC administration, and (4) review the potential use of wearable, on-body subcutaneous (SC) drug delivery systems that can be used to further facilitate the utility of OSCAT.

LIMITATIONS ASSOCIATED WITH INTRAVENOUS ADMINISTRATION OF ANTIBOTICS IN THE OUTPATIENT SETTING

Intravenous administration is the primary route used in OPAT. Every parenteral antibiotic can be administrated by this route, either by continuous, extended, or intermittent infusion. Some parenteral antibiotics are also approved for intramuscular injection. However, IM administration is impractical for longer courses of therapy due to pain. The treatment of BJI has been a common infection in which OPAT has been used as: (i) treatment courses often last several weeks; (ii) hospitalization is generally not needed; and (iii) oral agents may not be adequate (2).

Peripheral (midline) catheters or peripheral-inserted central catheters (PICC) have been extensively used for OPAT and are preferred due to their short-term use and lessened number of complications (1). They are mainly inserted through a radiological-guided procedure, simple to maintain, and easily removed. However, these types of intravenous access catheters have intrinsic disadvantages in this specific setting. Peripheral catheters must be changed every 4 days which is challenging for longer-term treatment, particularly in older patients who have poor venous network. Subclavicular or jugular central venous line exposes patients to unnecessary risks of infection and thrombosis and is prone to accidental withdrawal. Ports, while commonly used for outpatient administration of cancer chemotherapeutics, are not practical for patients who required parenteral antibiotics for a few weeks to months as their insertion and removal necessitate two surgical procedures.

Despite the clear advantages of midlines and PICC over other IV administration devices, they still associated with a number of potential complications (3). The most frequent is catheter occlusion which often requires an exchange of the catheter. The most concerning complications are IV catheter-related infections and thrombophlebitis (4, 5). In a systematic review of the literature, adverse event rates associated with vascular access devices ranged from 0 to 29% (6). In a single-center study evaluating 8,263 patients on OPAT over a 4-year period, 381 (4.6%) had at least one visit to the emergency department within 30 days of imitating OPAT and 104 ED visits (54% of OPAT-related ED visits and 27% of all ED visits) were due to occlusions and dislodgement of the intravenous catheter (7). Older patients are particularly vulnerable to experiencing complications during OPAT and influenced by the patient's cognition, mobility, and dexterity (1).

OPAT TRENDS AND THE CONCEPT OF OSCAT IN CLINICAL PRACTICE

To date, OPAT has largely been delivered at physicians' offices/clinics or at patients' homes by home healthcare agencies. To minimize the continued need for intensive healthcare services in the outpatient setting, there are two main emerging practices in OPAT (**Figure 1**). The first trend is patient's self-administration of parenteral antibiotics after a training course independent of a home healthcare worker. Self-administration of IV antibiotics requires a degree of patient skill and responsibility and may not be practical for populations such as IV drug users, geriatric patients, and patients with cognitive or physical impairments. While this OPAT practice is becoming more commonplace, the potential for line complications still exists. In a study of 1,464 patients who received 1,950 OPAT courses at home, 9% of courses had at least one vascular access problem requiring

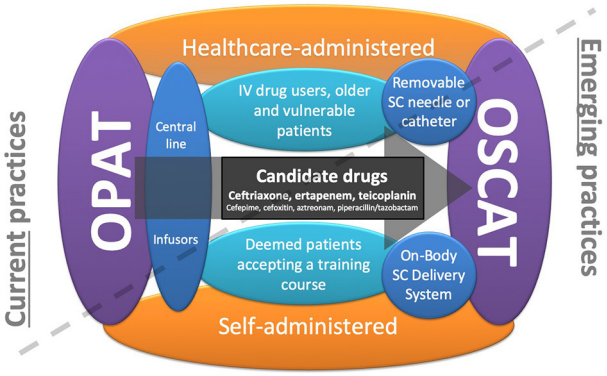

FIGURE 1 | Current trends in outpatient parenteral antimicrobial therapy (OPAT): OPAT currently needs central lines and daily consumption of healthcare professionals at home. The first trend concerns self-administered OPAT that makes the patient more independent, and the second trend concerns the outpatient subcutaneous antimicrobial therapy (OSCAT) concept that avoids line complications. By cumulating these two trends, on-body subcutaneous delivery systems seem to be particularly relevant, making the patient more independent and avoiding line complications and the constraint of the daily passage of health professionals.

clinical intervention. The most common complication was occlusion (49%), followed by accidental dislodgement (14%). Thrombosis and line infection occurred less frequently at rates of 0.34 and 0.16/1,000 OPAT days, respectively (8). However, recent studies of this method have not described increases in hospital readmissions nor complications in comparison with administration in the presence of a healthcare worker (3).

The second trend in OPAT is the use of SC administration. The major advantage of SC administration is that it minimizes the potential previously mentioned complications associated with IV catheter administration (9, 10). In addition, it is less demanding for nurses and could be performed at home or in long-term care facilities. There are a number of important considerations with the use of OSCAT as it is not yet FDA approved; however it is extensively used by French infectious diseases physicians and geriatricians (11) (**Figures 2A–C**). To facilitate OSCAT, there are now commercially available wearable, on-body SC delivery systems (**Figures 2D–I**). These devices make OSCAT more viable as they increase patient independence while avoiding line complications and remove the need for healthcare professionals. Below, we review the current published literature on SC administration of antibiotics in the outpatient setting, their PK properties by SC administration, identify the parenteral antibiotics best suited for SC administration, and review the potential use of wearable, on-body SC drug delivery systems that can be used to facilitate OSCAT.

SUBCUTANEOUS ADMINISTRATION OF ANTIBIOTICS: CURRENT STATE OF THE EVIDENCE

Main Antibiotics

The first studies describing the use of SC administration of antibiotics in humans were published in the 1970s (12). In a large French national survey, based on voluntary participation, 367/382 (96%) of ID physicians and geriatricians reported prescribing antibiotics to be administered *via* the SC route (11). Of those surveyed who reported prescribing SC antibiotics, 100% reported to using ceftriaxone, they also prescribed teicoplanin, aminoglycosides, ertapenem, and amoxicillin in 39, 35, 33, and 15% of cases, respectively. In a retrospective study of 368 patients (mean age, 87 years) hospitalized in an acute geriatric unit of a Spanish public hospital treated with SC antibiotics between January 2012 and December 2016, ceftriaxone (233/368) and ertapenem (98/368) were the most commonly prescribed SC antibiotics (13). Case series report the use of SC route for administrating piperacillin-tazobactam (14), ceftazidime (15–17), and fosfomycin (18).

Main Indications

The main reasons for utilizing the SC route were poor venous access, delirium, swallowing disorders, palliative care, tolerance, absence of oral active antibiotic drug, and facilitating hospital discharge or avoiding hospitalization (11, 19).

Tolerance

In a prospective study evaluating the local tolerance of subcutaneously administered antibiotics in 219 patients (mean age, 83 years), 163 (74%) patients received ceftriaxone, 30 (13.7%) received ertapenem, and 10 (4.6%) received teicoplanin (19). Overall, 50 (22,8%) patients experienced 74 adverse events (AE) receiving ceftriaxone ($n = 35/163$), ertapenem ($n = 7/30$), and teicoplanin ($n = 7/10$). Pain was the most frequently reported local AE ($n = 29$). Other local AEs reported included hematoma ($n = 16$), induration ($n = 17$), and erythema ($n = 6$). However, no skin necrosis was reported. There was one AE considered to be severe, resulting in hospital readmission due to persistent induration and pain at the injection site; otherwise, AEs were transient. Reconstitution with lidocaine was used in ∼30% of the cases and tended to decrease the occurrence of AEs (31% with lidocaine vs. 69% without) but not significantly ($p = 0.09$). Moreover, the use of a rigid catheter and a rapid infusion (<5 min) were associated with the occurrence of pain. In the abovementioned Spanish study, 3% of AE were reported (possibly underestimated due to the retrospective design of the study) mainly associated with aminoglycosides.

Considering all the SC ceftriaxone injections reported in the literature (∼440 patients), only two cases of skin necrosis were reported and the most common AE was pain. With ertapenem (∼200 patients), only one case of skin necrosis was reported. Local AEs have also been reported with teicoplanin (∼110 patients); however, high concentrations were reported to have been administered. Skin necrosis was frequently described with SC admiration of aminoglycosides in several case reports (20–25).

Main Infections

In a prospective evaluation of SC antibiotics conducted in France, the main sources of infection were urinary tract (44%), respiratory tract (33%), and BJI (7%). Several other publications from the same reference center for the management of complex

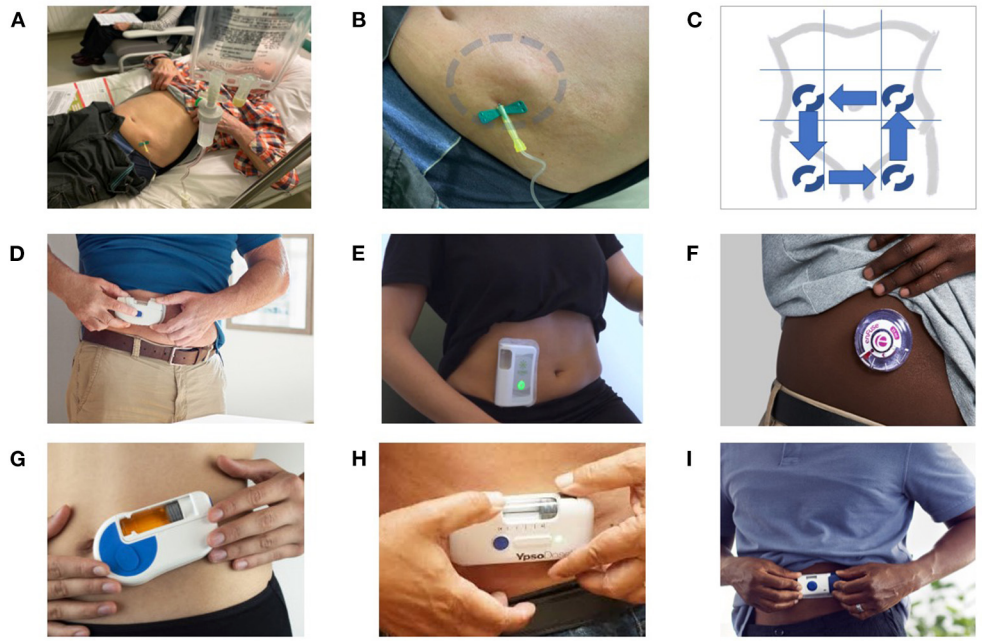

FIGURE 2 | SC administration of antibiotics: from the French experience to the use of on-body delivery systems for subcutaneous drug delivery. **(A–C)** Example of an off-label SC administration in the French reference center for the management of complex BJI. An 80-year-old patient with a prosthetic joint infection required prolonged ertapenem therapy, as no oral options were available due to multidrug resistance, and the use of a catheter was considered to be not appropriate and feasible. As a consequence, instead of using a catheter for a daily single injection, the patient was treated with SC administration, with dilution of the drug in 50 ml of saline, and gravity infusion using a removable butterfly needle **(A)**. At the end of the 30–45-min injection, a tumefaction appeared around the injection site (blue circle), which gradually disappears over 15–30 min due to diffusion of the antibiotics **(B)**. Each injection was performed on different sites, with rotating alternation of the SC injections on the left flank, right flank, anterior face of the right thigh, and anterior face of the right thigh **(C)**. This patient, treated for several weeks, did not experience any local injection site adverse events, such as inflammation or necrosis. **(D–I)** Examples of on-body delivery systems for subcutaneous drug delivery: SmartDose® Gen II 10 ml (West Pharmaceuticals) **(D)**; Wearable on-body device utilizing a vial (Sorrel Medical) **(E)**; EnFuse® On-Body Infusor (Enable Injections) **(F)**; Wearable On-Body Large Volume Injector (Sonceboz) **(G)**; YpsoDose® (Ypsomed AG) **(H)**; and SmartDose® Gen I 3.5 ml (West Pharmaceuticals) **(F)**.

BJI report the use of SC administration of antibiotics (26–28), especially, the authors described a prospective cohort of 10 patients (67–90 years) receiving SC prolonged suppressive antibiotic therapy for prosthetic joint infections or chronic osteomyelitis with a median treatment duration of 433 days, seven patients received SC ertapenem, three received SC ceftriaxone, and one received SC ceftazidime (one patient had sequential therapy with 8 days of ceftriaxone before switching to ertapenem). Six of the patients had a favorable outcome, for three patients, failure occurred after antibiotic cessation, one was lost to follow-up. One patient who received repeated direct injections of 2 g of ceftriaxone diluted to 2–5 ml final volume developed a skin necrosis (28).

Main Pharmacokinetic Data

There is an increasing number of publications evaluating the pharmacokinetics (PK) of numerous antibiotics administered subcutaneously, particularly beta-lactams. In general, based on available literature, absorption of antibiotics after SC administration is complete resulting in bioavailability comparable with the same dose administered intravenously; however, the time to Cmax is prolonged and the overall Cmax is reduced (29).

Among the five studies evaluating PK of SC ceftriaxone (∼80 subjects), three included healthy volunteers (30–32) and two were carried out in geriatrics and ID departments (33, 34). The average bioavailability of SC ceftriaxone across doses from 500 mg to 2 g was 96–107% compared with the IV route (30–32). The use of hyaluronidase before SC ceftriaxone injection increased the Cmax and shortened the time to Cmax (31).

Five studies collected PK data on SC ertapenem with patients (∼60 patients) hospitalized in intensive care unit (35), ID (26, 27), or geriatrics (36), they confirmed a decreased Cmax and increased time to achieve it. After a 1 g dose of ertapenem, the bioavailability was 99 ± 18% after SC administration compared with IV (35). In a population PK analysis and PK/PD simulation based on the pharmacokinetics of IV and SC ertapenem in patients with BJI in a geriatric population, SC administration resulted in slightly higher or comparable time above the MIC compared with IV (27, 36).

Five studies reported SC teicoplanin PK data in patients (∼80 patients) with suspected or confirmed nosocomial infections admitted to ICU, geriatric, or ID departments (37–41). AUC

and Cmin were lower when teicoplanin was administrated with SC compared with IV during the loading phase; however, these differences disappeared overtime, indicating that IV route should be preferred during the first days of treatment and SC could be used afterwards with adequate Cmin and AUC/MIC (40).

In healthy volunteers, a SC infusion of cefepime or temocillin demonstrated comparable PK profile as an intramuscular or IV injection, respectively (42). Data on aminoglycosides PK (∼60 patients) confirmed a decreased Cmax (12, 43–45).

CANDIDATE ANTIBIOTICS FOR SC DELIVERY

A suitable parenteral antibiotic candidate for SC administration is one that is absorbed completely, has a favorable PK/PD profile, and is well-tolerated. Due to the lower Cmax with SC administration, concentration-dependent antibacterials such as aminoglycosides are not good candidates; however, for time-dependent antibacterials such as beta-lactams, SC administration may be appropriate. A relatively long half-life is also a desired feature to enable OSCAT, as it may allow for less-frequent administration. With comparable PK profiles due to their complete absorption and longer half-lives, the pharmacodynamic profiles of ceftriaxone, ertapenem, and teicoplanin administered subcutaneously are comparable with those same profiles when the same antibiotic is administered intravenously making them ideal candidates from a PK and PK/PD perspective (27, 32, 37).

Another key consideration is local tolerability. The local tolerance of SC administration depends on several factors including the injection site, viscosity of the formulation, volume and rate of administration, pH, concentration, and osmolarity of the drug solution (46). In a study to evaluate local tolerability of SC administration of antibiotics, rapid infusion (<5 min), the use of a rigid catheter, and the class of antibiotic (teicoplanin) were significantly associated with a greater occurrence of AEs (19). However, during SC infusion of teicoplanin in patients with BJI, doses lower than 600 mg were better tolerated than higher doses (38). In a study evaluating the impact of injection volume on pain with SC injection, higher volumes were associated with increased pain (47). In a non-clinical study in Sprague-Dawley rats, tolerability of a SC infusion of ceftriaxone was concentration dependent and at high concentrations, no difference in tissue injury was observed between a bolus injection and a 2-h infusion (48). Based on the available literature, slower infusions of appropriate antibiotic concentrations could provide SC antibiotic therapies that are generally well-tolerated.

ENABLING OSCAT WITH WEARABLE, ON-BODY SUBCUTANEOUS DRUG DELIVERY SYSTEMS

With an increase in large-molecule drug development and the challenges of formulating a drug product that enables self-administration, there has been a surge in the development of wearable, on-body delivery systems. Nulasta® (pegfilgrastim, Amgen) and Repatha® (evolocumab, Amgen) are two examples of drugs that are administered subcutaneously *via* an on-body delivery system. There are many other systems that are in development that could be a potential solution to enable wider adoption of OSCAT (**Figures 2D–I**). Generally, these systems act as an infusion pump whereby they contain a medical-grade adhesive that is used to adhere the device to the skin of the arm, abdomen, or thigh after the drug is loaded into the system. Once placed, the device is activated, a needle is inserted into the SC space, and the drug delivery process begins. The rates of the infusions can be controlled either by pre-programmable electronics within the system, elastomeric tensions of drug reservoirs, or the inner diameter of the needle. Once the infusion is complete, the system is removed from the skin and discarded appropriately.

In order to ensure a particular on-body delivery system is appropriate for OSCAT, several considerations should be considered. First, since many parenteral antibiotics have limited stability after reconstitution, development of a novel, liquid formulation would be desirable. If this is not feasible, a specialized, simple, and patient-centric reconstitution system and method would be needed for patient self-administration. Second, the antibiotic must be compatible with the materials that it comes into contact within the on-body delivery system to ensure safety for the patient. Finally, the costs of the system should be appropriate to ensure that it is financially feasible for patients to be able to have access.

OTHER POTENTIAL TRENDS FOR OUTPATIENT MANAGEMENT

In addition to OSCAT, other innovative therapies have been developed to improve the management of serious bacterial infections in the outpatient setting. The use of oral antibiotics for some infections may offer an alternative to intravenous antibiotics in the outpatient setting. Recent randomized trials in patients with endocarditis (POET trial) and in BJI (OVIVA trial) demonstrated similar outcomes between treatment with oral antibiotics compared with intravenous antibiotics (49, 50). *In vitro* resistance could limit the utility of many oral antibiotics for infections caused by Gram-negative organisms; however, the availability of the oxazolidinones provide alternatives to intravenous routes for some serious infections caused by multidrug-resistant Gram-positive organisms (51). For serious bacterial skin infections requiring longer treatment durations, tedizolid may offer an advantage over linezolid due to the reduced potential to cause myelosuppression (52–54). In addition to oral antibiotics, the availability of long-acting lipoglycopeptides (e.g., dalbavancin and oritavancin) may also provide additional alternatives to OPAT as these drug half-lives ranged from 250 to 350 h (corresponding to 10–14 days), thus requiring only a few doses to provide a 4–6-week treatment course (55). As with OSCAT, additional data are required to further elucidate the utility of oral antibiotics and long-acting antibiotics in the management of serious bacterial infections where

intravenous antibiotics have been recognized as a standard of care.

CONCLUSIONS

In conclusion, OSCAT is an attractive alternative to the intravenous route of administration traditionally associated with OPAT. It has been used mainly in France primarily where prolonged courses are necessary and oral routes may not be feasible with some pathogens, such as in many BJI. Antibiotics with longer half-lives that are completely absorbed and are well-tolerated are ideal candidates for OSCAT. The concentration of the antibiotic, the osmolality of the solution, and the infusion rate contribute to the local tolerability of the SC infusion of the antibiotic. The availability of wearable, on-body SC drug delivery systems could improve the uptake of OSCAT while facilitating patient self-administration.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

TF initiated the project and wrote the first draft of the manuscript. All authors participated in the literature review and the improvement of the manuscript.

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Conflict of Interest: TF received speaker honorarium from scPharmaceuticals. TL served as a consultant for scPharmaceuticals. JG served as an advisory board member for scPharmaceuticals. VT received honoraria and research grants from scPharmaceuticals. JM was SVP of Clinical Development and Medical Affairs for scPharmaceuticals who was developing antibiotics for subcutaneous administration. CR-B received research grant from the French Health authorities (*Agence Nationale de Sécurité du Médicament et des produits de santé*, ANSM) and SG from the *Foundation Innovations en Infectiologie* (FINOVI) to perform a prospective study about the pharmacokinetic and safety of subcutaneous antimicrobial therapy in France (*Pharmacocinétique et tolérance des antibiotiques administrés par voie sous-cutanée chez le patient âgé de plus de 65 ans*; PHASAGE study).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 20 January 2021
doi: 10.3389/fmed.2020.576231

Antibiotics in Bone Cements Used for Prosthesis Fixation: An Efficient Way to Prevent *Staphylococcus aureus* and *Staphylococcus epidermidis* Prosthetic Joint Infection

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OPEN ACCESS

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: 25 June 2020
Accepted: 30 November 2020
Published: 20 January 2021

Citation:
Cara A, Ballet M, Hemery C, Ferry T, Laurent F and Josse J (2021) Antibiotics in Bone Cements Used for Prosthesis Fixation: An Efficient Way to Prevent Staphylococcus aureus and Staphylococcus epidermidis Prosthetic Joint Infection. Front. Med. 7:576231. doi: 10.3389/fmed.2020.576231

Prosthetic joint infections (PJIs) are one of the most frequent reasons for arthroplasty revision. These infections are mostly associated with the formation of biofilm, notably by staphylococci, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*. To minimize the rates of PJIs following primary or revision total joint arthroplasty, antibiotic-loaded bone cements (ALBCs) can be used for prosthesis fixation. However, its use is still debated. Indeed, various studies reported opposite results. In this context, we aimed to compare the prophylactic anti-biofilm activity of ALBCs loaded with two antibiotics with ALBC loaded with only one antibiotic. We compared commercial ready-to-use cements containing gentamicin alone, gentamicin plus vancomycin, and gentamicin plus clindamycin to plain cement (no antibiotic), investigating staphylococcal biofilm formation for 10 strains of *S. aureus* and *S. epidermidis* with specific resistance to gentamicin, vancomycin, or clindamycin. Firstly, we performed disk diffusion assays with the elution solutions. We reported that only the cement containing gentamicin and clindamycin was able to inhibit bacterial growth at Day 9, whereas cements with gentamicin only or gentamicin and vancomycin lost their antibacterial activity at Day 3. Then, we observed that all the tested ALBCs can inhibit biofilm formation by methicillin-susceptible staphylococci without other antibiotic resistance ability. Similar results were observed when we tested vancomycin-resistant or clindamycin-resistant staphylococci, with some strain-dependent significant increase of efficacy for the two antibiotic ALBCs when compared with gentamicin-loaded cement. However, adding vancomycin or clindamycin to gentamicin allows a better inhibition of biofilm formation when gentamicin-resistant strains were used. Our *in vitro* results suggest that using

commercially available bone cements loaded with gentamicin plus vancomycin or clindamycin for prosthesis fixation can help in preventing staphylococcal PJIs following primary arthroplasties, non-septic revisions or septic revisions, especially to prevent PJIs caused by gentamicin-resistant staphylococci.

Keywords: arthroplasty, prosthetic joint infection, biofilm, *Staphylococcus*, antibiotic loaded bone cement

INTRODUCTION

The number of primary and revision total joint arthroplasty (TJA) has risen over the last decades. In the US, primary total hip arthroplasties (THAs) and total knee arthroplasties (TKAs) are projected to reach 635,000 and 935,000 procedures, respectively, in 2030 (1). With aseptic loosening, infection is a major cause for arthroplasty revision, especially in early failures after TKA (2). In a recent French study on a cohort of 1,170 reinterventions after TKA, prosthetic joint infection (PJI) accounts for almost 50% of total revision (3). PJIs occur after 1–7% of TJA (4). The most incriminated pathogens are staphylococci, especially *Staphylococcus aureus*, mostly in early and delayed PJIs, and *Staphylococcus epidermidis*, mostly in late chronic or exacerbated PJIs (5, 6). Staphylococcal PJIs can be complicated to treat, and it is partially due to the ability of staphylococci to form biofilm. Biofilms are communities of bacteria embedded in an extracellular matrix. The formation of biofilm is classically described in three main phases: (i) initial attachment, (ii) production of extracellular matrix and cell proliferation, and (iii) biofilm structuring and cell detachment (7). The first phase, initial attachment, is critical for biofilm formation. Indeed, when staphylococci start to produce their extracellular matrix and structure as biofilm, it confers to bacteria some properties of tolerance against antibiotics (8). Indeed, a subpopulation of bacteria inside biofilms faces a lack of nutrients and oxygen. These conditions lead to a decrease of metabolic activity and an increase of antibiotic tolerance, explaining the difficulty to treat biofilm-associated infections (9). Biofilms were reported to be tolerant to antibiotic concentrations 10–1,000-fold superior to the minimal inhibition concentrations (MICs) determined for planktonic bacteria (10).

Preventing the adherence of planktonic bacteria to the prosthesis or the early steps of the formation of other biofilm-like structures that happen in the first hours or days after the prosthesis implantation is a key point to fight PJIs. During this early time, the race to the surface took place. Tissue cells have to colonize the implant before the bacteria to permit the prosthesis integration and prevent bacteria to form biofilm (11). To prevent PJIs following primary or revision TJA, antibiotic-loaded bone cements (ALBC) can be used for prosthesis fixation. Bone cements are composed of polymethyl methacrylate (PMMA). Initially, PMMA can only polymerize at high temperatures, so it cannot be used for medical applications. But, a new method for polymerizing PMMA at room temperature was developed in 1943, allowing its use for prosthesis fixation (12). In prophylactic situations, bone cements can be loaded with low doses of antibiotics (between 0.5 and 2 g antibiotics/40 g PMMA) to prevent PJIs with a limited impact on the mechanical properties of cement.

Prophylactic ALBCs are commonly used in Europe, especially in Scandinavian countries. It was mostly justified by previous studies based on the Norwegian arthroplasty register showing that systemic antibiotics combined with ALBCs for prosthesis fixation led to fewer revisions than systemic antibiotics or ALBC alone following THA (13). Moreover, another study from 2006 reported that the risk of THA revision due to PJI was equivalent for uncemented and for cemented arthroplasties with ALBC, but higher for cemented arthroplasties without antibiotic cement (14). Similar results were published in a recent meta-analysis about implant fixation and the risk of PJI in THA (15). The authors reported that all cemented prostheses (cemented fixations, hybrid fixations, reverse hybrid fixations) were each associated with an increase of PJI risk when compared with uncemented prosthesis. For ALBCs, the risk of PJI was reduced when compared with cemented fixations. When ALBCs were compared with uncemented fixations, the authors did not report any difference concerning the PJI risk. However, the same group performed a meta-analysis about implant fixation and the risk of infection in TKA. Their observation suggests that uncemented fixation may be associated with lower PJI risk in primary TKA than cemented fixation, and that the use of ALBC may be associated with increased PJI risk when compared with plain cement (16). To note, Sultan et al. highlighted that the question about the use of ALBCs in TJA is more relevant in TKA than in THA as most of the patients received cemented implants in TKA, whereas cementless prostheses were more and more used in THA (4). They also highlighted a potential bias regarding patient selection, suggesting that patients with high risk of infection (diabetes mellitus, obesity) were more subject to TJA with ALBCs.

However, the type of ALBCs (handmade or ready-to-use, which antibiotic(s) is/are loaded) is rarely questioned in these studies about primary TJA. Moreover, the choice of the antibiotics can largely influence the development of PJIs. Indeed, gentamicin is mostly used alone in ALBCs in primary TJA, and the percentage of gentamicin resistance is important in staphylococci. In 1999, Schmitz et al. investigated the prevalence of gentamicin resistance in staphylococci in 19 different European hospitals. Of the *S. aureus* isolates, 23% were resistant to gentamicin. They reported that resistance to gentamicin is more frequent in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates (75%) than in methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates (4%). Of the coagulase-negative staphylococci (CNS) isolates, 33% of the strains were reported to be resistant to gentamicin. For methicillin-resistant

TABLE 1 | List of the bacterial strains used in this study and their antibiotic resistance profiles.

	Cefotaxime	Gentamicin	Vancomycin	Clindamycin
MSSA	S	S	S	S
MRSA	**R**	S	S	S
GentaR MRSA	**R**	**R**	S	S
VancoR MSSA	S	S	**R**	S
ClindaR MSSA	S	S	S	**R**
MSSE	S	S	S	S
MRSE	**R**	S	S	S
GentaR MSSE	S	**R**	S	S
MRSE VancoR	**R**	S	**R**	S
ClindaR MRSE	**R**	S	S	**R**

Resistance profiles were determined with Vitek 2 in the routine laboratory. R, resistant; S, susceptible.

CNS (MRCNS) isolates, the prevalence of gentamicin resistance was 48%, whereas it was 7% for methicillin-susceptible CNS (MSCNS) (17). However, the strains were isolated from blood, hospital-acquired pneumonia, or skin and soft tissue infections, not from PJIs. In 2009, Hellmark et al. reported that, in a collection of 33 *S. epidermidis* isolated during revision surgery for PJI in two Swedish hospitals, 84% of the isolates were resistant to oxacillin, 79% were resistant to gentamicin, and 67% were resistant to clindamycin, whereas no isolate was reported to be resistant to vancomycin (18). The authors also suggested that the high gentamicin resistance could be related to the common use of gentamicin-loaded cement on previous surgeries.

In this context, we aim to investigate the prophylactic anti-biofilm activity of ALBCs with two antibiotics destined to prosthesis fixation. We compared commercial ready-to-use ALBCs containing gentamicin alone, gentamicin plus vancomycin, and gentamicin plus clindamycin to plain cement (no antibiotic), investigating staphylococcal biofilm formation in elution solutions from these four cements.

MATERIALS AND METHODS

Bacterial Strains

A collection of 10 strains of *S. aureus* and *S. epidermidis* was used in this study. We used the MSSA SH1000, a reference strain routinely used in our laboratory for biofilm experiments (considered as our MSSA strain) and nine clinical strains. These methicillin-susceptible or -resistant clinical strains were isolated during routine work performed at the Bacteriology Department of Hôpital de la Croix-Rousse, Hospices Civils de Lyon. These strains were selected for their specific antibiotic susceptibilities regarding gentamicin or vancomycin or clindamycin. Resistance profiles were determined with Vitek 2 (Biomérieux) by the routine Bacteriology laboratory. All the strains were tested with Crystal Violet method beforehand to ensure that they can form at least moderate biofilm regarding Stepanovic's classification (19). The strains are presented in **Table 1**.

TABLE 2 | List of the bone cements used in this study and their characteristics.

Cement	Antibiotic and quantity				Commercial name
Plain	–	–	–	–	PALACOS R
G	Gentamicin	0.5 g	–	–	PALACOS R + G
G + V	Gentamicin	0.5 g	Vancomycin	2 g	COPAL G + V
G + C	Gentamicin	1 g	Clindamycin	1 g	COPAL G + C

Antibiotic-Loaded Bone Cements

Four bone cements commercialized by Heraeus Medical were used in this study: plain cement (without antibiotic), cement loaded with gentamicin alone (G), cement loaded with gentamicin plus vancomycin (G + V), and cement loaded with gentamicin plus clindamycin (G + C). Disk-like specimens (diameter 2.5 cm, height 1.0 cm) were used. Specific antibiotic loads for each cement are presented in **Table 2**.

Preparation of ALBC Elution Solutions

To evaluate the effect of ALBCs against biofilm formation, we prepared elution solutions that contain antibiotics released from ALBCs. Disk-like specimens were incubated in 20 mL of Tryptic Soy Broth (TSB, Bacto) supplemented with 1% of glucose (an artificial medium that favors strong biofilm formation in 24 h) in Falcon tube 25 mL. The ALBCs were incubated for 1–9 days at 37°C (**Figure 1**). Indeed, most of prosthesis inoculation occurs during the surgery or during the few days after the surgery, as the scar and the joint cavity are not yet impervious. Consequently, eluted antibiotics that have prolonged effect to limit the formation of the biofilm could be of importance to prevent the bacterial inoculation immediately after the surgery. The media were changed daily. For the biofilm formation experiments, ALBC elution solutions from Day 1, Day 3, and Day 9 were used.

Disk Diffusion Assay With ALBC Elution Solutions

Bacterial suspensions were prepared in saline solution and adjusted at 0.5 McFarland for each strain. Then, the bacterial suspensions were swabbed on Muller Hinton agar plates over the entire agar surface. After inoculation, sterile disks with a diameter of 6 mm were applied on the inoculated plates. Disks were impregnated with 20 μL of each ALBC elution solution. Plates were incubated for 24 h before the measurement of the diameters of inhibition zones. Two independent experiments were performed in technical duplicate.

Determination of the Prophylactic Anti-biofilm Effect of ALBC Elution Solutions

Overnight cultures of *S. aureus* or *S. epidermidis* in liquid Brain Heart Infusion (BHI) were standardized to OD600 = 1 ± 0.05 before being diluted at 1:100 in ALBC elution solutions (Day 1, Day 3, and Day 9), and 100 μL was added in a 96-well plate (Greiner Bio-One) for 24 h of incubation at 37°C in

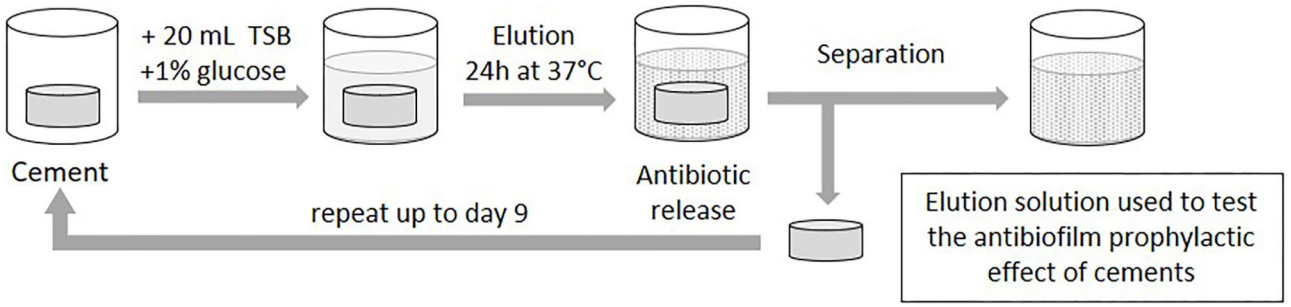

FIGURE 1 | Preparation of elution solutions. A specimen of cement was placed in 20 mL of TSB supplemented with 1% of glucose and incubated at 37°C. Each day, the media were discarded, and new media were added to the cements. Elution solutions for Day 1, Day 3, and Day 9 were used for biofilm experiments.

TABLE 3 | Disk diffusion assay with ALBC elution solutions.

	Day 1				Day 3				Day 9			
	Plain	**G**	**G + V**	**G + C**	**Plain**	**G**	**G + V**	**G + C**	**Plain**	**G**	**G + V**	**G + C**
MSSA	6 ± 0	14.5 ± 0.7	14 ± 0	27.5 ± 3.5	6 ± 0	6.5 ± 0.7	7.5 ± 0.7	21.5 ± 2.1	6 ± 0	6 ± 0	6 ± 0	21 ± 1.4
MRSA	6 ± 0	13 ± 2.8	14.5 ± 3.5	26 ± 1.4	6 ± 0	8 ± 1.4	8.5 ± 0.7	21.5 ± 0.7	6 ± 0	6 ± 0	6 ± 0	20 ± 1.4
GentaR MRSA	6 ± 0	6 ± 0	7.5 ± 0.7	25.5 ± 2.1	6 ± 0	6 ± 0	6 ± 0	20 ± 2.8	6 ± 0	6 ± 0	6 ± 0	19 ± 1.4
VancoR MSSA	6 ± 0	15.5 ± 0.7	14.5 ± 0.7	25.5 ± 0.7	6 ± 0	6.5 ± 0.7	7 ± 0	19 ± 1.4	6 ± 0	6 ± 0	6 ± 0	17.5 ± 0.7
ClindaR MSSA	6 ± 0	11.5 ± 2.1	12.5 ± 3.5	16 ± 1.4	6 ± 0	6 ± 0	7 ± 1.4	8.5 ± 2.1	6 ± 0	6 ± 0	6 ± 0	8 ± 1.4
MSSE	6 ± 0	21 ± 1.4	18.5 ± 0.7	25.5 ± 2.1	6 ± 0	10.5 ± 0.7	11 ± 1.4	20 ± 2.8	6 ± 0	6.5 ± 0.7	6.5 ± 0.7	19.5 ± 0.7
MRSE	6 ± 0	23 ± 4.2	23 ± 4.2	27 ± 4.2	6 ± 0	14 ± 1.4	15.5 ± 0.7	20 ± 2.8	6 ± 0	11 ± 1.4	10.5 ± 0.7	18.5 ± 2.1
GentaR MSSE	6 ± 0	6 ± 0	6 ± 0	27.5 ± 3.5	6 ± 0	6 ± 0	6 ± 0	20.5 ± 3.5	6 ± 0	6 ± 0	6 ± 0	18.5 ± 0.7
VancoR MRSE	6 ± 0	19 ± 1.4	19 ± 1.4	26 ± 2.8	6 ± 0	9.5 ± 0.7	10 ± 0	19 ± 1.4	6 ± 0	6 ± 0	6 ± 0	17 ± 0
ClindaR MRSE	6 ± 0	22 ± 2.8	20.5 ± 3.5	26 ± 2.8	6 ± 0	11.5 ± 0.7	13 ± 1.4	19 ± 1.4	6 ± 0	7 ± 1.4	7 ± 1.4	16.5 ± 0.7
Mean	6.0	15.2	15.0	25.3	6.0	8.5	9.15	18.9	6.0	6.7	6.6	17.6
SD	0.0	6.2	5.5	3.3	0.0	2.8	3.2	3.8	0.0	1.6	1.4	3.6

Results are presented as mean ± SD. Values are in mm.

humid atmosphere. After 24 h, the supernatant was removed, and biofilms were washed for 45 min using Biofilm Care (20). This smooth washing method favors the preservation of the biofilm that otherwise can be denatured with classic washing methods. Biofilms were then resuspended in 200 μL of phosphate buffer saline (PBS) by scraping the wells using sterile pipette tips and sonicating for 10 min at 40 Hz using Bactosonic (Bandelin). Finally, the number of viable bacteria inside the biofilm was evaluated with plate counting on COS agar plates (Biomérieux).

Graphical Representation and Statistical Analysis

For each condition, three independent experiments in technical experiments (three wells for each condition for each experiment) were performed. Results were presented as inhibition of biofilm formation by comparing G, G + V, and G + C data to plain cement (no antibiotic) data. Data (nine values per condition) were presented as histograms (median with range). Due to the number of values, non-parametric statistical analysis was performed. We performed Kruskal–Wallis tests comparing the data at each day. Then, we performed first tests to compare G, G + V, and G + C to the control condition (plain cement). Then, we performed second Dunn's multiple comparisons to compare G, G + V, and G + C with each other. All analyses were performed using Prism software (GraphPad, San Diego, CA, USA).

RESULTS

Effects of ALBC Elution Solutions Against Planktonic Bacteria

We first performed disk diffusion assays with the ALBC elution solutions to observe the effect of ALBCs against planktonic staphylococci (**Table 3**). For plain cements, the diameters were 6 mm, which is the diameter of the disk. It means that there was no antibacterial effect of the elution solutions from plain cement. At Day 1, we observed that almost all the ALBCs had an antibacterial effect. The only exceptions were for the gentamicin-resistant MRSA and methicillin-susceptible *Staphylococcus epidermidis* (MSSE) and the vancomycin-resistant when they are exposed to G and G + C cements. At Days 3 and 9, a decrease of antibacterial activity is observed for all ALBCs. Only G + C kept an antibacterial activity against all the strains except the clindamycin-resistant MSSA.

Inhibition of Biofilm Formation by ALBCs With Multi-Susceptible or Only Methicillin-Resistant Staphylococcal Strains

Then, we investigated the prophylactic anti-biofilm effect of ALBCs against methicillin-susceptible strains (**Figures 2A,B**). After 24 h of incubation in the elution solutions, 10^8 and 10^7 CFU were counted for plain cement for MSSA and MSSE, respectively. For both strains, all ALBCs (G, G + V, and G + C) decreased the biofilm formation without difference whatever the time of elution (Day 1, Day 3, or Day 9). Median values of 10^1 CFU were reported for all the three ALBCs. However, two exceptions were present: at Day 9 for MSSA, we observed an increase of CFU counts to 10^4 for G and a significant difference between the efficacy of G + C and G (**Figure 2A**); at Day 3 for MSSE, the number of CFU increased over 10^2 for G, and significant differences were observed between G + V and G + C when compared with G (**Figure 2B**).

Similar results were obtained when the methicillin-resistant strains [MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE)] were tested, with globally no significant difference between the biofilm inhibition effect of G, G + V, and G + C (**Figures 2C,D**). Again, we reported two exceptions: at Day 9 for MRSA, the CFU count increases to 10^5 and was not significantly different from G. Moreover, the CFU counts for G + V and G + C were statistically different from G (**Figure 2C**). The other exception was a significant difference between G and G + C for the MRSE strain at Day 1 that was not reproduced at Day 3 or Day 9 (**Figure 2D**).

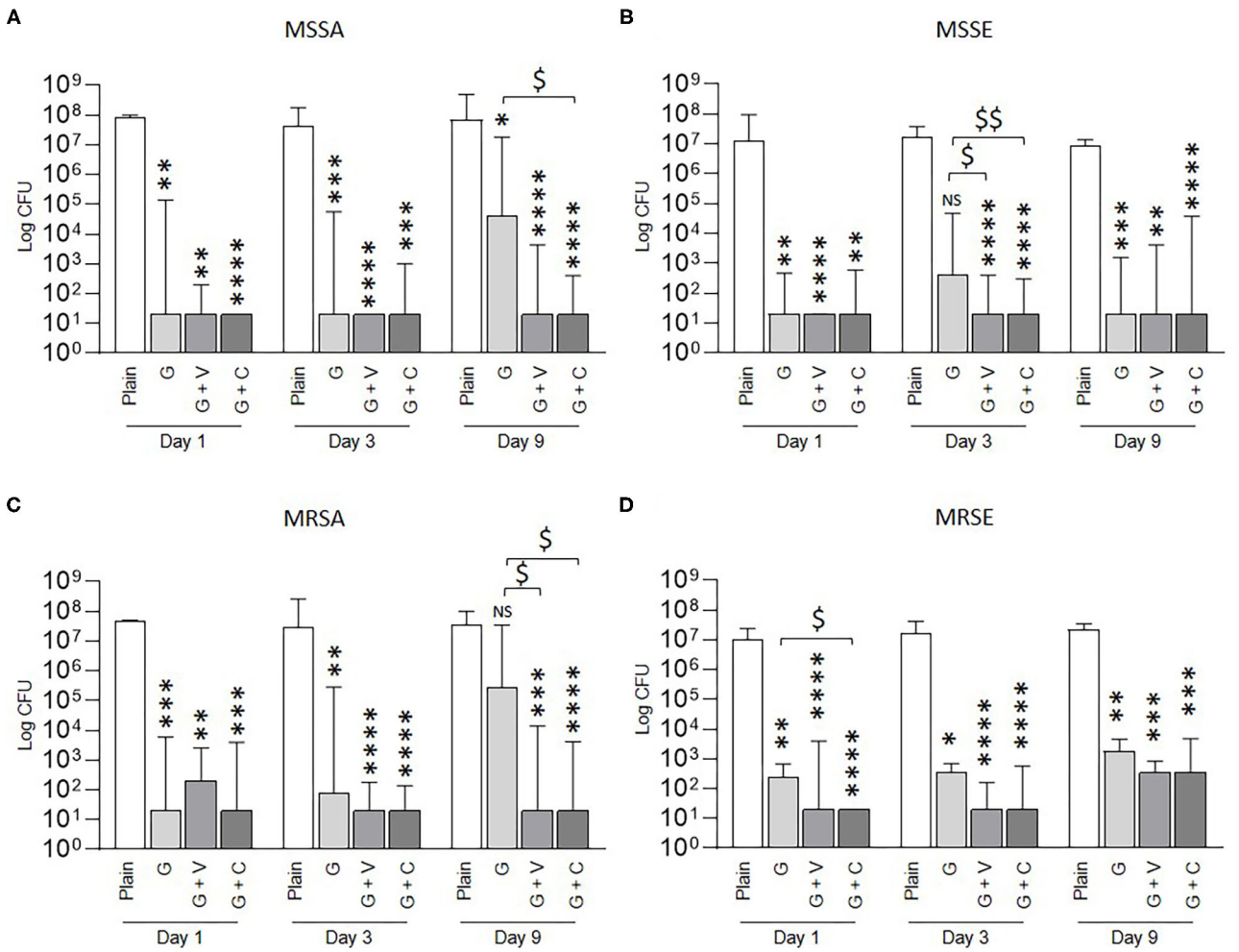

FIGURE 2 | Prophylactic anti-biofilm effect of ALBCs against MSSA strain **(A)**, MSSE strain **(B)**, MRSA strain **(C)**, and MRSE strain **(D)**. Three independent experiments in technical experiments (three wells for each condition for each experiment) were performed. Non-parametric Kruskal–Wallis tests were performed to compare the data at each day. A Dunn's multiple comparisons test was performed as follow-up test. For each day, *, **, ***, and **** mean $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, in comparison with plain cement (control without antibiotic). For each day, $ and $$ mean $p < 0.05$, $p < 0.01$, respectively, in comparison with G cement.

G, G + V, and G + C cements shared the same ability to inhibit biofilm formation from MSSA, MSSE, MRSA, and MRSE strains with some exceptions that are strain and time dependent.

Inhibition of Biofilm Formation by ALBCs With Gentamicin-Resistant Staphylococcal Strains

We then tested the ability of ALBCs to inhibit the formation of biofilm by gentamicin-resistant staphylococcal strains (**Figures 3A,B**). With elution solution from plain cement, the bacterial count was between 10^7 and 10^8 CFU. The G cement did not permit to inhibit the formation of biofilm by the gentamicin-resistant strains (**Figures 3A,B**) whatever the elution solutions used (Day 1, Day 3, or Day 9). The results for G were like the ones obtained with the plain cement. For G + V and G + C, CFU count was between 10^1 and 10^3, corresponding to at least a 4-Log decrease. An exception was observed at Day 9 for the gentamicin-resistant MSSE where G + C kept its ability to significantly decrease biofilm formation, whereas G + V had no effect on biofilm formation (**Figure 3B**).

G + V and G + C had a significant better ability to inhibit biofilm formation than G cement for the two gentamicin-resistant tested strains.

Inhibition of Biofilm Formation by ALBCs With Vancomycin-Resistant or Clindamycin-Resistant Staphylococcal Strains

We next tested the efficacy of ALBCs to inhibit biofilm formation by vancomycin- and clindamycin-resistant staphylococcal strains (**Figure 4**). Globally, all the ALBCs were able to significantly decrease biofilm formation for the four tested strains. As seen in **Figure 1** for the MSSA, MSSE, MRSA, and MRSE strains, we observed strain- and time-dependent exceptions. Indeed, the effect of G cement was not significant for the vancomycin-resistant and the clindamycin-resistant MSSA strains at Day 1 (**Figures 4A,C**). The same observations were made at Day 3 for the vancomycin-resistant and clindamycin-resistant MRSE strains (**Figures 4B,D**). In both cases, the CFU counts of G + V and G + C were significantly lower than that of G cement. In other specific situations, we observed significant differences between G + V or G + C and G (**Figures 4A,C,D**).

As observed in **Figure 1**, G, G + V, and G + C cements shared the same ability to inhibit biofilm formation from vancomycin-resistant and clindamycin-resistant staphylococci with some exceptions that are strain and time dependent.

Global Analysis With Pooled Results for *S. aureus* and *S. epidermidis* Strains

To have an overview of the anti-biofilm activity of ALBCs, the results from the 10 staphylococcal strains were pooled in one graph (**Figure 5**). At each day, all ALBCs were significantly able to decrease biofilm formation when compared with plain cement. Moreover, G + V had a significantly better anti-biofilm effect than G cement on gentamicin-resistant staphylococci that represent 20% of the total bacterial population.

DISCUSSION

The use of ALBC for prosthesis fixation in primary total arthroplasties or revision surgeries is still debated. Indeed,

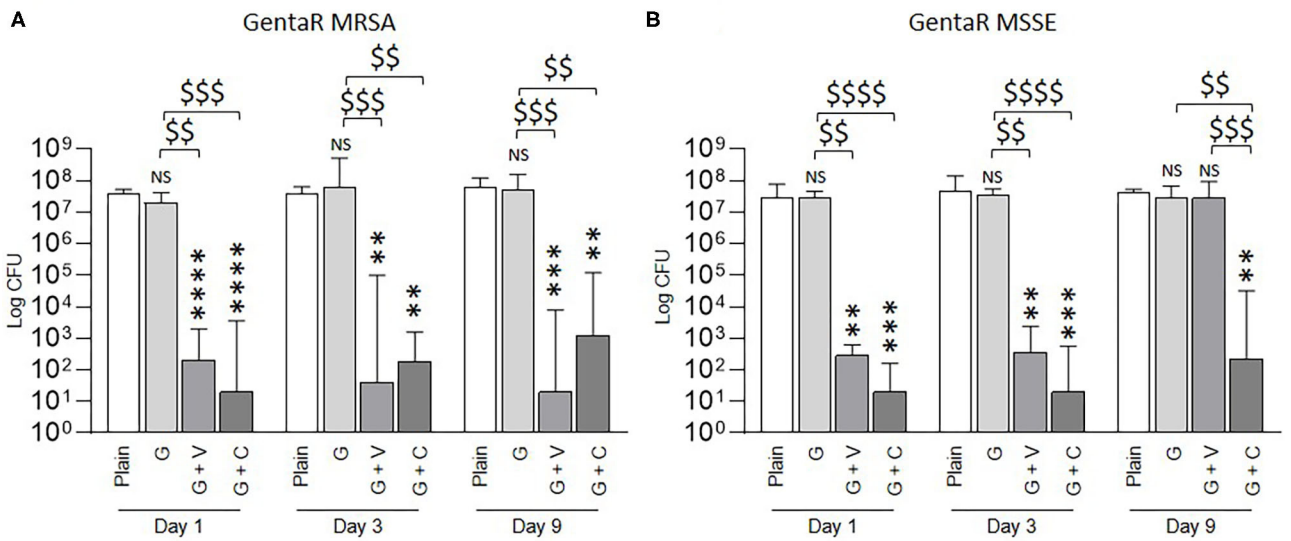

FIGURE 3 | Prophylactic anti-biofilm effect of ALBCs against gentamicin-resistant MRSA **(A)** strain and gentamicin-resistant MSSE strain **(B)**. Three independent experiments in technical experiments (three wells for each condition for each experiment) were performed. Non-parametric Kruskal–Wallis tests were performed to compare the data at each day. A Dunn's multiple comparisons test was performed as follow-up test. For each day, **, ***, and **** above the plot mean $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, in comparison with plain cement (control without antibiotic). For each day, $$, $$$, and $$$$ mean $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, in comparison with G cement.

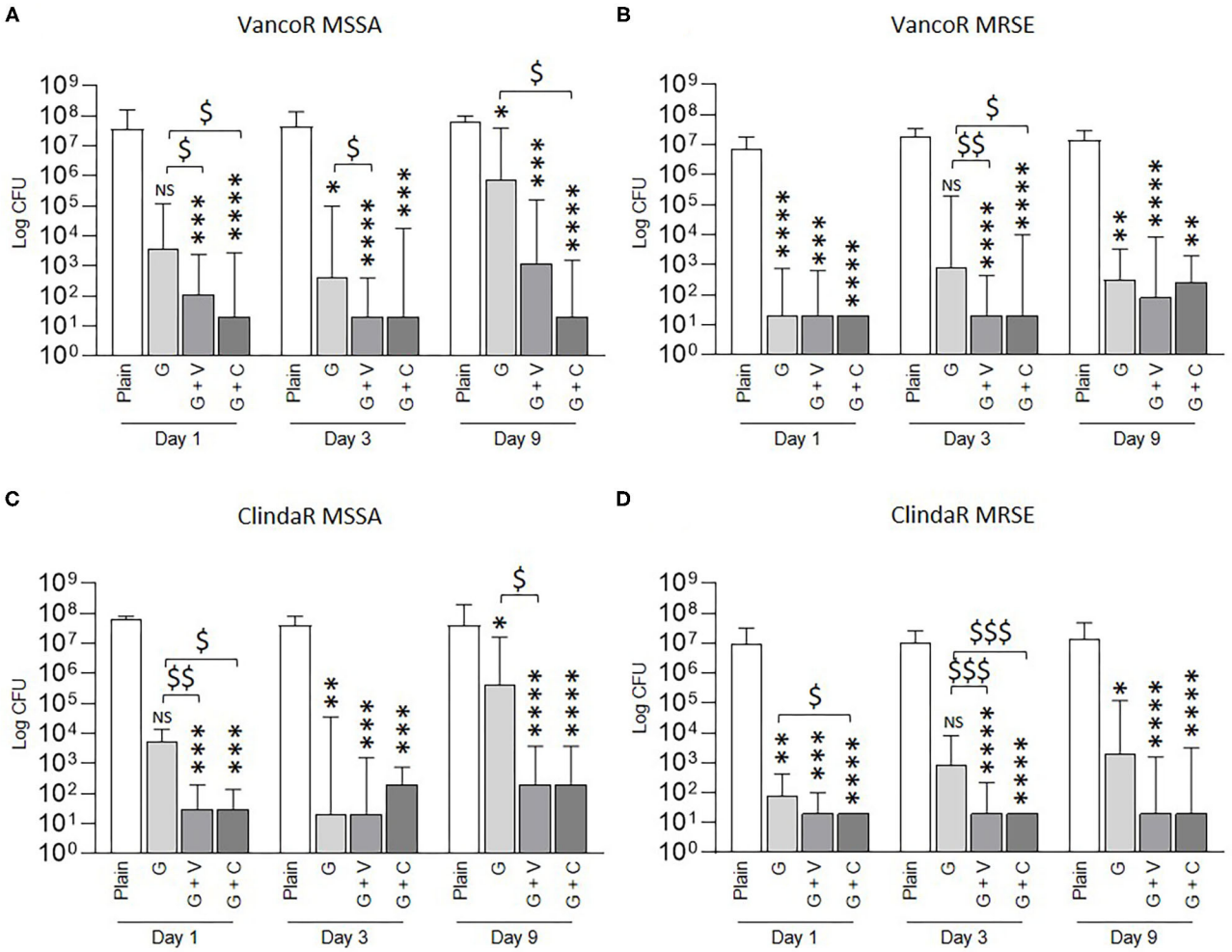

FIGURE 4 | Prophylactic anti-biofilm effect of ALBCs against vancomycin-resistant MSSA strain **(A)**, vancomycin-resistant MRSE strain **(B)**, clindamycin-resistant MSSA strain **(C)**, and clindamycin-MRSE strain **(D)**. Three independent experiments in technical experiments (three wells for each condition for each experiment) were performed. Non-parametric Kruskal–Wallis tests were performed to compare the data at each day. A Dunn's multiple comparisons test was performed as follow-up test. For each day, *, **, ***, and **** above the plot mean $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, in comparison with plain cement (control without antibiotic). For each day, $, $$, and $$$ mean $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, in comparison with G cement.

various studies reported opposite results. However, in these studies, the type of ALBC (handmade or commercial) and the type of antibiotics loaded in the cement (gentamicin alone, gentamicin coupled with another antibiotic) are rarely taken in consideration. In this study, we investigated the *in vitro* anti-biofilm activity of commercially available ALBCs with low doses of antibiotics. Using low-dosed ALBCs is primordial to minimize the negative effects on cement mechanical properties that can be observed with high-dosed ALBCs. However, it was reported that low-dosed ALBCs can favor the induction of resistance, especially when gentamicin alone is used (21).

In this study, we chose to use elution solutions to investigate the effect of ALBCs on biofilm formation. Previous studies mostly focused on biofilm formation directly on the cement, investigating the biofilm formation by microscopy or by direct interaction between cement and bacterial culture on agar plate (22). By studying the effect of elution solutions on biofilm formation on an independent material (in our case, the bottom of 96-well plates), we placed ourselves in the context that ALBCs have to prevent biofilm formation on themselves but also on the prosthesis (metallic or polyethylene components) or on bone or soft tissues. Moreover, we chose to use the classical conditions of biofilm formation, using a rich nutrient medium supplemented with glucose and a high inoculum, both favoring a rapid and intense development of biofilm. These conditions are not the best to easily prove the anti-biofilm effect of ALBCs and can explain why in most of our experiments, we did not reach a total inhibition of biofilm formation. Finally, the elution solutions were changed every day to mimic the depletion/elimination of antibiotics that happens

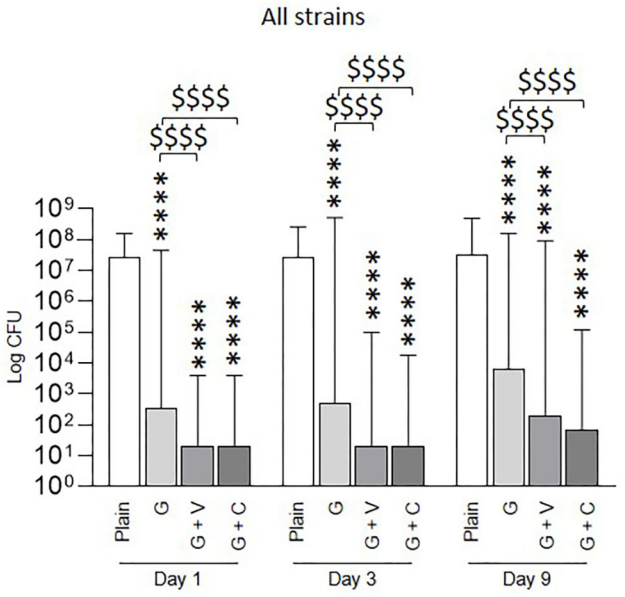

FIGURE 5 | Prophylactic anti-biofilm effect of ALBCs against all the tested staphylococcal strains (10 strains pooled). Three independent experiments in technical experiments (three wells for each condition for each experiment) were performed. Non-parametric Kruskal–Wallis tests were performed to compare the data at each day. A Dunn's multiple comparisons test was performed as follow-up test. For each day, **** above the plot means $p < 0.0001$ in comparison with plain cement (control without antibiotic). For each day, $$$$ means $p < 0.0001$, respectively, in comparison with G cement.

in the joint. It means that the concentrations of antibiotics are lower at Day 9 than at Day 1. However, our conditions can be considered as not clinically relevant. Indeed, the formation of biofilm takes several days in patients, and synovial fluid and bone environment can be considered as poor media for biofilm formation. The time and the type of media for biofilm formation could influence the biofilm structure and composition and then impact the susceptibility to antibiotics. Regarding our method, we chose to use CFU counting. This method is the standard for bacterial counting but suffers from low reproducibility. Confocal microscopy is the method of choice for imaging and determining biofilm formation, but as we tested 120 conditions (10 strains, four cements, and three conditions), CFU counting seemed more accurate.

Synergistic activities of gentamicin plus vancomycin or gentamicin plus clindamycin against staphylococci have been known for almost 40 years (23–25). Ensing et al. observed a higher effect of the G + C cement (COPAL G + C) than of the G cement (PALACOS R + G) (22). In their article, they observed that the antibiotic release from the G + C cement was more important than the one from the G cement, explaining the higher activity of G + C. The authors also reported that the gentamicin-susceptible *S. aureus* that they used for their study formed gentamicin-resistant small colony variants (SCVs) on the G cement (22). However, COPAL G + C contains more gentamicin (1 g) than PALACOS R + G (0.5 g). This difference could also explain the higher activity of G + C. In our study, we observed a difference of biofilm inhibition between G + V or G + C and G cement when we tested the gentamicin-resistant staphylococci. In the global population of staphylococci, *S. aureus* and CNS, such as *S. epidermidis*, the prevalence for gentamicin resistance can vary between 23 and 79% (17, 18). It means that staphylococcal PJIs have a non-negligible possibility to be caused by a gentamicin-resistant strain. In this context, using an ALBC combining gentamicin to another antibiotic appears warranted. In our study, we tested a gentamicin-resistant MRSA strain and a gentamicin-resistant MSSE strain. In both cases, gentamicin alone cannot prevent biofilm formation, even after Day 1 of elution, when the concentration of antibiotic is the most elevated. Indeed, even with a concentration of gentamicin around 100 μg/mL as it can be dosed at Day 1 (data not shown), the concentration is too low to overpass gentamicin resistance mechanisms and to prevent biofilm. However, for ALBCs loaded with gentamicin coupled with vancomycin or clindamycin, biofilm formation was prevented, even when elution solutions from Day 9 were used (**Figure 3**).

Regarding these previous results, it could be tempting to wonder that ALBCs with only vancomycin or clindamycin would be enough for preventing PJIs. However, PJIs are not only due to staphylococci, and vancomycin or clindamycin is not efficient against Gram-negative bacteria. Moreover, staphylococci can be resistant to vancomycin, even if it is not frequent, or to clindamycin, which concerned 79% of *S. epidermidis* strains in the study by Hellmark et al. (18). In our study, we tested two vancomycin-resistant strains (MSSA and MRSE) and two clindamycin-resistant strains (MSSA and MRSE). For these four strains, all ALBCs were able to prevent biofilm formation (**Figure 4**). However, anti-biofilm activity appears more pronounced for G + V and G + C than for gentamicin alone after 9 days of elution for clindamycin-resistant strains (**Figure 4**). For COPAL G + V cements, the higher anti-biofilm effect could be logically attributed to the presence of vancomycin. For the COPAL G + C cements, the higher dose of gentamicin could allow a better inhibition of biofilm formation than that of cement with gentamicin alone (**Figure 4**, **Table 2**). Our results highlight that the combination of antibiotics can potentialize the anti-biofilm effect even if the strain is resistant to one of the loaded antibiotics. Moreover, it is important to take into account that the chemicophysical properties of ALBCs differ between the G cement and the G + V and G + C cements. Indeed, PALACOS R + G and COPAL G + V and COPAL G + C have different porosities that impact the release of the antibiotics and can potentially explain the higher effect of G + V and G + C in specific conditions.

Regarding the mechanisms involved in the inhibition of biofilm, two mechanisms are identified: (i) killing the planktonic staphylococci that will not form biofilm thereafter and/or (ii) acting directly against adhering staphylococci during the early step of biofilm formation. In our study, the staphylococci are directly exposed to antibiotics as they grow in ALBC elution solutions, so we cannot differentiate which mechanism is involved. It seems logical that the antibiotics present in the solution first attack the planktonic bacteria, and that the

decrease of activity sometimes observed at Day 9 with the G cement is due to a lower concentration in antibiotics. However, when we compared our results between the disk diffusion assays and the biofilm inhibition assays, we observed difference regarding the activity. Indeed, even though G and G + V lost their activity in disk diffusion assay (**Table 3**), they kept a good activity against biofilm formation (**Figures 2–5**). G and G + V cements can inhibit biofilm formation at concentrations that are not sufficient to inhibit bacterial growth in disk diffusion assay. These results suggest that G and G + V cements have a specific activity against biofilm formation that is different from the bactericidal/bacteriostatic activity. Their activities not only are due to the killing of planktonic staphylococci before biofilm formation but also involve a specific effect against biofilm formation. Regarding G + C cement, we observed an antibacterial effect until Day 9 in disk diffusion. In this case, we can hypothesize that the killing of planktonic bacteria before biofilm formation has a more important role than with G and G + V cements in the global activity against biofilm formation.

Finally, concerning clinical evidence, recent meta-analysis did not highlight differences in PJI rates between primary plain-cemented and ALBC-cemented TJA and point out differences between primary TKA and primary THA. However, as the parameters of ALBCs (commercially available or handmade; only gentamicin or gentamicin with another antibiotic) were not taken into account, it appears essential to investigate the impact of using commercial ALBCs following cemented TJA and the impact of adding vancomycin or clindamycin in clinical trials.

CONCLUSION

Our *in vitro* results suggest that using commercially available ALBCs loaded with gentamicin added with vancomycin or clindamycin for prosthesis fixation can help in preventing staphylococcal PJIs following primary TJA, non-septic TJA revisions or septic TJA revisions, especially PJIs caused by gentamicin-resistant staphylococci. Moreover, our results suggest that elution solutions from ALBCs can prevent biofilm formation at concentrations that are not able to inhibit bacterial growth in disk diffusion assays.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AC, MB, and CH performed the experiments and analyzed the results. JJ defined the study plan, analyzed the results, and drafted the manuscript with TF and FL. All authors reviewed and accepted the manuscript.

FUNDING

This work was financially supported by Heraeus Medical, but the company was not involved in the experimental process and the data analysis.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 21 January 2021
doi: 10.3389/fmed.2020.539501

Bone and Joint Infection Involving *Corynebacterium* spp.: From Clinical Features to Pathophysiological Pathways

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Edited by:
Françoise Van Bambeke, Catholic University of Louvain, Belgium

Reviewed by:
Naveen Kumar Devanga Ragupathi, The University of Sheffield, United Kingdom
Jaime Esteban, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Spain

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *01 March 2020*
Accepted: *22 October 2020*
Published: *21 January 2021*

Citation:
Chauvelot P, Ferry T, Tafani V, Diot A, Tasse J, Conrad A, Chidiac C, Braun E, Lustig S, Laurent F and Valour F (2021) Bone and Joint Infection Involving Corynebacterium spp.: From Clinical Features to Pathophysiological Pathways. Front. Med. 7:539501. doi: 10.3389/fmed.2020.539501

Introduction: Corynebacteria represent often-neglected etiological agents of post-traumatic and/or post-operative bone and joint infection (BJI). We describe here clinical characteristics and bacteriological determinants of this condition.

Methods: A retrospective cohort study described characteristics, outcome and determinants of treatment failure of all patients with proven *Corynebacterium* spp. BJI (i.e., ≥2 culture-positive gold-standard samples). Available strains were further characterized regarding their antibiotic susceptibilies, abilities to form early (BioFilm Ring Test®) and mature (crystal violet staining method) biofilms and to invade osteoblasts (gentamicin protection assay).

Results: The 51 included BJI were mostly chronic (88.2%), orthopedic device-related (74.5%) and polymicrobial (78.4%). After a follow-up of 60.7 weeks (IQR, 30.1–115.1), 20 (39.2%) treatment failures were observed, including 4 *Corynebacterium*-documented relapses, mostly associated with non-optimal surgical management (OR 7.291; $p = 0.039$). Internalization rate within MG63 human osteoblasts was higher for strains isolated from delayed (>3 months) BJI ($p < 0.001$). Infection of murine osteoblasts deleted for the β1-integrin resulted in a drastic reduction in the internalization rate. No difference was observed regarding biofilm formation.

Conclusions: Surgical management plays a crucial role in outcome of BJI involving corynebacteria, as often chronic and device-associated infections. Sanctuarisation within osteoblasts, implicating the β1 cellular integrin, may represent a pivotal virulence factor associated with BJI chronicity.

Keywords: ***Corynebacterium*, osteoblasts, biofilm, bone and joint infection, intracellular**

INTRODUCTION

Bone joint infection (BJI), and especially prosthetic joint infection (PJI), represents a major public health concern (1), due to: (i) their prevalence, complicating 1 to 2% of arthroplasty procedures, with an important upcoming increase due to the projected rise in prosthetic joint replacement indications in the coming years (2, 3); (ii) their severity, associated with a 5% mortality rate and responsible for permanent disabilities in up to 40% of patients; and iii) their substantial economic burden estimated to be as high as 75,000 to 100,000 USD per episode attributed to protracted hospital course, re-operations, lengthened rehabilitation time and extended use of antimicrobials (4–7). Consequently, BJI has been pointed out as a priority axis of clinical and scientific research in many countries. The optima management requires a multidisciplinary approach combining both surgical procedure and extended antimicrobial therapy (8). Despite this complex management, they are associated with a high failure rate, exceeding 20% in some series, with frequent relapses and transition to a chronic state (9–14). This propensity to chronicity and relapse has been related to specific bacterial phenotypes responsible for subsequent emergence of bacterial reservoirs, protecting the pathogen from the extracellular host defenses and most antimicrobials (15, 16). These mechanisms have been well-characterized among *Staphylococcus aureus*, the main etiological agent of BJI (17–19), and consist in: (i) biofilm formation, an surface-adherant bacterial community living in a matrix of self-generated polymeric substances (20, 21); (ii) internalization and persistence within non-phagocytic bone cells, triggered by the interaction of staphylococcal fibronectin binding proteins (FnBP) with host Fibronectin that acts as a bridge with cellular α5β1 integrin to prompted bacterial endocytosis by an active cellular process (22–24); and (iii) phenotype switching to small colony variants (SCVs), a slow-growing bacterial phenotype which can emerge during intracellular or biofilm-associated lifestyles, and conferring enhanced resistance to antimicrobials (25, 26).

Corynebacteria are a highly heterogeneous group of Gram positive rods containing more than 110 species. Their pathogenic potential is species-dependent: some of them, as *Corynebacterium glutamicum* or *Cladosporium halotolerans*, have never been described in human pathology, when others have been implicated in various infectious disease, from urinary tract infection to infective endocarditis (27). Two type of virulence factor have been well-characterized in this genus. First, exotoxin production has been described in *Corynebacterium diphteriae, Corynebacterium ulcerans,* and *Corynebacterium pseudotuberculosis.* These three pathogenic strains can product diphteria toxin and/or phospholipase B, and therefore cause diphtheria, which is the best known corynebacteria-associated disease (27). Interestingly, even non-toxinogenic strains of *C. diphteriae* can cause invasive infections such as endocarditis, brain abscess or BJI (28). Secondly, some species have been shown to produce various adhesion molecules allowing interaction with eukaryote cells. A fibrinogen and fibronectin binding-like activity has been demonstrated from invasive strains of *Corynebacterium pseudodiphtericum* (29), interaction with fibronectin determines corynebacteria adhesion to vaginal epithelial cells (30), and *C. diphteriae* can invade epithelial cells, with an important role of a transmembrane protein called DIP0733, which possesses a fibrinogen and collagen binding activity (31–33).

As part of normal human skin microbiota, corynebacteria can be implicated in inoculation disease. They are especially involved in up to 3% of BJI (34–36). However, little is known about the specific aspects of *Corynebacterium* spp. BJI: epidemiologic data are lacking, their specific management is not addressed in current guidelines, and the pathophysiology of *Corynebacterium* spp. BJI has not been investigated so far (18, 37, 38). We report here the experience of our regional reference center with the management of *Corynebacterium* spp. BJI, aiming to describe patients' characteristics and treatment failure's determinants. Clinical isolates were further characterized for species distribution, antimicrobial susceptibility profile, ability to form biofilm and to invade bone cells.

PATIENTS AND METHODS

Ethical Statements

This study (ClinicalTrials.gov registration number NCT03081273) received the approval of the French South-East Ethics Committee (reference number QH20/2014). All patients received written information about the study. The requirement for written informed consent was waived by the Committee for the protection of persons (CPP) according to French legislation at time of the study.

Inclusion Criteria and Data Collection

This retrospective cohort study (2007–2016) included all patients followed-up in the infectious disease department of our tertiary care center for a proven *Corynebacterium* spp. BJI, i.e., with clinical, biological and/or radiological symptoms consistent with the diagnosis of BJI, with at least two *per* operative culture-positive samples yielding the same isolate (same species and same antibiotic susceptibility profile), and treated as such (1, 37, 38). Patients with diabetic foot- or pressure ulcer-related osteomyelitis were excluded because of their specific pathophysiology and management. For each patient, data were extracted from medical records by two of the study authors (infectious diseases specialists).

Microbiological diagnosis was performed according to international standards. For each patient, three to five intraoperative bone and/or periprosthetic tissue samples were collected under sterile conditions. They were then inoculated onto a Columbia sheep's blood agar plate (with reading at days 1, 2, and 3 before being thrown away), two PolyVitex chocolate agar plates (with reading at days 1, 2, and 3 before being thrown away for the first one and with reading at days 7 and 10 for the second one), two blood agar plates for anaerobic incubation (with reading at days 3 and 5 before being thrown away for the first one and with reading at days 7 and 10 for the second one) and into a Schaedler anaerobic liquid broth for which a daily reading was performed. If not

cloudy, the broth was systematically subcultured on day 10 onto chocolate and blood agar plates for anaerobic incubation, incubated for 5 days in 5% CO_2 and anaerobic atmosphere, respectively. Isolated bacteria were identified according to standard laboratory procedures (VITEK 2 system or VITEK matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; bioMerieux, Marcy l'Etoile, France). When several specimens were positive, the identification of each type of colony was performed for all specimens. Antimicrobial susceptibility profiles were determined at least twice for each type of bacteria after a random selection among the positive specimens. Results of superficial and/or soft tissue samples were excluded.

Definitions

BJIs were classified according to: (i) the potential presence of an orthopedic implant (i.e., joint prosthesis or osteosynthesis device); and (ii) the duration of progression from the presumed date of inoculation (i.e., date of device implantation for post-operative ODI, or date of symptom onset for native BJI) up to diagnosis, differentiating acute (≤4 weeks) vs. chronic (>4 weeks), and early (≤3months) vs. delayed (>3 months) infections (1, 19).

The surgical strategies considered as optimal were: (i) surgical debridement for chronic osteomyelitis; (ii) debridement with implant retention for acute ODI; and (iii) implant removal for chronic ODI. One-time exchange for chronic ODI was accepted if bacterial identification was previously known, without compromised local conditions (sinus tract, abscess and/or flap coverage requirement) (3, 11).

Treatment failure consisted in: (i) clinically persisting infection under appropriate antibiotherapy; or (ii) clinical relapse after the end of antibiotherapy; or (iii) septic indication for unplanned surgical revision more than 5 days after primary procedure; or (iv) superinfection; or (v) death related to the BJI or to a complication of its management.

Biological inflammatory syndrome referred to a plasmatic CRP level > 10 mg/L.

Strain Characterization and Susceptibility Testing

Baseline strain characterization was routinely performed at time of diagnosis and retrieved from patients' medical records, including (i) species identification using VITEK®2 MS (bioMérieux, version 2.8.4.20081127, Shimadzu Biotech) (39); and (ii) antimicrobial susceptibility profile using the disk diffusion method on Mueller-Hinton agar supplemented with 5% sheep blood, as recommended by the European Committee on Antimicrobial Susceptibility Testing. Most clinical isolates responsible for a BJI diagnosed at our institution had been stored in cryotubes at −80°C since 2007. Available *Corynebacterium* spp. strains isolated from the included patients were subcultivated on Colombia agar supplemented with 5% sheep blood (COS, bioMérieux, Marcy l'Etoile, France) at 37°C for 48 h for further bacteriological assessments.

Biofilm Formation

Early-stage biofilm formation was assessed using a protocol based on the BioFilm Ring test®, relying on the immobilization of magnetic beads by the growing biofilm matrix (13). Briefly, 96-well microplates were inoculated with a set of 10-fold serial dilution of standardized bacterial suspension in BHI mixed with 1% (v/v) toner solution containing magnetic beads (Biofilm Control, Saint Beauzire, France). A well without bacteria was used as negative control. After 5 h of static incubation at 37°C, each well was covered with 100 µL of white opaque oil (contrast liquid) and plates were placed for 1 min on a dedicated block for magnetization before being scanned with a specific plate reader (Pack BIOFILM, Biofilm Control): free beads were attracted at the center of the well to form a spot, of which intensity dropped down as beads were immobilized during biofilm formation. The adhesion strength of each strain was expressed as BioFilm Index (BFI), as previously described (40). The biofilm-forming potential (BP) was calculated using the formula: BP = [1 – (BFI sample/average BFI of negative control)] for each well. The cut-off value corresponded to three standard deviations above the mean of the negative control wells (BFIc = 0.53). Isolates with values of BP above 0.53 were considered significant biofilm formers. The last dilution above 2BFIc identifies the ability of the microorganism to form biofilm: poor (BP < 2BFIc at 10^{-1} dilution), weak (BP > 2BFIc at 10^{-1} and/or 10^{-2} dilution), moderate (BP > 2BFIc at 10^{-3} and/or 10^{-4} dilution), and high (BP > 2BFIc at 10^{-5} and/or 10^{-6} dilution) biofilm producers (41).

Ability to form mature biofilm was evaluated using the crystal violet staining test, as previously described (42). Briefly, 96-well microplates were inoculated with standardized bacterial suspension in BHI supplemented with 1% glucose, and incubated for 24 h at 37°C. After being washed, biofilm was colored with 100 µL of 0.1% crystal violet (Merck, Fontenay-sous-Bois, France). After new wash, dye bound to the biofilm was resolubilized with 100 µL of 33% acetic acid (VWR International) per well. The optical density at 490 nm, measured with a micro ELISA Auto Reader, Model 680 (BioRad, Hercules, USA), allows a quantitative measurement of formed biofilm. *S. aureus* 6850 was used as positive control in each experiment.

Invasion of Human Osteoblasts

The ability of gentamicin-susceptible isolates to invade osteoblasts was evaluated in a gentamicin-protection assay. MG63 osteoblastic cells (CRL-1427; LGC standard, USA) were seeded at 40,000 cells per well into 48-well tissue culture plates and cultured for 24 h. Osteoblasts were infected with bacterial suspensions standardized in BHI at a multiplicity of infection of 1:100. After 2 h of co-culture, cells were treated for 1 h with gentamicin (200 mg/L) to kill the remaining extracellular bacteria and subsequently lysed by a 10-min incubation in sterile water. Dilutions of cell lysates were spiral-plated on COS using an easySpiral® automated plater (Interscience, Saint-Nom-la-Bretèche, France). Colonies were enumerated using a Scan®1200 automated plate reader (Interscience).

Given that the internalization of *S. aureus* within osteoblasts requires bacterial binding to the cellular α5β1 integrin via

fibronectin (43, 44), *Corynebacterium* internalization was further investigated by infecting two murine osteoblastic cell lines with isolates able to invade MG63 cells in the above model: (i) OB-$\beta1^{+/+}$ expressing a functional integrin β1 subunit, (ii) OB-$\beta1^{-/-}$ deficient in the expression of the β1 integrin subunit after the conditional deletion of the *itgb1* gene by transfection (45, 46).

S. aureus laboratory strain 6850 was used as positive control in each experiment while *S. aureus* DU5883 strain, deleted for the *fnbA/B* genes (and so unable to invade osteoblasts), was used as negative control (47).

Statistical Analysis

Studied variables were described as percentages for dichotomous variables and as medians with interquartile range (IQR) for continuous variables. In percentage calculation, the number of missing values was excluded from the denominator. Non-parametric tests were used to compare groups (Fisher exact and Mann-Whitney U tests), as appropriate. Kaplan-Meier curves were compared between groups using the log-rank (Mantel-Cox) test. Determinants of treatment failure were assessed using stepwise binary logistic regression, and expressed as odd ratios (ORs) with their 95% confidence intervals (95%CI). Non-interacting variables with medical meaning and *p*-values obtained in univariate analysis <0.15 were included in the final multivariate model. Bacteriological data provide from three independent experiments in triplicate, and results are expressed as mean of the nine measure points and its 95%CI. Results were expressed relatively to *S. aureus* 6850. A value of $p < 0.05$ was considered significant. All analyses were performed using SPSS v19.0 (SPSS, Chicago, IL, USA) and GraphPad-Prism v5.03 (GraphPad, San Diego, CA, USA) softwares.

RESULTS

Characteristics of the Included Population

Fifty-one *Corynebacterium* spp. BJIs occurring in 49 patients were included, as two patients presented two consecutives independent BJI episodes (**Table 1**). All infections resulted from an inoculation mechanism, and were mostly chronic ($n = 45$, 88.2%) and ODI ($n = 38$, 74.5%). ODI included 23 (60.5%) osteosynthesis devices and 15 (39.5%) prosthetic joint infections (PJI) (**Table 2**).

Surgery was performed in 47 (92.2%) patients and considered as optimal in 39 (76.5%) cases. The total duration of antibiotherapy specifically directed against corynebacteria was 18.1 (IQR, 13.1–29.3) weeks, initially administrated intravenously for 14.1 weeks (IQR, 6.5–18.3) in 48 patients (94.1%).

Bacteriological Findings

As one patient presented a co-infection with two different *Corynebacteria*, 52 strains were considered for inclusion. Species identification and antimicrobial susceptibility testing were available in patients' medical records for 45 of them. The most frequent species were *Corynebacterium striatum* ($n = 18$, 37.5%) and *Corynebacterium tuberculostearicum* ($n = 6$, 12.5%). Antimicrobial susceptibility profiles are presented in **Figure 1**. Most infections were polymicrobial ($n = 40$, 78.4%), including co-infections with coagulase-negative staphylococci ($n = 20$, 50.0%), *Enterobacteriaceae* ($n = 14$, 35.0%), *S. aureus* ($n = 8$, 20.0%), anaerobes ($n = 7$, 17.5%), enterococci ($n = 5$, 12.5%), *P. aeruginosa* ($n = 4$, 10.0%), streptococci ($n = 4$, 10.0%) and/or *Candida* ($n = 1$, 2.5%). A detailed description of the eleven patients with a monomicrobial *Corynebacterium* spp. BJI is provided in **Supplementary Table 1**.

Outcome and Determinants of Treatment Failure

After a median follow-up of 60.7 weeks (IQR, 30.1–115.1) including 38.0 weeks (IQR, 10.1–85.7) after completion of the antibiotherapy, 20 (39.2%) treatment failures were observed in a median delay of 14.3 weeks (IQR, 9.1–18.6) after treatment initiation, including 13 (65.0%) persistent infections, 6 (30%) relapses, 10 (50%) superinfections and one infection-related death. Seventeen (85.0%) cases required an additional surgical procedure, including one limb amputation. Four (20.0%) treatment failures were documented with the same *Corynebacterium* spp. strain; no documentation was obtained in 8 (40.0%) patients. Comparison of patients with and without treatment failure is presented in **Table 1**, as well as univariate analysis for risk factor for treatment failure. In multivariate analysis, among male gender, initial biological inflammatory syndrome, non-optimal surgical management, and corynebacteria-directed combination therapy, independent determinants for treatment failure were an initial biological inflammatory syndrome (OR, 15.119; 95%CI, 1.189–192.205; $p = 0.036$) and non-optimal surgical management (OR, 7.291; 95%CI, 1.107–48.016; $p = 0.039$) (**Figures 2A,B**). Interestingly, the 3 (5.9%) patients who received daptomycin (6 to 8 mg/kg/day) as first-line regimen relapsed (**Figure 2C**), despite an optimal surgical management. Of note, two of these patients had a polymicrobial infection. The three *Corynebacterium spp*. Isolates were fully susceptible do daptomycin, with MICs of 0.5, 0.094, and 0.032 mg/L. The choice of daptomycin was based on the polymicrobial nature of the infection in one patient, and previous antimicrobial intolerances in the two others. Finally, daptomycin was used as part of a combination therapy in two of these three patients.

Concerning specifically ODI, 16 (42.1%) treatment failures were observed. No significant risk factors was highlighted (**Table 2**), but treatment failure-free survival curve analysis suggested a significantly poorer outcome in patients with PJI compared to osteosynthesis device infection, in case of non-optimal surgical management, and if daptomycin was used as first-line regimen (**Figures 2D–F**).

Bone Cell Invasion

Among the 52 potential strains isolated from the patient study, 22 had not been conserved, five had been isolated in other institutions before patient referral to our reference center, seven were resistant to gentamicin preventing to perform gentamicin-protection assay, three could not be formally identified at the species level, and two *C. tuberculostearicum* strains had cultural aspect with tiny colonies preventing their enumeration on blood

TABLE 1 | Comparison of patients with favorable and unfavorable outcome and determinants of treatment failure in all patients with *Corynebacterium* spp. BJI (univariate analysis).

	Outcome				Univariate analysis	
	Total population	**Favorable**	**Failure**	***p*-value**	**OR (95%CI)**	***p*-value**
n	**51**	**31**	**20**			
Demographics						
Male gender	36 (70.6%)	19 (61.3%)	17 (85.0%)	0.115	3.579 (0.861–14.871)	0.079
Age (median, 95%CI), years	54.2 (44.2–68.8)	52.5 (46.3–67.6)	56 (41.6–69.0)	0.862	0.996 (0.963–1.030)*	0.810
Comorbidities						
BMI (median, 95%CI), kg/m²	26.9 (23.5–28.6)	25.3 (23.7–27.5)	27.3 (23.3–28.8)	0.378	1.112 (0.938–1.319)	0.220
ASA score (median, 95%CI)	1 (1–2)	2 (1–2)	1 (1–2)	0.697	0.908 (0.477–1.729)	0.770
CCI (median, 95%CI)	0 (0–2)	0 (0–2)	0.5 (0–2)	0.687	1.068 (0.737–1.547)	0.728
***Corynebacterium* species**						
C. striatum	18 (37.5%)	12 (38.7%)	6 (36%)	0.764	0.7731 (0.218–2.444)	0.611
C. tuberculostearicum	6 (12.5%)	3 (9.7%)	3 (17.6%)	0.661	1.750 (0.315–9.716)	0.522
C. simulans	5 (10.4%)	4 (12.9%)	1 (5.9%)	0.637	0.375 (0.039–3.633)	0.397
C. jekeium	4 (8.3%)	2 (6.5%)	2 (11.8%)	0.629	1.706 (0.220–13.243)	0.610
C. minutissimum	4 (8.3%)	2 (6.5%)	2 (11.8%)	0.629	1.706 (0.220–13.243)	0.610
C. amycolatum	3 (6.3%)	3 (9.7%)	0 (0.0%)	1.000	0.519 (0.050–5.379)	0.582
Corynebacterium urealyticum	2 (4.2%)	1 (3.2%)	1 (5.9%)	1.000	1.667 (0.098–28.320)	0.724
Others	5 (10.4%)	3 (9.7%)	2 (11.8%)	0.661	1.750 (0.315–9.716)	0.522
Type of BJI						
Native chronic osteomyelitis	13 (25.5%)	9 (29.0%)	4 (20.0%)	0.529	0.611 (0.160–2.339)	0.472
ODI						
PJI	15 (39.5%)	6 (27.3%)	9 (56.3%)	0.099	3.429 (0.078–13.390)	0.076
Osteosynthsesis device	23 (60.5%)	16 (72.2%)	7 (48.3%)	0.099	0.292 (0.075–1.139)	0.076
BJI mechanism						
Superinfection	24 (47.1%)	13 (41.9%)	11 (55.0%)	0.402	1.692 (0.545–5.257)	0.363
Inoculation mechanism	51 (100%)	31 (100%)	20 (100%)	1.000	NC	NC
Post-operative	48 (94.1%)	30 (96.8%)	18 (90.0%)	1.000	0.300 (0.025–3.549)	0.340
Post-traumatic	23 (45.1%)	14 (45.2%)	9 (45.0%)	0.553	0.994 (0.321–3.075)	0.991
BJI chronology						
Early infection (<3 months)	34 (69.4%)	21 (70.0%)	13 (68.4%)	1.000	0.929 (0.268–3.219)	0.907
Chronic infection (>4 weeks)	45 (88.2%)	26 (83.9%)	19 (95.0%)	0.384	3.654 (0.394–33.880)	0.254
Diagnostic features						
Sinus tract	29 (63.0%)	18 (60.0%)	11 (68.8%)	0.750	1.467 (0.406–5.301)	0.559
Abscess	9 (20.0%)	5 (17.2%)	4 (25.0%)	0.700	1.600 (0.362–7.073)	0.535
Biological inflammatory syndrome	30 (69.8%)	17 (58.6%)	13 (92.9%)	0.033	9.176 (1.054–79.892)	0.045
Initial plasmatic CRP level (mg/L)	37.3 (15.3–96.2)	30.0 (14.5–91.5)	45.0 (21.7–93.9)	0.565	0.998 (0.990–1.007)	0.724
Polymicrobial infection	40 (78.4%)	26 (83.9%)	14 (70.0%)	0.304	0.449 (0.116–1.736)	0.246
Surgical management	47 (92.2%)	29 (93.5%)	18 (90.0%)	0.640	0.621 (0.080–4.804)	0.648
Inappropriate surgical management	12 (23.5%)	5 (16.1%)	8 (35.0%)	0.178	2.800 (0.743–10.553)	0.128
Flap coverage requirement	8 (15.7%)	3 (9.7%)	5 (25.0%)	0.237	3.111 (0.652–14.845)	0.155
Medical management						
Antimicrobial therapy duration						
Total treatment duration (weeks)	24.7 (14.1–54.4)	18.1 (13.1–33.9)	37.1 (22.4–59.4)	0.080	1.018 (0.997–1.039)	0.088
Corynebacterium-specific treatment duration	16.3 (13.1–22.8)	14.9 (12.9–18.9)	20.0 (16.0–33.9)	0.039	1.070 (1.004–1.141)	0.038
Corynebacterium-specific intravenous treatment	48 (94.1%)	29 (93.5%)	19 (95.0%)	1.000	0.310 (0.111–15.479)	0.830
Intravenous treatment duration	14.1 (6.5–18.3)	13.1 (5.9–15.0)	18.1 (14.9–27.9)	0.130	1.095 (1.007–1.190)	0.034
Oral switch	26 (54.2%)	20 (69.0%)	6 (31.6%)	0.018	0.208 (0.060–0.723)	0.013

(Continued)

TABLE 1 | Continued

	Outcome				Univariate analysis	
	Total population	**Favorable**	**Failure**	***p*-value**	**OR (95%CI)**	***p*-value**
n	**51**	**31**	**20**			
Corynebacterium-specific combination therapy	36 (75.0%)	24 (82.8%)	12 (63.2%)	0.176	0.357 (0.093–1.365)	0.132
Combination therapy duration	12.9 (6.8–16.6)	12.1 (4.3–13.9)	19.3 (10.6–22.5)	0.491	1.107 (0.977–1.255)	0.112
First line antimicrobial regimen						
Initial oral antimicrobial therapy	18 (35.3%)	14 (45.2%)	4 (20.0%)	0.080	0.304 (0.082–1.119)	0.073
Betalactam	24 (50.0%)	15 (50%)	9 (50.0%)	1.000	1.000 (0.311–3.218)	1.000
Glycopeptide	35 (68.6%)	23 (74.2%)	12 (60.0%)	0.360	0.522 (0.157–1.738)	0.289
Clindamycin	5 (10.0%)	4 (13.3%)	1 (5.0%)	0.636	0.342 (0.035–3.311)	0.354
Linezolid	0 (0.0%)	0 (0.0%)	0 (0%)	NC	NC	NC
Daptomycin	3 (5.9%)	0 (0.0%)	3 (15.0%)	0.055	NC	NC
Posterior antimicrobial regimen						
Betalactam	20 (40.8%)	11 (36.7%)	9 (47.4%)	0.555	1.555 (0.484–4.995)	0.459
Glycopeptide	19 (37.3%)	10 (32.2%)	9 (47.4%)	0.358	1.718 (0.539–5.475)	0.360
Clindamycin	8 (15.7%)	5 (16.1%)	3 (15.0%)	0.496	0.555 (0.125–2.469)	0.439
Linezolid	9 (17.6%)	7 (22.6%)	2 (10.0%)	0.512	1.643 (0.442–6.102)	0.458
Daptomycin	5 (9.8%)	4 (12.9%)	1 (5.0%)	1.000	0.722 (0.119–4.372)	0.723
Daptomycin-containing regimen	8 (15.7%)	4 (12.9%)	3 (15.0%)	0.696	1.687 (0.370–7.697)	0.499

*95%CI, 95% confidence interval; ASA, American society of anesthesiologists; BMI, Body mass index; CCI, Charlson comorbidity index; CRP, C-reactive protein;NC, Not calculable; ODI, Orthopedic device-related infection; OR, Odd ratio; PJI, Prosthetic joint infection. *Calculated for 10 additional years.*

agar plates. Consequently, ability to invade human osteoblasts could be assessed for 13 corynebacteria strains (seven *C. striatum*, three *Corynebacterium simulans*, two *Corynebacterium amycolatum/xerosis*, and one *Corynebacterium minutissimum*) isolated from different patients (**Table 3**).

In comparison with *S. aureus* DU5883, all but one strain were significantly able to invade MG63 osteoblasts (**Figure 3**). The internalization rate was roughly comprised between 1 and 10% of positive control (*S. aureus* 6850). One *C. amycolatum/xerosis* strain (n°15) isolated from a delayed BJI even presented a very high internalization rate (200% of positive control).

Strains isolated from delayed BJI had a significantly higher internalization rate compared to early ones (**Figure 3A**).

The internalization rate of each species are provided in **Supplementary Figure 1A**. The little number of isolates per species did not allow to provide pertinent statistical comparison.

Strains able to invade MG63 human osteoblasts, including isolate n°15, were challenged in OB-$\beta1^{-/-}$ murine osteoblasts, resulting in a drastic reduction of the internalization rate compared to OB-$\beta1^{+/+}$ cells (**Figure 3B**).

Biofilm Formation

Early-stage biofilm formation was assessed for 11 of the 13 isolates used in the cellular infection model. The two other Cor 10 and 13° formed aggregates under the culture conditions specifically required for the BioFilm Ring test®. All but two corynebacteria had a poor BP. The two last strains Cor 5 and Cor 8b had a weak BP (**Table 3**).

All the 13 isolates were evaluable regarding their mature biofilm formation by the crystal violet staining method. Six of them formed mature biofilm, with a rate ranging from 8.6 to 42.4% compared to *S. aureus* 6850 (**Table 3**).

The little number of isolates per species prevented providing relevant interspecies comparison (**Supplementary Figure 1B**).

Early or mature biofilm formation abilities were not correlated with any relevant clinical feature.

Of note, neither internalization nor biofilm formation ability had a significant impact on patient outcome.

DISCUSSION

Representing more than 3% of PJI etiologic agents (34–36), *Corynebacterium* spp. have been largely neglected in this field. Despite the limitations inherent to the retrospective and unicentric nature of our study, it provides major clinical and therapeutic insights regarding corynebacteria BJI. Our results are reinforced by the attempt to minimize the risk of considering commensal *Corynebacterium* spp. strains isolated as contaminants by including only BJI with at least two concordant positive *per* operative samples and excluding contiguous infections such as decubitus ulcer- and diabetic foot-related osteomyelitis that are associated with a high risk of sample contamination. Indeed, conclusions of some previously published series must be interpreted with caution as including more than 50% of patients with contiguous BJI and based on the culture results of superficial samples (48, 49).

TABLE 2 | Comparison of patients with favorable and unfavorable outcome and determinants of treatment failure in patients with *Corynebacterium* spp. orthopedic device-related infection (univariate analysis).

	Outcome			Univariate analysis		
	ODI	**Favorable**	**Failure**	***p*-value**	**OR (IC95%)**	***p*-value**
Demographics						
Male gender	25 (65.8%)	12 (54.5%)	13 (81.3%)	0.165	3.611 (0.798; 16.347)	0.096
Age (median, 95%CI), years	53.1 (44.1;69.0)	52.2 (47.5;69.0)	54.0 (41.6;69.0)	0.679	0.991 (0.956; 1.027)	0.615
Comorbidities						
BMI (median, 95%CI), kg/m²	25.4 (22.9;28.6)	24.5 (22.5;26.0)	28.0 (23.5;28.9)	0.100	1.199 (0.969; 1.484)	0.095
ASA score (median, 95%CI)	1 (1.0;2.0)	1 (1.0;2.0)	1.5 (1.0; 2.3)	0.589	1.250 (0.605; 2.584)	0.547
CCI (median, 95%CI)	0 (0.0;1.8)	0 (0.0;1.0)	0.5 (0.0;2.0)	0.453	1.325 (0.809; 2.171)	0.263
BJI mechanism						
Superinfection	16 (42.1%)	8 (36.4%)	8 (50.0%)	0.511	1.750 (0.472; 6.483)	0.402
Inoculation mechanism	38 (100%)	22 (100%)	16 (100%)			
Post-operative	37 (97.4%)	22 (100%)	15 (93.8%)	0.421	NC	NC
Post-traumatic	16 (42.1%)	10 (45.5%)	6 (37.5%)	0.744	0.720 (0.193; 2.681)	0.624
BJI chronology						
Early infection (<3 months)	25 (69.4%)	13 (61.9%)	12 (80.0%)	0.295	2.462 (0.527; 11.500)	0.252
Chronic infection (>4 weeks)	33 (86.8%)	18 (81.8%)	15 (93.8%)	0.374	3.333 (0.336; 33.113)	0.304
Diagnostic features						
Sinus tract	21 (63.6%)	13 (61.9%)	8 (66.7%)	1.000	1.231 (0.278; 5.454)	0.785
Abscess	6 (18.8%)	2 (10.0%)	4 (33.3%)	0.165	4.500 (0.679; 29.808)	0.119
Biological inflammatory syndrome	26 (78.8%)	14 (70.0%)	12 (92.3%)	0.202	5.143 (0.540; 48.943)	0.154
Initial plasmatic CRP level (mg/L)	30.0 (15.0;93.9)	20.0 (14.0;46.0)	52.4 (20.1;96.2)	0.414	0.999 (0.991; 1.007)	0.824
Surgical management	36 (94.7%)	22 (100%)	14 (87.5%)	0.171	NC	NC
Inappropriate surgical strategy	9 (25.0%)	4 (18.2%)	5 (35.7%)	0.147	3.500 (0.808–15.163)	0.094
Surgical strategy DAIR/debridement	14 (38.9%)	7 (31.8%)	7 (50.0%)	0.314	2.143 (0.539; 8.512)	0.279
One-stage exchange	1 (2.8%)	1 (4.5%)	0 (0.0%)	1.000	NC	NC
Two-stage exchange	11 (30.6%)	9 (40.9%)	2 (14.3%)	0.142	0.241 (0.043; 1.346)	0.105
Definitive device ablation	10 (27.8%)	5 (22.7%)	5 (37.5%)	0.462	1.889 (0.430; 8.295)	0.400
Two-stage exchange OR definitive device ablation	21 (58.3%)	14 (63.6%)	7 (50.0%)	0.499	0.571 (0.147; 2.228)	0.420
Flap coverage requirement	5 (13.2%)	2 (9.1%)	3 (18.8%)	0.632	2.308 (0.338; 15.750)	0.393

95%CI, 95% confidence interval; ASA, American society of anesthesiologists; BMI, Body mass index; CCI, Charlson comorbidity index; CRP, C-reactive protein; NC, Not calculable; ODI, Orthopedic device-related infection; OR, Odd ratio; PJI, Prosthetic joint infection.

Our results confirm that proven *Corynebacterium* BJI occur mainly by inoculation after trauma, mostly after road crash-related open fractures. Indeed, a predominance of young men was noted, with up to 70% of chronic osteomyelitis. PJI were less frequent than previously described (48), and mostly corresponded to superinfections during complex PJI managements. These differences can be explained by our stringent bacteriological definition of cases. Finally, the species distribution slightly differed from previous studies, with a predominance of *C. striatum* and *C. tuberculostearicum*, and less *C. amycolatum* and *Corynebacterium jekeium* than previously described (36, 48).

The management of BJI involving *Corynebacterium* is complex. First, optimal surgical management appeared as a crucial determinant for treatment outcome, as previously described for chronic and/or ODI (50, 51), including removal of orthopedic device and extensive bone curettage when necessary (37, 38). However, this theoretical optimal management can be impaired by fracture stabilization requirements, and sometimes leads to major tissue loss. Choice of antimicrobial therapy is also challenging. As shown by our results and previous series (48), *Corynebacterium* isolates can be resistant to most of the antibiotics commonly used in BJI, including amoxicillin which remains the drug of choice for susceptible isolates. Moreover, polymicrobial infection prevalence, vancomycin toxicity and/or patient's antibiotic intolerances can raise the need of off-label use of alternative drugs. In this setting, daptomycin has been increasingly used in Gram positive BJI (52). Interestingly, all patients treated by daptomycin experienced treatment failure, leading to highlight the use of this antimicrobial as a significant risk factor for poor outcome. Although based on a limited number of patients, this finding is coherent with treatment failures and daptomycin resistance selection observed during other chronic conditions such as infective endocarditis (53–55)

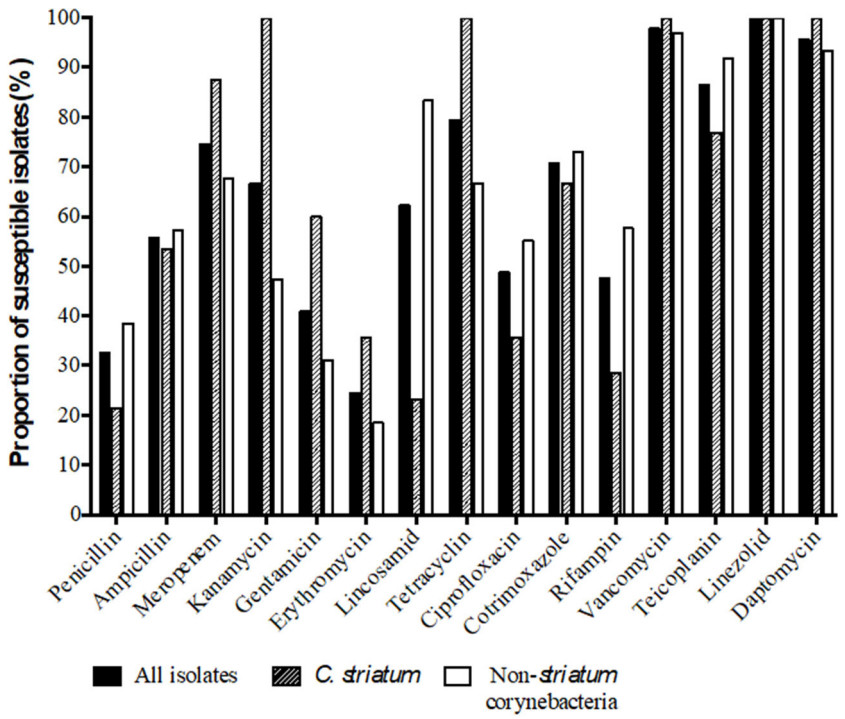

FIGURE 1 | Susceptibility profile of the 51 BJI *Corynebacterium* spp. isolates.

and raises the question of reconsidering the use of daptomycin as a first-line agent. Finally, the higher rate of treatment failure of ODI compared to PJI was not explained by statistically significant differences between patients or their management. However, ODI mostly occurred following the management of severe limb trauma, requiring flap coverage in more than 20% of cases. Even if not highlighted by our results, the complexity of orthopedic situations observed in such kind of patients might have led to this poor outcome.

Overall, and despite a complex surgical and medical management, BJI with *Corynebacterium* spp. are difficult-to-treat infections, as evidenced by (i) the failure rate approaching 40%, (ii) the frequent need of iterative surgical procedures including surgical flap reconstruction in 15% of patients, and (iii) the prolonged courses of antimicrobial therapies. If polymicrobism has been highlighted as a risk factor for treatment failure in some studies (56), this point is still controversial (57), and polymicrobial infections were not associated with a poorer outcome in our series. Associated with a dramatically increase of morbidity and medical/societal cost (58), BJI chronicity and relapse have consequently to be investigated, including underlying mechanisms leading to bacterial escape from the action of the host immune system and/or the antibiotics. The extensive evaluation of *Staphylococcus aureus* BJI pathomechanisms highlighted three main phenotypic bacterial factors associated with BJI chronicity (59): (i)internalization and persistence in non-professional phagocytic bone cells (osteoblasts), which had been confirmed to be clinically associated with BJI chronicity (15), (ii) biofilm formation (60), and (iii) emergence of small colony variants (25). We provide here the first assessment of these mechanisms toward a collection of clinical *Corynebacterium* isolates responsible for BJI. We demonstrated that almost all *Corynebacterium* isolates were able to invade osteoblasts and that their internalization rate was correlated with BJI chronicity, even if *in fine* the cure rate was not impacted. This ability to sanctuarize in bone cells emphasized the importance of surgical debridement in chronic BJI with *Corynebacterium* spp. and pleads for including the ability of antibiotics to eradicate the intracellular reservoir of corynebacteria in the choice of antimicrobial therapy strategies, as suggested for *S. aureus* (15, 61, 62). Interestingly, the infection of murine osteoblasts deficient in the expression of β1 integrin abolished the cellular invasion ability of the evaluated strains. This strongly suggest that corynebacteria osteoblastic invasion relies on mechanisms similar to *S. aureus*, of which fibronectin binding proteins A and B link to fibronectin of the bone matrix that acts as bridges between *S. aureus* and osteoblasts through the cellular α5β1 integrin (43, 44). The ligand of the cellular β1 integrin remains to be described in corynebacteria, as representing a future potential therapeutic target. Regarding biofilm formation, all investigated strains of our study were poor biofilm formers as most Gram positive bacteria except *S. aureus* (40). A few studies have however suggested that biofilm formation could be a determinant of *Corynebacterium* spp. hospital acquired infections (63, 64). Unfortunately, we were not able to perform the biofilm and intracellular assays on the whole series, which might represent a bias. Indeed, the ability of corynebacteria to form biofilm seems strain-related, as shown by the differences observed toward a same species according to their sequence types (ST) (63, 65). However, no clinical differences

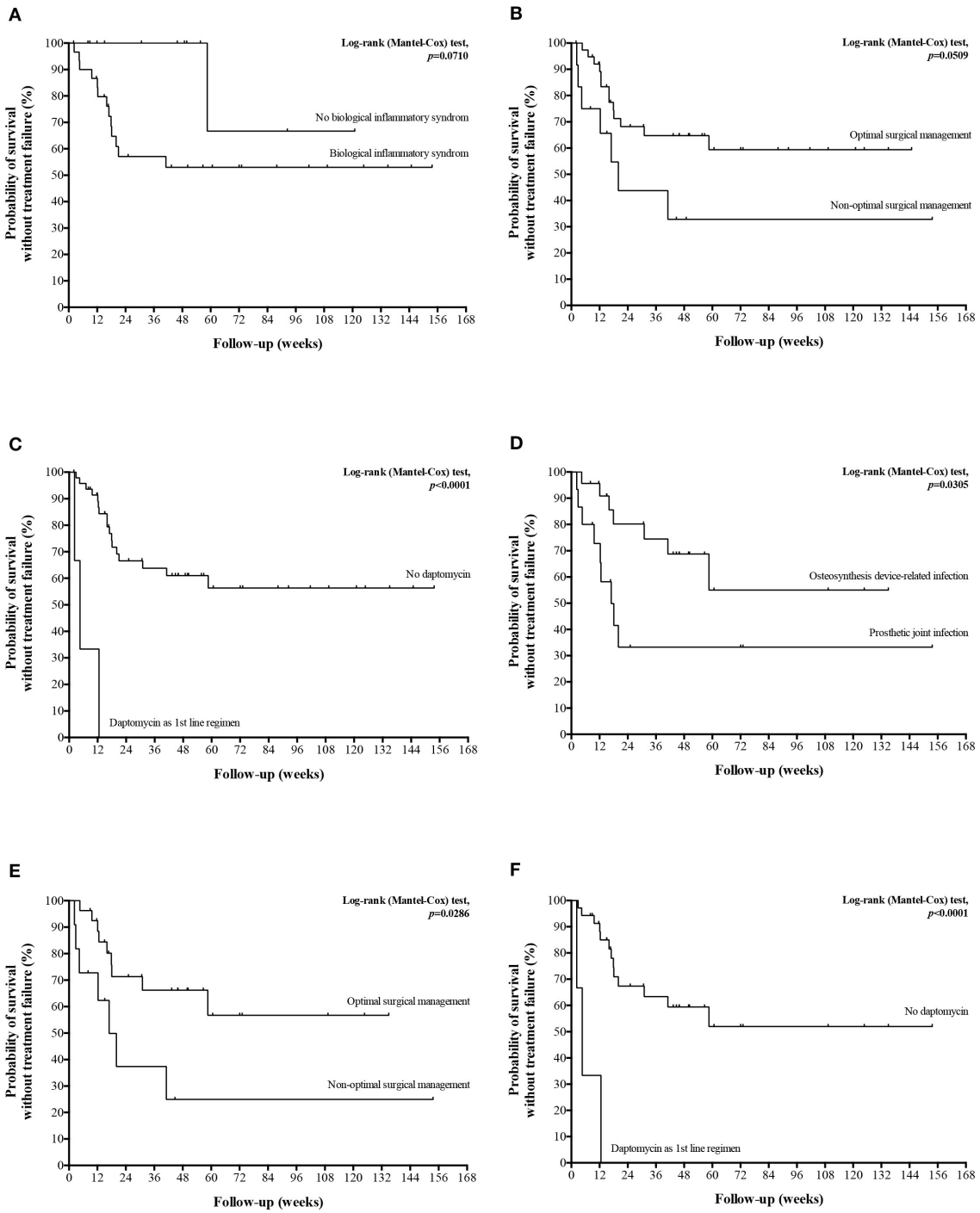

FIGURE 2 | Kaplan-Meier curves for the cumulative risk of treatment failure in all patients **(A–C)** and in patients with ODI panel **(D–F)** according to the major determinants of treatment failure.

were noted in our series between the patients for which the strain was available and the others (data not shown). Additionally, the comparison of isolates coming from mono and polymicrobial infection would have been interesting, but only three strains of our series were isolated from monomicrobial infection, making the comparison irrelevant.

The short time of follow-up (less than a year) of patients without treatment failure is not enough to affirm treatment success and represent another limitation to this study. However, even if relapses have been described several months/years after the end of therapy, this represents a rare event.

TABLE 3 | Description of the isolates evaluated in the osteoblastic cell infection model and biofilm formation assays.

Strain identification	*Corynebacterium* species	Type of BJI	Chronology of infection	Internalization rate (95%CI)*	Biofilm-forming potential	Mature biofilm formation (95%CI)*
Cor 1b#	*C. striatum*	Osteosynthesis infection	Early	0.34% (0.06–0.62)	POOR	0.36% (−0.08–0.79)
Cor 4	*C. striatum*	Native osteomyelitis	Early	3.63% (1.47–5.80)	POOR	35.71% (26.26–45.17)
Cor 5#	*C. simulans*	Prosthetic joint infection	Early	0.60% (0.30–0.89)	WEAK	1.64% (−0.84–4.13)
Cor 8b	*C. striatum*	Osteosynthesis infection	Delayed	4.29% (0.71–7.88)	WEAK	42.39% (30.65–54.13)
Cor 9b	*C. striatum*	Prosthetic joint infection	Early	1.30% (0.36–2.25)	POOR	5.66% (1.79–9.52)
Cor 10	*C. amycolatum/xerosis*	Prosthetic joint infection	Delayed	1.35% (0.37–2.32)	N/A	8.58% (3.78–13.38)
Cor 11a	*C. minutissimum*	Native osteomyelitis	Delayed	55.6% (28.99–82.24)	POOR	1.04% (−0.27–2.34)
Cor 12#	*C. simulans*	Prosthetic joint infection	Delayed	5.99% (3.11–8.87)	POOR	4.61% (−1.70–10.92)
Cor 13	*C. striatum*	Native osteomyelitis	Early	15.3% (9.67–20.91)	N/A	13.59% (6.11–21.06)
Cor 14	*C. simulans*	Native osteomyelitis	Delayed	2.84% (0.75–4.93)	POOR	8.56% (−0.04–17.15)
Cor 15	*C. amycolatum/xerosis*	Osteosynthesis infection	Delayed	206% (131.08–281.86)	POOR	18.06% (2.89–33.22)
Cor 16	*C. striatum*	Osteosynthesis infection	Early	35.2% (-3.23–73.647)	POOR	2.38% (−0.17–4.93)
Cor 18	*C. striatum*	Prosthetic joint infection	Delayed	7.29% (3.03–11.54)	POOR	0.01% (−0.01–0.04)

95%CI, 95% confidence interval; BJI, Bone and joint infection; CO, Chronic osteomyelitis; ODI, Osteosynthesis device-associated infection; PJI, Prosthetic joint infection.
**Results are given as mean and its 95% confidence interval (95%CI), compared to S. aureus 6850. #Designated monomicrobial infections.*

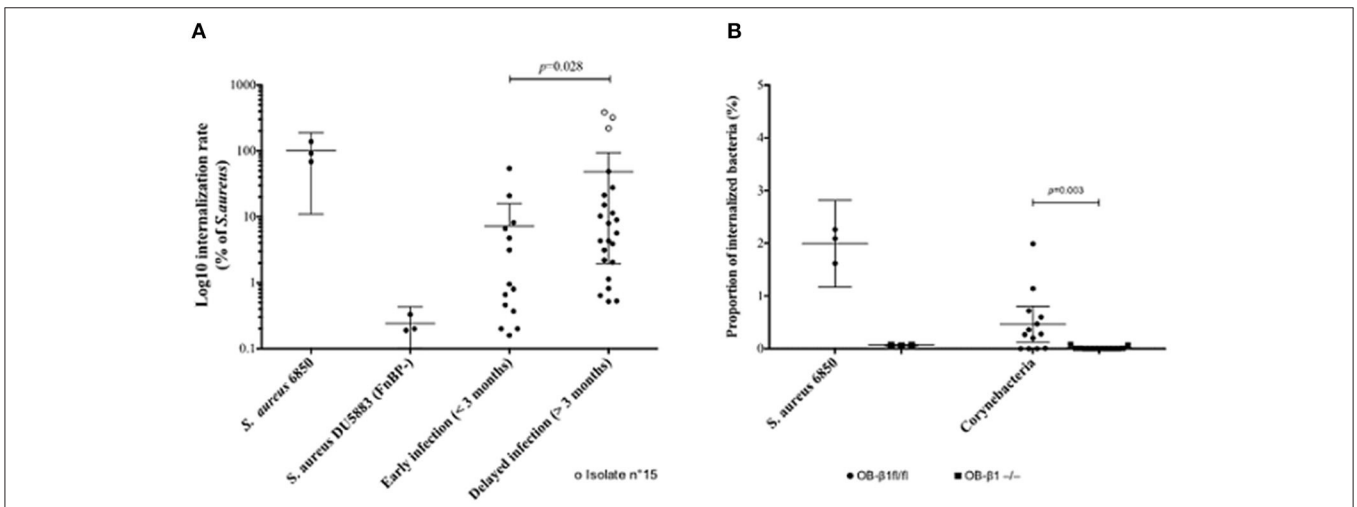

FIGURE 3 | Ability of *Corynebacterium* spp. isolates to invade osteoblastic cells. **(A)** Internalization rates of *Corynebacterium* isolates in MG63 human osteoblasts according to bone and joint infection (BJI) evolution delay, in comparison with *S. aureus* 6850 (positive control) and *S. aureus* DU5883 strain, inactivated for the *fnbA/B* genes (FnBP, negative control). **(B)** Internalization rates of the *Corynebacterium* isolates in murine osteoblasts with functional (OB-$\beta1^{fl/fl}$) or deficient (OB-$\beta1^{-/-}$) expression of the integrin β1 subunit, in comparison with *S. aureus* 6850.

This series of proven *Corynebacterium* BJI allows to better understand this neglected disease. Most often presenting as a post-traumatic or post-surgical chronic infection, this difficult-to-treat condition requires a complex and collaborative medical-surgical management due to its poor prognosis which is mostly driven by the initial surgical debridement. Furthermore, if

biofilm formation did not appear as a pivotal physiopathological mechanism of *Corynebacterium* in BJI, bone cells invasion via the cellular β1 integrin allows the formation of an intracellular reservoir that leads to chronic infection.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The requirement for written informed consent from participants for the usage of clinical isolates in the study was waived by the Committee for the protection of persons (CPP) according to French legislation at time of the study.

AUTHOR CONTRIBUTIONS

PC collected the data, conducted most of the experiment, and wrote the manuscript under the supervision of FV. FV designed the study, analyzed the results and helped to perform the experiments. TF and FL helped to design the study. VT and AD reviewed the manuscript. JT provided the protocols for biofilm experiments. AC, CC, EB, and SL provided clinical data. All authors revised and edited the manuscript and read and approved the final manuscript.

FUNDING

The clinical part of the study was carried out as part of our routine work. The bacteriological work was supported by an institutional grant to FV by the Hospices Civils de Lyon.

ACKNOWLEDGMENTS

Lyon Bone and Joint Infection Study Group

Coordinator – TF; **Infectious Diseases Specialists** – TF, FV, Thomas Perpoint, Patrick Miailhes, Florence Ader, Agathe Becker, Sandrine Roux, Claire Triffault-Fillit, AC, Alexie Bosch, Marielle Perry, Fatiha Daoud, Johanna Lippman, EB, CC; **Surgeons** – SL, Elvire Servien, Romain Gaillard, Antoine Schneider, Stanislas Gunst, Cécile Batailler, Michel-Henry Fessy, Yannick Herry, Anthony Viste, Philippe Chaudier, Cyril Courtin, Lucie Louboutin, Sébastien Martres, Franck Trouillet, Cédric Barrey, Emmanuel Jouanneau, Timothée Jacquesson, Ali Mojallal, Fabienne Braye, Fabien Boucher, Hristo Shipkov, Joseph Chateau, Philippe Céruse, Carine Fuchsmann, Arnaud Gleizal; **Anesthesiologists** – Frédéric Aubrun, Mikhail Dziadzko, Caroline Macabéo; **Microbiologists** – FL, Jean-Philippe Rasigade, Laetitia Beraut, Céline Dupieux, Camille Kolenda, Jérôme Josse; **Imaging** – Fabien Craighero, Loic Boussel, Jean-Baptiste Pialat; **Nuclear Medicine** – Isabelle Morelec, Marc Janier, Francesco Giammarile; **PK/PD specialists** – Michel Tod, Marie-Claude Gagnieu, Sylvain Goutelle; **Clinical research assistant and database manager** – Eugénie Mabrut.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.539501/full#supplementary-material

Supplementary Figure 1 | Comparison of *Corynebacterium* species regarding their ability to invade MG63 human osteoblasts **(A)** and to form mature biofilm **(B)**.

Supplementary Table 1 | Clinical characteristics of the eleven patients with monomicrobial *Corynebacterium* spp. BJI.

Supplementary Table 2 | Description of patients according to the BJI types, and comparison of osteosynthesis device-related and prosthetic joint infection.

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Conflict of Interest: Biofilm Control provided support in the form of salaries for JT but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 05 March 2021
doi: 10.3389/fmed.2021.586875

Impact on the Gut Microbiota of Intensive and Prolonged Antimicrobial Therapy in Patients With Bone and Joint Infection

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Edited by:
Richard V. Goering, Creighton University, United States

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Catherine Davis, Creighton University, United States

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *24 July 2020*
Accepted: *25 January 2021*
Published: *05 March 2021*

Citation:
Levast B, Benech N, Gasc C, Batailler C, Senneville E, Lustig S, Pouderoux C, Boutoille D, Boucinha L, Dauchy F-A, Zeller V, Maynard M, Cazanave C, Le Thi T-T, Josse J, Doré J, Laurent F and Ferry T (2021) Impact on the Gut Microbiota of Intensive and Prolonged Antimicrobial Therapy in Patients With Bone and Joint Infection. Front. Med. 8:586875. doi: 10.3389/fmed.2021.586875

There is a growing interest in the potentially deleterious impact of antibiotics on gut microbiota. Patients with bone and joint infection (BJI) require prolonged treatment that may impact significantly the gut microbiota. We collected samples from patients with BJI at baseline, end of antibiotics (EOT), and 2 weeks after antibiotic withdrawal (follow-up, FU) in a multicenter prospective cohort in France. Microbiota composition was determined by shotgun metagenomic sequencing. Fecal markers of gut permeability and inflammation as well as multi-drug-resistant bacteria (MDRB) and *Clostridioides difficile* carriage were assessed at each time point. Sixty-two patients were enrolled: 27 native BJI, 14 osteosynthesis-related BJI, and 21 prosthetic joint infections (PJI). At EOT, there was a significant loss of alpha-diversity that recovered at FU in patients with native BJI and PJI, but not in patients with osteosynthesis-related BJI. At EOT, we observed an increase of Proteobacteria and Bacteroidetes that partially recovered at FU. The principal component analysis (PCoA) of the Bray–Curtis distance showed a significant change of the gut microbiota at the end of treatment compared to baseline that only partially recover at FU. Microbiota composition at FU does not differ significantly at the genus level

when comparing patients treated for 6 weeks vs. those treated for 12 weeks. The use of fluoroquinolones was not associated with a lower Shannon index at the end of treatment; however, the PCoA of the Bray–Curtis distance showed a significant change at EOT, compared to baseline, that fully recovered at FU. Levels of fecal neopterin were negatively correlated with the Shannon index along with the follow-up ($r^2 = 0.17$; $p < 0.0001$). The PCoA analysis of the Bray–Curtis distance shows that patients with an elevated plasma level of C-reactive protein (≥5 mg/L) at EOT had a distinct gut microbial composition compared to others. MDRB and *C. difficile* acquisition at EOT and FU represented 20% (7/35) and 37.1% (13/35) of all MDRB/*C. difficile*-free patients at the beginning of the study, respectively. In patients with BJI, antibiotics altered the gut microbiota diversity and composition with only partial recovery, mucosal inflammation, and permeability and acquisition of MDRB carriage. Microbiome interventions should be explored in patients with BJI to address these issues.

Keywords: gut microbiota, antimicrobial therapy, antibiotics, bone and joint infection, dysbiosis

INTRODUCTION

Bone and joint infections (BJI) are a public health issue in industrialized countries (1). Different kinds of BJI exist, depending on the pathophysiology and the route of bone contamination. BJI may occur spontaneously, such as hematogenous spondylodiscitis, septic arthritis, or diabetic foot infection with osteomyelitis, and these BJI can be grouped as "native BJI" (2, 3). Osteosynthesis-associated BJI frequently involves the long bones, especially the tibia. Fracture-related infections are major contributors to this group of BJI (4). Prosthesis joint infection (PJI) is the last group of BJI that mainly occurs in an elderly population in whom a hip or a knee prosthesis becomes infected after its implantation (5). Whatever the mechanism of acquisition, a BJI is considered as one of the most difficult-to-treat bacterial infections, as the eradication of the pathogen is challenging (1). The multidisciplinary management and a medico-surgical approach are crucial in all these patients. Surgery is essential in most of them, and upon diagnosis of BJI immediately after surgery, an intravenous, broad-spectrum empirical antimicrobial therapy is usually started and secondarily adapted to the microbiological culture results that reveal the pathogens involved and their susceptibilities to antibiotics. Depending on the type of BJI and the clinical presentation, oral antibiotics are frequently prolonged for a total of 6 or 12 weeks of therapy (2, 3, 5, 6). As it is one of the longest duration of antibiotics for a bacterial infection, it could be associated with significant side effects, such as a huge impact on the gut microbiota and the promotion of acquired antimicrobial resistance (7–9).

It has been demonstrated that short-term antibiotic usage strongly affects the gut microbiota. Extensive literature describing alteration of the gut microbiota by the use of antibiotics has bloomed over the recent years (10–13). The most frequent manifestation is antibiotic-associated diarrhea (14), which may be due to the direct toxin effects of antibiotics on the intestine, altered digestive function secondary to reduced concentrations of gut bacteria ("functional diarrhea"), or overgrowth of pathogenic microorganisms, such as *Clostridioides difficile* infection. The latter can account for most of the severe antibiotic-associated diarrhea observed and is characterized by a loss of the gut microbiota barrier properties, leading to frequent *C. difficile* infection recurrence with high morbidity. Of note is that one of the most effective treatments of recurrent *C. difficile* infection is fecal microbiota transplantation, which aims at restoring the fecal microbial ecosystem by transferring into the gut a preparation of feces from a healthy donor (15).

Nowadays, the gut microbiota is considered a major factor involved in the pathophysiology of many diseases. The development of novel molecular technologies coupling high-throughput metagenomic sequencing and bioinformatics/biostatistics has overcome the limitation of culture-based analysis of stool samples, opening a new way for the exploration of the gut microbiota in various diseases such as inflammatory bowel diseases (16), metabolic disorders (17), infectious diseases (18), or, more recently, central nervous system diseases (19). In all these conditions, an altered composition of the gut microbiota has been described, suggesting the deleterious effect on host physiology of modified gut microbial communities.

Infections and the use of antibiotics can cause significant and sometimes irreversible effects on the gut microbial composition throughout life (20, 21). The manner in which antibiotics affect gut microbial communities can vary according to different parameters: the route of antibiotic administration (22, 23), the duration of treatment (24), the broad or narrow spectrum of action of bacterial species-targeted antibiotics (25), the use of antibiotic combinations, or the repetition of antimicrobial treatment (26). The impact caused by antibiotics on gut microbiota results in alteration of the bacterial diversity and gut functions. These alterations could be transitory or could last over time (21). After antibiotic withdrawal, the gut microbiota has, in theory, the potential ability to return to its base state by a mechanism called resilience (26). However, resilience after prolonged antibiotic exposure has been poorly evaluated.

Antibiotics and gut microbiota modifications have also been associated with local alterations in gut physiology with

inflammation and increased permeability (27). To explore these aspects within the clinical model of BJI treatment, we assessed the dynamics of various markers of intestinal inflammation such as fecal neopterin and calprotectin, which are increased in an inflammatory context (28, 29) and zonulin, the only known protein that regulates intercellular tight junctions (30).

The main concern with prolonged antibiotic treatment is the emergence of antimicrobial resistance and its spread into a patient's environment (31). Extensive use of antibiotics in the last 40 years has systematically led to the emergence of bacterial resistance and development of nosocomial infections, mainly due to methicillin-resistant *Staphylococcus aureus* (MRSA) in the 1990s and now particularly due to commensals of the gut microbiota such as multidrug-resistant (MDR) Enterobacteriaceae. These hospital-acquired infections are causes of considerable morbidity and mortality in many industrialized countries and are one of the major concerns of public health issues and threats. In the last 10 years, MDR Enterobacteriaceae, particularly MDR *Escherichia coli* and *Klebsiella pneumoniae*, also emerged as community-acquired infections (i.e., infections contracted outside of a healthcare setting) (32, 33). Antibiotic treatments may facilitate the acquisition of MDR bacteria (MDRB) in the gut that can disseminate in the patient's environment (31).

In this study, we aimed to investigate for the first time the microbiological, clinical, and biological consequences on the gut microbiota and its host of prolonged antibiotic treatment in patients with different types of BJI. Antibiotic-related gut microbiota modifications and its resilience were assessed using shot-gun metagenomic sequencing after different treatment durations (6 weeks compared to 12 weeks) and different types of antibiotics, while potential association of the gut microbiota composition was investigated with (i) gut markers of inflammation and permeability, (ii) C-reactive protein (CRP), a systemic marker of inflammation that usually followed during BJI, and (iii) the acquisition of MDRB or *C. difficile* fecal carriage.

MATERIALS AND METHODS

Study Description

We performed a multicentric prospective interventional study in France called Interventi*O*nal *S*tudy of Bone and Joint *I*nfections *R*elated Gut Dysbios*IS* (OSIRIS) (NCT03011502; EudraCT 2016-003247-10) from January 2017 to September 2017. Five recruiting centers belonging to the CRIOAc network, a nationwide network of clinical centers dedicated to the management of complex BJI, were selected: Lyon (Hospices Civils de Lyon, CRIOAc Lyon), Bordeaux (CRIOAc GSO), Nantes (CRIOGO), Paris (CRIOAc Paris), and Lille-Tourcoing (CRIOAc G4).

Ethics

Patients highly suspected of BJI were informed and enrolled after their signature of written consent in the OSIRIS protocol. Patient follow-up was designed as part of standard care, with some interventions dedicated to the specific needs of the protocol. This study was reviewed and approved by a regional ethics committee (Comité de Protection des Personnes SUD-EST II; 69HCL16_0623). The study was also approved by the French Health authority (Agence Nationale de Sécurité du Médicament et des produits de Santé, ANSM).

Data Collection

Clinical data and stool collection were performed at baseline visit (B) within 24 h before starting the antibiotics, at the end of the treatment (EOT), and at 2 weeks after antibiotic withdrawal during a follow-up visit (FU). An electronic case report form (e-CRF) was created, and clinical data and the results of the serum CRP measurements were prospectively collected during follow-up using the ClinSight™ software.

Data that support the findings of the study are available from the corresponding author upon reasonable request.

Stool Collection and Fecal Microbiome Analysis

The patients collected their stools using a dedicated clean container system, ensuring stool conservation (Fecotainer®) within 48 h prior to freezing. The samples were then snap-frozen in triplicates of 1 g and stored at−80°C. At the end of the follow-up of the last patient, the samples were sent to Eurofins Inc., for DNA extraction and shotgun analysis. Genomic DNA was extracted from the fecal samples using the Qiagen QIAamp Fast DNA stool mini-kit after bead beating. Positive (*Escherichia coli*) and negative (no template, i.e., water) controls have been added throughout the process, from DNA extraction to sequencing, to validate the successful completion of each step. Sequencing library was constructed for each DNA sample using the TruSeq Nano DNA Library Prep kit (Illumina) according to the manufacturer's instructions. Libraries were then sequenced in paired-end (2 × 125 bp) HiSeq2500 v4 (Illumina) runs.

Bioinformatics analyses were performed on the Gut Print® platform with the in-house MgRunner v1.1.2 pipeline. In brief, after quality filtering using Trimmomatic (34), host sequence removal was performed using Bowtie2 (35). To ensure comparability, all samples were rarefied to the same sequencing depth, i.e., 5,000,000 paired-end sequences per sample. Taxonomic profiling was then performed with Kraken v.0.10.5-beta (36) and the RefSeq genomic database (2015 release, http://www.ncbi.nlm.nih.gov/refseq/). The measurement of α- and β-diversity indexes was performed in R Statistical Software ((37), version 3.4.4, http://www.R-project.org) using vegan and phyloseq packages. Identification of marker taxa for the different groups was achieved through differential abundance analysis using linear discriminant analysis effect size (38).

Neopterin, Calprotectin, Zonulin, and IgA Quantification in Stools

Biological markers of gut permeability and inflammation were monitored at each time point. At the end of the follow-up of the last patient, the samples were sent to the biochemistry laboratory of the HCL Centre Hospitalier Lyon Sud for ELISA-related techniques of analysis to be performed. Supernatants were obtained in the laboratory and then run to quantify sIgA (ImmunoChrom kit RIC6100—BioVendor), neopterin (kit neopterin ELISA Ref59321—IBL

International), calprotectin (fCAL RefEKCAL2—Bülhmann), and zonulin (RefK5600—ImmunDiagnostik), according to the manufacturer's instructions.

Fecal MDRB Cultures and Antibiogram Analysis

Fecal swabs were sampled at each specified patient visit and then sent prospectively to the IAI for fresh cultures on specific gelose media to evaluate the fecal portage of MDRB such as extended-spectrum beta-lactamases-producing Enterobacteriaceae (ESBL), carbapenemase-producing Enterobacteriaceae, MRSA, and *C. difficile*. When positive (i.e., detection of a growth) in selective chromogenic media (ChromID?: MRSA, ESBL, OXA48 and CARBA SMART, Biomérieux, Marcy-l'étoile, France), antibiograms and mass spectrometry were done on isolates to specify the minimum inhibitory concentrations of specific antibiotics and specify the isolates' identification (genus/species). When positive for ESBL, patient samples were thawed to quantify ESBL and total Gram-negative bacteria.

Statistical Analysis

Linear regression, Wilcoxon, and Mann–Whitney test were performed with Prism (GraphPad®, version 8.4.3, GraphPad Software, La Jolla California, USA; www.graphpad.com). *P* values are indicated in the graphs unless specified in the legend. Permutational multivariate analysis of variance (PERMANOVA) was performed with the R Statistical Software using the Adonis package. Analyses were performed both at the genus and OTU levels with no qualitative differences between the two taxonomic levels.

RESULTS

BJI Population

At the end of the inclusion period, 62 patients were enrolled, including 27 with a native BJI, 14 with an osteosynthesis-related BJI, and 21 with a PJI. The patients' characteristics are detailed in **Table 1**, and the patients' characteristics during the study are presented in **Table 2**. Each patient received personalized antimicrobial therapy, from empirical to targeted treatment, depending on the microbiological culture results and local and recommended treatment strategy. A total of 16 different types of antibiotic were used. The most frequent one was fluoroquinolone (FQ) ($n = 47$), a category that includes ofloxacin, levofloxacin, and ciprofloxacin (**Figure 1A**). After excluding extreme values, the duration of antimicrobial treatment was divided into two different groups corresponding to the two mainly recommended antibiotic duration in BJI: a "6 weeks" group ($n = 20$; between 41 to 60 days) and a "12 weeks" group ($n = 15$; between 81 and 100 days; **Figure 1B**).

Antibiotics Alter Gut Microbiota Diversity and Composition With Partial Recovery 2 Weeks After Antibiotic Withdrawal

BJI antimicrobial therapies alter the gut microbiota diversity as demonstrated by a decrease of the Shannon index and richness between B and EOT (**Figures 2A,B**). However, after 2 weeks of antibiotic withdrawal, the microbiota diversity increased but remained at lower values than those before treatment (**Figures 2A,B**). Interestingly, resilience differed between the three different subpopulations of BJI (**Figure 2C** and **Supplementary Figure 1**). Indeed native BJI showed no significant difference between B and FU for the Shannon Index and richness, whereas osteosynthesis-related BJI presented only a partial recovery at FU for both parameters. Patients with PJI presented a lower Shannon index at FU (mean $= 3.0 \pm 0.6$) compared to EOT (mean $= 3.2 \pm 0.4$) and B (mean $= 3.6 \pm 0.6$), even if it did not reach statistical significance probably because of the small sample size (**Figure 2C**). Interestingly, when considering the gut microbiota composition using taxonomic analysis, we observed an increase of Proteobacteria that partially recovered at FU (relative abundance at B: 7.2%, EOT: 13.5%, and FU: 10.2%).

TABLE 1 | Patients' characteristics.

BJI population	Native BJI ($n = 27$)	Osteosynthesis-related BJI ($n = 14$)	PJI ($n = 21$)	Total ($n = 62$)
Male (*n*, %)	17 (63)	10 (71.5)	13 (62)	40 (64.5)
Age (years)[a]	56.1 (13.2)	51.8 (17.6)	65.3 (9.1)	58.6 (14.1)
Antibiotic duration (days)[a]	58.8 (26.7)	69.8 (28.4)	68.3 (29.3)	64.5 (27.8)
BMI (mean)[a]	25.6 (6.5)	28.1 (5.8)	29.5 (7.0)	27.5 (6.6)
MDRB carriage at baseline (*n*, %)				
- MRSA	3 (11.1)	1 (7.1)	5 (23.8)	9 (14.5)
- ESBL-producing	0	0	0	0
- Enterobacteriaceae	3 (11.1)	1 (7.1)	5 (23.8)	9 (14.5)
- HREB	0	0	0	0
Clostridioides difficile carriage at baseline (*n*, %)	1 (3.7)	0	0	1 (1.6)

[a] *Data are expressed as mean (standard deviation).*

BJI, bone joint infection; PJI, prosthesis joint infection; BMI, body mass index; MDRB, multidrug-resistant bacteria; MRSA, methicillin-resistant Staphylococcus aureus; ESBL, extended-spectrum beta-lactamases; HREB, highly resistant emergent bacteria.

TABLE 2 | Patients' characteristics along the study.

BJI population	Baseline	End of treatment	Follow-up	Normal values
Feces weight (g)	60.5 ($n = 54$)	61.5 ($n = 46$)	72 ($n = 46$)	100–200
MDRB fecal carriage (%)	17.54	25.00	39.13	NA
DNA extraction	1.04 ± 0.14	0.69 ± 0.12	0.84 ± 0.14	NA
Neopterin[a] (pmol/g)	97.7	494.7[b]	285.4[b]	55
Calprotectin[a] (μg/g)	128.6	49.4[b]	53.5[b]	<50
Zonulin[a] (ng/mL)	85.2	128.1[b]	131.0[b]	61 ± 46
IgA[a] (μg/g)	2,187.6	2,235.5	2,204.7	2,000
CRP[c] (mg/L)	50.3	6	6.6	<5

[a] *Quantification in fecal supernatants.*
[b] *Significant p value <0.05, Wilcoxon test.*
[c] *Blood quantification.*
BJI, bone and joint infection; MDRB, multidrug-resistant bacteria; IgA, immunoglobulin A; CRP, C-reactive protein; NA, not applicable.

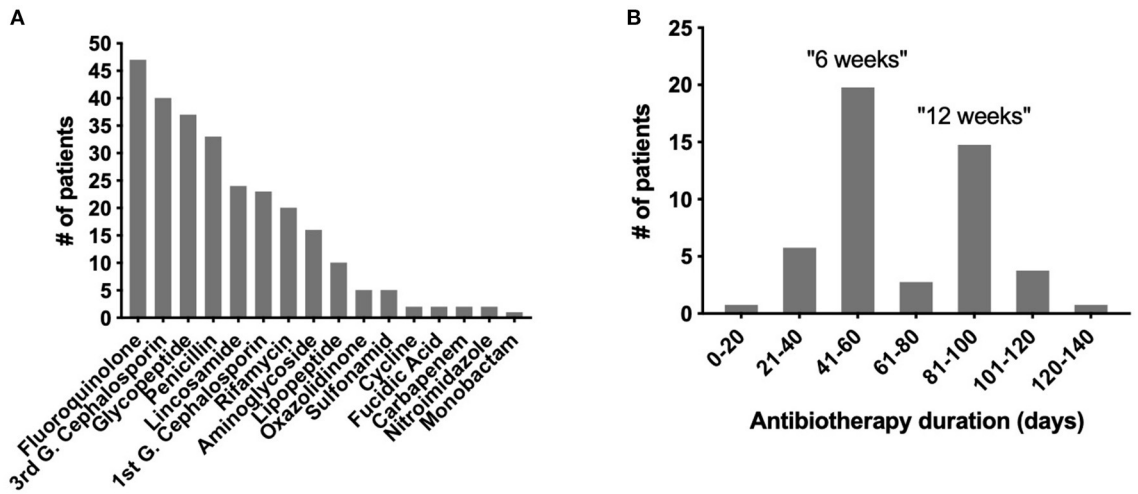

FIGURE 1 | Types of antibiotics and treatment duration in the OSIRIS cohort. **(A)** Distribution of the different types of antibiotics used. **(B)** Distribution of the antibiotic durations used.

In PJI, there was also a decrease of Firmicutes relative abundance compared to baseline (relative abundance at B: 36.1%, EOT: 28.4%, and FU: 26.5%; **Figure 2D**). Considering the whole population of BJI, the principal coordinate analysis (PCoA) of the Bray–Curtis distance showed significant changes of the gut microbiota at the end of treatment that only partially recovered at FU (**Figure 2E**). The compilation of the 20 bacteria that varied the most in relative abundance between B and EOT and B and FU for each group of patients is presented in **Supplementary Figures 2A,B**, respectively. A linear discriminant analysis effect size showing species that supported the differences between baseline and EOT is also shown **Supplementary Figure 3**. As PJI usually concern older patients for whom the microbiota may show a lower alpha-diversity, we evaluated if the age at baseline could be correlated with the Shannon index at different times of treatment. No correlation was found between age and Shannon index at B, EOT, and FU nor between body mass index and Shannon index (**Supplementary Figure 4**).

No Difference of Gut Microbiota Diversity and Recovery After Antibiotic Withdrawal Between 6 and 12 Weeks of Treatment

Taxonomic analysis showed that the microbiota composition at FU did not differ significantly at the phylum level when comparing patients treated for 6 and 12 weeks with antibiotics (**Figure 2D**). Considering alpha-diversity, there was no significant difference between 6 and 12 weeks at EOT and FU (**Figures 3A,B**). Moreover, the Shannon index at EOT and FU did not correlate with antibiotic duration (**Figure 3C**). Accordingly, the PCoA of the Bray–Curtis distance showed that there was no difference in terms of recovery when comparing FU to baseline, whether antibiotic withdrawal occurred after 6 or 12 weeks of treatment (**Figures 3D,E**). However, analysis at the species level showed actual differences underlying potentially distinct pathophysiological functions (**Supplementary Figure 2**). Of note is the fact that no difference was found when comparing the composition of the gut

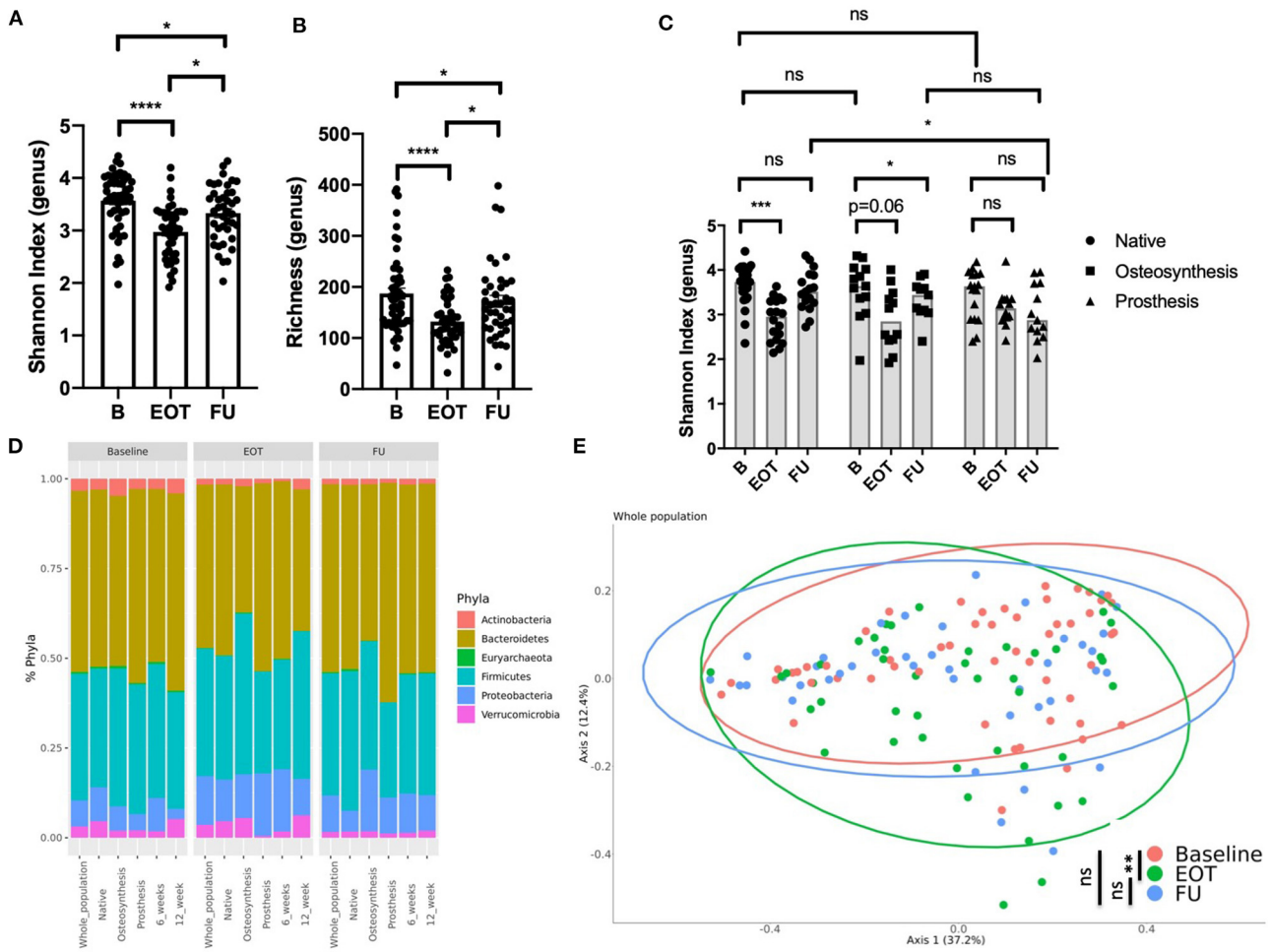

FIGURE 2 | Effect of antibiotics on the gut microbiota in bone joint infection (BJI). **(A)** Shannon index (genus level) at three times of sampling; Wilcoxon test. **(B)** Richness (genus level) at three times of sampling; Wilcoxon test. **(C)** Shannon index at three times of sampling according to the type of BJI; Wilcoxon test for paired comparison, Mann–Whitney analysis for inter-group comparisons. **(D)** Global composition of bacterial microbiota at the phylum level. **(E)** Beta diversity. Principal coordinate analysis of Bray–Curtis distance at three different times of sampling; permutational multivariate analysis of variance. B, baseline; EOT, end of treatment; FU, follow-up. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

microbiota for 6 and 12 weeks of treatment at any timepoint (data not shown).

Treatment by Fluoroquinolone Was Associated With Significant Changes of Gut Microbiota Composition That Rapidly Recover After Antibiotic Withdrawal

The use of FQ was associated with a lower richness and Shannon index at the end of antibiotic treatment (**Figures 4A,B**). However, diversity rapidly recovered after FQ withdrawal, suggesting a high but transient impact of FQ on gut microbiota. Accordingly, the PCoA of the Bray–Curtis distance showed significant changes of the gut microbiota composition at the end of treatment in the FQ-treated group that recovered at FU, whereas no difference was found between baseline, EOT, and FU for patients that did not receive FQ (**Figures 4C,D**).

Fecal Markers of Mucosal Inflammation Were Increased at the End of Treatment and Correlated With Microbiota Alpha-Diversity

Fecal neopterin and fecal calprotectin, two markers of mucosal inflammation, were significantly increased at the end of treatment, with sustained changes that persisted at 2 weeks after antibiotic withdrawal (**Figures 5A,B**). However, only fecal neopterin reached a clinically relevant range of variation (≥200 pmol/g). Fecal zonulin also showed a significant increase at the end of treatment that persisted after 2 weeks, suggesting an increased intestinal permeability that lasted after exposure to antibiotics (**Figure 5C**). In line with a possible modification of mucosal immunity, fecal immunoglobulin A was also significantly modified at FU (**Figure 5D**). Strikingly, the level of fecal neopterin negatively correlated with the Shannon index

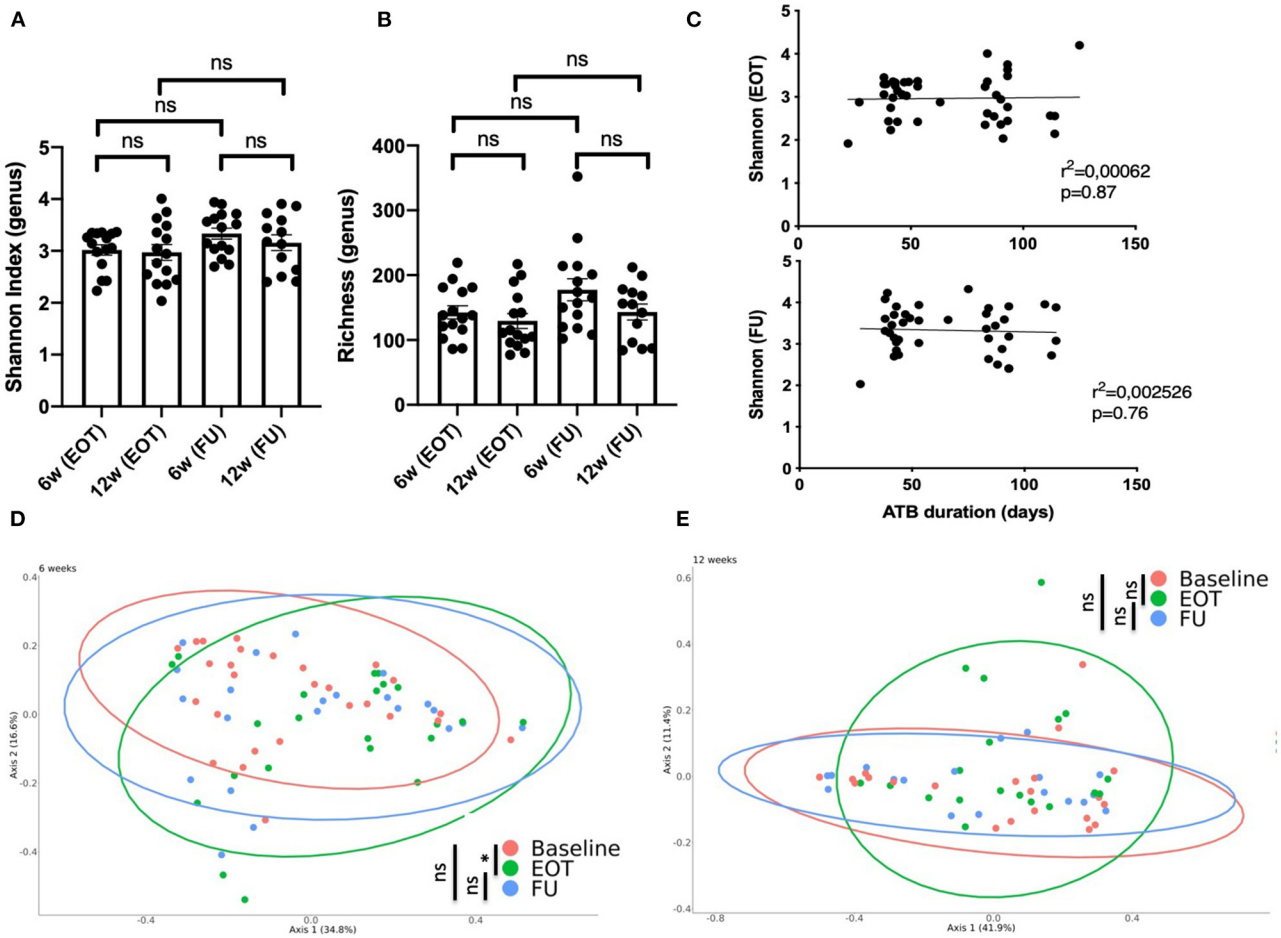

FIGURE 3 | Impact of antibiotic duration on the gut microbiota composition. **(A)** Shannon index distribution (genus level) at the end of treatment and at follow-up (15 days after antibiotic withdrawal) according to antibiotic duration; Wilcoxon test for paired comparison and, Mann-Whitney test for inter-group comparisons. **(B)** Richness distribution (genus level) at the end of treatment and at follow-up (15 days after antibiotic withdrawal) according to antibiotic duration; Wilcoxon test for paired comparison and, Mann-Whitney test for inter-group comparisons. **(C)** Correlation between the Shannon index and antibiotic duration at EOT and FU; simple linear regression. Principal coordinate analysis of Bray–Curtis distance at three different times of sampling for **(D)** patients treated for 6 weeks with antibiotics (41 to 60 days) or **(E)** 12 weeks with antibiotics (81 to 100 days); permutational multivariate analysis of variance. B, baseline; EOT, end of treatment; FU, follow-up. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

all along the follow-up (**Figure 5E**, $r^2 = 0.17$; $p < 0.0001$). The evolution of fecal markers in each subpopulation of BJI is detailed in **Supplementary Figure 5**.

Systemic CRP Decreased With Antimicrobial Treatment, but Elevated CRP at EOT Correlated With Fecal Neopterin and Was Associated With a Distinct Gut Microbiota Composition

Systemic CRP significantly decreased from B to ETO with antimicrobial treatment and from B to FU (**Figure 6A**). As we hypothesized that residual systemic inflammation evaluated by CRP could be associated with microbiota alterations, we assessed the correlation between CRP and fecal neopterin, the markers of inflammation that varied the most at EOT. Of note is the fact that 28 patients had CRP ≥5 mg/L at EOT despite a favorable outcome of the BJI. We found that CRP at EOT correlated with fecal neopterin, and this suggests that residual systemic inflammation could be associated with gut inflammation rather than with a relapse of the BJI (**Figure 6B**). Moreover, the PCoA analysis of the Bray–Curtis distance showed that patients with elevated CRP at EOT presented a distinct gut microbial composition (PERMANOVA, $p = 0.034$) with an increase in *Fusobacterium* species (**Figure 6C**, **Supplementary Figure 6**).

MDRB Fecal Carriage in the Fecal Microbiota of Patients Can Appear After Antibiotic Withdrawal

Among the BJI population, nine patients were positive for MDRB fecal carriage at baseline (9 ESBL, 0 MRSA, 0 VRE; **Figure 7A**, **Table 1**). Among the 35 patients negative for MDRB at baseline and who performed MDRD screening at EOT, acquisition of ESBL was detected for six patients (6/35; 17.1%).

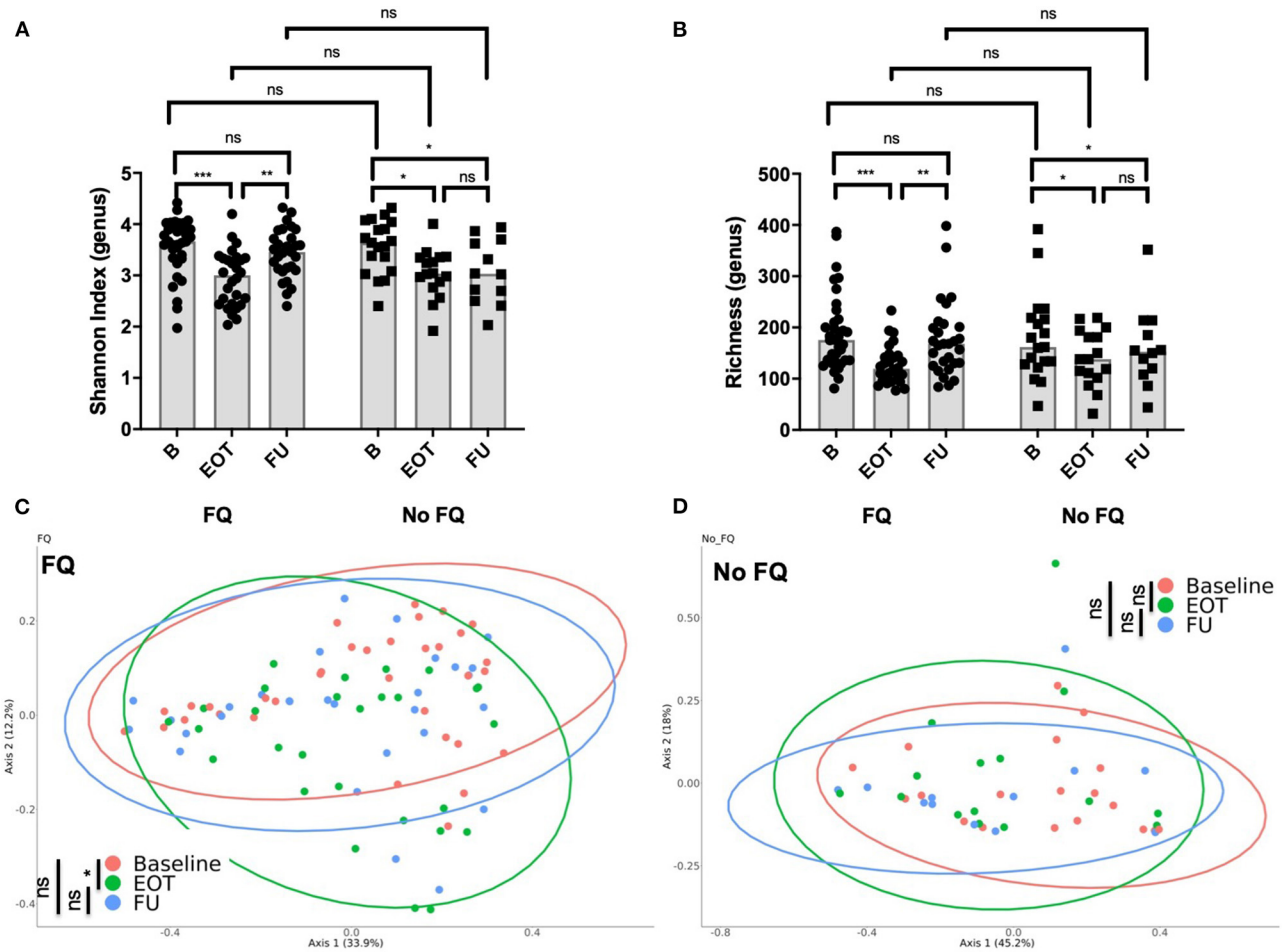

FIGURE 4 | Impact of the use of fluoroquinolone (FQ) on gut microbiota composition. **(A)** Shannon index distribution (genus level) at the end of treatment and at follow-up (15 days after antibiotic withdrawal) according to exposition to FQ; Wilcoxon test for paired comparison and, Mann-Whitney test for inter-group comparisons. **(B)** Richness (genus level) at the end of treatment and at follow-up (15 days after antibiotic withdrawal) according to exposition to FQ; Wilcoxon test for paired comparison and, Mann-Whitney test for inter-group comparisons. Principal coordinate analysis of Bray–Curtis distance at three different times of sampling for **(C)** patients treated with FQ or **(D)** without FQ; permutational multivariate analysis of variance. B, baseline; EOT, end of treatment; FU, follow-up. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Additional acquisition of MDRB at FU was observed for five patients (5/35, 14%: three ESBL, one carbapenem-resistant *Enterobacter*, and one rectal MRSA). Concerning *C. difficile*, one patient had an asymptomatic carriage at baseline and two other patients acquired C. *difficile* during the study (2/35, 5.7%). Overall, MDRB and *C. difficile* acquisition at EOT and FU represented 20% (7/35) and 37.1% (13/35) of all MDRB/*C. difficile*-free patients at the beginning of the study, respectively (**Figure 7A**). Of interest is that the quantification of MDRB-positive samples at FU in comparison to baseline clearly indicated an increased proportion of ESBL bacteria among fecal gram-negative bacteria (**Figure 7B**). Interestingly, among the nine patients with an ESBL carriage at baseline, five were still positive at EOT (5/9, 55.6%) and six at FU (6/9, 66.7%). MDRB carriage was not associated with differences in term of resilience (Bray–Curtis index between B and FU; **Figure 7C**) nor with different levels of fecal neopterin (**Figure 7D**).

DISCUSSION

This study explores, for the first time, how prolonged antibacterial therapy can disrupt the gut microbiota composition in the context of BJI. First, our data show that antibiotic treatment induced a significant loss of microbiota diversity that rapidly recovered at 2 weeks after the end of treatment for native and PJI but not for osteosynthesis-related BJI. These modifications were associated with distinct variations of bacterial phyla, in particular, with an increase of Proteobacteria and Bacteroidetes that did not fully recover at 2 weeks after antibiotic withdrawal. Second, comparing 6 to 12 weeks of antibiotic treatment did not show a major impact of treatment duration on the gut microbiota composition at the genus level and on microbiota diversity or resilience after treatment. In contrast, FQ was associated with a greater impact on microbiota diversity compared to other antibiotics, with a high resilience at FU. Third, fecal markers of inflammation were increased after antibiotic treatment, with a

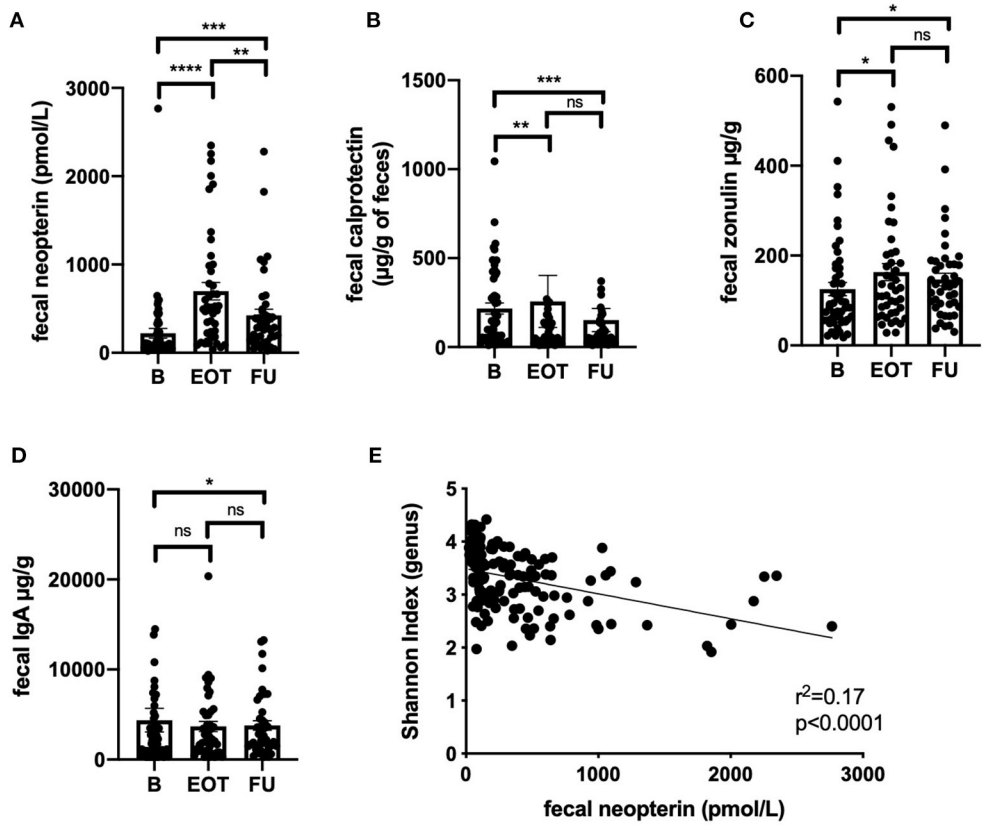

FIGURE 5 | Correlation between markers of gut inflammation, permeability, and microbiota alpha-diversity. Values of fecal neopterin **(A)**, fecal calprotectin **(B)**, fecal zonulin **(C)**, fecal immunoglobulin A **(D)** at different time points; Wilcoxon test. **(E)** Correlation between fecal neopterin and the Shannon index (genus level) all along the study; simple linear regression. B, baseline; EOT, end of treatment; FU, follow-up. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

correlation between fecal neopterin and both microbial alpha-diversity and serum level of CRP. Moreover, patients with an elevated CRP presented a distinct gut microbial composition when compared to the others. This suggests that modifications of the gut microbiota by antibiotics could be associated with low-grade mucosal inflammation and residual systemic inflammation that possibly persisted at least 2 weeks after antibiotic withdrawal. Finally, as expected, antibiotic treatment was associated with MDRB acquisition and, particularly, ESBL emergence.

The potential effects of antibiotics on gut microbiota communities have been described for various treatments but only on small cohorts and rarely after prolonged antibiotic treatments (39). As observed in our work, loss of bacterial diversity was commonly reported particularly for molecules that target anaerobes with possible long-lasting effects even after a short course of antibiotic exposure (40). Indeed Jernberg and colleagues reported significant durable changes in *Bacteroides* clonal diversity up to 2 years after 7 days of clindamycin treatment (25), whereas others reported limited changes at 4 weeks after a short-term ciprofloxacin treatment (41).

Moreover, the effect of antibiotics on bacterial communities varies between individuals. For example, repetitive ciprofloxacin exposure amplifies microbial changes but only for some subjects (26). Thus, pre-treatment microbial diversity may account for differences in microbial communities' resilience and long-term effects of antibiotics. This initial dysbiosis/eubiosis state at the beginning of treatment may account for the rapid recovery of the gut microbial diversity of patients with native BJI compared to others. Indeed osteosynthesis-related BJI and PJI involved patients with complex infections and often previous antibiotic exposures. Even if the overall gut microbiota diversity can recover after treatment, definitive loss of some bacterial strains persists over time (21). Indeed, in our data, alpha-diversity seemed to be almost back to initial levels at 2 weeks after the end of treatment, but permanent changes in the abundance of specific species remained, of which the pathophysiological consequences remain unknown. However, functional redundancy supported by different bacterial species may counteract the possible effects of these permanent changes in microbiota composition (42).

When comparing the 6- and 12-week groups by PCoA analysis of the Bray–Curtis distance or when considering the correlation between antibiotic duration and alpha-diversity, the duration of treatment did not seem to affect the overall microbiota diversity or the resilience of the gut microbiota after antibiotic withdrawal. One possible explanation could be that 6 weeks of treatment is a sufficient amount of time to reach a microbial steady state that may persist with only small variations if antibiotics are prolonged. Indeed doses of antibiotics in BJI are, most of the

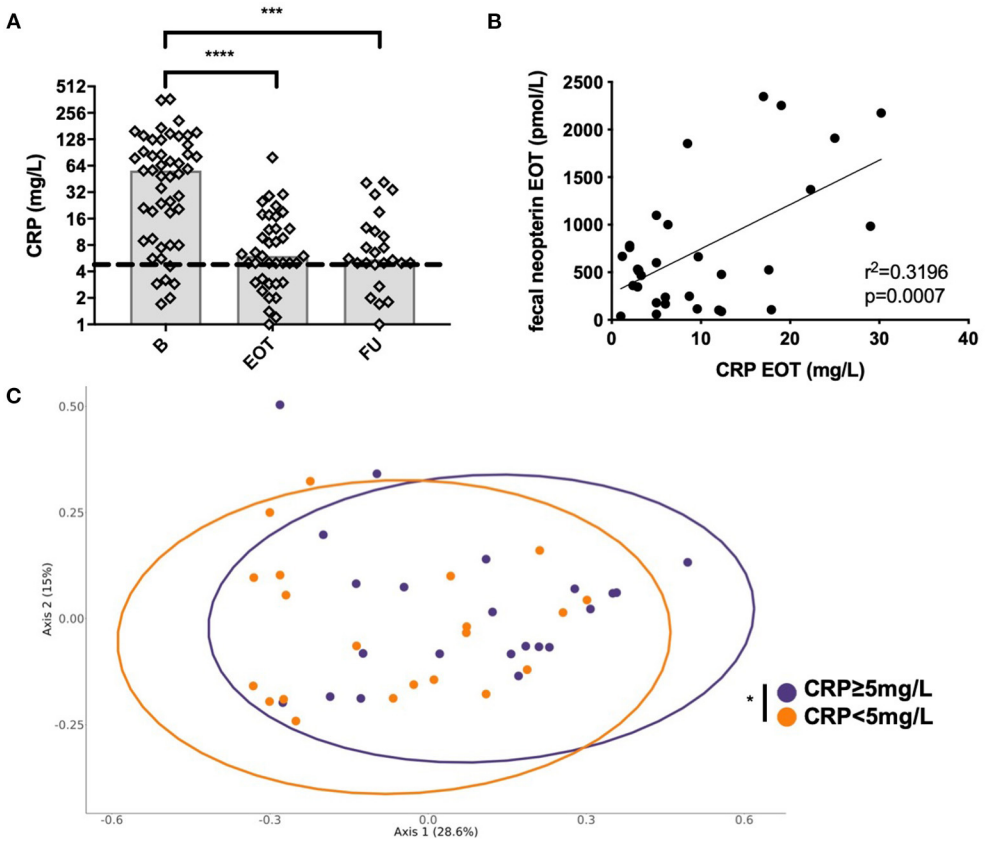

FIGURE 6 | Correlation between systemic and mucosal markers of inflammation. **(A)** Evolution of the plasmatic level of C-reactive protein (CRP) at different time points. **(B)** Correlation between fecal neopterin and plasmatic CRP; simple linear regression. **(C)** Principal coordinate analysis of Bray–Curtis distance between patients with each sample colored according to plasmatic CRP level at the end of treatment; permutational multivariate analysis of variance. B, baseline; EOT, end of treatment; FU, follow-up. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

time, high because of the limited diffusion into the bones of the majority of antibacterial therapies. This combination of high dose and prolonged exposure may favor a rather rapid achievement of a steady state of the gut microbiome that could be stable over time as the treatment is continued.

As described in numerous works, antibiotic exposure was associated with the selection of MDRB in our population. Notably in our cohort, the proportion of patients positive for MDRB or *C. difficile* reached almost 40% at FU. Even if MDRB decolonization is known to occur spontaneously after antibiotic withdrawal (43), increasing the amount of data suggests that genes for antibiotic resistance can persist for a long time once selected (44), which suggests a long-lasting effect of antibiotic treatement for patients with BJI.

One other striking result of this study is the identification of a correlation between markers of gut inflammation such as fecal neopterin and microbiota diversity. In rats, antibiotic exposure has already been associated, as we observed, with an increase of gut permeability and increased plasma levels of haptoglobin, a precursor of zonulin (45). Interestingly, the authors reported similar modifications of the gut microbiota with an increase of Proteobacteria and a general decrease of microbiota diversity. In the same line, Feng et al. (27) reported similar modifications of gut permeability after antibiotic treatment in mice, associated with the activation of the NLRP3 inflammasome and autophagy.

We also found that residual systemic inflammation evaluated by CRP correlated with fecal neopterin and, in consequence, with the potential persistence of microbiota alterations at the end of antibiotic treatment. It is of importance, as CRP is usually monitored to evaluate the BJI's response to antibiotics. Indeed some physicians consider that if CRP is still elevated at the end of treatment, it could be due to bacterial persistence at the site of bone infection, leading to consideration of prolongation of the antibiotic treatment. However, data indicate that the CRP level at the end of treatment is not predictive of a persistent infection (46, 47). Thus, our results raise the hypothesis that abnormal CRP at the end of the treatment could be a potential marker of gut barrier dysfunction associated with microbial dysbiosis. Further data are required to confirm this hypothesis.

Antibiotic impact on the gut microbiota has potential long-term effects which suggest several measures to correct or prevent these changes. The best way would be to minimize the use of antibiotics, preferentially by using *in situ* antibiotics or using antibiotics with a narrow spectrum to limit the impact on

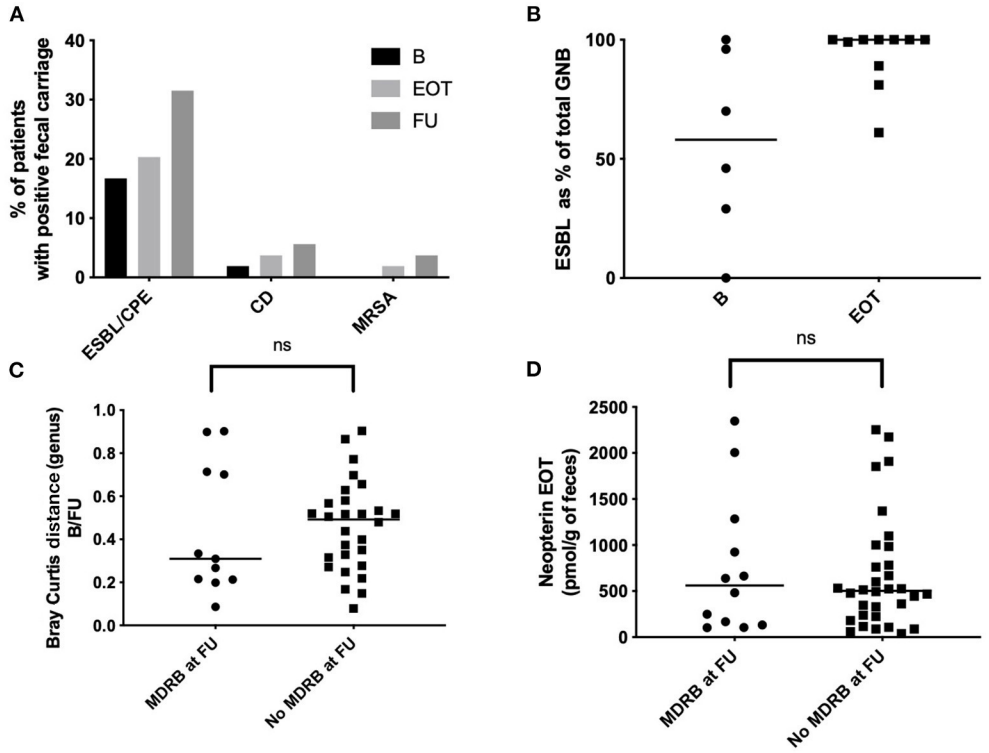

FIGURE 7 | Fecal multi-drug-resistant bacteria (MDRB) and *Clostridioides difficile* carriage. **(A)** Proportion of patients with a positive fecal carriage (culture) at different time points. **(B)** EBSL as percentage of the total of all Gram-negative bacteria (aerobic culture). **(C)** Bray Curtis distance between baseline and 15 days after antibiotic withdrawal (FU) according to the carriage of MDRB; Mann–Whitney test. **(D)** Fecal neopterin at baseline and 15 days after antibiotic withdrawal (FU) according to the carriage of MDRB; Mann–Whitney test. B, baseline; EOT, end of treatment; FU, follow-up; ESBL, extended-spectrum beta-lactamases; MRSA, methicillin-resistant *Staphylococcus aureus*. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

the gut microbiota. However, in many infections such as BJI, antibiotics cannot be replaced, and long-term systemic treatment at high dosage is mandatory to cure patients. In these situations, microbe-based therapy to counteract the deleterious ecological effects of such treatments could be of interest, especially in selected populations at risk of non-recovery (elderly, personal medical history of *C. difficile* infection, low microbiota diversity, possibly osteosynthesis-related BJI, etc.). When specifically targeting the gut microbiota, different tools are commonly used. Either selected microbes (bacteria and/or fungi) can be added as probiotics or specific molecules (prebiotics) can be used to promote specific species of interest, but as the gut microbiota is a complex ecosystem, such tools may miss significant network interactions at the level of bacterial species or between different kingdoms (bacteriophages and fungi, for example).

Moreover, there are some concerns about probiotics, as they are composed of only a few bacterial species, and their capacity to positively impact antibiotic-associated dysbiosis is debatable.

Fecal microbiota transplantation is nowadays the only treatment that permits the engraftment of a complex ecosystem with proven functional benefits. It consists of the transfer of the fecal microbial ecosystem of a healthy donor to a recipient in order to restore gut homeostasis. Evaluation of fecal microbiota transplantation in various pathological conditions is now blooming, with contrasting results extending the need to validate the administration modality and long-term safety (48).

Our study has some limitations. First, the relatively low number of evaluated patients may account for a lack of power especially in subgroup analysis. Moreover, some patients did not perform stool sampling at all time points, which may also have induced some bias. However, it is, to our knowledge, one of the largest clinical studies evaluating the effects of prolonged antimicrobial therapy on the gut microbiota. The use of rectal swabs may facilitate recruitment and increase patient adherence to develop larger studies. Furthermore, evaluation of microbial composition and inflammatory markers at a more distant time point after antibiotic withdrawal would be of great interest to assess the long-term impact of antibiotics on the gut ecosystem and mucosal physiology.

In conclusion, to our knowledge, this is the first study that explores the impact of prolonged antibiotic treatment on gut microbiota in the context of BJI. As expected, antibiotics significantly altered the gut microbiota diversity and composition, with a rapid but partial recovery observed at 2 weeks after antibiotic withdrawal. Antibiotic duration or the use of FQ did not seem to affect this resilience. These modifications were associated with an increase in markers of mucosal inflammation and gut permeability and elevated levels

of CRP. Further studies are needed to explore these possible links and their impact on resilience. Finally, as illustrated in our cohort, acquisition of MDRB remains one the most challenging side effects of long-term exposure to antibiotics. Innovative microbe-based therapies could be a promising tool to address these issues.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of legal reasons. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Protection des Personnes SUD-EST II. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TF designed the study with BL and CC. TF, CB, SL, CP, DB, F-AD, VZ, ES, TF, and CC managed the patients. JJ and FL generated resistances data. MM and T-TL managed the centralization and biobanking of samples. BL, NB, and TF performed the literature review and wrote the first draft of the manuscript. BL, NB, and CG performed the data analysis. All authors contributed to the improvement of the manuscript.

FUNDING

This study was funded by MaaT Pharma (http://www.maatpharma.com) as part of the Concours Mondial de l'Innovation funded by Banque Publique d'Investissement—France.

ACKNOWLEDGMENTS

OSIRIS Study group (list of collaborators): Coordinator, TF; CRIOAc Lyon: TF, CB, FL, SL, CP, A. Becker, C. Triffault-Fillit, S. Roux, NB, A. Viste, Y. Herry, P. Chaudier, JJ, C. Kolenda, A. Monteix, E. Mabrut; CRIOAc Nantes: DB, G. Bart, P. Bemer, A.S. Lecompte, J. Brochard, L. Khatchatourian, N. Asseray; CRIOAc GH Diaconesses Croix Saint Simon: VZ, S. Marmor; CRIOAc Grand Sud-Ouest: F-AD, V. Dubois, CC; CRIOAc Nord-Ouest G4, Lille-Tourcoing: ES, N. Blondiaux; study coordination, CRC and CRB Croix-Rousse Hospital: MM, C. Brochier, I. Delfour, S. Thevenon, T-TL; and HCL Biochemistry—laboratory which generated the fecal supernatant protein quantification: A. Mialon, C. Cuerq; MaaT Pharma: BL, CG, Corentin Le Camus, Lilia Boucinha. INRAE Jouy en Josas: JD.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.586875/full#supplementary-material

Supplementary Figure 1 | Richness evaluated at each time of sampling according to the type of bone joint infection. Wilcoxon test for paired comparison and Mann–Whitney analysis for inter-group comparisons were used. B, baseline; EOT, end of treatment; FU, follow-up.

Supplementary Figure 2 | Bacterial species that vary the most after antibiotic treatment. Variations correspond to the ratio of relative abundance between **(A)** baseline and the end of treatment and **(B)** between baseline and follow-up (15 days after antibiotic withdrawal). Descriptions of the 20 bacteria that varied the most in relative abundance between B and EOT and B and FU for each group of patients are presented. B, baseline; EOT, end of treatment; FU, follow-up.

Supplementary Figure 3 | Linear discriminant analysis effect size showing species that support differences between baseline and end of treatment.

Supplementary Figure 4 | Correlation between the Shannon index and clinical parameters. Correlation between the age at baseline and the Shannon index at baseline **(A)**, end of treatment **(B)**, and 15 days after antibiotic withdrawal **(C)**. Correlation between the body mass index at baseline and the Shannon index at baseline **(D)**, end of treatment **(E)**, and 15 days after antibiotic withdrawal **(F)**. *r*, Pearson correlation coefficient; B, baseline; EOT, end of treatment; FU, follow-up.

Supplementary Figure 5 | Correlation between markers of gut inflammation, permeability, and microbiota alpha-diversity according to the type of bone joint infection. Values of fecal neopterin **(A)**, fecal calprotectin **(B)**, fecal zonulin **(C)**, and fecal immunoglobulin A **(D)** at different time points; Wilcoxon test. B, baseline; EOT, end of treatment; FU, follow-up.

Supplementary Figure 6 | Linear discriminant analysis effect size showing species that support differences between patients with an elevated C-reactive protein (≥5 mg/L) at the end of treatment and others.

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Conflict of Interest: BL and CG are employed by the commercial company MaaT Pharma. LB was employed by the commercial company MaaT Pharma. NB declared a travel grant from Maat. JD is co-founder of MaaT Pharma. TF received advisory honorarium from MaaT Pharma and was the principal investigator of this study.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declare that this study received funding from MaaT Pharma. The funder had the following involvement in the study: design, management and analysis.

frontiers in Medicine

BRIEF RESEARCH REPORT
published: 16 March 2021
doi: 10.3389/fmed.2021.565555

Experience With the Use of the MicroDTTect Device for the Diagnosis of Low-Grade Chronic Prosthetic Joint Infections in a Routine Setting

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Infectious Diseases – Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: 25 May 2020
Accepted: 21 January 2021
Published: 16 March 2021

Citation:
Kolenda C, Josse J, Batailler C, Faure A, Monteix A, Lustig S, Ferry T, Laurent F and Dupieux C (2021) Experience With the Use of the MicroDTTect Device for the Diagnosis of Low-Grade Chronic Prosthetic Joint Infections in a Routine Setting. Front. Med. 8:565555. doi: 10.3389/fmed.2021.565555

Background: In prosthetic joint infections (PJIs), identification of the causative microorganisms is critical to successfully adapt and optimize treatment. However, microbiological diagnosis of PJIs remains a challenge notably because bacteria are embedded in biofilm adhered to the prosthetic material. Recently, dithiothreitol (DTT) treatment of prosthesis has been proposed as a new strategy to release bacteria from biofilm and to improve the yield of microbiological diagnosis. In this study, we evaluated the interest of a commercial device using DTT, the MicroDTTect system (Heraeus, Hanau, Germany), for the diagnosis of low-grade chronic PJIs, compared to the conventional culture of periprosthetic tissue (PPT) samples.

Methods: Twenty patients undergoing a surgery procedure for removal of prosthetic material because of a suspicion of low-grade PJI without pre-operative microbiological documentation were included (NCT04371068). Bacteriological results using the fluid obtained after prosthesis treatment with the MicroDTTect system were compared to results obtained with conventional culture of PPT samples.

Results: All the bacteria considered as responsible for PJIs recovered from culture of PPT samples were also detected using the MicroDTTect device. For one patient, an additional bacterial isolate (*Staphylococcus haemolyticus*) suspected to be involved in a polymicrobial PJI was identified using DTT treatment. Time to positivity of the cultures was also reduced using the MicroDTTect system, notably in case of *Cutibacterium acnes* infection. However, probable bacterial contaminants were found (MicroDTTect system, $n = 5$; PPT samples, $n = 1$).

Conclusion: This study showed that DTT treatment of the prosthetic component using the MicroDTTect device could improve the microbiological diagnosis of low-grade PJIs.

Keywords: dithiothreitol, prosthetic joint infection, bone and joint infection, microbiological diagnosis, biofilm, implant failure

INTRODUCTION

Prosthetic joint infections (PJIs) are one of the major causes of implant failure after joint arthroplasty with an average incidence of 1.1% for primary total hip replacement and 0.6% for primary total knee replacement (1). These infections are associated with high morbidity rates and health-care costs (2). The diagnosis of PJIs can be very challenging, notably in case of infection due to low-virulence organisms, as clinical features and microbiological diagnostic results may be conflicting (3). A definition of periprosthetic infection has been proposed by the MusculoSkeletal Infection Society (MSIS), including major and minor criteria (4). However, in some forms of low-grade PJI, several of these criteria may not be met despite the presence of a true infection, such as the identification of the microorganism involved after culture of periprosthetic tissue (PPT) samples, whereas it is pivotal for antimicrobial susceptibility tests and subsequently for treatment adaptation. Among others, biofilm formation, which corresponds to a complex microbial community adherent to various surfaces and protected by self-produced matrix, is a major limiting factor for culture of bacterial pathogens in the context of PJIs (5). Traditional sampling techniques may fail to detach viable biofilm-embedded bacteria from prosthetic surfaces and periprosthetic tissues, thus leading to false negative results in culture and to a possible diagnostic conclusion of aseptic failure, especially when clinical signs are confounding, as in low-grade infections or in case of recent antibiotic treatment.

To overcome these difficulties, different methods have been developed to improve the microbiological diagnosis yield in PJIs by disrupting the bacterial biofilm before culture. For instance, superiority of culture of sonication fluids obtained from explanted prosthesis over conventional culture of PPT samples has been reported in several studies (6–9). However, sonication is limited by the need of specific instrumentation, which is neither available nor affordable in all laboratories, the difficulties in managing large implants, and the risk of sample contamination, caused by improperly sealed sample containers and/or bacteria proliferation in sonication water. Recently, an alternative method for biofilm detachment using a chemical agent, namely DL-dithiothreitol (DTT), has been proposed (10). DTT is a sulfhydryl compound that reduces disulfide bonds and destroys intra- and inter-molecular bonds between cysteine residues in proteins. It can alter the extracellular matrix of biofilm and release bacteria from it, without affecting bacterial viability allowing further bacterial growth before identification and antibiotic susceptibility testing with traditional methods (11). Drago et al. notably showed that DTT treatment could be a reliable alternative to sonication for the microbiological diagnosis of PJIs because it is easier to use, as the procedure does not require any specific laboratory instruments and it was associated with a better sensitivity and the same specificity compared to sonication (12).

A commercial device containing a DTT solution has been developed, namely MicroDTTect (4i for infection, Monza, Italy), to simplify the process, reduce the multiple transfers and steps with technical manipulations, and thus to limit risks of contamination. To evaluate the added value of this approach, we focused our study to patients presenting with a suspicion of low-grade chronic PJI, clinical context in which the MicroDTTect system could be particularly of interest compared to conventional culture of PPT samples.

MATERIALS AND METHODS

Patient Recruitment

Between February 2018 and August 2019, a total of 20 patients undergoing a surgery procedure for prosthetic material removal in the surgery department of Croix Rousse hospital (Lyon University Hospital, CRIOAc Lyon, France) because of painful prosthesis, prosthesis loosening happening <10 years after the implantation, or suspicion of chronic PJI with negative joint puncture or with no joint puncture performed in the previous 3 months, were enrolled in this study. Exclusion criteria included mechanical explanation for the pain or loosening, and clinical evidence of infection (fistula, abscess, discharge, or local inflammation). The study group included 12 men and 8 women, undergoing surgery for total knee ($n = 15$) or hip ($n = 5$) prosthesis replacement (one- or two-stage).

Samples Collection

Prosthetic implants were aseptically collected in the operating room and immediately placed in the MicroDTTect collection system (**Figure 1**). In parallel, PPT samples (between 5 and 7 per patient) were also collected according to the usual protocols of the hospital and put into plastic sterile containers Ultra-Turrax® (Labelians, Nemours, France) containing metallic beads, immediately sealed and transported to the laboratory for standard microbiological analysis.

Microbiological Procedures

The MicroDTTect procedure was performed according to the manufacturer's recommendations. The MicroDTTect device containing the prosthesis was placed on a mechanical shaker for 15 min to increase contact between DTT and prosthesis, in order to detach bacteria and biofilm from the material surface. The obtained DTT suspension was transferred into dedicated test tubes. Three BACT/ALERT® aerobic FA Plus, anaerobic FN Plus, pediatric PF plus blood culture bottles (bioMérieux) were inoculated with 5 mL of this suspension. The tubes were then centrifuged at 3,200 rpm for 10 min and then, all the supernatant except 1 mL was discarded. After resuspension of the pellet in the remaining DTT solution, 100 µL were plated onto: one sheep blood agar plate (bioMérieux, Marcy l'Etoile, France) incubated during 2 days in aerobic atmosphere, two chocolate blood agar plates (bioMérieux) incubated for 2 and 5 days under 5% CO_2, and two Schaedler agar plates (bioMérieux) incubated for 5 and 14 days in anaerobic conditions. In addition, one Schaedler broth was also inoculated with 100 µL of the resuspended pellet. Bacterial growth was followed automatically by a BACT/ALERT® VIRTUO® system (bioMérieux) for the bottles and every day visually for the broth during 14 days. If positive, broth or bottles were subcultured onto one sheep blood agar, one chocolate agar, and one Schaedler agar plates incubated for 2 days in CO_2 and anaerobic condition, respectively.

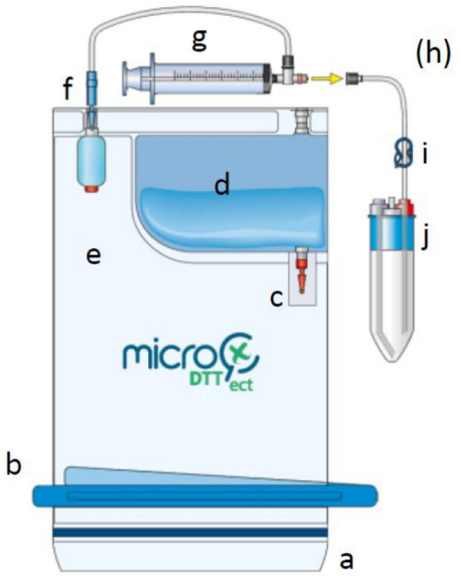

FIGURE 1 | Drawing showing the different components of the MicroDTTect device. The MicroDTT system is composed of: (a) closing system with minigrip, (b) clamp for the second closure, (c) unbreakable red valve, (d) dithiothreitol solution at 0.1% (w/v), (e) PVC two-compartment bag, (f) unbreakable blue valve, (g) syringe, (h) injection point with needle (optional, for direct inoculation into blood culture bottles), (i) blue clip, and (j) liquid collection test tube(s). Prosthetic implant was aseptically collected in the operating room and immediately placed in the MicroDTTect device (compartment e). After mechanical agitation, the DTT suspension was transferred into several sterile tubes (j). This figure is published with the permission of Heraeus.

In parallel, PPT samples were homogenized using a specific grinder Ultra-Turrax® Tube Drive Ika® (Labelians) during 1 min followed by plating of 20 µL on the same solid agar media as described above and inoculation of 100 µL into a Schaedler liquid broth.

When cultures were positive, each morphology of colony was identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry using the VITEK® MS device (bioMérieux). Results obtained with both approaches (MicroDTTect device and PPT samples) were compared in terms of number and types of bacterial species, time to positivity, and semi-quantitative analysis of the bacterial inoculum in the samples (number of colonies on plates).

Ethics

For this observational study, which did not require the approval of an ethics committee, written information was given to all patients. The study was registered as a clinical trial (no. NCT04371068) and was approved by the National Data Protection Commission (no. 18–265), as required by national ethics rules.

RESULTS

Bacteriological growth was observed for 11 out of 20 (55%) prosthetic samples treated using the MicroDTTect system, while PPT samples were positive in culture for only 8 out of 20 patients (40%) (**Table 1**). Isolated bacteria were mostly Gram-positive cocci: coagulase-negative staphylococci (n=10 strains/8 patients using MicroDTTect; $n = 4$ strains/4 patients in PPT samples) and *Enterococcus faecalis* (1 strain in both DTT and PPT samples). *Cutibacterium acnes* isolates were also recovered for two patients with both techniques, and one additional isolate was found in one PPT sample.

Agreement between the results obtained with MicroDTTect prosthesis processing vs. conventional culture of PPT samples was observed for 13 out of 20 patients (65%) (same bacteria identified, $n = 5$; negative culture, $n = 8$). For seven out of eight patients with culture-positive PPT sample(s), bacteria recovered from PPT samples were also detected using the MicroDTTect procedure. However, for two patients, if one species was recovered using both approaches (*Staphylococcus epidermidis* and *C. acnes,* respectively), an additional species was collected only using the MicroDTTect procedure: for patient n°10, numerous colonies of *S. haemolyticus* grew on all culture media using the MicroDTTect procedure and was considered as a likely pathogen involved in a polymicrobial infection, undetected with the conventional culture of PPT samples; for patient n°12, *S. epidermidis* was recovered on only one out of the nine inoculated media (five colonies). For patient n°16, a single colony of *C. acnes* was found for only one out of five PPT samples, while the MicroDTTect culture was negative and was considered as a contaminant by clinicians. For four patients (n°11, 13, 19, and 20), likely skin flora contaminants (coagulase-negative staphylococci, *Micrococcus luteus*) were isolated after a culture of MicroDTTect suspension on one out of the nine media, while the culture of PPT samples remained negative. For these, patient records were discussed during a multidisciplinary meeting, which concluded that there was no evidence of PJI based on bacteriological and histopathological analyses of PPT samples.

When considering only likely true culture positive samples, time to positivity using the MicroDTTect system was at least equivalent to PPT culture and, in most cases (six out of seven), decreased by 24 h and up to 9 and 11 days for patients with a true *C. acnes* infection (n°9 and 12, respectively). Of note, the bacterial inoculum recovered after the culture of the prosthetic material was also higher than the one obtained with conventional culture of PPT samples (**Table 1**).

DISCUSSION

Diagnosis of PJIs remains a major challenge for microbiology laboratories. Despite the continuous development of innovative microbiological techniques, none of them is considered as a definitive gold standard, and the diagnosis of bone and joint infections is based on a combination of clinical, biological, and/or microbiological arguments. Biofilm formation during chronic PJI is considered as one of the reason of the insufficient sensitivity of classical culture approach using PPT samples, especially when patients received antimicrobial chemotherapy before sampling (7). In order to improve the accuracy of microbiological diagnostic methods, specific approaches allowing detachment of

TABLE 1 | Comparison of microbiological results obtained by culture of the prosthetic material using the MicroDTTect device vs. classical culture of periprosthetic tissue samples.

Patient	Type of surgery	Routine lab results				MicroDTTect results				Final diagnosis of infection (Yes/No)
		Bacterial species	No. of pos samples	Time to positivity	No. of col/plate	Bacterial species	No. of pos media	Time to positivity	No. of col/plate	
1	2-stage TKP replacement	*S. caprae*	5/5	24 h	EBO-50 CFU	*S. caprae*	9/9	24 h	50 CFU	Y
2	2-stage TKP replacement	–	0/5	–	–	–	–	–	–	N
3	1-stage TKP replacement	–	0/6	–	–	–	–	–	–	N
4	1-stage THP replacement	–	0/5	–	–	–	–	–	–	N
5	1-stage TKP replacement	–	0/5	–	–	–	–	–	–	N
6	2-stage THP replacement	*S. epidermidis*	2/5	48 h	1 CFU	*S. epidermidis*	9/9	24 h	100 CFU	Y
7	2-stage TKP replacement	*S. epidermidis*	5/5	48 h	2–10 CFU	*S. epidermidis*	9/9	24 h	25–100 CFU	Y
8	2-stage THP replacement	*E. faecalis*	4/5	48 h	EBO-4 CFU	*E. faecalis*	9/9	24 h	100–200 CFU	Y
9	2-stage THP replacement	*C. acnes*	2/5	14 days	10 CFU	*C. acnes*	4/9	5 days	100 CFU	Y
10	2-stage TKP replacement	*S. epidermidis*	4/5	48 h	EBO-10 CFU	*S. epidermidis* *S. haemolyticus*	9/9 9/9	24 h 24 h	200–300 CFU 200–300 CFU	Y
11	1-stage TKP replacement	–	0/5	–	–	*S. epidermidis* *S. pettenkoferi*	1/9 1/9	5 days 5 days	10 CFU 10 CFU	N
12	1-stage THP replacement	*C. acnes*	4/5	14 days	1–10 CFU	*S. epidermidis* *C. acnes*	1/9 7/9	48 h 3 days	5 CFU 200–500 CFU	Y
13	1-stage TKP replacement	–	0/7	–	–	*S. epidermidis*	1/9	7 days	50 CFU	N
14	1-stage TKP replacement	–	0/5	–	–	–	–	–	–	N
15	1-stage TKP replacement	–	0/5	–	–	–	–	–	–	N
16	1-stage TKP replacement	*C. acnes*	1/5	14 days	1 CFU	–	–	–	–	N
17	1-stage TKP replacement	–	0/5	–	–	–	–	–	–	N
18	1-stage TKP replacement	–	0/5	–	–	–	–	–	–	N
19	1-stage TKP replacement	–	0/5	–	–	*S. haemolyticus*	1/9	7 days	5 CFU	N
20	1-stage TKP replacement	–	0/5	–	–	*M. luteus*	1/9	30 h	EBO	N

Pos, positive; col, colonies; CFU, colony-forming unit; EBO, enrichment broth only.

bacteria from biofilm formed and stuck on prosthetic implants have been developed in the last decade including mechanical (low-frequency ultrasounds) and chemical treatment (DTT). In this study, we evaluated a commercial standardized system using DTT, the MicroDTTect device for the diagnosis of low-grade PJIs, in comparison with conventional culture of PPT samples. The data obtained showed that this device could be a valuable tool for the diagnosis of such infections and to improve the yield of microbiological diagnosis. All bacteria responsible of true PJI (same bacteria isolated at least in two different PPT samples from the same patient) were detected using culture of the prosthesis placed in the MicroDTTect device. Moreover, for one patient, the use of MicroDTTect allowed us to detect a likely polymicrobial infection due to *S. epidermidis* and *S. haemolyticus*, while only *S. epidermidis* was isolated with conventional culture of PPT samples. The results also showed that this device could be of interest to reduce significantly the time to positivity of culture and thus accelerate the microbiological diagnosis and optimize the management of patients, notably in case of infection by low-virulent bacteria, such as *C. acnes*.

A few other studies highlighted that treatment with DTT could be of interest for the diagnosis of PJIs (13–15). Calori et al. reported a higher sensitivity using the same MicroDTTect device for collection of both prosthesis and PPT samples compared to conventional cultures (15). However, the comparative method used consisted in the collection of flocked swabs during the surgery, specimens that are not recommended by the various international guidelines for the diagnosis of PJIs due to lack of sensitivity (16, 17). This could have introduced a major bias in the results. Sambri et al. compared DTT and sonication treatment of prosthetic material in a large cohort of patients undergoing prosthesis revision and showed that both technics were more sensitive than PPT sample cultures (13). Moreover, DTT treatment was superior to sonication among patients in whom infection was not suspected preoperatively, whereas the authors did not observe any difference between DTT and sonication in patients with suspicion of infection before surgery. Finally, in the study of De Vecchi et al., treatment of PPT samples with DTT allowed an increase in sensitivity (14). However, homogenization of samples not treated with DTT was simply performed in sterile saline, without grinding them using beads, which is well-known to improve the microbiological diagnosis of PJI (18).

Contrary to the studies previously mentioned, we chose to evaluate the interest of the MicroDTTect device in a specific subgroup of patients with PJI, namely low-grade PJI, and so excluding patients for whom classical approach for microbiological diagnosis, that is, culture of PPTs, is likely enough sensitive. So, we decided not to include patients if the diagnosis of PJI was certain or strongly suspected, for example, if they presented a fistula communicating with the prosthesis or if a microbiological evidence of infection was available before the surgery. Indeed, we believe that this kind of diagnostic technologies, which trigger additional costs to the classical technics used for PJI diagnosis, should be dedicated to patients for whom the diagnosis is the most difficult and for whom supplementary technics could improve the sensitivity of conventional PPT samples culture. This point of view is also supported by the data of Sambri et al. showing that DTT treatment of prosthetic material was especially relevant when PJI was not suspected preoperatively, that is to say for patients with low-grade infections, presenting no clinical and biological signs of infection. These restrictive criteria account for the limited number of patients included, which is a limitation of the present study. The strict selection of patients also probably explains the absence in our study of some bacterial species frequently involved in PJIs, such as *Staphylococcus aureus* and Gram-negative bacilli. We mainly recovered coagulase-negative staphylococci and *C. acnes* isolates, frequently involved in delayed chronic and low-grade PJIs (19).

The major advantages of the MicroDTTect system are that it is quick and easy to use. It is also a closed system providing a completely sterile transportation of the prosthesis and theoretically reducing the risk of sample contamination as minimal manipulations are required. However, cultures of the MicroDTTect samples of five patients in the present study showed growth of one or two skin commensal coagulase-negative staphylococci, but recovered on only one out of nine seeded media, while culture of PPT samples remained negative. These isolates were considered as likely contaminants. However, it is impossible to know if these contaminations have been "acquired" during manipulation of the prosthesis or during plating. Other studies reported good specificity rates, comparable to those of conventional culture, but some of the authors considered that samples were positive only if at least five colonies grew on agar plates after 24 h, whereas this threshold has not been validated by other studies (12, 13, 15). Finally, Romano et al. also suggested that the use of the MicroDTTect device may allow a substantial economic balance or advantage (20). Although the use of MicroDDTect induces an increase of the direct costs compared to culture of PPT samples, the authors showed that this technology was cost-effective notably thanks to the reduction of the time required for sample treatment and the improvement of diagnostic accuracy compared to tissue cultures combined or not with sonication. The present study was a non-interventional study (results not provided to the clinicians) and did not allow us to evaluate the medico-economic impact of this device. Nevertheless, the impact of such diagnostic tools on the cost of PJIs management deserves to be evaluated more deeply in further studies because the results may be highly variable from one country or hospital to another. These studies should focus on low-grade infections, infections for which the diagnosis may be difficult and for which the MicroDTTect device could represent a real gain in diagnostic sensitivity.

In conclusion, in this study, we showed that treatment of the prosthetic component with DTT using the MicroDTTect device improves the microbiological diagnosis of low-grade PJIs by allowing the identification of additional bacteria and reducing the time required to detect them. For optimal interpretation of results, only patients with several positive media should be considered as infected. The added economic value of this diagnostic device has now to be evaluated in real-life conditions.

DATA AVAILABILITY STATEMENT

All data supporting the findings of the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JJ, CD, TF, and FL supervised the project. AF and AM carried out the bacteriological analyses. CB, SL, and TF included the patients. CK analyzed the results and wrote the manuscript with support from CD and FL. All authors discussed the results and contributed to the final manuscript.

FUNDING

This work was supported by Heraeus, who provided the MicroDTTect devices for the present study but had no control or authority over the analysis, interpretation, and presentation of the results.

ACKNOWLEDGMENTS

We thank Christine Durand and Miguel Julienne for the help in coordinating the interaction between orthopedic rooms and the department of Bacteriology.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

BRIEF RESEARCH REPORT
published: 31 March 2021
doi: 10.3389/fmed.2021.583086

Pharmacokinetic/Pharmacodynamic Dosage Individualization of Suppressive Beta-Lactam Therapy Administered by Subcutaneous Route in Patients With Prosthetic Joint Infection

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Infectious Diseases – Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *14 July 2020*
Accepted: *18 February 2021*
Published: *31 March 2021*

Citation:
Goutelle S, Conrad A, Pouderoux C, Braun E, Laurent F, Gagnieu M-C, Cohen S, Guitton J, Valour F and Ferry T (2021) Pharmacokinetic/Pharmacodynamic Dosage Individualization of Suppressive Beta-Lactam Therapy Administered by Subcutaneous Route in Patients With Prosthetic Joint Infection. Front. Med. 8:583086. doi: 10.3389/fmed.2021.583086

Suppressive parenteral antibiotic therapy with beta-lactams may be necessary in patients with Gram-negative bone and joint infection (BJI). Subcutaneous drug administration can facilitate this therapy in outpatient setting, but there is limited information about this practice. We have developed an original approach for drug dosing in this context, based on therapeutic drug monitoring (TDM) and pharmacokinetic/pharmacodynamic (PK/PD) principles. The objective of this study was to describe our approach and its first results in a case series. We analyzed data from patients who received suppressive antibiotic therapy by subcutaneous (SC) route with beta-lactams as salvage therapy for prosthetic joint infection (PJI) and had TDM with PK/PD-based dose adjustment. Ten patients (six women and four men with a mean age of 77 years) were included from January 2017 to May 2020. The drugs administered by SC route were ceftazidime ($n = 4$), ertapenem ($n = 4$), and ceftriaxone ($n = 2$). In each patient, PK/PD-guided dosage individualization was performed based on TDM and minimum inhibitory concentration (MIC) measurements. The dose interval could be prolonged from twice daily to thrice weekly in some patients, while preserving the achievement of PK/PD targets. The infection was totally controlled by the strategy in nine out the 10 patients during a median follow-up of 1,035 days (~3 years). No patient acquired carbapenem-resistant Gram-negative bacteria during the follow-up. One patient presented treatment failure with acquired drug resistance under therapy, which could be explained by late MIC determination and insufficient exposure, retrospectively. To conclude, our innovative approach, based on model-based TDM, MIC

determination, and individualized PK/PD goals, facilitates, and optimizes suppressive outpatient beta-lactam therapy administered by SC route for PJI. These encouraging results advocate for larger clinical evaluation.

Keywords: prosthetic-joint infection, antimicrobial therapy, pharmacodynamics, pharmacokinetics, beta-lactam, subcutaneous administration

INTRODUCTION

Prolonged suppressive antimicrobial therapy (SAT) is necessary in some patients with prosthetic joint infection (PJI). This may be the only option to control the infection in patients for whom surgical removal of the prosthesis cannot be performed for various reasons (1). Those patients are often old, with multiple co-morbidities. In most cases, SAT is administered in outpatient setting. Oral antibiotics active against Gram-positive bacteria are the most frequently prescribed drugs in this indication (1). However, in case of infections caused by fluoroquinolone- and cotrimoxazole-resistant Gram-negative pathogens, parenteral administration may be necessary with beta-lactams usually used intravenously such as ceftriaxone, ceftazidime, and even ertapenem.

In case of prolonged parenteral antibiotic therapy with injectable beta-lactams, important questions are the dosage regimen that should be administered and the route of administration. Conventional dosing of beta-lactams consists on daily (e.g., ertapenem and ceftriaxone) or multiple daily intravenous administrations (e.g., ceftazidime). The dosage regimen is governed by the pharmacokinetic/pharmacodynamic (PK/PD) properties of those agents that often have a short half-life and exhibit time-dependent antibacterial effect (2). Thus, frequent administration of beta-lactam is thought to be necessary to maintain antibiotic concentration above the minimum inhibitory concentration (MIC) over a sufficient time between two administrations.

Frequent administration of intravenous (IV) drugs has several limitations in the outpatient setting. Long-term venous access should be maintained and requires specific care. Frequent IV administration is laborious for nurses, uncomfortable for patients, and costly. Spacing drug administration is desirable in this setting, but it should respect the PK/PD requirements of each drug to ensure treatment efficacy. It has been shown that the subcutaneous (SC) route may facilitate drug administration in patients compared with IV route, while preserving the PK/PD objectives of beta-lactams (3–8). Combining infrequent administration and SC route could be a way to facilitate prolonged suppressive outpatient therapy with beta-lactams, but there is limited information on this practice.

The objective of this work was to report the principles and first results of our salvage dosing approach for suppressive outpatient SC antibiotic therapy with beta-lactams based on PK/PD monitoring.

METHODS

Data Collection and Patients' Therapy

We analyzed data from patients who received suppressive antibiotic therapy by SC route with beta-lactam as salvage therapy and had therapeutic drug monitoring with PK/PD-based dose adjustment from January 2017 to May 2020 in our reference center for bone and joint infection called CRIOAc Lyon (http://www.crioac-lyon.fr). Part of the data have been reported in a previous article that focused on safety and outcome (7). The present study focuses on PK/PD and dosage individualization, in patient with PJI. All patients gave their consent to be included in the Lyon BJI cohort study that is registered on the website clinicaltrial.gov (NCT02817711). Collecting data on the efficacy and safety of off-label antibiotic in BJI is one of the objectives of this cohort study.

Three beta-lactams were used as suppressive therapy by SC route: ertapenem, ceftriaxone, and ceftazidime. Subcutaneous administration of those three drugs is still off-label in France but is supported by several clinical reports and studies (6, 7, 9–11). The decision of suppressive antibiotic therapy was taken by a multidisciplinary team including infectious disease physicians, surgeons, and microbiologists. Parenteral drugs were used when no oral drug could be administered because of the pathogen's resistance profile and/or polymicrobial infection and/or history of drug-related adverse events. The SC route was selected in order to facilitate outpatient care and the patient's acceptance of prolonged therapy, especially as suppressive intravenous therapy was not considered as feasible (benefit/risk ratio was considered in favor of the SC instead of intravenous administration). SC administration consisted in a 30–45-min gravity infusion of the diluted antibiotic (50 ml of isotonic saline) via a disposable butterfly needle inserted in the anterior side of the thigh or in the abdominal flank. Patients were followed-up at least every month at CRIOAc Lyon. The suppressive parenteral antibiotic therapy was started during hospitalization, after conventional primary intravenous antimicrobial therapy, based on microbiology data (type of bacteria and drug susceptibility). The initial dosing regimen was conventional with daily or multiple daily administrations depending on the beta-lactam considered and patients' characteristics. As for all patients receiving a prolonged beta-lactam in our institution, screening for rectal carriage for third cephalosporin-resistant of carbapenem-resistant *Enterobacteriaceae* was performed during the follow-up.

TABLE 1 | Characteristics of patients who received SC outpatient beta-lactam therapy and model-based TDM results.

Patient number (sex)	Age (years)[a]	Type of PJI	Weight (kg)[a]	CL_{CR} (ml/min)[a]	Drug monitored	Targeted pathogen	Pathogen MIC (target concentration) in mg/l	TDM #1		TDM #2		TDM #3	
								Dosage (route)	C_{min} in mg/l (time > target)	Dosage (route)	C_{min} in mg/l (time > target)	Dosage (route)	C_{min} in mg/l (time > target)
1 (M)	83	Hip	74	60	Ceftazidime	*E. coli*	0.5 (2.5)	8 g/24 h (IV, CI)	NA	2 g/24 h (SC)	5.6 (100%)	1 g/24 h (SC)	2.8 (100%)
2 (M)	81	Knee	110	58	Ceftazidime	*P. aeruginosa* *E. coli*	0.75 (3.75)[b]	2 g/24 h (SC)	4.9 (100%)	2 g TW (SC)	<1 (50%)	–	–
3 (M)	69	Knee	80	96	Ceftazidime	*P. aeruginosa*	2 (10)	3 g/12 h (SC)	22.4 (100%)	3 g/24 h (SC)	2.0 (58%)	–	–
4 (M)	78	Hip	73	35	Ceftazidime	*P. aeruginosa*	2 (10)	1 g/12 h (SC)	20.7 (100%)	1 g/24 h (SC)	7.0 (83%)	1 g Mon 1 g Wed 2 g Fri (SC)	2.7 (40%)
5 (F)	75	Knee	74	118	Ertapenem	*E. cloacae*	0.38 (7.6)[c]	1 g/24 h (SC)	3.7 (NA)	1 g Mon 1 g Wed 2 g Fri (SC)	<1 (37%)	–	–
6 (F)	78	Hip	68	90	Ertapenem	*E. cloacae*	0.064 (1.28)	1 g/24 h (SC)	3.9 (100%)	1 g/48 h (SC)	2.7 (100%)	–	–
7 (F)	75	Hip	90	61	Ertapenem	*E. coli*	≤0.5 (10)[d]	1 g/12 h (SC)	43.9 (100%)	1 g/24 h (SC)	17.0 (100%)	1 g Mon 1 g Wed 2 g Fri (SC)	3.5 (55%)
8 (F)	63	Hip	80	63	Ertapenem	*E. asburiae*	0.032 (0.64)	1 g/12 h (SC)	35.4 (100%)	1 g/24 h (SC)	14.2 (100%)	1 g TW (SC)	2.3 (100%)
9 (F)	80	Knee	70	56	Ceftriaxone	*S. marcescens*	≤1 (16)[e]	2 g/24 h (SC)	71.7 (100%)	1 g Mon 1 g Wed 2 g Fri (SC)	20.3 (100%)	–	–
10 (F)	74	Knee	115	80	Ceftriaxone	*E. coli*	0.023 (0.5)	2 g/12 h (SC)	61.7 (100%)	2 g TW	6.6 (100%)	–	–

[a] *Values at the time of the first TDM.*

[b] *Both bacteria had the same MIC.*

[c] *The MIC was not available when thrice weekly dosing was started after the first TDM results. Later, it was reported as 0.38 mg/l (see main text).*

[d] *The MIC was initially not available for this patient. We assumed a maximal MIC of 0.5 mg/l, based on the MIC distribution from EUCAST. Thereafter, the initial MIC was measured at 0.032 mg/l. The MIC measured on samples collected after relapse was 0.023 mg/l.*

[e] *The MIC was not measured for this patient and there is no epidemiological cut-off (ECOFF) defined for Serratia marcescens with ceftriaxone. We considered the ECOFF of cefotaxime (1 mg/l) provided by EUCAST.*

BJI, bone and joint infection; CI, continuous infusion; Fri, Friday; MIC, minimum inhibitory concentration; Mon, Monday; NA, not applicable; PJI, prosthetic joint infection; TDM; therapeutic drug monitoring, TW, thrice weekly; Wed, Wednesday.

Pharmacokinetic/Pharmacodynamic Dosage Individualization

Therapeutic drug monitoring (TDM) of the drug was first performed under conventional dosing, at the steady state. Blood samples were obtained during a planned follow-up visit in the BJI center. A typical PK profile included three samples: one pre-dose (trough or C_{min}), one 30 min after the end of the SC infusion (C_{max}), and one about 5–6 h after the end of the administration. The sampling times were precisely recorded for each patient, as well as body weight and renal function at the date of TDM. Drug concentrations of ertapenem, ceftriaxone, and ceftazidime were measured by validated liquid chromatography methods that are available in routine analysis in our institution.

The results were then analyzed by PK modeling. We used the BestDose^{TM} software to perform Bayesian estimation of individual PK parameters (e.g., clearance and volume of distribution) in each patient (12). Once the model had been fit to data and provided acceptable results, it was used to simulate a future dosing regimen. Future dosing regimens with standard and increased dosing interval (e.g., every 48 h or three administrations per week) were examined. The achievement of the PK/PD objective was calculated based on predicted concentrations and the MIC of the pathogen identified in bone samples, when available. When the MIC of the bacteria was not available, we used the MIC distribution of the bacteria provided by EUCAST. For beta-lactam, the usual objective is to maintain free (i.e., unbound to plasma protein) concentrations above the MIC ($fT > MIC$) over 50% to 100% of the dosing interval (2). In patients treated for BJI, this objective may be revised according to bone penetration. For ceftriaxone, available data suggest that bone to plasma concentration ratios are similar to the plasma free fraction of the drug, about 5–10% (13). For ertapenem, Boselli et al. (14) reported bone to plasma concentration ratios ranging from 0.1 to 0.4, which is higher than the free fraction in plasma (5–10%). For ceftazidime, Leigh et al. (15) reported mean bone-to-serum concentration ratios ranging from 0.20 to 0.30, depending on the site and bone tissue. This is lower than ceftazidime free fraction in plasma that is about 80%. We considered the worst-case scenario in terms of bone penetration for each agent and set the target plasma concentration to be achieved as 10 to 20 × MIC for ertapenem and ceftriaxone (i.e., assuming that bone concentration is equal to the free fraction in plasma) and 5 × MIC for ceftazidime. An individualized drug dosage, with increased dosing interval, was suggested to the clinicians whenever possible. The achievement of the PK/PD objectives was controlled by TDM and modeling on subsequent visits when feasible.

RESULTS

Ten patients with PJI received SC suppressive antibiotic therapy and had dosage based on TDM and PK/PD on the study period. This case series included six women and four men, with median (min–max) age, body weight, and creatinine clearance of 77 years (63–83), 77 kg (68–115), and 62 ml/min (35–118), respectively. Their characteristics are shown in **Table 1**, as well as the PK/PD results and dosage adjustment. The drugs administered as suppressive therapy were ceftazidime ($n = 4$), ertapenem ($n = 4$), and ceftriaxone ($n = 2$). In each patient, PK/PD-guided dosage individualization was performed, with changes in drug amount and/or dose interval based on TDM and MIC measurements.

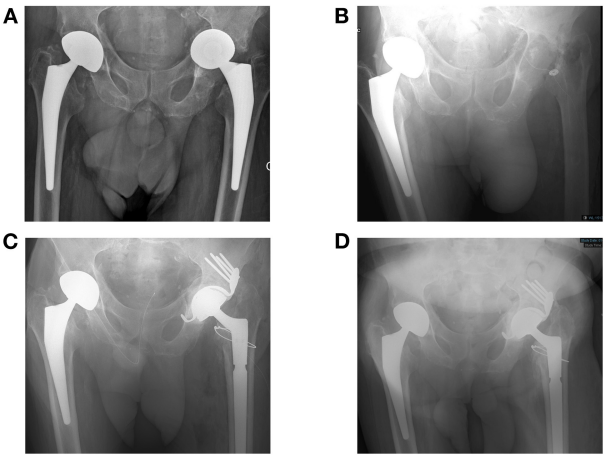


FIGURE 1 | Clinical description of the patient #4, a 77-year-old man. He had a past history of anal cancer and congestive heart failure with arrhythmia. Right and left hip prostheses were implanted in 2013 and 2014, respectively, both following femoral head fracture. As prosthesis loosening occurred with migration of the prosthesis in the pelvis **(A)**, a prosthetic joint infection (PJI) of the left hip was suspected. Explantation was performed in 2017 **(B)**, revealing *P. aeruginosa* chronic infection. Unfortunately, the strain was resistant to ciprofloxacin, but remained susceptible to ceftazidime. Intravenous (IV) ceftazidime was administered after explantation and stopped 15 days before reimplantation. At the time of reimplantation 3 months later, a complex acetabular reconstruction with the Burch–Schneider antiprotrusio cage and allografts was performed **(C)**, without any occurrence of loosening during the prolonged follow-up of 2 years **(D)**. As the cultures were still positive with persistence of *P. aeruginosa* in culture with the same susceptibility, IV ceftazidime 2 g/8 h was prescribed again. The dose was then reduced to 1 g/12 h as chronic kidney injury occurred (creatinine clearance 30 ml/min), before performing the first therapeutic drug monitoring (TDM) 6 months after the reimplantation, after switch from IV to SC ceftazidime 1 g/12 h. The outcome was favorable with a total control of the infectious disease (i.e., without occurrence of any sign of infection) during the follow-up. Unfortunately, the patient died ∼2 years after de reimplantation (727 days) following trauma and hemorrhagic shock.

An illustrative case of our dosing approach is that of a 77-year-old man who had a chronic PJI of the hip (patient #4; **Figure 1**). The pathogen identified was *Pseudomonas aeruginosa* with a measured MIC of 2 mg/l for ceftazidime. At the time of the first TDM, he had renal impairment with estimated glomerular filtration rate of 34 ml/min/1.73 m^2. His weight was 73 kg. He was initially administered SC ceftazidime 1 g/12 h. The target concentration for this patient was set at 10 mg/L (5 × MIC). Lowering the number of administration was examined to facilitate outpatient therapy. **Figure 2** summarizes how his dosage regimen was changed from 1 g/12 h to a thrice weekly regimen based on TDM and PK/PD modeling.

A second case illustrates the importance of MIC in the dosing decision (patient #5, **Figure 3**). This was a 75-year-old

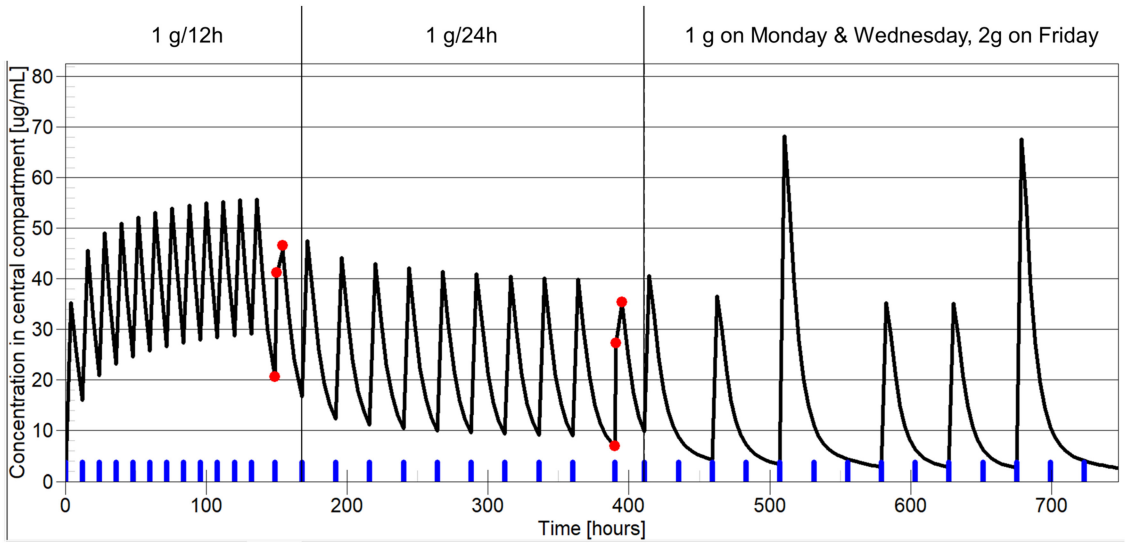


FIGURE 2 | Example of dosage individualization based on pharmacokinetic/pharmacodynamic (PK/PD) in patient #4, treated with suppressive ceftazidime for persistent *P. aeruginosa* PJI. The *x*-axis shows the time, the *y*-axis represents ceftazidime plasma concentration. Of note, this is not the real time of drug therapy. The time scale has been altered to show the three dosage periods on the same plot. The blue marks on the *x*-axis show drug administrations. The red dots represent the patient-measured ceftazidime concentrations. The black line represents model prediction. The vertical line separates the three dosage periods: 1 g/12 h, 1 g/24 h, and 1 g on Monday and Wednesday + 2 g on Friday. On the first TDM occasion, under a dosage of 1 g/12 h, the measured ceftazidime C_{min} was 20.7 mg/l, well above the target concentration of 10 mg/l (5 × MIC) for this patient. The model predicted that a dosage of 1 g/24 h would result in C_{min} of 8.2 mg/l and 88% of time above the target level. The dosage was adjusted as suggested. TDM was performed a second time, 2 months later, under a dosage of 1 g/24 h. The measured ceftazidime C_{min} was 7 mg/l, in good agreement with the model prediction 2 months before. The model predicted that a dosage of 1 g on Monday and Wednesday and 2 g on Friday would result in C_{min} of 2.7 mg/l and 40% of time spent above the target level of 10 mg/l (5 × MIC).

woman who had a complicated chronic PJI of the knee. Six months after surgery, ertapenem administered as 1 g/24 h by SC route was continued as suppressive therapy targeting the multidrug resistant *Enterobacter cloacae*. The patient had no signs of uncontrolled infection at this time but experienced a poor functional outcome with irreductible flessum and mild lucencies on X-ray (**Figure 3A**). This targeted bacteria was reported to be susceptible to ertapenem, but the MIC was not available and it was unknown when TDM was performed. TDM was first performed about 4 months after SC ertapenem was started, as the patient was inquiring about the possibility of less frequent SC injections. At the time of TDM, the patient weighted 74 kg and had creatinine clearance of 118 ml/min. **Figure 4** shows the estimated PK profile obtained after Bayesian estimation of PK parameters based on three measured concentrations, the alternative dosage adjustment examined, and the predicted value of the PK/PD objective ($fT > MIC$). The target concentration was set at 20 times the MIC, as explained above. As the MIC was unknown, we considered three putative MIC values based on the ertapenem MIC distribution of *Enterobacter cloacae* provided by EUCAST: a low MIC of 0.015 mg/l, an intermediate MIC of 0.064 mg/l, and a high MIC of 0.5 mg/l. The achievement of the PK/PD target under thrice weekly dosage regimens strongly depended on the MIC. The results were acceptable for MIC $\leq$ 0.064, with $fT > MIC$ greater than 40% and up to 100%. However, the exposure was clearly not sufficient for the high MIC. Of note, 1 g/24 h was associated with more favorable PK/PD, with $fT > MIC$ of about 60% for a MIC of 0.5 mg/l. Based on this simulation, the dosage of SC ertapenem was adjusted with 1 g on Monday and Wednesday and 2 g on Friday. Unfortunately, 7 months after this dosage adjustment, the patient showed treatment failure, with total prosthesis loosening (**Figure 3B**) and purulent discharge with acquired resistance of *Enterobacter cloacae* to ertapenem. The MIC of the original strain that was finally retrieved was at 0.38 mg/l, a high value associated with insufficient $fT > MIC$ of the thrice weekly regimen, retrospectively.

Except for this latter patient, in whom the failure was predictable *a posteriori*, the infection was totally controlled by the strategy in nine out the 10 patients during a median follow-up of 1,035 days (~3 years) (extreme values 251 and 1,664 days; interquartile range 372–1,291 days); eight of them were followed >2 years without any recurrence, except for one patient (patient #7) in whom ertapenem was stopped when COVID-19 was diagnosed. Unfortunately, 2 weeks after the withdrawal of ertapenem, the patient presented a clinical failure with the same pathogen (*E. coli*) that remained susceptible to ertapenem (MIC = 0.023 mg/l), demonstrating that our model-based TDM SC outpatient beta-lactam therapy was efficient as long as the treatment was continued. Concerning the potential acquisition of resistant bacterial carriage in the gut microbiota, nine patients were already colonized with 3rd generation cephalosporin-resistant Gram-negative bacteria before suppressive therapy, and one of them lost it during the follow-up. One patient

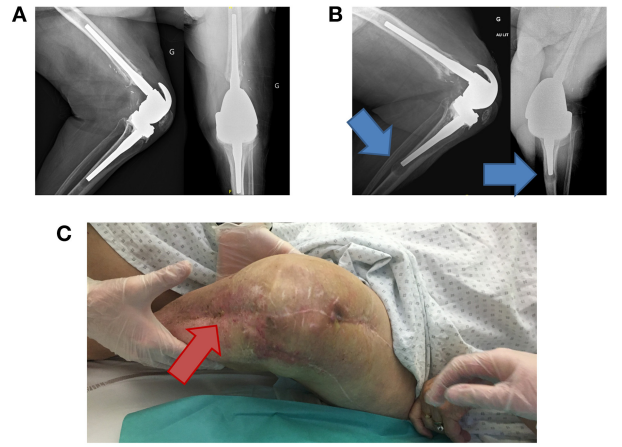


FIGURE 3 | Clinical description of patient #5, a 75-year-old woman. She had a past history of diabetes, Parkinson's disease, and hypertension. A left knee prosthesis was implanted in 2009. In 2017, she experienced a distal femoral fracture requiring osteosynthesis and then debridement for acute infection. As a pseudarthrosis occurred, a two-stage exchange was performed. *Enterobacter cloacae* producing extended-spectrum beta-lactamases (ESBL) was found, and imipenem was prescribed and was stopped after the reimplantation. Unfortunately, the patient developed signs of acute infection and a new debridement revealed *Pseudomonas aeruginosa* and *Candida albicans* superinfection, with persistence of the ESBL *Enterobacter cloacae*. The initial therapy included IV imipenem, oral ciprofloxacin, and oral fluconazole. After 6 weeks, imipenem was replaced by IV ertapenem (1 g/12 h), and irreductible flessum persisted with mild prosthesis loosening on X-ray **(A)**. Ciprofloxacin and fluconazole were stopped after 12 weeks and 6 months, respectively. Six months after surgery, ertapenem administered as 1 g/24 h by SC route was continued as suppressive therapy targeting the multidrug-resistant *Enterobacter cloacae*. Unfortunately, prosthesis loosening **(B)** and purulent discharge occurred **(C)** (the red arrow points to the fistula from which purulent discharge occurred) revealing the persistence of the ESBL *Enterobacter cloacae* into the joint, despite ertapenem therapy. It became resistant to ertapenem.

never acquired any resistant bacterial carriage. No patient acquired carbapenem-resistant Gram-negative bacteria during the follow-up.

DISCUSSION

Prolonged suppressive outpatient parenteral antimicrobial therapy is demanding for patients and health care professionals. This case series illustrates how the route of administration and the dosage regimen can be individualized to facilitate this therapy. Our approach for route and dosage individualization of beta-lactam in patients with PJI is basically based on four principles: SC administration; drug TDM; pathogen MIC determination; and model-based, goal-oriented dose adjustment.

First, the subcutaneous route facilitates drug administration in such setting compared with IV route. The venous access required for IV administration may be difficult to maintain in the long term and is associated with a higher risk of infection (17). The SC route also appears to be preferred by patients, as it reduces discomfort and facilitates home care compared with IV route (18).

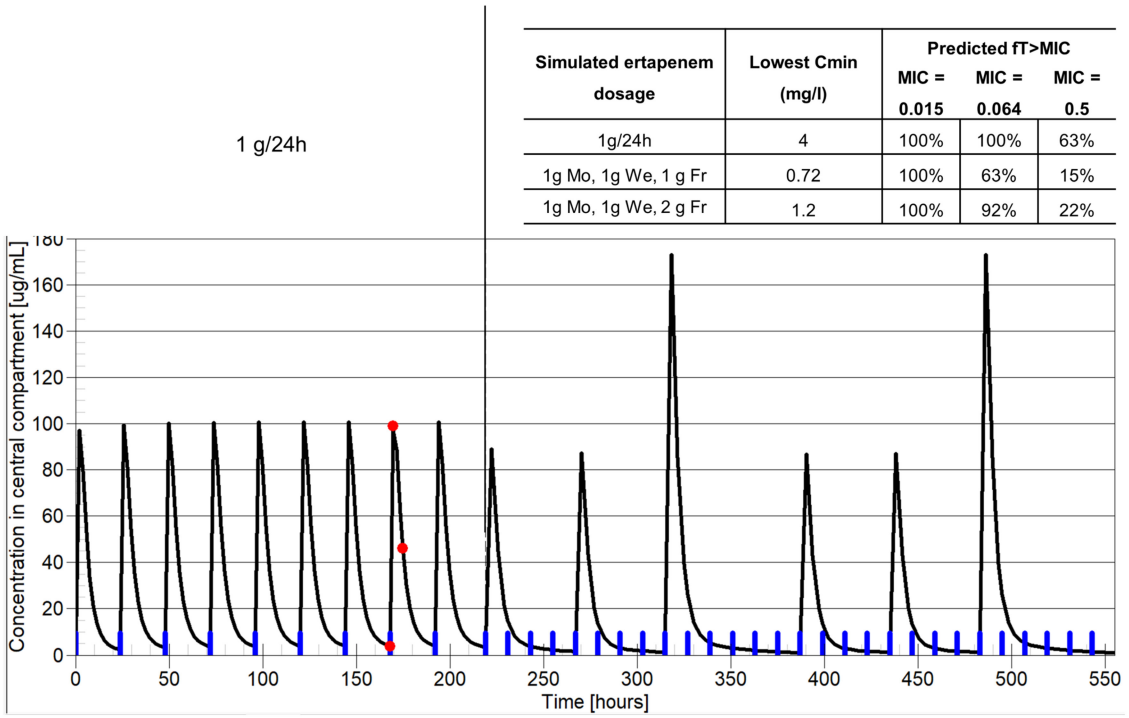
From a PK/PD perspective, SC administration is especially interesting for the administration of beta-lactam drugs, as it results in time above the MIC similar if not greater than IV administration of the same dose (3–5, 8). This suggests that SC administration of beta-lactams should be as efficient as IV administration, although there is a dearth of high-level clinical evidence.

The second principle is to perform TDM, with plasma concentration measurement of beta-lactams. In our approach, TDM is key to perform model-based dose adjustment. It is also useful to check that the target concentration is achieved after increasing the dose interval. Of note, TDM of beta-lactam is often performed as a trough-only approach in other settings (16, 19). A trough level is sufficient provided that its value is greater than the target concentration, which is most often a multiple of the MIC, so that the time spent above the target level is 100%. However, in the case of trough concentration lower than the MIC, the result cannot be interpreted. The time spent above the MIC is lower than 100% but remains unknown. Because our goal is to space drug administration for facilitating prolonged antibiotic therapy, we use a different TDM approach with three drug samples. This allows to better estimate the individual PK parameters of the drug with the model (including half-life) and to calculate the time spent above the MIC in all cases, even when it is lower than 100%.

Third, as illustrated in **Figure 2**, the determination of the pathogen MIC is important in our approach for dosage individualization. Basically, the MIC determines the individual requirements in terms of drug exposure and so determines the therapeutic margin. As shown in **Table 1**, infrequent administration of beta-lactams, even those with short half-life such as ceftazidime, is possible when the MIC is low, because the PK/PD target ($fT > MIC$ of 50–100%) can still be achieved. For example, in patients #6, 8, and 10, the observed $fT > MIC$ was still 100% even under thrice weekly dosage. By contrast, when the bacterial MIC is high, increasing the dosage interval is not possible, because $fT > MIC$ will be insufficient to ensure efficacy.

It has been suggested elsewhere that the MIC epidemiological cut-off (ECOFF) of the pathogen should be used to interpret TDM results and perform dose adjustment of antibacterials, because the precision of MIC assay is often low (20). We believe that this is not justified in all situations, especially when the measured MIC is much lower than the ECOFF (21). Using the ECOFF for PK/PD-based dose adjustment consists in considering the worst-case scenario and the need for high dosage in all patients infected by a given pathogen. Basically, this assumption preclude PK/PD dosage individualization. By contrast, as shown in **Table 1**, using the measured MIC permits to set individualized goals in each patient and adjust the dosage to patients' condition and needs.

Our approach for dosage individualization is based on Bayesian PK modeling and dose adjustment. The use of PK models permits to interpret TDM results most efficiently, as one can calculate the individual PK parameters, estimate the value of the PK/PD objective (e.g., $fT > MIC$ for beta-lactams),



Simulated ertapenem dosage	Lowest Cmin (mg/l)	Predicted fT>MIC		
		MIC = 0.015	MIC = 0.064	MIC = 0.5
1g/24h	4	100%	100%	63%
1g Mo, 1g We, 1 g Fr	0.72	100%	63%	15%
1g Mo, 1g We, 2 g Fr	1.2	100%	92%	22%

FIGURE 4 | Example of dosage individualization based on PK/PD in patient #5, treated with suppressive ertapenem for a persistent *E. cloacae* PJI. The *x*-axis shows the time and the *y*-axis represents ertapenem plasma concentration. Of note, this is not the real time of drug therapy, as past therapy before TDM was much longer. The blue marks on the *x*-axis show drug administrations. The red dots represent the patient-measured ertapenem concentrations. The black line represents model prediction. The vertical line separates the past therapy with 1 g/24 h and predicted future therapy. The inserted table shows the predicted C_{min} and PK/PD objective for three candidate dosage regimens and three possible MIC values.

and simulate future dosage regimens achieving the individual target. Bayesian dosing programs outperform empirical and other dose adjustment methods (22, 23). This approach is especially useful to predict the adequacy of infrequent drug administration of beta-lactams in our setting, which would be virtually impossible without models. In our case series, most model predictions have been confirmed by subsequent concentration measurements when the patients were stable (data not shown).

Finally, the tolerance and safety of prolonged suppressive subcutaneous antibiotic therapy is also a major challenge, considering the off-label characteristic of this procedure and the potential risk of acquisition of carbapenem-resistant bacterial carriage in the gut microbiota. All patients received therapy over several months or years, corresponding to ∼4,000 SC injections, without any serious adverse event at the site of injection. None of our patients acquired a carbapenem-resistant bacteria detectable in stools during the follow-up, which is reassuring in a safety point of view.

There is a number of limitations in this study. The clinical results should be interpreted cautiously because of the limited sample size. We used conventional PK/PD targets for beta-lactam therapy ($fT > MIC$ of 50% to 100%) but those have not been evaluated in patients with PJI. Limited data was available from each patient, as TDM was performed infrequently. The long-term efficacy and safety of subcutaneous suppressive beta-lactam therapy administered by SC route remains to be evaluated in prospective clinical trials.

To conclude, this case series shows that suppressive outpatient beta-lactam therapy administered by SC route in patients with PJI is feasible. We have developed an innovative approach to facilitate and optimize this therapy based on model-based TDM, MIC determination, and individualized PK/PD goals. This approach has shown encouraging results so far for these patients requiring salvage therapy but needs further clinical evaluation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided

their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SG and TF conceived the study and wrote the manuscript. TF, AC, CP, EB, and FV managed all the patients and initiated the proposal for subcutaneous therapy as suppressive treatment during multidisciplinary meetings. M-CG, SC, and JG performed drug therapeutic drug monitoring. SG performed PK/PD analysis of TDM data. FL provided microbiology results with MIC determination. All the authors participated in manuscript editing with significant intellectual inputs. All authors approved the final version of the manuscript.

FUNDING

This study was based on the routine care of patients managed in our regional reference center and received no dedicated funds.

ACKNOWLEDGMENTS

The authors would like to acknowledge the following: coordinator—*TF*; infectious disease specialists—*TF*, *FV*, *Thomas Perpoint*, *Patrick Miailhes*, *Florence Ader*, *Sandrine Roux*, *Agathe Becker*, *Claire Triffault-Fillit*, *AC*, *CP*, *Nicolas Benech*, *Pierre Chauvelot*, *Marielle Perry*, *Fatiha Daoud*, *Johanna Lippman*, *EB*, *Christian Chidiac*; surgeons—*Elvire Servien*, *Cécile Batailler*, *Stanislas Gunst*, *Axel Schmidt*, *Matthieu Malatray*, *Elliot Sappey-Marinier*, *Michel-Henry Fessy*, *Anthony Viste*, *Jean-Luc Besse*, *Philippe Chaudier*, *Lucie Louboutin*, *Quentin Ode*, *Adrien Van Haecke*, *Marcelle Mercier, Vincent Belgaid*, *Arnaud Walch*, *Sébastien Martres*, *Franck Trouillet*, *Cédric Barrey*, *Ali Mojallal*, *Sophie Brosset*, *Camille Hanriat*, *Hélène Person*, *Nicolas Sigaux*, *Philippe Céruse*, *Carine Fuchsmann*; anesthesiologists—*Frédéric Aubrun*, *Mikhail Dziadzko*, *Caroline Macabéo*; microbiologists—*FL*, *Laetitia Beraud*, *Tiphaine Roussel-Gaillard*, *Céline Dupieux*, *Camille Kolenda*, *Jérôme Josse*; pathology—*Marie Brevet*, *Alexis Trecourt*; imaging—*Fabien Craighero*, *Loic Boussel*, *Jean-Baptiste Pialat*, *Isabelle Morelec*; PK/PD specialists—*Michel Tod*, *M-CG*, *SG*; and clinical research assistant and database manager—*Eugénie Mabrut*.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 03 May 2021
doi: 10.3389/fmed.2021.550095

Risk Scores and Machine Learning to Identify Patients With Acute Periprosthetic Joints Infections That Will Likely Fail Classical Irrigation and Debridement

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: 08 April 2020
Accepted: 23 March 2021
Published: 03 May 2021

Citation:
Wouthuyzen-Bakker M, Shohat N, Parvizi J and Soriano A (2021) Risk Scores and Machine Learning to Identify Patients With Acute Periprosthetic Joints Infections That Will Likely Fail Classical Irrigation and Debridement. Front. Med. 8:550095. doi: 10.3389/fmed.2021.550095

The most preferred treatment for acute periprosthetic joint infection (PJI) is surgical debridement, antibiotics and retention of the implant (DAIR). The reported success of DAIR varies greatly and depends on a complex interplay of several host-related factors, duration of symptoms, the microorganism(s) causing the infection, its susceptibility to antibiotics and many others. Thus, there is a great clinical need to predict failure of the "classical" DAIR procedure so that this surgical option is offered to those most likely to succeed, but also to identify those patients who may benefit from more intensified antibiotic treatment regimens or new and innovative treatment strategies. In this review article, the current recommendations for DAIR will be discussed, a summary of independent risk factors for DAIR failure will be provided and the advantages and limitations of the clinical use of preoperative risk scores in early acute (post-surgical) and late acute (hematogenous) PJIs will be presented. In addition, the potential of implementing machine learning (artificial intelligence) in identifying patients who are at highest risk for failure of DAIR will be addressed. The ultimate goal is to maximally tailor and individualize treatment strategies and to avoid treatment generalization.

Keywords: debridement, implant retention, risk score, machine learning, failure, periprosthetic joint infection

INTRODUCTION

Success rates of the "classical" debridement, antibiotics, irrigation and implant retention (DAIR) for acute periprosthetic joint infections (PJI) vary widely, ranging from 30 to 90% (1–5). Apart from a thorough surgical debridement with exchange of modular components, many factors contribute to the success of DAIR; that includes shorter duration of symptoms, lack of patient comorbidities, a low bacterial inoculum and/or degree of inflammation at clinical presentation, a causative microorganism that is susceptible to antibiotics with anti-biofilm properties and many others (6–25). For this reason, being able to identify a category of patients who are likely to fail DAIR is essential, either to choose a different surgical procedure, to intensify antimicrobial

treatment or to apply new innovative treatment strategies to increase the chance of treatment success. In this overview we will outline the current recommendations for DAIR treatment and discuss the limitations of these recommendations. In addition, we will address preoperative risk classification systems and the potential of machine learning to predict DAIR failure. These latter two show great potential to be used in clinical practice and may aid in clinical decision making.

WHO SHOULD RECEIVE DAIR ACCORDING TO THE IDSA GUIDELINES

Since many different factors have been identified in literature as independent predictors for DAIR failure (**Table 1**) (6–25), it is a great challenge to select those patients who are the best candidates for DAIR. According to the IDSA guidelines published in 2013 (26), a DAIR is advised for patients with acute PJI, defined as a symptom duration of <3 weeks or, in case of early post-surgical infections, within 4 weeks of index arthroplasty. In addition, the prosthesis needs to be well-fixed, a sinus tract should be absent and the microorganism needs to be susceptible to oral antimicrobial agents with anti-biofilm activity. If these conditions are met, a DAIR is recommended, and in other situations revision of the implant is advised. Although this approach seems legitimate, it entails important limitations. First, it excludes a large subgroup of patients that may still benefit from DAIR. For example, in post-surgical cases it is advised to remove the infected implant when the index arthroplasty occurred more than 4 weeks ago. However, the process of mature biofilm formation varies substantially according to the type of causative microorganism and the inoculum size that contaminates the joint during surgery (27, 28). To therefore, exclude these patients as a candidate for a DAIR procedure is not justified. Indeed, Löwik et al. demonstrated an acceptable outcome of DAIR in patients presenting more than 4 weeks after the index arthroplasty as long as DAIR was performed within 4 week after the onset of symptoms and modular components were exchanged (29). In this category of patients, the prosthesis could still be retained in around 80% of patients without the need for life long suppressive antibiotic treatment. A second limitation of the IDSA recommendation concerning the indication for DAIR is the lack of distinction between early acute (post-surgical) and late acute (hematogenous) PJIs. This distinction may be critical, since several studies demonstrated a worse outcome in late acute PJIs treated with DAIR compared to early acute PJIs, in particular when caused by staphylococci (8, 13, 23, 30). Considering the difference in pathogenesis, and the chance of continuous seeding to the prosthetic joint in case of hematogenous infections (e.g., endocarditis), it is reasonable to assume that these infections should be approached differently as well. A third limitation of the IDSA guideline, is that the causative microorganism(s) and its susceptibility to antibiotics are often not known prior to surgery. A final limitation is the fact that implant- and host-related factors are not included in the decision-making model to determine appropriateness of DAIR. This may result in misclassifying a patient as a good candidate for DAIR while existing comorbidities may expose the patient to an increased risk for complications and failure. In addition, as the microorganism and its susceptibility to antibiotics is often not known prior to surgery, these implant- and host-related factors are of utmost importance to take into consideration.

PREOPERATIVE RISK SCORES TO PREDICT DAIR FAILURE

To identify patients who are likely to fail DAIR, two preoperative risk scores have been proposed in literature; one for early acute (post-surgical) and one for late acute (hematogenous) PJIs (8, 16). These risk scores include only those variables that are known preoperatively without taking into account the causative microorganism and its susceptibility to antibiotics, mimicking the situation mostly encountered in clinical practice.

KLIC-score for Early Acute (Post-Surgical) PJI

In 2015, Tornero et al. published the KLIC-score as preoperative risk score for predicting DAIR failure in early acute PJI (16). The authors of this study examined a cohort of 222 patients who were within 3 months after the index surgery and who had no more than 3 weeks of symptoms prior to DAIR. DAIR failure was defined as the need for a second DAIR, implant removal, suppressive antibiotic treatment or infection-related death within 60 days after the initial irrigation and debridement. They analyzed in a univariate model several variables that were known preoperatively, like host-related factors, duration of symptoms, characteristics of the infected implant and serum inflammatory parameters, and developed a risk stratification score according to the beta-coefficients of the multivariate analysis (**Figure 1A**). Chronic **K**idney disease, **L**iver cirrhosis, the **I**ndex surgery (revision surgery or prosthesis indicated for a fracture), a **C**emented prosthesis and a **C**-reactive protein > 115 mg/l (**KLIC**) appeared to be the most prominent preoperative variables associated with failure. The score demonstrated 100% DAIR failure when having a preoperative score of more than six, and 4.5% when having a score lower than two. After this publication, three additional studies from other institutions validated the KLIC-score in their cohort of patients (18, 31, 32). All three institutions demonstrated the predictive power of the KLIC-score in patients with a very low or a very high score, but the score was less useful in patients with average scores. In addition, one study identified that other variables appeared to be more predictive in their cohort of patients compared to those defined in the KLIC (18), stressing the importance of differences in local epidemiology when implementing risk scores from an external cohort of patients.

CRIME80-score for Late Acute (Hematogenous) PJI

Following the KLIC-score, Wouthuyzen-Bakker et al. performed the same statistical analysis in a large multicenter cohort of 340 patients with late acute PJIs (8). Late acute PJI was defined as the appearance of acute symptoms of infection occurring

TABLE 1 | Summary of studies depicting independent predictors of DAIR failure in acute PJIs by using multivariate analysis.

Reference	Author et al.	Year	*N*	Host, implant and surgical factors (known preoperatively)	aOR/aHR	Microorganism and antibiotics (known postoperatively*)	aOR/aHR
(6)	Lora-Tamayo	2013	345	Immune suppressive drugs	2.23	Polymicrobial	1.77
				Serum CRP	1.22	Levofloxacin and rifampin[a]	0.42
				Exchange modular components	0.65	Vancomycin and rifampin[b]	0.29
				≥2 debridements	1.63	Bacteremia	1.81
(7)	Lora-Tamayo	2017	462	Rheumatoid arthritis	2.36		
				Revision prosthesis	1.37		
				Late post-surgical infection	2.20		
				Exchange modular components	0.60		
(8)	Wouthuyzen-Bakker	2018	340	Male sex	2.02	*S. aureus*	3.52
				Age > 80 years	2.60		
				COPD	2.90		
				Rheumatoid Arthritis	5.13		
				Fracture	5.39		
				Serum CRP > 150 mg/L	2.00		
				Exchange modular components	0.35		
(10)	Urish	2017	206	Symptoms > 7 days	1.68	*S. aureus*	0.59
(9)	Marculescu	2006	99	Sinus tract	2.84		
				Symptoms > 8 days	1.77		
(48)	Tornero	2016	143			Suboptimal antibiotic treatment[c]	4.92
(12)	Puhto	2015	113	Leukocytes > 10×10^9/L	3.70	Ineffective empirical antibiotics	3.20
(13)	Vilchez	2011	65	Late acute PJI	2.57		
				≥2 debridements	4.61		
(14)	El Helou	2010	91			Rifampin in staphylococci PJI	0.11
(15)	Martínez-Pastor	2009	47	Serum CRP > 150 mg/L	3.57	No fluoroquinolone in Gram negative	9.09
(16)	Tornero	2015	222	Chronic renal failure	5.92		
				Liver cirrhosis	4.46		
				Femoral neck fracture	4.39		
				Revision prosthesis	4.34		
				Cemented prosthesis	8.71		
				Serum CRP > 115 mg/L	12.3		
(17)	Rodriguez-Pardo	2014	174	Chronic renal failure	2.56	Fluoroquinolone in Gram negative	0.23
(18)	Löwik	2018	386	Male sex	2.03		
				Left-sided prosthesis	1.80		
				Ischemic heart disease	1.84		
(19)	Tornero	2014	160	Liver cirrhosis	12.4	No fluoroquinolone in Gram negative	6.5
				Serum CRP > 120 mg/L	1.06		
(20)	Bergkvist	2016	35	Hip fracture	8.30		
(21)	Byren	2009	112	Revision prosthesis	3.10	*S. aureus*	2.9
				Arthroscopic procedure	4.20		
(22)	Vilchez	2011	53	Serum CRP > 220 mg/L	20.4		
				≥2 debridements	9.80		
(23)	Rodriguez	2010	50			*S. aureus*	5.3
(24)	Letouvet	2016	60	Number of prior surgeries	6.30	*S. aureus*	9.4
						Antibiotic treatment < 3 months	20.0
(25)	Soriano	2006	47			*Enterococcus* spp. or MRSA	17.6

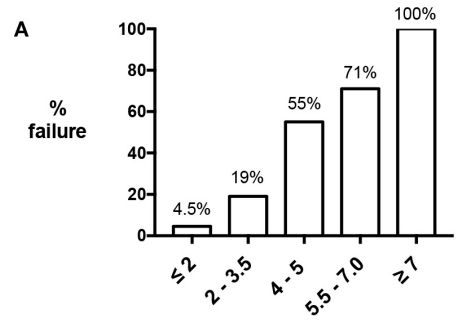
**The presence of bacteremia, the causative microorganism and its susceptibility to antibiotics are sometimes known prior to DAIR, but in most cases not.*

[a] *Sub-group analysis of patients with a post-surgical PJI due to methicillin-susceptible S. aureus (MSSA).*

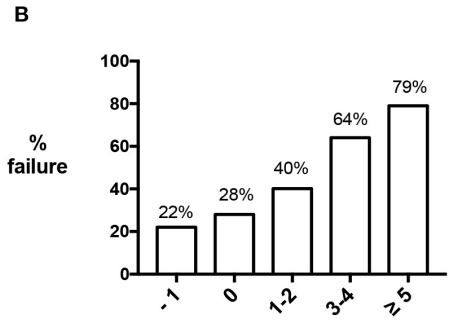
[b] *Sub-group analysis of patients with a post-surgical PJI due to methicillin-resistant S. aureus (MRSA).*

[c] *No rifampin for Gram positives and no fluoroquinolone for Gram negatives.*

CRP, C-reactive protein; COPD, Chronic Obstructive Pulmonary Disease.



K	Chronic renal failure (Kidney)	**2**
L	Liver cirrhosis	**1.5**
I	Index surgery: indication prosthesis: fracture OR revision prothesis	**1.5**
C	Cemented prosthesis	**2**
	CRP > 115 mg/L	**2.5**



C	COPD	**2**
	CRP > 150 mg/L	**1**
R	Rheumatoid arthritis	**3**
I	Indication prosthesis: fracture	**3**
M	Male	**1**
E	Exchange of mobile components	**-1**
80	Age > 80 years	**2**

FIGURE 1 | Preoperative risk scores for DAIR failure. KLIC-score for predicting DAIR failure in early acute (postsurgical) PJI **(A)** and CRIME80-score for predicting DAIR failure in late acute (hematogenous) PJI **(B)**.

more than 3 months after the index arthroplasty, in a prior asymptomatic prosthetic joint. Patients with a sinus tract and/or patients with symptoms existing for longer than 3 weeks before DAIR were excluded. In contrast to the study of Tornero et al., a second DAIR procedure was not considered as failure, and failure could occur even 60-days after the initial debridement. In addition, the authors also included the exchange of mobile components as a valid preoperative variable, as the possibility to exchange it can be known prior to surgery as well. According to this analysis, **C**hronic obstructive pulmonary disease, a **C**-reactive protein > 150 mg/L, **R**heumatoid arthritis, fracture as **I**ndication for the prosthesis, **M**ale sex, not **E**xchanging the mobile components and an age > **80** years (**CRIME80**), were the strongest preoperative variables associated with failure (**Figure 1B**). The strength of prediction of the CRIME80-score was lower than the KLIC-score, starting with a baseline failure rate of 22%, and increasing to 79% with a score higher than four. It is important to note that the isolation of *Staphylococcus aureus* was one of the major predictors of failure in the late acute cohort. When *S. aureus* was the causative microorganism, the baseline failure rate was 43%, and the preoperative variables turned out to be less predictive in these cases. For this reason, the authors stress the importance of isolating the microorganism prior to deciding the surgical procedure. Unlike the KLIC-score, the CRIME80-score has not yet been validated in an external cohort of patients.

Potential of Machine Learning (Artificial Intelligence) in Predicting DAIR Failure

Considering the complex interplay of factors associated with DAIR failure, regular statistical methods lack the finesse for more accurate and individualized predictions. The advantage of machine learning over regular statistical methods, like multivariate analysis, is its ability to actually learn from data input. Where multivariate analysis examines the correlation of variables and the strength of these correlations, machine learning learns from observations by using decision trees. The subsequently created algorithm is then able to process new input that has not been seen before. By this means, machine learning models are able to process more complex data, and by building precision models they are able to make more accurate predictions. Machine learning has become more and more popular in infection management (33). Recently, Shohat et al. used random forest analysis as a machine learning model to predict DAIR failure (34). The authors of this study analyzed more than 1,000 patients that underwent irrigation and debridement of a hip or knee prosthesis for acute PJI. The created algorithm had good discriminatory power, with an area under the curve of 0.74. Cross-validation, a model validation technique assessing the ability to process an independent dataset, showed similar probabilities, indicating a high accuracy of the model. Although the model still needs to be validated in an external cohort of patients, the created algorithm has great potential

to be used in daily practice by easily entering patient data in a computer-based software or smartphone application, and may aid in clinical decision making and patient counseling. As the causative microorganism is of great influence on treatment outcome (7, 8), the authors of this study decided to include this variable in the analysis as well. Although its inclusion improves its predictive power, the microorganism needs to be entered to ensure the highest accuracy of the model, and thus, ideally should be known prior to surgery. The same holds for the presence or absence of bacteremia.

TO TAILOR AND INDIVIDUALIZE TREATMENT STRATEGIES

The described preoperative risk scores and machine learning model, can be applied in daily clinical practice, and may aid in the decision making process. When a patient has a high a priori risk for DAIR failure, immediate implant removal should be considered; not only to avoid surgery that is very likely to fail, but also to reduce the adverse effect of DAIR on subsequent surgical procedures (35, 36). Wouthuyzen-Bakker et al. analyzed the treatment outcome of immediate implant removal vs. DAIR in late acute PJIs by matching patients according to their preoperative CRIME80 score (37). The authors found that implant removal resulted in 83% treatment success in patients with a CRIME80 score $\geq$ 3, while the success was only 35% when treated with DAIR. No clear difference was observed between one- and two-stage exchange arthroplasties. Although a high CRIME80 score was logically associated with the presence of more comorbidities and old age, immediate implant removal was associated with a lower—instead of higher—mortality rate compared to DAIR. These data suggest that immediate implant removal is safe, even though the surgery in general is more aggressive.

A promising technique to potentially increase the success rate of DAIR, especially for difficult to treat microorganisms (e.g., multidrug resistant Gram negatives or rifampin resistant staphylococci), is to locally inject a selected cocktail of bacteriophages during surgery. Although future studies are needed to endorse this practice, its clinical success has been described as salvage therapy in relapsing *S. aureus* PJI (38). A main disadvantage though, is that the microorganism(s) causing the infection not only needs to be known prior to surgery, but the corresponding targeted bacteriophages need to be produced in the laboratory, before they can be applied. Since delaying DAIR increases the risk of treatment failure, the use of bacteriophage therapy for this indication is therefore, probably less feasible.

When a DAIR has been performed in a patient with a low preoperative risk score for failure, but the infection turns out to be caused by a microorganism that is resistant to biofilm active drugs, antibiotic duotherapy can be considered, particularly during the initial period. Adding fosfomycin to the antibiotic regimen as a second drug in infections with multidrug resistant Gram negatives or Gram positive microorganisms, or adding daptomycin for Gram positive infections, have shown great promise: both of the latter antibiotics have shown good antibiofilm properties *in vitro* and *in vivo* when used as part of a combination treatment (39–45). An alternative option is life-long antibiotic suppressive therapy, especially if patients are not eligible for additional surgery. According to a recent large multicenter cohort study with a follow-up period of 5 years, PJI can be controlled with antibiotic suppressive therapy in around 50% of cases (46). Another alternative strategy would be to apply new and more innovative treatments to control infection, like applying subcutaneous antibiotics for patients who do not tolerate oral antibiotics or for infections caused by multidrug resistant bacteria that lack an oral alternative. This proof of concept was demonstrated by Ferry et al. and was successful in 6 out of 10 patients (47). Considering the low chance of success for both treatment strategies, isolating the microorganism prior to surgery and choosing for implant removal in high risk patients would be preferable.

CONCLUSION

Selecting those patients who are good candidates for a DAIR procedure is essential. Current IDSA recommendations for DAIR entail important limitations, and tools that also take into account other variables that are associated with DAIR failure are needed. Preoperative risk scores like the KLIC-score for early acute (post-surgical) and CRIME80-score for late acute (hematogenous) PJI could be helpful, especially when the microorganism is not known prior to surgery. In addition, machine learning shows great potential to predict failure more accurately compared to regular statistical methods. Implementing the aforementioned tools in daily care will help physicians tailor and individualize treatment strategies. Both described risk classification systems as well as the recently published machine learning model need clinical evaluation in larger external cohorts of patients to validate its predictive power.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
published: 05 May 2021
doi: 10.3389/fmed.2021.569159

Case Report: Arthroscopic "Debridement Antibiotics and Implant Retention" With Local Injection of Personalized Phage Therapy to Salvage a Relapsing *Pseudomonas Aeruginosa* Prosthetic Knee Infection

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: 03 June 2020
Accepted: 18 March 2021
Published: 05 May 2021

Citation:
Ferry T, Kolenda C, Batailler C, Gaillard R, Gustave C-A, Lustig S, Fevre C, Petitjean C, Leboucher G, Laurent F and the Lyon BJI Study group (2021) Case Report: Arthroscopic "Debridement Antibiotics and Implant Retention" With Local Injection of Personalized Phage Therapy to Salvage a Relapsing Pseudomonas Aeruginosa Prosthetic Knee Infection. Front. Med. 8:569159. doi: 10.3389/fmed.2021.569159

Bacteriophages are viruses that specifically target bacteria. They are considered to have a high potential in patients with prosthetic joint infection (PJI), as they have a synergistic anti-biofilm activity with antibiotics. We report here the case of an 88-year-old man (63 kg) with relapsing *Pseudomonas aeruginosa* prosthetic knee infection. The patient had severe alteration of the general status and was bedridden with congestive heart failure. As prosthesis explantation and/or exchange was not feasible, we proposed to this patient the use of phage therapy to try to control the disease in accordance with the local ethics committee and the French National Agency for Medicines and Health Products Safety (ANSM). Three phages, targeting *P. aeruginosa*, were selected based on their lytic activity on the patient's strain (phagogram). Hospital pharmacist mixed extemporaneously the active phages (initial concentration 1 ml of 1×10^{10} PFU/ml for each phage) to obtain a cocktail of phages in a suspension form (final dilution 1×10^{9} PFU/ml for both phages). Conventional arthroscopy was performed and 30 cc of the magistral preparation was injected through the arthroscope (PhagoDAIR procedure). The patient received intravenous ceftazidime and then oral ciprofloxacin as suppressive antimicrobial therapy. Under this treatment, the patient rapidly improved with disappearance of signs of heart failure and pain of the left knee. During the follow-up of 1 year, the local status of the left knee was normal, and its motion and walking were unpainful. The present case

suggests that the PhagoDAIR procedure by arthroscopy has the potential to be used as salvage therapy for patients with *P. aeruginosa* relapsing PJI, in combination with suppressive antimicrobial therapy. A Phase II clinical study deserves to be performed to confirm this hypothesis.

Keywords: bacteriophages, phage therapy, prosthetic-joint infection, *P aeruginosa*, phagotherapy

CASE REPORT

An 88-year-old man (63 kg) had a past history of arrhythmia with severe cardiomyopathy and bilateral arthroplasties several years ago. A colonoscopy was performed and was followed a few days later by clinical signs of septic arthritis of the left knee. The patient did not have fever, but CRP was ∼200 mg/L. Echocardiography disclosed no signs of endocarditis. Analysis of joint puncture showed infiltration by polymorphonuclear cells (57,000/mm^3) and *Pseudomonas aeruginosa* susceptible to ceftazidime and ciprofloxacin grew in culture. Open (i.e., by arthrotomy) Debridement Antibiotics and Implant Retention (DAIR) procedure was performed (1), followed by treatment with intravenous ceftazidime 6 g/day plus oral ciprofloxacin (500 mg bid). Three weeks after the surgery, the outcome seemed to be favorable, ceftazidime was stopped, and ciprofloxacin was prolonged for a total duration of 12 weeks. Six months later, the patient experienced a relapse of the joint knee effusion (**Figure 1A**), with heart failure. CRP was ∼100 mg/L. X-ray disclosed no loosening of the prosthesis (**Figure 1B**). A knee joint puncture showed *P. aeruginosa* persistence, with the same antimicrobial susceptibility profile. The patient was totally bedridden with severe alteration of the general status. As general anesthesia was contraindicated to explant the prosthesis or to perform a new open DAIR, we proposed to this patient the use of phage therapy to try to control the disease. After multidisciplinary meetings in our reference center (which is certified by the French ministry of health for the management of complex bone and joint infection), (2) and in accordance with the local ethics committee, this case was individually discussed with the French National Agency for Medicines and Health Products Safety (ANSM), to validate that no other options could be proposed without excessive risk of death. Phages, targeting *P. aeruginosa*, were selected from the Pherecydes Pharma library based on their lytic activity on the patient's strain (3). The phages have been produced in a non-GMP facility but have undergone a thorough quality evaluation with multiple quality control tests. Phagograms were performed using kinetic assay and the plaque assay, to calculate the efficiency of plating score (EOP) as previously described (**Figures 1C,D**) (4). Three bacteriophages (PP1450, PP1777, and PP1792) were selected, as they were totally or partially active for at least one technique. PP1450 and PP1777 belong to the *Myoviridae* family, and their closest relative in public database (Genbank) belong to the *Pbunavirus* genus (ICTV 2018). PP1792 belongs to the *Podoviridae* family and *Bruynoghevirus* genus. The patient signed a written consent, explaining the procedure and the risk/benefit ratio. Hospital pharmacist mixed extemporaneously the active phages [initial concentrations 1 ml of 1 × 10 (5) PFU/ml for each phage] to obtain a cocktail of phages in a suspension form [final concentration of 1 × 10 (6) PFU/ml for both phages]. Conventional arthroscopy was performed (**Figure 1E**) using anteromedial and anterolateral entry points and washing of joint with saline. After drainage of the arthroscopic liquid, 30 cc of the phage suspension was injected through the arthroscope. Then, entry points were closed to be waterproof. No other bacteria grew in culture. The patient received again 3 weeks of intravenous ceftazidime (6 g/day) and oral ciprofloxacin (500 mg bid). The patient rapidly improved with disappearance of signs of heart failure and pain of the left knee (**Supplementary Video 1**). The CRP reached normal values quickly. A subcutaneous nodule that has spontaneously ulcerated appeared on the external side of the knee (**Figure 1F**), without discharge or any communication with the joint, and then disappeared spontaneously. At 6 months, the local status of the left knee was normal (**Figure 1G**) and its motion and walking were unpainful (**Supplementary Videos 2**, **3**). The dose of ciprofloxacin was reduced to 250 mg bid as suppressive antimicrobial therapy to prolong the remission of symptoms (7). One year after the phage administration, the patient unfortunately died from lithiasic pancreatitis, without any clinical signs of prosthetic joint infection (PJI).

Bacteriophages are viruses that specifically target bacteria. They are considered to have a high potential in patients with PJI, as they have a synergistic anti-biofilm activity with antibiotics (8, 9). In several patients with relapsing chronic PJI due to *S. aureus* for whom explantation was not Possible, we already performed open DAIR and used selected bacteriophages that were injected into the joint (PhagoDAIR procedure) with a good clinical response (6, 10). Moreover, recent data from animal models provided further support for phage therapy as effective adjunctive treatment for PJI (5). In the present case, arthroscopic DAIR was the only possible surgery, to limit the risk of perioperative death, whereas this procedure is considered to have no place in the management of PJI due to (i) an incomplete debridement (peroperative dislocation is not feasible), (ii) an inability to exchange the polyethylene part of the prosthesis, and (iii) an extremely low success rate. In counterpart, it is easy to inject into the joint the bacteriophages preparation during arthroscopy, and the joint remained perfectly tight (6). The opportunity to target the biofilm is a potential key determinant in such patients if the prosthesis cannot be explanted. By using personalized phage therapy as adjuvant therapy, the aim is to act locally on bacteria embedded in biofilm sticked on the implant surface into the joint cavity, as demonstrated recently in animal and *in vitro* models (11).

This case report leads to question the intrinsic capacity of the phage therapy to improve the outcome of the patient, as

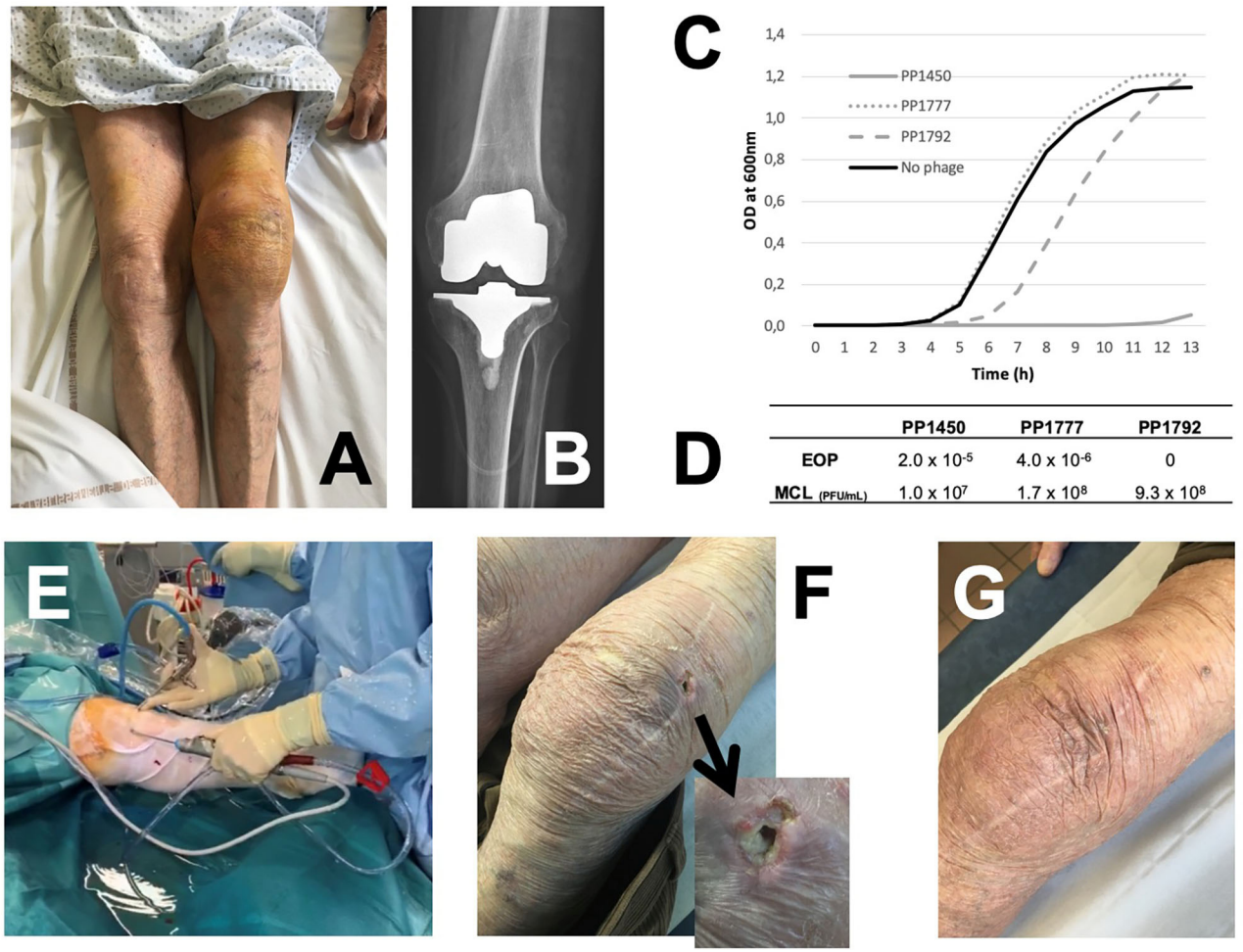

FIGURE 1 | (A) Left knee joint effusion due to relapsing *P. aeruginosa* prosthesis knee infection; **(B)** X-ray showing no prosthesis loosening. The susceptibility of the patient's strain to the bacteriophages PP1450, PP1777, and PP1792 (phagogram) was performed using two complementary techniques: **(C)** For the kinetic assay, phages were incubated at a theorical multiplicity of infection (MOI, ratio of phages/bacteria) equal to 100 with the patient's strain. PP1450 was able to inhibit the bacterial growth (gray full line); PP1792 delayed the bacterial growth (gray dotted line) and PP1777 had no impact (gray dashed line). **(D)** For the plaque assay, titers obtained with the patient's strain and the reference strain are determined to calculate the efficiency of plating score (EOP) score (the closer to 1 is the score, the more efficient the phage is). Phages PP1450 and PP1777 were active on the patient's strain with an EOP score of 2.0×10^{-5} and 4.0×10^{-6}, respectively. Partial lysis without PFU were observed for PP1792 (considered to have a weak bactericidal or bacteriostatic activity in this assay). **(E)** Arthroscopic DAIR with administration of the phage cocktail at the end of the procedure through the arthroscope. **(F)** Ulceration of a subcutaneous nodule on the external side of the knee observed 2 months after the arthroscopy. **(G)** Finally, a favorable outcome under suppressive antimicrobial therapy.

he was also managed with surgery and antibiotics. However, as the patient presented relapsing PJI after previous standard of care treatments, the expected success rate of iterative DAIR procedure performed by arthroscopy and followed by suppressive antimicrobial therapy was very limited if the bacteriophages had no effect on the biofilm. Indeed, arthroscopic DAIR is usually contraindicated in patients with PJI, as (i) the risk of relapse is particularly high if the polyethylene part cannot be changed, likely because such plastic surface promotes biofilm formation; (ii) the reduction of the bacterial load is significantly lower in comparison with open DAIR; and (iii) the evidence and guidelines discourage its use as too much worse outcomes were reported (1, 7, 11–16). Finally here, we hypothesized that the phage administration has helped the suppressive antimicrobial therapy to succeed in the control of the infection, i.e., to prolong the remission (15, 16).

The present data suggest that the PhagoDAIR procedure by arthroscopy has the potential to be used as salvage therapy for patients with *P. aeruginosa* relapsing PJI, in combination with suppressive antimicrobial therapy. A Phase II clinical study deserves to be performed to confirm this hypothesis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospices Civils de Lyon Ethic Committee. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

TF managed the patients and coordinated the treatment procedure. TF wrote the draft of the manuscript. CB, RG, and SL participated to the patient care. CK, C-AG, CF, and CP participated to the microbiological work. GL prepared the phage mix. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The patient was treated under the routine care in our regional reference center. The patients were treated under the supervision of the French Health ministry. Pherecydes Pharma provided the bacteriophages.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.569159/full#supplementary-material

Supplementary Video 1 | Video showing the patient walking without any pain 3 weeks after the surgery.

Supplementary Video 2 | Video showing the motion of the left knee without any pain 3 months after the surgery.

Supplementary Video 3 | Video showing the patient walking without any pain 3 months after the surgery.

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Conflict of Interest: CF and CP are employed by Pherecydes Pharma.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 10 May 2021
doi: 10.3389/fmed.2021.552669

Economic Study of 2-Stage Exchange in Patients With Knee or Hip Prosthetic Joint Infection Managed in a Referral Center in France: Time to Use Innovative(s) Intervention(s) at the Time of Reimplantation to Reduce the Risk of Superinfection

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Objective: Chronic prosthetic joint infections (PJI) are serious complications in arthroplasty leading to prosthesis exchange and potential significant costs for health systems, especially if a subsequent new infection occurs. This study assessed the cost of chronic PJI managed with 2-stage exchange at the Lyon University Hospital, CRIOAc Lyon reference center, France. A threshold analysis was then undertaken to determine the reimbursement tariff of a hypothetical preventive device usable at the time of reimplantation, which possibly enables health insurance to save money according to the risk reduction of subsequent new infection. This analysis was also performed for a potential innovative device already available on the market, a dual antibiotic loaded bone cement used to fix cemented prosthesis that releases high concentrations of gentamicin and vancomycin locally (G+V cement).

Method: Patients >18 years, admitted for a hip or knee chronic PJI managed with 2-stage exchange, between January 1, 2013, and December 31, 2015, were retrospectively identified. Following, resource consumption in relation to inpatient hospital stay, hospitalization at home, rehabilitation care, outpatient antibiotic treatments, imaging, laboratory analysis, and consultations were identified and collected from patient records and taken into account in the evaluation. Costs were assessed from the French health insurance perspective over the 2 years following prosthesis reimplantation.

OPEN ACCESS

Edited by:
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Reviewed by:
Carlo Luca Romano', University of Milan, Italy
Jesse Kuiper, Spaarne Gasthuis, Netherlands
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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *16 April 2020*
Accepted: *22 March 2021*
Published: *10 May 2021*

Citation:
Serrier H, Julien C, Batailler C, Mabrut E, Brochier C, Thevenon S, Maynard-Muet M, Henry A, Lustig S, Huot L, Ferry T and the Lyon BJI Study group (2021) Economic Study of 2-Stage Exchange in Patients With Knee or Hip Prosthetic Joint Infection Managed in a Referral Center in France: Time to Use Innovative(s) Intervention(s) at the Time of Reimplantation to Reduce the Risk of Superinfection. Front. Med. 8:552669. doi: 10.3389/fmed.2021.552669

Results: The study included 116 patients (median age 67 y; 47% hip prosthesis). Mean cost of chronic PJI was estimated over the 2 years following prosthesis reimplantation at €21,324 for all patients, and at €51,697 and €15,745 for patients with ($n = 18$) and without ($n = 98$) a subsequent new infection after reimplantation, respectively. According to the threshold analysis the reimbursement tariff (i) should not exceed €2,820 for a device which can reduce the risk of a new infection by 50% and (ii) was between €2,988 and €3,984 if the G + V cement can reduce the risk of a new infection by 80% (this reduction risk is speculative and has to be confirmed by clinical trials).

Conclusion: This study revealed that chronic PJI requiring a 2-stage revision is costly, with significant costs in relation to the reimplantation procedure (about 15 k€). However, following reimplantation the rate of subsequent new infection remained high, and the cost of reimplantation following a new infection is considerable, reaching 50k€ per patient. These first cost estimates of managing chronic PJI with 2-stage exchange in France underline the economic interest of preventing new infections.

Keywords: prosthetic-joint infection, cost analysis, prevention, antibiotics, cement, healthcare system, superinfection, bone and joint infection

INTRODUCTION

Infection is the most drastic complication following arthroplasty. In general, the risk of infection is considered to be low (1–2%), but increases by up to 50% in patients with a wide range of cumulative morbidities (1, 2). Debridement and implant retention with mobile part exchange of the prosthesis is the recommended treatment for patients with an acute prosthetic joint infection (PJI) (2, 3). In patients experiencing a relapse following debridement and implant retention or in patients with chronic PJI, a prosthesis revision, i.e., a 1-stage or a 2-stage exchange is recommended, to eradicate the bacteria embedded in biofilm at the surface of the implant. Two-stage exchange is the recommended strategy in the USA and remains a frequent strategy proposed in Europe for knee PJI and for most complex cases, despite more and more surgeons opting to perform 1-stage exchange, especially in France (2–8). PJI is considered to be one of the most costly infectious diseases to treat, as it requires at least one surgery, prolonged hospitalization, rehabilitation care, prolonged antibiotherapy, and extended absence from work in working-age patients. The mean total cost for the management and treatment of septic knee revision in Germany has been calculated to be $12,224 (€11,282) (9), while in the United Kingdom, the mean total costs associated with septic hip revision has been calculated as £21,937 (€24,117) (10). In a study undertaken within a Turkish University Hospital, the median cost of general arthroplasty procedures without PJI (including total hip, total knee, and shoulder) is estimated at $5,937 (€5,479) and increases to $16,999 (€15,689) when PJI occurs (11). When focusing on a two-stage revision, in the Portuguese context, the mean cost of PJI is €11,415 and €13,793 for hips and knees, respectively (12). In contrast, the additional cost associated with the treatment of a hip or knee PJI is estimated at €44,600 for a two-stage revision in Finland (13). Cost also seems to vary considerably depending on the type of pathogen involved, its resistance profile, and if the patient experienced a failure. For instance, in the USA in 2009, the estimated mean cost associated with methicillin-susceptible *Staphylococcus aureus* PJI was $68,053 (€62,823), whereas methicillin-resistant *S. aureus* PJI costs were significantly higher, at a mean of $107,264 (€99,021) per case (14). In Australia, the median cost of treating PJI per patient was AU$34,800 (€19,469), with a 156% increase in case of treatment failure (15). Finally, it is expected that the global cost of PJI will increase in coming decades, especially due to an increase in the absolute number of PJI cases, as the need for joint arthroplasty is expected to increase substantially with population demographic aging. In the USA, the annual cost to hospitals of revision surgery for infection increased from $320 million (€295 million) in 2001 to $566 million (€522 million) in 2009, and was projected to exceed $1.62 billion (€1.49 billion) by 2020 (16).

In this context, it seems essential to prevent septic failures in patients with PJI. These failures are mainly dominated by the onset of a new infection (also called superinfection) that occurs after the reimplantation in 15–30% of the patients for whom a 2-stage exchange was performed (7, 8, 17). To reduce the risk of superinfection, optimization of the classical measures of prevention such as systemic antimicrobial prophylaxis are mandatory at the time of reimplantation (2), and local additional interventions that may further decrease this risk have to be evaluated. In recent years, innovative prevention devices have been developed to prevent PJI. For example, some devices incorporate antibiotics into a bio-absorbable hydrogel or a cement, which can thus be delivered *in situ* (18, 28). Usable during the treatment of PJI or failure, they may increase the probability to avoid certain new infections and therefore reduce the costs of overall treatment. From a payer perspective, these devices could even be profitable, given the high cost of PJI and

Abbreviations: PJI, Prosthetic Joint Infection; HaH, hospitalization at home; PCM, primary care mode; ACM, associated care mode.

particularly chronic PJI. In this context, it is important to have high-quality analysis cost data (19) to show the economic impact of PJI, chronic or not, and to estimate costs that could be avoided by using an infection prevention device.

The aim of this study is to assess the cost of knee or hip chronic PJI managed with 2-stage exchange at the CRIOAc Lyon Reference Center. This center belongs to the French CRIOAc network, a nation-wide network with dedicated activity to manage complex bone and joint infection (20). A threshold analysis was then conducted to determine the reimbursement tariff of a hypothetical device usable at the time of reimplantation that would prevent new infection to a point which French health insurance saves money according to the risk reduction. In addition, as the G + V cement is a device already available on the French market and a candidate of interest in such a patient population to fix the cemented prosthesis and potentially contributes to reduce the rate of new infection, the threshold analysis was also performed for this potential innovative device.

METHODS

Study Characteristics and Data Collection

Patients aged 18 and over, admitted to the CRIOAc Lyon Reference Center for a hip or knee chronic PJI managed with 2-stage exchange, between January 1, 2013, and December 31, 2015, were retrospectively identified. Exhaustivity was checked using the data from the Lyon BJI cohort study. Information about the clinical (infection localization, new infection after reimplantation), demographic (age and gender), and data on resource consumption was collected directly from eligible patients' hospital records. In addition, information on patient care pathway and the outpatient resource consumption which is collected prospectively and recorded in the medical electronic charts as routine care in our institution was included. Information on the management of the osteoarticular infection was also collected, and patients were categorized as follows: explantation then reimplantation (category 1); 1st surgery (usually debridement and implant retention also called DAIR procedure), explantation then reimplantation (category 2); explantation, 2nd look (usually spacer exchange), then reimplantation (category 3); 1st surgery (usually DAIR), explantation, 2nd look (usually iterative DAIR), then reimplantation (category 4). A septic failure was defined in the study as the occurrence, after the reimplantation, of signs of infection (clinical signs of septic arthritis, discharge), leading to the diagnosis of a new episode of PJI (by joint puncture or need for revision). The Ethical Committee of the hospital approved the study (approval No. 17-089); clinical trial number NCT03612076.

Cost Analysis

A cost study on the 2-stage management of patients with hip or knee PJI at our institution was conducted from the perspective of the French health insurance. Only direct costs, related to the management and treatment of a 2-stage hip or knee procedure, were therefore taken into account and valued using tariffs. Even if the main part of the costs is accumulated during the first year following the reimplantation, a time horizon of 2 years from the reimplantation of the prosthesis was retained in order to take into account the entire impact on resource consumption.

To be exhaustive, our analysis took into account in- and also out-hospital costs including hospital stay, hospitalization at home (HaH), rehabilitation care, outpatient parenteral antimicrobial therapy (OPAT), oral antibiotic treatments, imaging, laboratory analysis, and consultations.

Hospital Stay

Data collected from patient files were used to extract information for each patient on all hospital stays from the medico-administrative database of the Hospices Civils de Lyon (program for medicalization of the information systems) during the 2 years following the reimplantation. This method allows us to have exhaustive data on hospital stay and information on the reimbursement tariff of each stay. Only stays related to the management of a hip or knee prosthesis including stays for recurrence and patient follow-up were included in this study. Each stay tariff includes the corresponding diagnosis related group tariff which pays for all the resources consumed during the stay (personnel, implant, laboratory analysis, and imaging) as well as expensive drugs and implantable medical devices that are not included in the diagnosis related group tariff. Of note, hospitalization of patients after the reimplantation is common in France, especially to remove the catheter used for intravenous antimicrobial therapy.

Hospitalization at Home and Rehabilitation Care

For HaH and rehabilitation care, the number of days for all stays was available but not the coding used to define the corresponding tariff.

For the HaH, the combination of codes that corresponded to the management of a hip or knee prosthesis was used to define a daily cost. This estimation was then used to value all HaH stays in our study. This association of codes correspond to one of the lowest tariffs possible for HaH, thus a conservative estimation.

For the rehabilitation care, an analysis of the medico-administrative database of two hospitals (Hospices Civils de Lyon and the Val Rosay Hospital) was undertaken corresponding to 311 stays, to estimate a daily cost. This estimation was then used to value all rehabilitation care stays in our study.

The assessment methodology of daily costs in HaH and rehabilitation care is detailed in **Supplementary Material 1**. These calculation assumptions were also tested in the sensitivity analysis.

Out-Hospital Costs

Outpatient oral and/or intravenous antibiotic treatments, consultations, imaging, and laboratory analysis were retained only if they were related to the management of the PJI and if they did not correspond to an episode of in-hospital care, as these costs would be included in the diagnosis related group tariff. Resource consumptions were valued using the current reimbursement tariff of the French health insurance.

Although there were a substantial number of laboratory analyses for each patient in the database, some of them would

have only a negligible impact on the overall result (tariff < 1€). Moreover, it was also difficult to determine which specific laboratory analyses were related to the disease of interest. Therefore, we chose to focus on the five biological checkups that are most frequently used for the management of hip or knee prosthesis: standard biology including complete blood count or hemogram, blood electrolytes, creatinine, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, bilirubine, and c reactive protein; cytochemistry of joint fluid; bacteriological examination; anatomopathological examination; antibiotic dosage. These five biological checkups were included in the analysis only when they were not included in a hospital stay and also valued according to the current reimbursement tariff of the French health insurance.

Statistical Analysis

Descriptive analysis was performed on the main characteristics of the population and cost results. A deterministic sensitivity analysis was carried out to test the impact of a modification of the main hypothesis on the result of the evaluation in order to test the uncertainty surrounding these choices.

Pricing information was not available for all stays in a rehabilitation hospital. A mean daily cost of around €255 was estimated based on available data and assigned to all rehabilitation care. The impact of a change of this value to €200 and €300 on the result was tested via the sensitivity analysis.

The discount rate used is 4%; the impact of a modification of this rate to 0% and 6% on our results was also tested.

Finally, to estimate the cost of HaH stays, coefficients corresponding to PCM 04 (post-surgical treatment), ACM 03 or ACM 11 (intravenous treatments or orthopedic rehabilitation), and to a Karnofsky index between 70 and 80% were used. To study the uncertainty around this choice, a more conservative assumption was tested with the same PCM but lowest coefficients for the ACM and the Karnofsky index.

Threshold Analysis

The objective of this exploratory analysis is to determine the reimbursement tariff per patient for a hypothetical innovative device, usable at the time of reimplantation, below which savings would have been made by the French health insurance if all the patients in our cohort had benefited from the product. However, this evaluation is not realistic since such a device cannot necessarily be used for all patients and will not offer the same effectiveness in all patients.

We have therefore also chosen to carry out this threshold analysis with a concrete example, the use of a cement that releases high concentrations of gentamicin and vancomycin (0.5 g of gentamicin and 2 g of vancomycin per bag of cement powder), referred to here as G+V cement, that has demonstrated *in vitro* its capacity to reduce biofilm formation (18). This device is a bone cement that could be used to fix prosthesis (21). To perform the threshold analysis, we first considered in our cohort, only patients with a cemented prosthesis and removed the cost of the cement used. Then, for patients with a new infection after reimplantation of the prosthesis, the pathogen in question were studied to identify among these infections, those for which the G + V cement could have been active. However, the fact that the product is active does not always mean that the infection would have been avoided. In the lack of clinical studies and *in vivo* data, we hypothesized that G+V cement avoids 80% of the infections for which it is active, based on *in vitro* analysis (22), and based on the wide spectrum of action of this antimicrobial combination (that is potentially active against most of Gram positive and Gram negative pathogens). We thus obtained a number of infections avoided whose cost of care gives us avoided cost attributable to G + V cement in our cohort, based on the drug susceptibility of the pathogens found to be responsible for the superinfection. Assuming that this device had been used for all patients with a cemented prosthesis of our cohort, we can thus estimate the reimbursement tariff of G + V cement below which the French health insurance saves money.

RESULTS

Patients' Characteristics

The number of patients included in the study is 116 with a mean age of 66 years old (see **Table 1**); all patients for whom a 2-stage exchange was performed during the study period were included, except one patient who declined consent. The population is composed of almost as many men ($n = 57$) as women ($n = 59$) and of slightly more patients with a knee ($n = 61$) than a hip prosthesis ($n = 55$). The vast majority (66%) of patients belong to the category 1 of osteoarticular infection management corresponding to "explantation then reimplantation." During the 2-stage procedure, 71 patients (61%) had a spacer; including 55 patients (77%) for a knee infection, and 16 patients (23%) for a hip infection. Of the 116 patients, 18 patients had a new infection after reimplantation. The main characteristics of the study population are detailed in **Table 1**. Of note, among the patients with a septic failure, we detailed their management and in particular the surgeries that had been undertaken for these patients in **Supplementary Material 2** [median time from reimplantation to failure: 8 weeks (IQR ± 25)]. Concerning the 98 patients without a septic failure, a new surgery was performed in four of them: hip dislocation and revision with a constraint liner at day 20 for one patient; tibial tubercle osteotomy screw removal at month 8 for another patient; patellar resurfacing and soft tissue repair at month 8 for another patient; and 1-stage revision for a mechanical issue at month 18 for the fourth patient.

Cost Analysis

The mean cost of 2-stage management care of patients with knee or hip PJI at the Hospices Civils de Lyon is estimated at €21,324 over 2 years from the reimplantation of the prosthesis (see **Table 2**). Hospital stays and rehabilitation care are the two main cost items. They represent, respectively, 34 and 61.52% of the total cost. Cost of antibiotics is very low because it only concerns antibiotics not included in a hospital stay. Most of them are delivered at the hospital and consequently included in the tariff of the stay.

There was at least one hospitalization for 75 patients in the first year of follow-up and for 21 patients in the second year. Only

TABLE 1 | Main characteristics of the study population ($n = 116$).

Characteristics	*N* (%) or mean or median, as appropriate	
Age		
	Mean (SD)	66 (13)
	Median (IQR)	67 (61–74)
Gender		
	Female	59 (50.86%)
	Male	57 (49.14%)
Charlson score		
	Mean (SD)	3.5
	Median (IQR)	3
Score ASA		
	Mean (SD)	2
	Median (IQR)	2
	1	25 (21%)
	2	61 (52%)
	3	29 (25%)
	4	3 (2%)
BMI		
	Mean (SD)	29
	Median (IQR)	28
Type of infection		
	Monomicrobial infection	76 (66%)
	Polymicrobial infection	21 (18%)
	Undocumented infections	18 (16%)
Type of the initial pathogen*		
	S. aureus	31 (27%)
	Coagulase-negative Staphylococci	26 (22%)
	Streptococcus spp.	13 (11%)
	Cutibacterium acnes	15 (13%)
	Enterococcus faecalis	5 (4%)
	Corynebacterium spp.	4 (3%)
	Pseudomonas spp.	1 (1%)
	Burkholderia spp.	1 (1%)
	Actinomyces neurrii	1 (1%)
	Veillonela spp.	1 (1%)
Osteoarticular infection categories		
	Category 1	76 (65.52%)
	Category 2	21 (18.1%)
	Category 3	13 (11.21%)
	Category 4	6 (5.17%)
Infection localization		
	Hip	55 (47.41%)
	Knee	61 (52.59%)

**at the time of explantation or at the time of DAIR before explantation.*

SD, standard deviation; IQR, interquartile range; ASA, American society of anesthesiologists; BMI, body mass index; DAIR, debridement antibiotics and implant retention.

Osteoarticular categories: explantation, then reimplantation (category 1); 1st surgery (usually debridement and implant retention also called DAIR procedure), explantation, then reimplantation (category 2); explantation, 2nd look (usually spacer exchange), then reimplantation (category 3), 1st surgery (usually DAIR), explantation, 2nd look (usually iterative DAIR), then reimplantation (category 4).

35 patients had no hospitalization during the 2 years of follow-up. The average duration of a new hospitalization is ∼8 days with a median of 5 days.

Only 11 patients had no stay in a rehabilitation hospital during the first year compared with 111 in the second year. The average length of stay in a rehabilitation hospital is 66 days with a median of 42 days.

The main part (80.69%) of the costs is accumulated in the first year following the reimplantation of the prosthesis (see **Table 2**). Mean cost of patient care is estimated at €17,207 for the first year and €4,117 euros for the second year.

Expensive drugs and implantable medical devices not included in diagnosis related group only represent 1.09% and 3.61% of the mean cost of hospital stays, respectively.

In terms of gender, the average cost of care is estimated at €22,932 for females and €19,660 for males (see **Table 3**).

Among the categories of osteoarticular infections management, category 4 corresponding to a 1st surgery, 2nd look, explantation, then reimplantation is the most expensive with a mean cost estimated at €47,611 per patient (see **Table 3**). The mean cost for category 1 patients (explantation, then a reimplantation), estimated at €18,329 per patient, is the lowest.

Mean costs are relatively close for patients with a knee infection and for those with a hip infection.

The mean cost is estimated at €51,697 for patients with a new infection after reimplantation and at €15,745 for patients without.

Threshold Analysis

Considering that all 116 patients of our cohort could have benefited from an innovative device that may prevent new infection, we estimated, according to the number of new infections avoided, the reimbursement tariff below which the French health insurance saves money (see **Table 4**). For example, if this innovative device avoids 50% of infections, nine patients would have had no infection. Therefore, the French health insurance could save money for a reimbursement tariff below €2,820 per patient.

Of the 18 patients with the new infection after reimplantation, based on the antibiogram, the G + V cement could have been active for 12 (see **Table 5**); however, only nine had a cemented prosthesis (3 hip and 6 knee prosthesis). In the hypothesis that G + V cement avoids 80% of the infections for which it is active, we estimate that the infection could have been avoided for 6 to 8 patients.

By removing the cost of the cement, the average cost of care is €52,020 for patients with infection after reimplantation against €15,669 for patients without. The cost that could have been avoided if the infection had been avoided is therefore estimated at €36,351 per patient.

According to these assumptions, the G + V cement cost per patient below which the avoided costs are higher than the extra costs is estimated between €2,988 and €3,984 (see **Table 6**).

TABLE 2 | Cost of care by follow-up year and type of resource consumption per patient.

	Year 1		Year 2 (discounted)		Two years cost per patient in €	
	Mean cost in € (SD)	**%**	**Mean cost in € (SD)**	**%**	**Mean cost in € (SD)**	**%**
Hospital stays	5,173 (8,988)	71.35	2,077 (6,799)	28.65	7,250 (12,713)	34
HaH	431 (1,949)	73.02	159 (1,714)	26.98	590 (2,569)	2.77
Rehabilitation care	11,313 (18,883)	86.24	1,805 (12,164)	13.76	13,118 (26,637)	61.52
Out-hospital costs						
Antibiotics	16 (45)	80.29	4 (24)	19.71	20 (50)	0.09
Consultations	61 (45)	75.65	20 (28)	24.35	81 (64)	0.38
Biology	165 (229)	81.57	37 (100)	18.43	203 (252)	0.95
Imagery	48 (70)	76.28	15 (29)	23.72	62 (84)	0.29
Total	17,207 (24,661)	80.69	4,117 (15,270)	19.31	21,324 (33,457)	100

TABLE 3 | Subgroup analysis of costs.

			Two years cost in €		
		N	**Mean (SD)**	**Median (IQR)**	**Min-Max**
Total		116	21,324 (33,457)	11,677 (5,033–24,325)	743–253,742
Gender					
	Female	59	22,932 (31,492)	14,307 (8,559–24,484)	743–204,917
	Male	57	19,660 (35,580)	8,957 (4,701–16,783)	897–253,742
Osteoarticular infection					
	Category 1	76	18,329 (19,005)	12,269 (5,938–24,325)	743–117,977
	Category 2	21	22,506 (55,524)	3,966 (2,556–13,363)	1,838–253,742
	Category 3	13	24,792 (19,149)	21,131 (10,678–36,342)	1,078–64,438
	Category 4	6	47,611 (78,184)	109,56 (8,502–34,977)	8,167–204,917
Infection localization					
	Hip	55	22,152 (32,516)	14,208 (5,782–23,269)	897–204,917
	Knee	61	20,577 (34,535)	10,678 (4,981–24,802)	743–253,742
New infection after reimplantation					
	Without	98	15,745 (18,144)	10,369 (4,957–17,756)	753–117,977
	With	18	51,697 (67,361)	36,623 (18,050–45,025)	1,078–253,742

Osteoarticular categories: explantation, then reimplantation (category 1); 1st surgery (usually debridement and implant retention also called DAIR procedure), explantation, then reimplantation (category 2); explantation, 2nd look (usually spacer exchange), then reimplantation (category 3), 1st surgery (usually DAIR), explantation, 2nd look (usually iterative DAIR), then reimplantation (category 4).

TABLE 4 | Reimbursement tariff of the preventive innovative device per patient in € below which health insurance saves money depending on the number of avoided infections.

Numbers of patients with avoided infection	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Threshold per patient in €	313	627	940	1,253	1,567	1,880	2,194	2,507	2,820
Numbers of patients with avoided infection	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Threshold per patient in €	3,134	3,447	3,760	4,074	4,387	4,701	5,014	5,327	5,641

TABLE 5 | Description of the pathogens and their susceptibility to gentamicin + vancomycin combination for the 18 patients with a failure after prosthesis reimplantation.

ID patient	Pathogen identified	S* to G + V	Pathogen identified	S* to G + V	Pathogen identified	S* to G + V	Pathogen identified	S* to G + V	Pathogen identified	S* to G + V	Conclusion about the potential activity of the G + V cement
1	*P. aeruginosa*	No									No
2	*Streptococcus***	Yes									Yes
3	Without documentation	UNK									UNK
4	MRSE	Yes									Yes
5	*E. aerogenes*	Yes	*P. aeruginosa*	Yes							Yes
6	*MDR E. cloacae*	Yes	*E. faecalis*	Yes							Yes
7	*MDR K. pneumoniae*	No									No
8	MRSE	Yes									Yes
9	*C. albicans*	No	*K. pneumoniae*	Yes	*C. tuberculostearicum*	Yes					No
10	*E. cloacae*	No	*E. faecium*	Yes							No
11	MRSE	Yes									Yes
12	MSSA	Yes	MSSE	Yes							Yes
13	*S. agalactiae*	Yes									Yes
14	MSSA	Yes									Yes
15	*S. lugdunensis*	Yes									Yes
16	MRSE	Yes									Yes
17	MSSA	Yes	*S. agalactiae*	Yes	*F. magna*	yes					Yes
18	MSSA	Yes	*B. fragilis*	No	*C. koserii*	Yes	*P. mirabilis*	No	*F. magna*	Yes	No

*MRSE, methicillin-resistant S. epidermidis; MDR, multidrug-resistant; MSSA, methicillin-susceptible S. aureus; MSSE, methicillin-susceptible S. epidermidis; *Susceptible to the combination gentamicin plus vancomycin; **obtained by PCR.*

TABLE 6 | G + V cement cost threshold per patient in € below which health insurance saves money depending on the number of avoided infections.

Numbers of patients with avoided infection	1	2	3	4	5	6	7	8	9
Threshold per patient in €	498	996	1,494	1,992	2,490	2,988	3,486	3,984	4,482

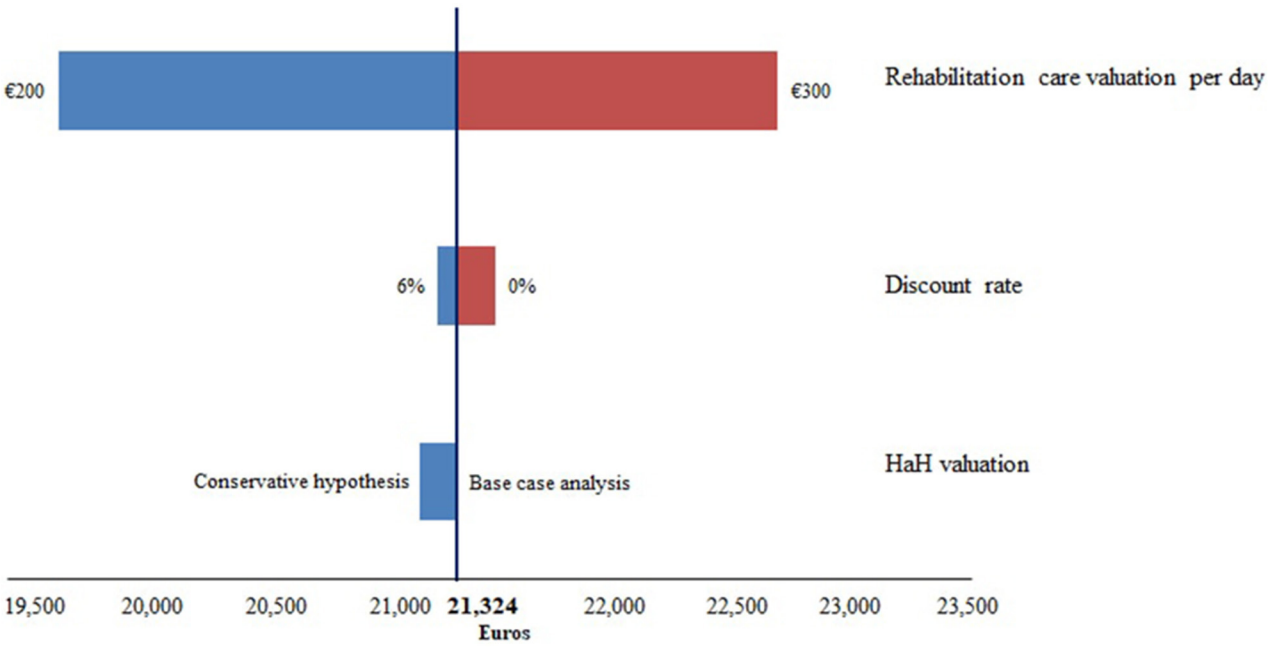

FIGURE 1 | Tornado diagram on the impact of methodological choices on the mean cost of care per patient.

Deterministic Sensitivity Analysis

The discount rate has a low impact on the mean cost of patient care. If the rate varies from 6 to 0% the mean cost increases from €21,246 euros to €21,488 (see **Figure 1**).

The choice concerning the HaH valuation also has little impact on the results. The transition to a conservative hypothesis reduces the average cost of €157.

The choice that impacts the most the results is the valuation of rehabilitation care. For daily costs of rehabilitation care of €200 and of €300, the mean costs per patient are estimated, respectively, at €19,635 and €22,688.

DISCUSSION AND CONCLUSION

The mean cost of 2-stage management care of patients with knee or hip PJI at the CRIOAc Lyon was estimated at €21,324 over 2 years from the reimplantation of the prosthesis. Through the exhaustive collection of all in- and out-hospital resource consumption, we demonstrate that the main component of the costs is accumulated in the first year following the reimplantation of the prosthesis. Hospital stays and rehabilitation care are by far the two main cost items. We find that the mean cost of management care is estimated at €51,697 for patients with a subsequent new infection after reimplantation and at €15,745 for patients without infection after reimplantation. Even if the sample size of patients with a new infection is small ($N = 18$), the difference in the cost of treating a patient with a new infection compared to a patient without a new infection is substantial (€35,952). Beyond the individual consequences for the patient that includes a potential loss of function due to the management of a septic relapse, this cost is considerable for the French health care system.

Our study is the only one to assess the cost of PJIs in the French context from a health insurance perspective. Our results are not comparable with those of the literature. Without being exhaustive, we have, however, attempted to discuss the consistency of our result by comparing them first with available French studies and then foreign studies whose objective and methodology are closest to this study.

A study previously evaluated the cost of hip PJI in France (23), but this study was conducted from the perspective of the hospital and not of the payer as in this study. Costs are therefore valued using the production cost when possible and not the tariff. The mean cost of hip PJI was thus estimated at €32,546 against €22,152 in our study (including patient with and without new infection after reimplantation). We estimated

the mean cost of hip or knee PJI at €15,745 for patients without new infection after reimplantation. The mean total costs for septic revision were £21,937 (€24,117) for total hip replacements in United Kingdom (10) and $12,224 (€11,282) for total knee arthroplasty in Germany (9). The median cost of arthroplasty (including total hip, total knee, but also shoulder) was estimated at $16,999 (€15,689) with PJI in a Turkish University hospital (11). Focusing on a two-stage revision, in the Portuguese context the mean cost of PJI was €11,415 and €13,793 for, respectively, hips and knees (12). Despite the differences in term of context and perspective, our mean hip or knee PJI cost estimate seems to be consistent with the literature.

We estimated the excess mean cost at €51,697 for hip or knee patients with a new infection after reimplantation. In the study of Puhto et al. (13), 8 patients who failed on debridement, antibiotics, and implant retention were treated in a two-stage revision. The excess cost of a hip or knee PJI was estimated at €44,600 for these patients in Finland. Peel et al. (15) estimated the mean cost of hip or knee PJI with a failure at AU$66,426 (€36,462). The costs are evaluated from the payer perspective, over a period of 3 years, but does not take into account rehabilitation care costs, which our study found to be a significant consumption of resources. The use of rehabilitation care is undoubtedly more important in the event of a new infection, which could partly explain this difference.

Furthermore, the sensitivity analysis shows that the valuation of rehabilitation care has the greatest impact on the mean cost of care. However, the value we retained is robust since it is based on the data of 311 stays from administrative databases of two hospitals (Hospices Civils de Lyon and at the Val Rosay hospital). The uncertainty, relative to our assumptions, which surrounds our results is therefore relatively low.

One of the main limitations of our results is the low number of patients with a new infection after reimplantation, which makes the comparison with patients without a new infection statistically fragile. Only a bootstrap would have enabled statistically robust comparisons to be made but our sample of patients with a new infection is also too low to perform resampling in a robust way. However, the difference between the two groups is substantial in terms of cost (€35,952) and could probably be confirmed in a future study on a larger sample.

In line with the perspective retained in our analysis, that of the French health insurance, we do not take into account the loss of patient productivity or the costs of informal care provided to the patient. Our estimates therefore do not reflect the societal burden of PJI, which is undoubtedly much greater.

As a consequence, prevention of infection is crucial in patients managed for a PJI. As our Reference Center has already set up all the recommended prophylaxis guidelines by using a checklist that includes WHO SSI prevention recommendations (24), innovative approaches by using particular devices that have the ability to act locally to reduce the rate of post-operative infection is now required for such patients. New generations of cement that release a combination of high doses of antimicrobials are candidates for that purpose. Based on clinical data from arthroplasty registers published in the early 2000s, systemic antibiotics combined with gentamicin loaded cement in patients for whom a cemented prosthesis is required are considered to be the most effective prophylaxis against deep infection (25). Recently, high dose dual antibiotic impregnated cements have been developed, such as a cement that releases a combination of high concentrations of gentamicin and clindamycin antibiotics. A quasi-randomized study showed that the rate of infection was lower when using this cement in comparison with standard low dose gentamicin cement in patients for whom hemiarthroplasty was performed following hip fracture, a patient group generally susceptible to PJI (26). In patients with PJI managed by a 2-stage approach, the rate of clindamycin-resistant and multidrug-resistant pathogens is particularly high and the spectrum of activity of the combination of gentamicin plus vancomycin seems to be more appropriate. *In vitro*, recent results suggest that the G + V cement, which is a bone cement available on the market that could be used to fix prosthesis and release a combination of high concentrations of gentamicin and vancomycin antibiotics, increases the anti-biofilm prophylactic effect compared to cement loaded with gentamicin alone (22). These findings were especially relevant for clinical strains of *S. aureus* and gentamicin-resistant staphylococci. A gentamicin + clindamycin (G + C) cement was also tested *in vitro* in this study and of note, G + C cements are also available in the market. The tested G + C cement has also anti-biofilm prophylactic effect *in vitro*, but clindamycin resistance is much more common than vancomycin resistance (27). As a consequence, the spectrum of activity of G + V cements seems to be more interesting, even if the dose of gentamicin, in the tested G + C cement, is higher in comparison with that of the G + V cement. Here, based on the antibiogram of the pathogen responsible for the new infection in patients managed with a 2-stage approach, there would be an added value in using the G + V cement. As a consequence, this cement is of interest in such a population, to fix the cemented prosthesis and to potentially contribute to reduce the rate of new infection.

The threshold analyses performed here are of importance as depending on the preventive efficacy of the intervention, the money saved could be calculated. For instance, if all patients within our cohort had benefited from an innovative device that prevents 50% of new infection, the French health insurance would have saved money for a reimbursement tariff at or below €2,820 per patient, while G + V cement is cost effective if it is less than between €2,988 and €3,984 per patient, depending on the hypothesis based on the number of new infections avoided. Therefore, the additional cost related to the use of G + V cement in the population studied would have been offset by the new infections avoided and their associated costs.

This study represents to date the only assessment of the cost of chronic hip or knee PJI in France, but it has some limitations. Comparisons of our results with the literature are somewhat complicated because the methodologies (perspective, time horizon, costs taken into account, etc.) and health systems are different. Although the sample size is small and even if our study is monocentric, the data presented here are the first cost estimates of 2-stage management care of patients with knee or hip chronic PJI in France, and underline the economic interest of preventing new infections after reimplantation. Finally, clinical studies are crucial to confirm the measurable efficacy of a

device of interest, and the proposed calculator will be a valuable tool to set the correct price for such a medical device in this specific application.

To conclude, this study revealed that chronic PJI requiring a 2-stage revision is a costly indication, with a significant cost of the reimplantation procedure alone (∼15 k€ in patients without a new infection). However, the rate of new infection continues to remain high, and the additional cost of reimplantation following a new infection is considerable, reaching ∼50 k€ per patient. These first cost estimates of knee or hip chronic PJI managed in 2-stage exchange in France underline the economic interest of preventing subsequent new infections, especially by using cost effective innovative devices that need to be evaluated in prospective studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospices Civils de Lyon ethic committee. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HS, CJ, EM, CBa, ST, MM-M, LH, and TF worked on the study conception and design. CJ, EM, CBr, ST, MM-M, and TF contributed to acquire data. HS analyzed data. HS and TF have been involved in drafting the manuscript. CBa, AH, and SL revised critically the manuscript. All authors read and approved the manuscript final version to be published.

FUNDING

Hospices Civils de Lyon received a research grant by Heraeus Medical GmbH to perform the study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.552669/full#supplementary-material

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Conflict of Interest: TF received a speaker honorarium from Heraeus Medical GmbH in 2017 [Symposium EBJIS 2017; Rationale for the use of local antibioticS in patients with septic pseudarthrosis (septic non-union)].

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 14 May 2021
doi: 10.3389/fmed.2021.550853

Arthroscopic "Debridement and Implant Retention" With Local Administration of Exebacase (Lysin CF-301) Followed by Suppressive Tedizolid as Salvage Therapy in Elderly Patients for Relapsing Multidrug-Resistant *S. epidermidis* Prosthetic Knee Infection

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Exebacase, a recombinantly produced lysin has recently (i) reported proof-of-concept data from a phase II study in *S. aureus* bacteremia and (ii) demonstrated antibiofilm activity *in vitro* against *S. epidermidis*. In patients with relapsing multidrug-resistant (MDR) *S. epidermidis* prosthetic knee infection (PKI), the only surgical option is prosthesis exchange. In elderly patients who have undergone several revisions, prosthesis explantation could be associated with definitive loss of function and mortality. In our BJI reference regional center, arthroscopic debridement and implant retention with local administration of exebacase (LysinDAIR) followed by suppressive tedizolid as salvage therapy is proposed for elderly patients with recurrent MDR *S. epidermidis* PKI with no therapeutic option or therapeutic dead end (for whom revision or transfemoral amputation is not feasible and no other oral option is available). Each use was decided in agreement with the French health authority and in accordance with the local ethics committee. A written consent was obtained for each patient. Exebacase (75 mg/mL; 30 mL) was administered directly into the joint during arthroscopy. Four patients (79–89 years old) were treated with the LysinDAIR procedure. All had several previous prosthetic knee revisions without prosthesis loosening. Three had relapsing PKI despite suppressive antibiotics following open DAIR. Two had clinical signs of septic arthritis; the two others

OPEN ACCESS

Edited by:
Marc Jean Struelens, Université libre de Bruxelles, Belgium

Reviewed by:
Carol Bernhard Strahm, Kantonsspital St. Gallen, Switzerland
Melchor Riera, Hospital Universitario Son Espases, Spain

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *10 April 2020*
Accepted: *31 March 2021*
Published: *14 May 2021*

Citation:
Ferry T, Batailler C, Souche A, Cassino C, Chidiac C, Perpoint T, le Corvaisier C, Josse J, Gaillard R, Roger J, Kolenda C, Lustig S, Laurent F and the Lyon BJI Study Group (2021) Arthroscopic "Debridement and Implant Retention" With Local Administration of Exebacase (Lysin CF-301) Followed by Suppressive Tedizolid as Salvage Therapy in Elderly Patients for Relapsing Multidrug-Resistant S. epidermidis Prosthetic Knee Infection. Front. Med. 8:550853. doi: 10.3389/fmed.2021.550853

had sinus tract. After the LysinDAIR procedure, no adverse events occurred during arthroscopy; all patients received daptomycin 8 mg/kg and linezolid 600 mg bid (4–6 weeks) as primary therapy, followed by tedizolid 200 mg/day as suppressive therapy. At 6 months, recurrence of the sinus tract occurred in the two patients with sinus tract at baseline. After >1 year follow up, the clinical outcome was favorable in the last two patients with total disappearance of clinical signs of septic arthritis even if microbiological persistence was detected in one of them. Exebacase has the potential to be used in patients with staphylococci PKI during arthroscopic DAIR as salvage therapy to improve the efficacy of suppressive antibiotics and to prevent major loss of function.

Keywords: lysin, prosthetic-joint infection, tedizolid, staphylococci, *S. epidermidis*, bacteriophage

INTRODUCTION

Prosthetic joint infection (PJI) is the most dramatic complication after joint arthroplasty. *S. aureus* and coagulase-negative staphylococci are frequently involved in patients with PJI (1). These bacteria could be involved in recurrence as they can produce biofilm and persist at the implant surface (2). In patients with acute staphylococci PJI, the recommended medico-surgical strategy is to perform an open debridement antibiotics and implant retention (DAIR) with exchange of the mobile polyethylene part, followed by an antibiotic regimen that includes rifampin, which demonstrates antibiofilm activity (3–5). Arthroscopic DAIR is contraindicated in patients with PJI as (i) the risk of relapse is particularly high if the polyethylene part cannot be changed, likely because such a plastic surface promotes biofilm formation, and (ii) the reduction of the bacterial load is significantly lower in comparison with open DAIR (6–8). In patients with chronic PJI, the recommended strategy is to exchange the prosthesis, in a one- or two-stage procedure, to mechanically eradicate the biofilm (3–5). In patients with relapsing or chronic staphylococci PJI, prosthesis explanation is sometimes not feasible, especially for the knee location in elderly patients with multiple comorbidities for whom explantation could be associated with a dramatic loss of function, reduction of the bone stock, fracture or peroperative death. Indeed, explantation without reimplantation, also called resection arthroplasty or the Girldestone procedure, is possible for the hip but not for the knee. Open DAIR is sometimes proposed for patients with relapsing or chronic staphylococci PJI, but as the risk of relapse is particularly high due to the bacterial persistence in biofilm, these patients are candidates for suppressive antibiotic treatment (SAT) (3–5). SAT consists of daily oral intake of active antibiotic to suppress the infection, i.e., to alleviate the symptoms and prevent the progression of the infection without hope for eradication. In cohort studies, the outcome is favorable in 30–70% of patients, depending on the patient profile, the pathogen involved, the drug used, and the duration of follow up (9–14). Doxycycline, cotrimoxazole, or cephalexin are the most frequently used drugs for SAT in staphylococcal PJI (9, 11, 12, 14). In patients with multidrug-resistant (MDR) coagulase negative staphylococci PJI, linezolid is frequently the only oral active drug. However, its use is associated with a significant toxicity when prescribed for >28 days (15, 16). In this context, the use of new adjuvant therapies is of great interest and may improve the stabilization of medical conditions of patient with PJI.

Lysins are cell wall hydrolase enzymes produced by bacteriophage during their lytic circle (17). As recombinantly produced proteins, lysins trigger rapid peptidoglycan hydrolysis, osmotic lysis, and cell death upon contact with bacteria. In contrast, antibiotic-mediated killing may require up to several hours. Lysin exebacase (CF-301) is an anti-staphylococcal lysin with potent bactericidal activity against *S. aureus* and additionally against coagulase-negative staphylococci. Exebacase is, furthermore, shown to disrupt mature biofilms formed by a wide range of methicillin-sensitive and -resistant *S. aureus* (MSSA) isolates as well as coagulase-negative staphylococci (18). The ability of exebacase to both eradicate biofilm biomass and kill bacteria in biofilm is demonstrated on a variety of surfaces, including catheters (19). Recently, a phase 2 superiority design clinical study, performed in adult patients to evaluate safety, tolerability, efficacy, and PK of exebacase when used in addition to standard-of-care (SoC) antibiotics for the treatment of *S.aureus* bacteremia, including endocarditis, revealed an improved outcome in patients receiving exebacase (20).

In France, 10 years ago, the ministry of health implemented a network of nine reference regional centers called "CRIOAc" to promote the research and management in the field of complex bone and joint infections. In our center, various strategies have been developed to try to control chronic infections in patients with PJI for whom prosthesis revision is not feasible (21). Some patients have been treated with therapeutic GMP/GMP-like-produced bacteriophages targeting *P. aeruginosa* or *S. aureus*. Unfortunately, no therapeutic bacteriophages active against *S. epidermidis* are available for compassionate treatment in France (22, 23), whereas we have had patients with relapsing MDR *S. epidermidis* prosthetic knee infection (PKI) who experienced iterative relapses, sometimes under SAT, after open DAIR. For such patients at a therapeutic dead end, we proposed arthroscopic DAIR with local administration of exebacase (LysinDAIR procedure) based on its antibiofilm activity against *S. epidermidis*, as compassionate treatment, followed by suppressive tedizolid as salvage therapy.

METHODS

Based on the use of exebacase in France for the phase 2 study in bacteremia, individual requests were done for successive patients to the French Health Authority, *Agence Nationale de Sécurité du Médicament et des Produits de Santé* (ANSM), to gain approval to perform LysinDAIR. In accordance with the local ethics committee, each case was discussed individually during multidisciplinary meetings in our CRIOAc center and then with ANSM to be sure that no other options associated with considerable loss of function or risk of death could be proposed. Exebacase MICs were evaluated for the *S. epidermidis* PJI clinical strains isolated from patient samples, using the Clinical and Laboratory Standards Institute (CLSI)-approved medium CAMHB-HSD composed of cation-adjusted Mueller-Hinton broth (CAMHB, BD BBL^{TM}) supplemented with 25% horse serum (Sigma-Aldrich) and 0.5 mM DTT (Dithiothreitol, Sigma-Aldrich) (24). MICs were determined after 18 h of incubation at 37°C as previously described (25). After authorization for compassionate use of exebacase from health authorities, a written consent was obtained for each patient, and the surgery planned. Conventional arthroscopy was performed, using anteromedial and anterolateral entry points, allowing sampling for bacterial cultures (joint fluid, synovial, and bone tissue) and washing of the joint with saline. In patients with a sinus tract, its resection (fistulectomy) was systemically performed. After drainage of the arthroscopic liquid, 30–50 cc of a solution containing Exebecase diluted in glucose 5% (final concentration 0.075mg/mL) were injected. Then, entry points were closed to be waterproof.

RESULTS

Four patients (79–89 years old) with significant comorbidities were treated with the LysinDAIR procedure as salvage therapy (**Figures 1**–**4**). All had undergone several previous prosthetic knee revisions without prosthesis loosening (**Figures 1A**–**4A**). Three had relapsing PKI despite suppressive antibiotics following open DAIR. Two had clinical signs of septic arthritis (**Figures 2B**, **4B**); the two others had sinus tract (**Figures 1B**, **3B**). All patients were infected only by *S. epidermidis* that expressed different drug susceptibilities over time, likely due to small colony variant phenotype and/or co-infection with different strains of *S. epidermidis* (**Table 1**). Despite the fact that past isolates were no more available for further drug susceptibility testing, based on the previous and current antimicrobial susceptibility test and patients' comorbidities, tedizolid was the only drug candidate to be used as potential SAT. Exebacase MIC values are detailed in **Table 1**. No adverse events occurred during arthroscopy (**Figure 1C**). The biofilm was clearly visible during arthroscopy for one patient (**Figure 1D**). All patients received intravenous daptomycin (8 mg/kg) immediately after arthroscopy and oral linezolid (600 mg bid) for 4–6 weeks, followed by oral tedizolid 200 mg/day (one pill) as suppressive therapy. During the treatment, two patients developed eosinophilic pneumonia attributed to daptomycin, one patient experienced diarrhea under linezolid therapy (without *C. difficile* infection), and another patient developed worsening of a previous thrombopenia under linezolid therapy. No adverse event was noticed under tedizolid treatment, in particular, neither myelotoxicity nor neurotoxicity. At 6 months, under tedizolid therapy, recurrence of the sinus tract occurred in the two patients with sinus tract at baseline (**Figure 1E**, **Figure 3C**). After >1 year of follow up (respectively, 14 and 16 months), the clinical outcome was decidedly favorable for the two last patients with complete disappearance of clinical signs of septic arthritis (**Figures 2C**, **4C**). As a mild joint effusion persisted in one of them (patient 2), a joint puncture was performed. Surprisingly, it revealed the persistence of the *S. epidermidis*, that remained susceptible to linezolid and tedizolid.

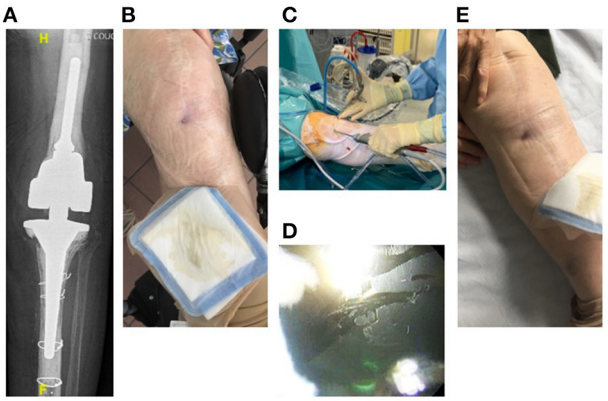

FIGURE 1 | Patient 1 was an 89-year-old woman with past history of recurrent lymphoma, splenectomy, and iterative prosthetic left knee revisions due to relapsing MDR *S. epidermidis* infection (04/02/2016). She had large constrained cemented prosthesis without loosening **(A)** and sinus tract **(B)**. She experienced a relapse under SAT (pristinamycin plus doxycyclin) following open DAIR 1 year ago. She was treated according to the LysinDAIR procedure **(C)**. The biofilm was visible at the surface of the implant during the DAIR procedure [08/11/2018; **(D)**]. She received daptomycin intravenously and linezolid orally, followed by tedizolid as SAT. At 6 months, still receiving tedizolid therapy, a new discharge occurred through the sinus tract **(E)**.

DISCUSSION

We report the compassionate use of exebacase administered locally during arthroscopy in four patients with relapsing MDR *S. epidermidis* PKI. This use is based on the crucial need for adjuvant therapeutic innovation for the management of patients with PKI, especially if MDR staphylococci are involved and if prosthesis revision is not feasible. Indeed, explantation without reimplantation (resection arthroplasty, also called the Girdlestone procedure) is, in theory, not acceptable for the knee location, whereas it is a possible option in patients with chronic prosthetic hip infection. Goldman et al. recently reports the functional outcome of patients with definitive resection arthroplasty of the knee, and even if this procedure facilitated the cure of the infection, all patients had residual pain, instability, and needed hinged orthosis with limited mobility. Arthrodesis using a silver-coated Arthrodesis implant or performing transfemoral

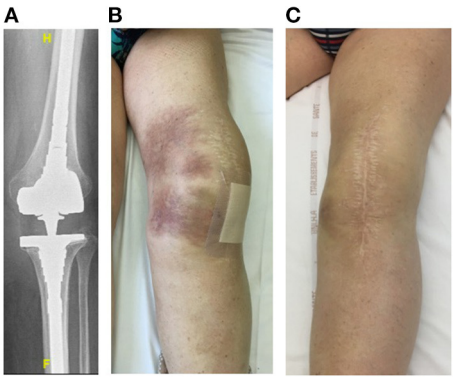

FIGURE 2 | Patient 2 was a 79-year-old man with history of severe ankylosing spondylitis under corticosteroids who presented a chronic left PKI due to *S. hominis* that was treated with a one-stage exchange. A postoperative infection occurred due to MDR *S. epidermidis* (02/12/3013) treated with open DAIR and SAT (minocycline followed by cotrimoxazole due to occurrence of a clinical relapse under minocycline therapy; 10/11/2013). He had a cementless revision prosthesis with long stem with no loosening **(A)** and clinical signs of septic arthritis (large joint effusion, pain during mobilization, skin inflammation without sinus tract) **(B)** and *S. epidermidis* grew from joint puncture (9/13/2018). He was treated according to the LysinDAIR procedure (08/11/2018), and a septic collection communicating with the joint was drained. He received daptomycin intravenously and linezolid orally and experienced eosinophilic pneumonia attributed to daptomycin and diarrhea attributed to linezolid. Then, tedizolid was prescribed as SAT, and the outcome was favorable with disappearance of the clinical signs of septic arthritis **(C)**. At 12 months, as a mild joint effusion persisted, a joint puncture was performed, and surprisingly, *S. epidermidis* was still present in culture. At the time of writing (16 months of follow-up after the LysinDAIR procedure), the clinical outcome was still favorable under tedizolid therapy, and the patient was able to resume golf.

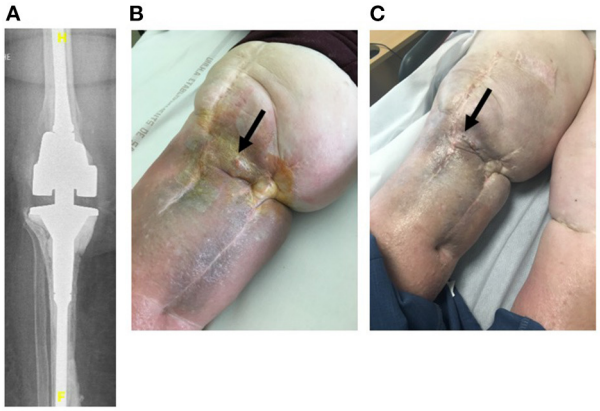

FIGURE 3 | Patient 3 was an 88-year-old woman with a current history of active chronic myeloid leukemia under imatinib therapy requiring iterative blood perfusions. She also had chronic kidney disease with indication of dialysis that the patient refused. She also had chronic lymphoedema. She had a right-cemented revision prosthesis **(A)** following a two-stage exchange for PKI due to MDR *S. epidermidis* (04/02/2011). Unfortunately, *S. epidermidis* persistence was diagnosed (6/7/2018), and a sinus tract occurred **(B)**. Cotrimoxazole as SAT was contraindicated due to the kidney disease. The patient received intermittent antimicrobial therapy with pristinamycin when skin and soft tissue inflammation occurred around the sinus tract **(B)**. She was treated according to the LysinDAIR procedure (10/01/2019). She received daptomycin intravenously and linezolid orally and experienced severe thrombopenia (30 G/L) leading to discontinuation of linezolid and a switch to tedizolid. Unfortunately, at 6 months, under tedizolid therapy, a new discharge occurred trough the sinus tract **(C)**.

amputation are other surgical options (26, 27). The latter option is associated to a catastrophic outcome and needs to be absolutely avoided (27).

SAT is seen as an alternative strategy for cases of PJI in which prosthesis explantation, i.e., biofilm eradication, could not be performed. SAT consists of the indefinite administration of antibiotics, and its goal is to control the infection, i.e., to reduce and ideally make disappear the clinical symptoms and slow down the occurrence of mechanical complications, such as prosthesis loosening. SAT is an infrequent therapeutic option but could be of importance in the elderly (9–14). The Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) states that the following conditions need to be met for the indication of SAT: (i) identification of the microorganism causing the infection, (ii) availability of oral antibiotics that are not toxic when administered over long periods of time, and (iii) possibility of close follow-up of the patient. This group states that it is reasonable to think that reducing the bacterial inoculum and debriding the infected tissues may favor the success of SAT and that a new debridement would allow the taking of good-quality tissue samples for culture before starting SAT (5). It is not known if the use of antibiofilm agents just after surgery and before prescribing SAT could facilitate the rate of success of SAT. The benefit of using rifampin at the initial phase of treatment of SAT is not clear as discussed in the Infectious Diseases Society (IDSA) guidelines that proposed to use cotrimoxazole or minocycline or doxycycline as SAT in patients with MDR staphylococci PJI (3).

Here, in this context, patients experiencing relapsing PKI despite previous prosthesis revision and open DAIR followed by SAT were selected for an innovative DAIR approach in including local phage therapy. We proposed arthroscopic DAIR to limit the risk of perioperative complications and the risk of superinfection. Indeed, arthroscopic DAIR is usually contraindicated in patients with PKI, but in the present cases, it facilitates the use of an antibiofilm agent that could be injected during the DAIR procedure. Thus, it is easy to inject into the joint a solution during arthroscopy, and the tightness of the joint is considerably better after arthroscopy in comparison with arthrotomy with less leakage of joint fluid through the scar. The use of exebacase is based on the fact that it has demonstrated an *in vitro* antibiofilm activity on *S. aureus* and against *S. epidermidis* strains in various models, such as *in vitro* models on polystyrene, glass, surgical mesh, and catheter (19). Moreover, it demonstrated *in vitro* synergy with a broad range of antibiotics against both methicillin-susceptible and -resistant *S. aureus* (18). Exebacase also is shown to be more active in combination with daptomycin than daptomycin or exebacase alone to treat methicillin-resistant *S. aureus* acute osteomyelitis in rats (28). Notably, the exebacase MIC values reported for isolates in this study

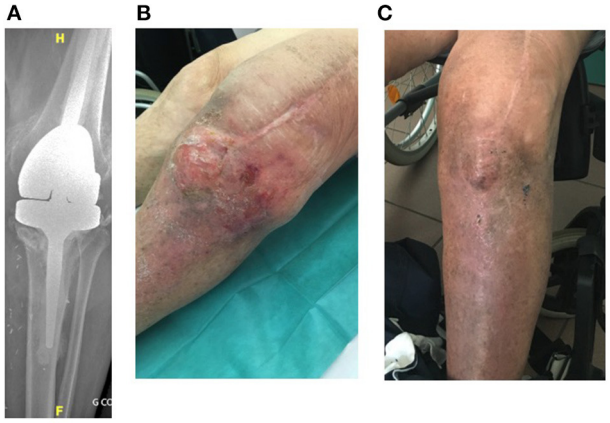

FIGURE 4 | Patient 4 was an 83-year-old man with a history of severe cardiomyopathy requiring anticoagulation, dyslipidemia, and diabetes. He had a left-cemented revision prosthesis **(A)** following a two-stage exchange for PKI due to *Streptococcus* spp. He experienced postoperative chronic septic arthritis due to *S. epidermidis* (5/23/2018), and he was treated by open DAIR, skin and soft tissue flap, and SAT (clindamycin plus levofloxacin followed by clindamycin plus cotrimoxazole). Under SAT, the patient experienced a relapse of the septic arthritis **(B)** due to MDR *S. epidermidis* (19/07/2018). He was treated according to the LysinDAIR procedure (10/01/2019) and received daptomycin intravenously and linezolid orally. He developed eosinophilic pneumonia attributed to daptomycin before the switch to tedizolid. During the follow-up, the clinical signs of septic arthritis totally disappeared. At the time of writing (14 months of follow-up after the LysinDAIR procedure), the clinical outcome was still favorable under tedizolid **(C)**.

were within the range of 0.125–2 μg/mL previously reported for *S. epidermidis* (18).

The choice of tedizolid for oral SAT is based on the fact that this drug has a strong potential in patients with PJI as several case reports and case series report its safe prolonged use (29, 30). Moreover, this drug remains active in MDR staphylococci and could have potential activity against persisters (31). However, PJI is an off-label use of tedizolid, and this antibiotic is a costly option for SAT as a one-year supply of this drug is approximately $127,000 in the United States and €75,000 in France (16).

As the selected patients here already experienced a relapse despite open DAIR and SAT, the rate of expected success, if exebacase had no effect on the biofilm, was close to zero. Even though we observed a relapse in the two patients with sinus tract, the impressive significant clinical outcome in the two other patients make the LysinDAIR procedure a potential innovative approach that need to be investigated. In the study of Prendki et al., (10)experiencing a sinus tract before the implementation of SAT was a risk factor for failure, but no surgery was performed in most of these patients from this study, and other studies (9, 11–14), as per published guidelines in the field (3–5), did not suggest that sinus tract should contraindicate the performance of DAIR followed by SAT in patients with chronic PJI.

Based on the present data, exebacase showed the potential to be used as salvage therapy administered during arthroscopic DAIR procedure in patients with staphylococci PKI, to improve the efficacy of SAT and to avoid considerable loss of function.

TABLE 1 | Antibiograms and exebacase MIC of the patients' *S. epidermidis* isolates.

Patient	PJI episode	Sampling date	Exebacase MIC (mg/L)	Antibiotics											
				OXA	K	GM	NN	E	L	TE	OFX	SXT	FA	RA	VA	LZD
Patient 1	Previous episode	04/02/2016	NT	R	R	R	R	R	R	I	R	R	R	R	S	S
	Joint puncture before surgery	17/11/2017	1	R	R	R	R	R	R	I	R	R	R	R	S	S
	At the time of surgery	08/11/2018	2	R	R	R	R	R	R	I	R	R	R	R	S	S
Patient 2	Previous episode	02/12/2013	NT	R	S	S	S	R	R	S	S	R	R	R	S	S
	Previous episode	11/10/2013	NT	S	R	R	R	R	R	S	R	R	R	R	S	S
	Joint puncture before surgery	13/09/2018	0.125	S	S	S	S	S	S	S	S	S	S	NT	S	S
	At the time of surgery*	08/11/2018	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Patient 3	Previous episode	04/02/2011	NT	R	R	R	R	R	R	R	R	S	R	NT	S	S
	Joint puncture before surgery	07/06/2018	0.25	S	R	R	R	R	R	R	R	S	R	R	S	S
	At the time of surgery	10/01/2019	2	S	R	R	R	R	R	R	R	I	R	R	S	S
Patient 4	Previous episode	23/05/2018	NT	S	S	S	S	R	S	S	S	S	R	S	S	S
	Joint puncture before surgery	19/07/2018	ID	S	S	S	S	R	S	S	S	S	S	S	S	S
	At the time of surgery	10/01/2019	NT	R	R	R	R	R	R	S	R	R	R	S	S	S

OXA, oxacillin; K, kanamycin; GM, gentamicin; NN, tobramycin; E, erythromycin; L, Lincomycin; TE, tetracycline; OFX, ofloxacin; SXT, cotrimoxazole; FA, fusidic acid; RA, rifampin; VA, vancomycin; LZD, linezolid; NA, strain not available; ID, impossible to determine as no bacterial growth was observed in the specific media (CaMHB-HSD composed of cation adjusted Mueller-Hinton broth) used for MIC determination; NT, not tested; R (in red), resistant; I (in orange), intermediate; S (in green), susceptible.

**The patient had surgery under cotrimoxazole therapy that probably inhibited the growth of S. epidermidis from peroperative samples.*

The observed initial clinical response in all patients and sustained clinical responses in two of the four suggests that the use of exebacase intra-articularly for PJI warrents further study to refine dosing and frequency of administration. The fact that exebacase was well-tolerated with no adverse events related to the arthroscopic administration and no events of hypersensitivity to the drug is encouraging, and this, together with the early signals of clinical response, warrant further investigation to refine dosing in a Phase 1 B design clinical study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written, informed consent was obtained from each patients for the publication of any potentially identifiable images or data included in this article.

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AUTHOR CONTRIBUTIONS

TF managed all the patients, directly interacted with the French Health authority, and wrote the manuscript. CB, SL, RG, and JR performed the arthroscopic lavage. FL, JJ, AS, and CK performed bacteriological experiments. All authors participated to the literature review and the improvement of the manuscript.

FUNDING

This study was based on the routine care of patients managed in our regional reference center. The patients were treated under the supervision of the French Health Ministry, and Contrafect provided the drug used without any charge.

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Conflict of Interest: CCa is employed by the company Contrafect. TF received honorarium as speaker from Contrafect to present these results to the company members. The Hospices Civils de Lyon - Institut des Agents Infectieux received financial support for a research project aimed at evaluating *in vitro* Lysin CF-301 activity on a collection of clinical strains.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 17 May 2021
doi: 10.3389/fmed.2021.553965

Challenging Methicillin Resistance Detection in Bone and Joint Infections: Focus on the MRSA/SA SSTI® Strategy

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The genus *Staphylococcus* is the main causative agent of bone and joint infections (BJI) in which outcomes are impacted by both effective surgical and appropriate antimicrobial management. In this context, methicillin resistance (MR) detection is a microbiological challenge to optimize the anti-staphylococcal drug coverage and to secure the surgical procedure. During the last decade, molecular tools have been developed to rapidly detect bacterial-resistant strains in clinical samples. The GeneXpert MRSA/SA SSTI® assay (Cepheid, Sunnyvale, CA, USA) is a real-time PCR method aimed at detecting methicillin-resistant *Staphylococcus aureus* (MRSA) in skin and soft tissues infections. In the literature, this test has been reported to be diverted from its original purpose to be evaluated in surgical samples. Within the current review, we update the GeneXpert MRSA/SA SSTI® assay performance in staphylococcal species determination (i.e., *S. aureus* vs. coagulase-negative species) together with MR genotype detection, when performed in osteoarticular infections.

Keywords: bone and joint infections, methicillin resistance, PCR, conventionnal culture, Xpert MRSA/SA SSTI®

OPEN ACCESS

Edited by:
Alex Soriano,
Hospital Clínic de Barcelona, Spain

Reviewed by:
Dolors Rodriguez-Pardo,
Vall d'Hebron University Hospital, Spain
Laura Morata,
Hospital Clínic de Barcelona, Spain

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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *20 April 2020*
Accepted: *08 April 2021*
Published: *17 May 2021*

Citation:
Titécat M, Loïez C, Demaeght F, Leclerc J-T, Martin T, Dezèque H, Migaud H and Senneville E (2021) Challenging Methicillin Resistance Detection in Bone and Joint Infections: Focus on the MRSA/SA SSTI® Strategy. Front. Med. 8:553965. doi: 10.3389/fmed.2021.553965

INTRODUCTION

Bone and joint infections (BJI) encompass a heterogenous group combining native joints and device-associated infections, covering children osteomyelitis, adults' septic arthritis, spondylodiscitis, and prosthetic joint infections (PJI). They require a complex management involving a multidisciplinary approach associating orthopedic surgeons, infectiologists, and microbiologists (1). *Staphylococcus* spp., the main bacterial genus involved in BJI, is reported to be a risk factor associated with inpatient mortality (2). Today, methicillin-resistant coagulase-negative staphylococci (MRCoNS) have become unavoidable in chronic PJI (3, 4) justifying empirical use of glycopeptides (5); while efficiently targeting methicillin-sensitive *Staphylococcus aureus* (MSSA) in native BJI is the key to avoid recurrence and complications (6, 7). In this context, providing rapid bacterial susceptibility results is crucial to guide efficient antimicrobial adaptation in the peri-operative time. Until now, the "gold-standard" method still relies on conventional microbial cultures that require 2–15 days to identify bacterial strains (8) and additional 24–72 h longer to

ascertain the antibiotic susceptibility pattern. Moreover, the efficacy of culture methods is partly questionable with a 62.6% sensitivity (9) in PJI cases, and importantly, methicillin resistance (MR) determination may be delicate to rapidly discriminate a heterogenous phenotypical feature and a "borderline" resistance with conventional methods. Hence, there is a need to improve diagnosis methods to reduce the time frame for appropriate antimicrobial management, since total hip and total knee revisions are anticipated to increase by 137 and 601%, respectively, between 2005 and 2030 (10) with 25% attributed to infection (11). During the last decade, the global bacterial resistance burden, including the spread of the virulent community-acquired methicillin-resistant *S. aureus* (CA-MRSA) clone US300 (12), triggered the development of molecular tools aimed at targeting bacterial pathogen DNA and their main resistance determinants. In this attempt, the GeneXpert MRSA/SA SSTI® test (Cepheid, Sunnyvale, CA, USA) was originally designed to detect *S. aureus* (SA) and methicillin-resistant SA (MRSA) directly in clinical samples of skin and soft tissue infections (SSTI). This test was efficiently evaluated in wound and blood culture specimens (13) engaging to divert its former use for osteoarticular applications by distinct clinical units worldwide. Beyond SA and MRSA identification, the assay allows for the specific detection of the genetic support of MR, the *mec*A gene, and interestingly provides the possibility to detect the presence of an MR staphylococcal (MRS) strain from the surgical site, whatever the species. We propose herein to review the performance of the GeneXpert MRSA/SA SSTI® assay for SA, MRSA, and MRCoNS detection and discuss the reliability of such use in several BJI contexts throughout recent articles.

BASIS OF THE GENEXPERT MRSA/SA SSTI® CONCEPT

MR is acquired by horizontal transfer and chromosomal integration of a mobile genetic element designated staphylococcal cassette chromosome mec (SCCmec) (14). The *mec*A gene encodes an alternative penicillin-binding protein (PBP2a), an enzyme responsible for crosslinking the peptidoglycans in the bacterial cell wall, resulting in poor affinity for β-lactams and global resistance to this class of antibiotics (15). The GeneXpert MRSA/SA SSTI® assay is a commercial real-time PCR-based method which relies on the simultaneous detection of three targets: the SA protein A (*spa*) gene, the gene supporting MR (*mec*A), and the SA SCC*mec* chromosomal insertion site which is located at the 3′ end of an unknown function open reading frame, *orfX* (16). All PCR steps (i.e., extraction, amplification, and detection) take place in a single-use cartridge which contains all the reagents necessary for the detection of the three abovementioned bacterial targets together with an internal sample processing control (SPC) (*Bacillus globigii* spores). According to the manufacturer's recommendations, clinical samples may be collected on Copan swabs, discharged in elution buffer, vortexed for 10 s, and then transferred into Xpert MRSA/SA cartridges. The overall analysis is complete in <1 h, and amplification curves are automatically read by the GeneXpert Dx System in terms of MRSA and SA positive or negative, respectively. A comprehensive look may lead to additional interpretations: (i) an isolated amplification of the *spa* gene assesses the presence of MSSA, (ii) the simultaneous detection of the three targets (i.e., *spa*, *mec*A, and SCC*mec*) attests the presence of MRSA, (iii) a unique amplification of the *mec*A gene can be interpreted as the presence of MRCoNS, (iv) the simultaneous detection of both the *spa* and the *mec*A genes supposes a mixed infection containing both MSSA and MRCoNS strains, and (v) the amplification of the *spa* gene and SCC insertion site without *mec*A signal may be interpreted as an MSSA empty cassette variant. The limits of detection reported by the manufacturer are 150 and 300 CFU/swab for positive SA and MRSA results, respectively.

RATIONALE FOR EVALUATING XPERT MRSA/SA SSTI® IN BJI

The literature points out only seven publications dealing with the performances of the MRSA/SA SSTI® real-time PCR assay (Cepheid, Sunnyvale, CA) in BJI diagnosis according to distinct protocols. Four out of seven were prospective studies; all but one (17) were led on adult cohorts. These studies were mainly conducted on PJI patients (18–21); unspecified BJI (22); a combination between PJI, spondylodiscitis, and septic arthritis (23); and children suffering from musculoskeletal infections (17). The number of patients included varied from 30 to 213 patients tested for one (21) to at least three distinct samples (18, 19), including joint aspiration, tissue or bone specimens, and prosthetic sonicates in one case (21). Tests of patients diagnosed with staphylococcal BJI were performed either on fresh samples (18, 19, 23) or frozen stored ones (−80°C) (17, 20–22). Biopsies were either directly vortexed (20), grinded, or crushed in saline buffer (17, 22, 23) or even cultured according to beadmill processing (18, 19, 21, 24). In all studies, the liquid phase of the samples was absorbed onto a swab (Copan, Cepheid) from 5 s (23) to 1 min (22) and then discharged in the elution buffer according to the manufacturer's recommendations. Another strategy consisted of directly collecting one Eswab from the periprosthetic tissue during the surgery and vortexing it into a reagent vial from the Xpert kit (21). RT-PCR results obtained from those swabs were compared to identification and resistance patterns reached from corresponding standard (17, 18, 23) and enriched cultures in blood culture bottles (17, 20) in Schaedler (22) or Rosenow broth (19), which were incubated from 5 (17) up to 14 (18, 19, 21–23) or 15 days (20). The main evaluation criteria were the accuracy of MR detection (18–20), the ability of the GeneXpert MRSA-SA SSTI restricted to the identification of SA and MRSA (17, 22), or the latter associated with MRCoNS detection (21, 23).

Appraising Xpert's Performance in SA/MRSA Detection

SA is the predominant causal pathogen involved in native infections which represent the most frequent clinical form of BJI, accounting for 68% of cases (2). SA also remains the main cause

TABLE 1 | Comparison of studies evaluating the GeneXpert MRSA/SA SSTI® in BJI diagnosis.

References	Dubouix-Bourandy et al. (23)	Titécat et al. (18)	Valour et al. (22)	Lourtet-Hascoët et al. (20)	Titécat et al. (19)	Sambri et al. (21)	Searns et al. (17)
Study design	Prospective	Prospective	Retrospective	Retrospective	Prospective	Prospective	Retrospective
Aim	MSSA	MR detection	SA/MRSA detection	MR detection	False negative in MR detection	MSSA	SA/MRSA detection
	MRSA					MRSA	
	MRCoNS detection					MRCoNS detection	
Populations studied	BJI (PJI—septic arthritis spondylodiscitis)	Chronic PJI	Adults BJI	PJI	PJI	Chronic PJI	Pediatrics musculoskeletal infections
No. of patients	105	30	76	62	213	70	184
No. of samples	135	104	91	72	NA	70	125
Samples characteristics	Fresh samples	Fresh samples	Frozen samples	Frozen samples	Fresh samples	Peri-operative Eswab	Frozen samples
SA detection in positive samples	18/18	37/37	68/72	NA	NA	11/11	51/59
False positive SA detection	0	0	3[a]	NA	NA	0	2[a]
SA detection performance	Se 100%	Se 100%	Se 94.4%	NA	NA	Se 100%	Se 85.4%
	Sp 97.8%	Sp 91.2%	Sp 100%			Sp 100%	Sp 98%
	PPV 90%	PPV 92.5%	PPV NA			PPV 100%	PPV 93%
	NPV 100%	NPV 100%	NPV NA			NPV 100%	NPV 95%
MSSA detection in positive samples	16/16	28/28	59/63	NA	NA	7/7	41/48
MSSA detection performance	Se 100%	Se 100%	Se 93.6%	NA	NA	Se 100%	Se 85.4
	Sp 98.3%	Sp 100%	Sp 100%			Sp 100%	Sp 98.5%
	PPV 88.9%	PPV 100%	PPV NA			PPV 100%	PPV 95.3%
	NPV 100%	NPV 100%	NPV NA			NPV 100%	NPV 95%
MRSA detection in positive samples	2/2	7/7	9/9	NA	NA	4/4	9/11
MRSA detection performance	Se 100%	Se 100%	Se 100%	NA	NA	Se 100%	Se 81.8
	Sp 100%	Sp 99%	Sp 100%			Sp 100%	Sp 100%
	PPV 100%	PPV 87.5%	PPV NA			PPV 100%	PPV 100%
	NPV 100%	NPV 100%	NPV NA			NPV 100%	NPV 98.9%
MRCoN detection	19/19	13/17	NA	9/25	NA	14/16	NA
MRCoN detection performance	Se 100%	Se 76.5%	NA	Se 36%	NA	Se 87.5%	NA
	Sp 95.3%	Sp 95.4%		Sp 98%		Sp 100%	

(Continued)

TABLE 1 | Continued

References	Dubouix-Bourandy et al. (23)	Titécat et al. (18)	Valour et al. (22)	Lourtet-Hascoët et al. (20)	Titécat et al. (19)	Sambri et al. (21)	Searns et al. (17)
	PPV 85.2%	PPV 76.5%		PPV 90%		PPV 100%	
	NPV 100%	NPV 95.4%		NPV 74%		NPV 96.4%	
False positive MR detection	4	5[a]	0	1[a]	NA	0	NA
False negative MR detection	0	4 (1 MRSA + 3 MRCoN)	0	16 MRCoN	6 (1 MRSA + 5 MRCoN)	2 MRCoN	2 MRSA
MR detection Xpert's overall performance	Se 100%	Se100%	Se 94.4%	Se 36%	Se 75%	Se 90%	Se 81.8%
	Sp 96.3%	Sp 91.2%	Sp100%	Sp 98%	Sp 93%	Sp 100%	Sp 100%
	PPV 86.2%	PPV 92.5%	PPV NA	PPV 90%	PPV 58%	PPV 100%	PPV 100%
	NPV 100%	NPV 100%	NPV NA	NPV 74%	NPV 97%	NPV 96%	NPV 98.9%

NA, not available.

[a]*Re-interpreted as "true positive" according to patient's history or other results of sample's culture.*

of acute hematogenous BJI in children, representing >90% of methicillin-sensitive strains (25). On the one hand, while most of the studies focus on MRSA, MSSA native BJI are associated with a high rate of treatment failure and further functional sequalae (6), as reported by a three times higher risk of recurrence following vancomycin therapy than observed with β-lactam antibiotics (7). On the other hand, when considering device-associated infections, SA is more likely to be involved in early acute infections (4, 26), while MR phenotype is subject to geographical discrepancies (3). In the USA, SA accounts for 38.6% of surgical site infections following orthopedic surgery, including 38% of MR strains (27), leading to an evaluated cost of $107,264 per case in comparison with $68,053 in case of MSSA (28). In Europe, SA is involved in a similar proportion of PJI (31.9 and 38.7% of total hip and knee arthroplasty infections, respectively) (29), whereas MR proportion follows a downward trend, as confirmed within German (30) and French PJI cohorts (4). When compared to conventional culture (**Table 1**), the Xpert's test shows attractive performances in SA detection with high sensitivity and specificity ranging from 85.4% (17) to 100% (18, 21, 23) and 91.2% (18) to 100% (21, 22), respectively. Moreover, positive PCR cases related to sterile cultures had to be reconsidered in light of the patient's history (i.e., proven SA infection) supporting the demonstrated sensitivity of the molecular assay. Interestingly, MRSA detection displays an analogous level of efficiency and is associated with a high negative predictive value (NPV 98.9–100%) allowing unambiguous use of β-lactams when SA is detected in osteoarticular samples. Further studies considering both the clinical outcome and the economic impact of such early screening would be of major interest, as suggested by the recent report from AlQahtani et al. (31) in the context of SA bacteremia.

Appraising Xpert's Performance in MRCoNS Detection

MRCoNS have gained global attention in recent years and are responsible for a large proportion of PJI in European countries (3). While CoNS are less pathogenic than SA, they are able to adhere and colonize orthopedic devices by producing a biofilm. This structure encloses low amounts of slow-growing bacteria and causes delayed infections characterized by subtle symptoms, making their clinical and microbiological diagnosis challenging. Moreover, these infections involving a large proportion of MRCoNS (3, 4) justify empirical use of vancomycin in combination with a broad-spectrum β-lactam (5, 32). High dosage regimens are required to ensure an effective bone diffusion of the antibiotics, but this strategy leads to a significant rate of adverse effect (33) that could be spared by directly detecting MRCoNS in the surgical site. Xpert's accuracy was addressed in this aim by targeting the *mec*A gene in solid and liquid osteoarticular samples (18–21, 23). The test was first validated by Dubouix-Bourandy et al. (23) on 25 samples isolated from various BJI conditions with sensitivity, specificity, positive predictive value (PPV), and NPV values of 100, 95.3, 85.2, and 100%, respectively. These results were further corroborated on chronic PJI patients (18, 22) with an acceptable sensitivity (76.5 and 87%) and a high NPV (95.4 and 96.4%). Nevertheless,

Lourtet-Hascoët et al. (20) questioned these performances due to a significant number of false negative results (16/25) and highlighted inconsistent sensitivity and NPV, respectively, 36 and 74%. These 16 samples were all frozen and originated from 14 periprosthetic tissues and 2 articular fluids. In contrast, the three other series were performed on fresh tissue samples or extemporaneous periprosthetic tissue swabs directly processed in the peri-operative time. Furthermore, when applied to a larger prospective cohort of 213 patients and 639 osteoarticular specimens (19) and considering Xpert's performance from a patient point of view, only 6 out of 213 patients (2.8%) were misdiagnosed, among whom 5 were infected by MRCoNS strains. It is worth noting that rapid and appropriate antimicrobial adaptation could be delivered to 194 out of 213 patients (91%).

FEW RESTRICTIONS OF THE TEST

Reaching 100% accuracy for a diagnostic tool is unrealistic in routine practice, especially when the microbiologist must deal with all the bacterial subtleties related to BJI contexts. Although we highlighted the good performances of the MRSA/SA SSTI above, the limits of this test in terms of false positive and false negative results must be discussed, owing to their potential impact on the antimicrobial strategy. Indeed, a false positive assay may lead to an inappropriate prescription of a broad-spectrum molecule with individual and ecological side effects, and a loss of opportunity to heal in case of MSSA infection. Interestingly, false positive tests have rarely been observed in the seven studies (**Table 1**) including 5 and 10 reported cases of SA and MR detection, respectively. These cases were mainly related to bacterial DNA detection in patients with an anteriority of infection or who had prior antimicrobial therapy leading to sterile cultures. Finally, these false positive cases could be re-interpreted as a true positive one, highlighting the limit of the gold standard used for the evaluation of this molecular assay. Secondly, worse than a false positive result, a false negative one may jeopardize surgery and the implanted device, implying revision procedure and prolonged hospital cost and length of stay associated with a morbidity increase. These false negative cases were more frequent in MRCoNS infections (18–20). This slightly lower accuracy comparative to SA infections must be interpreted according to the assay's content (i.e., specific probes targeting SA and MRSA) and also physiopathological considerations relative to acute SA infections involving high inoculum of bacteria and chronic MRCoNS infections involving low amounts of biofilm embedded bacteria, not evenly distributed at the implant's surface. Consequently, in a chronic context, the number of samples analyzed should not be restricted to a single one for correct interpretation. Multiplying samples and PCR tests may entail a financial burden for clinical laboratories that should counterbalance the economic consequences of misdiagnosis for healthcare settings. So far, the number and the kind of osteoarticular specimens required for a contributive analysis have to be defined. Moreover, regarding the software's interpretation algorithm, the latter is configured for MRSA/SA detection in skin and soft tissue samples with positivity reports related to abundant bacterial load resulting in low PCR Ct values. Consequently, these criteria cannot be extrapolated to osteoarticular or MRCoNS infection contexts. Accordingly, in BJI indication, the microbiologist's appraisal is required to interpret amplification curves, and particularly late Ct values of the *mec*A gene, in order to not miss a positive sample (18, 19). Finally, other false negative results were also reported in cases of staphylococcal small colonies variants or polymicrobial infections, without any obvious explanations yet.

RELEVANCE OF THE MRSA/SA SSTI® IN ROUTINE PRACTICE?

Although conventional culture is a perfectible gold standard, it remains the key method to document the infection and to provide an exhaustive antibiogram. The use of blood culture bottles has significantly reduced the time for micro-organism detection with a sensitivity increased to 87% (9) allowing, in the most favorable conditions, phenotypical antimicrobial data in 48 h. The aim of the MRSA/SA SSTI® assay is to reduce this time frame to a couple of hours by targeting the main resistance determinant, i.e., the *mec*A gene, which is decisive for empirical antimicrobial therapy adaptation. In contrast to the 16S rRNA PCR (34, 35) or other home-designed multiplex PCR panels (36), the Xpert's test targets a single bacterial genus but delivers results in 72 min (23) vs. 2 days and half a day, respectively. Xpert is also easily implementable in the routine workflow of a clinical laboratory, with a hand-on time of 2 min (23), and does not involve the use of complex molecular facilities nor dedicated technical supports. Alternatively, an equivalent concept is proposed by the automated multiplex PCR Unyvero i60 ITI (Curetis, Holzgerlingen, Germany). This cartridge system targets 52 pathogens at the genus level, among which are 15 bacteria and yeasts at the species level, and 19 antimicrobial resistance markers delivering available results in 5 h. This assay has been evaluated in PJI diagnosis in three studies (37–39), although MR detection accuracy was only addressed by Malandain et al. (38). Unfortunately, when tested on culture-positive samples, no more than 35% of bla_{mecA} gene amplifications were detected.

Collectively, these data fully support the relevance of the Xpert's assay in osteoarticular infections for rapid antimicrobial adaptation in the peri-operative time along with its applicability in routine practice. One may assume that this strategy of early and accurate diagnosis is cost-effective; however, this point has to be fully demonstrated for acute and chronic indications, respectively. Nowadays, the room for such new molecular methods in the diagnostic strategy of BJI remains to be clearly defined in clinical and microbiological guidelines.

AUTHOR CONTRIBUTIONS

MT drafted the manuscript. ES, CL, HM, J-TL, FD, TM, and HD reviewed the article and provided critical insights. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We warmly thank Madeleine Engilof for her valuable advice.

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Conflict of Interest: ES declares personal honoraria from Cepheid for speaker activity.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Surgery

ORIGINAL RESEARCH
published: 20 September 2021
doi: 10.3389/fsurg.2021.551814

Rotating Hinge Knee Arthroplasty for Revision Prosthetic-Knee Infection: Good Functional Outcomes but a Crucial Need for Superinfection Prevention

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OPEN ACCESS

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Specialty section:
This article was submitted to Orthopedic Surgery, a section of the journal Frontiers in Surgery

Received: *14 May 2021*
Accepted: *16 August 2021*
Published: *20 September 2021*

Citation:
Bourbotte-Salmon F, Ferry T, Cardinale M, Servien E, Rongieras F, Fessy M-H, Bertani A, Laurent F, Buffe-Lidove M, Batailler C, Lustig S and The Lyon Bone and Joint Infections Study Group (2021) Rotating Hinge Knee Arthroplasty for Revision Prosthetic-Knee Infection: Good Functional Outcomes but a Crucial Need for Superinfection Prevention. Front. Surg. 8:551814. doi: 10.3389/fsurg.2021.551814

Introduction: Management of chronic infection following total knee arthroplasty (TKA) is challenging. Rotating hinged prostheses are often required in this setting due to severe bone loss, ligamentous insufficiency, or a combination of the two. The nature of the mechanical and septic complications occurring in this setting has not been well-described. The aim of this study was to evaluate patient outcomes using a hinge knee prosthesis for prosthetic knee infections and to investigate risk factors for implant removal.

Methods: This was a retrospective cohort study that included all patients treated in our tertiary level referral center between January 2009 and December 2016 for prosthetic knee infection with a hinge knee prosthesis. Only patients with a minimum 2-year of follow-up were included. Functional evaluation was performed using international knee society (IKS) "Knee" and "Function" scores. Survival analysis comparing implant removal risks for mechanical and septic causes was performed using Cox univariate analysis and Kaplan-Meier curves. Risk factors for implant removal and septic failure were assessed.

Results: Forty-six knees were eligible for inclusion. The majority of patients had satisfactory functional outcomes as determined by mean IKS scores (mean knee score: 70.53, mean function score: 46.53 points, and mean knee flexion: 88.75°). The 2-year implant survival rate was 89% but dropped to 65% at

7 years follow-up. The risk of failure (i.e., implant removal) was higher for septic etiology compared to mechanical causes. Patients with American society of anesthesiologists (ASA) score>1, immunosuppression, or with peripheral arterial diseases had a higher risk for septic failure. Patients with acute infection according to the Tsukayamaclassification had a higher risk of failure. Of the 46 patients included, 19 (41.3%) had atleast one infectious event on the surgical knee and most of these were superinfections (14/19) with new pathogens isolated. Among pathogens responsible for superinfections (i) cefazolin and gentamicin were both active in six of the cases but failed to prevent the superinfection; (ii) cefazolin and/or gentamicin were not active in eight patients, leading to alternative systemic and/or local antimicrobial prophylaxis consideration.

Conclusions: Patients with chronic total knee arthroplasty (TKA) infection, requiring revision using rotating hinge implant, had good functional outcomes but experienced a high rate of septic failure, mostly due to bacterial superinfection. These patients may need optimal antimicrobial systemic prophylaxis and innovative approaches to reduce the rate of superinfection.

Keywords: arthroplasty, total knee arthroplasty, knee prosthesis, prosthetic-joint infection, septic revision, superinfection, prevention

INTRODUCTION

Prosthetic-joint infection (PJI) is a devastating complication after total knee arthroplasty (TKA). The rate of PJI following primary TKA is ∼1–2% (1–4). The rate of bacterial resistance or a *de novo* infection (also called superinfection) is significantly higher in patients with chronic infection requiring prosthesis revision. Management is challenging, requiring a multi-disciplinary approach to determine the optimal strategy for prosthesis choice (non-constrained or constrained), staging surgery or not (single vs. two stage), the duration and delivery of systemic antimicrobial therapy, and the choice of antimicrobial prophylaxis at the time of reimplantation.

Hinged knee prostheses are often used in the revision of TKA (5). The indications for a hinged TKA are restricted to limb-salvage procedures such as tumor, complex fracture, or revision surgery with significant bone loss or collateral ligaments failure (6–11). In limited situations, the hinged knee prosthesis may be indicated in a primary setting, such as severe deformity (12).

The longevity of hinged TKAs remains a major concern, with high rates of mechanical complications being widely reported (8, 13–15). In order to limit such complications, prosthesis design has evolved and the third generation of rotating hinged TKA (RHTKA) has been available since 1999 (16, 17). The addition of a rotating platform allows increased freedom of movement compared to previous designs with the rationale of reducing force transmission at the implant-cement-bone interface. This implant could be used in the revision setting for the treatment of infected TKA, but data regarding outcomes when used for this indication remain limited and are heterogeneous (18–20).

Since 2009, third-generation rotating hinge knee prosthesis has been used in our institution for septic TKA revision surgery. The purpose of this study was to analyze the outcomes of patients with the use of this prosthesis for septic TKA revisions and to determine risk factors for mechanical and septic failures.

MATERIALS AND METHODS

This retrospective study was conducted at our regional referral center for the management of complex bone and joint infection called CRIOAc Lyon (http://www.crioac-lyon.fr). Patients who underwent RHTKA for septic revisions from 2009 to 2016 were included. This study received local institutional ethics approval. Patients were selected from the Lyon BJI cohort study (NCT02817711), and a dedicated data collection was performed for this study (NCT02856971).

Diagnostic Criteria for TKA Infection

The diagnosis was made using the criteria of TKA infection according to the International Consensus Meeting on Prosthetic Joint Infections (21). Prosthetic joint infection was classified according to the Tsukayama and Zimmerli classifications that have been well-described (2, 22).

Therapeutic Strategy

All prosthetic infections are discussed at a weekly multidisciplinary meeting. Our institution was responsible for the recommended prophylaxis guidelines included in the WHO surgical site infection (SSI) prevention recommendations (23). Cephazolin was routinely used for antimicrobial prophylaxis (in addition to the antimicrobial therapy used to treat the current infection) during prosthesis removal and reimplantation, according to national guidelines (24). For revision surgery, the scar was routinely excised and a trans-quadricipital tendon approach was used for arthrotomy. Additional exposure was achieved with an anterior tibial tuberosity (ATT) osteotomy, if required ($n = 7$). A 4.5 mm hole was drilled in the anterior

cortex of both femur and tibia to mark the joint line for later reconstruction (25). Well-fixed prostheses were removed with a combination of sharp osteotomes, and cement was removed with the OSCAR® system (Orthosonics, Edimburg, United Kingdom). Numerous surgical samples were taken before administering antimicrobials (approved during the multi-disciplinary meeting), and seven samples were taken for bacteriological analysis and one for pathology. Then, extensive debridement and synovectomy were made, including the posterior cruciate ligament if there was any remaining stump after removal of implants. Pulsed lavage irrigation of the joint was performed with at least 6 L of saline solution. In patients for whom a 2-stage procedure was proposed, a gentamicin-loaded cement spacer was implemented with PALACOS® R+G (high viscosity), containing 0.8 g of gentamicin per 40 g of cement. The spacer was either articulating or static, depending on the condition of the local bone and soft tissues. ATT osteotomies were stabilized with non-resorbable transosseous sutures. The wound was closed with drainage left in place for 3 days. Patients wore a molded resin cast after implant removal. Patients were made strictly non-weight bearing until the second-stage surgery. Intensive physiotherapy began the day after the surgery, based on gait rehabilitation with walking aids. The second-stage surgery (reimplantation) was scheduled in patients with favorable local conditions and for whom the infection was deemed to be controlled. It was carried out under antibiotics or after an antibiotic window depending on the time since the explantation.

For the second-stage surgery, a large synovectomy was repeated. Collateral ligaments were dissected but not excised. Bone defects were managed either with bone cement or with wedges. All reimplanted hinged TKAs were fixed with high viscosity gentamicin-loaded cement (PALACOS® R+G). ATT osteotomies were secured with two cortical screws. The drainage was removed the day after the surgery. Physiotherapy started on the first post-operative day. Full weight bearing was allowed for single-stage exchange patients. Bacterial cultures were performed, and antibiograms were generated for all cultured bacteria. The antibiotic prescription was managed by infectious disease specialists during multidisciplinary meetings, with empirical antimicrobial therapy (no fixed protocol), and then targeted antimicrobial therapy prescribed according to the French and international guidelines. A total course of 3 months of antimicrobial therapy is the standard period of systemic therapy in our institution.

Outcome Assessment

The aim of the study was to evaluate patient outcomes and implant survival. We evaluated the survival rates of rotating hinge knee prosthesis by comparing the risk for failure (i.e., implant removal) due to mechanical vs. septic causes, using a Cox univariate analysis (Hazard ratio, HR; 95% confidence interval, CI) and Kaplan-Meier curves (Log-rank test) (26). Risk factors for prosthesis removal were identified regardless of the cause. Patients with septic failure were additionally assessed with antibiograms of the strains responsible for the relapse to determine sensitivity to cefazolin (used as systemic antimicrobial prophylaxis) and gentamicin (used as local antimicrobial prophylaxis in the cement). Finally, risk factors for septic failure (i.e., need for subsequent surgery such as Debridement Antibiotics and Implant Retention [DAIR] or implant removal due to clinical signs of infection occurrence) were specifically evaluated with univariate Cox analysis. Risk factors for infectious events were analyzed using the following items: "age," "ASA score $>$ 1," "immunosuppression," "acute infection as initial clinical presentation according to Tsukayama classification," "acute infection as initial clinical presentation according to Zimmerli classification," "peripheral arterial disease." IKS $\ll$ knee $\gg$ and $\ll$ function $\gg$ scores (International Knee Surgery) (27) were calculated for all patients who still had their prostheses at the last medical examination.

Statistical Analysis

Multivariate Cox analyses were performed using the most significant determinants ($p < 0.05$) identified in the univariate analysis with another determinant. Due to the low sample size of the population, we did not include >2 variables into a single multivariate model. A p-value <0.05 was considered significant. Statistical analyses were performed using SPPS Statistics Base 17.0 (Softonic International, San Francisco, CA, USA). Percentages of patients with or without characteristics of interest were compared using chi-square or Fisher's exact test, as appropriate.

RESULTS

During the study period, 230 patients were treated in our institution for infected TKAs. The indications for hinged TKAs used are presented in **Table 1**. Patients who underwent a revision of septic TKA by any other type of prosthesis than hinged prostheses ($n = 180$) and patients who underwent TKA revision with a hinged prosthesis for mechanical problems ($n = 35$) were excluded. Fifty patients who underwent revisions with hinged TKA for septic revision were eligible for inclusion. The population characteristics are presented in **Table 2**. Four patients were lost to follow-up before 24 months, including one patient who died after the revision (prostatic cancer). Another patient died after 2 years of follow-up (pulmonary embolism). This patient was included in the analysis and considered as lost to follow-up at the date of death. The number of hinged TKAs followed over 2 years was, therefore, 46, with a mean follow-up of 38.1 months [10; 88].

Out of the 46 patients, 43 (93.5%) were managed with two-stage revision surgery. A cement spacer was used in 40 cases (static, $n = 13$; articulated, $n = 27$), and 3 patients were not given a spacer during the implant removal surgery because soft tissues did not allow. The average time between implant removal and second-stage reimplantation was 9.3 weeks. Thirty-six patients (81.2%) underwent second-stage reimplantation before 12 weeks, and most ($n = 28$) were reimplanted with adequate antimicrobial treatment. In these latter patients, the average time between implant removal and second-stage reimplantation was 8.9 weeks. Among the patients for whom a two-stage approach was performed, an antibiotic window before reimplantation was

TABLE 1 | Main indications of the use of hinged total knee arthroplasty (TKA) ($n = 50$ knees).

Indication	*n* (%)
Hinged TKA revision	15 (30%)
Collateral ligaments deficiency	12 (24%)
Bone losses (AORI III)	15 (30%)
Femur	8 (16%)
Tibia	3 (6%)
Femur + Tibia	4 (8%)
Patella baja with ATT osteotomy required	6 (12%)
Complex periprosthetic open fracture	2 (4%)

TABLE 2 | Population characteristics (50 patients).

Item	
Males (*n*, %)	22 (44)
Females (*n*, %)	28 (56)
Mean age[a] in years (± SD)	73.04 ± 10.19
Medical history / risk factors for infection related to the host (n, %)	
- TKA previous infection	17 (34)
- Immunosuppression[b]	10 (20)
- Diabetes	16 (32)
- Rheumatoid arthritis	5 (10)
- Pre-operative anticoagulant	15 (30)
- Cirrhosis	1 (2)
- Antecedent of surgery on the index knee	22 (44)
Mean ASA[c] score	2.36
Mean number of surgeries before the index TKA[d](±SD)	0.87 ± 1.56
Mean number of surgeries before the hinged TKA[d] (±SD)	5.04 ± 2.47
Type of infection (n, %)	
- Early infection <1 month	17 (34)
- Sub-acute infection <3 months	4 (8)
- Chronic infection	22 (44)
- Acute hematogenous infection	5 (10)
- Unknown	2 (4)

[a] *Mean age at the time of the hinged TKA implantation.*
[b] *Immunosuppression: any cause except diabetes, including long-term corticosteroids intake, Rheumatoid arthritis with cortioids and/or Methotrexate, cirrhosis, malignant hemopathy, chronic renal failure with cockroft <30 μmol/mL, solid cancer with immunomodulators, or chemotherapy.*
[c] *Physical status score of the American Society of Anaesthesiologists (ASA).*
[d] *Any surgery including arthroscopies.*

planned in 15/43 patients (34.9%) with an average time between implant removal and the reimplantation of 10.1 weeks.

Patients were selected for single-staged exchange ($n = 4$) if they had severe co-morbidities rendering an unfavorable risk-benefit ratio from two-stage management. One patient had a prosthetic loosening for which the septic origin was not suspected, until the results of intraoperative bacteriological samples returned positive.

The rotating hinged prostheses used are presented in **Table 3**. The distribution of the pathogens responsible for the initial TKA infection is presented in **Table 4**. No organism was found

TABLE 3 | Hinged prostheses used (50 patients).

Prothesis	*n* (%)
OSS™ RHK[a] (Biomet Zimmer®)	32 (64)
AXEL II (BBraun®)	13 (26)
LEXA (C2F®)	4 (8)
ROTAX (Lépine®)	1 (2)
Distal femoral replacement	12 (24)
Proximal tibial replacement	3 (6)
Both distal femoral and proximal tibial replacement	4 (8)
"Standard" Hinged TKA	31 (62)

[a] *Rotating hinge knee.*

TABLE 4 | Distribution of the pathogens responsible for index TKA infections (50 patients).

Pathogens	*n* (%)
Staphylococcus	15 (32.6)
Methicillin-susceptible *S. aureus*	4 (8.7)
Methicillin-resistant *S. aureus*	1 (2.3)
Methicillin-susceptible CNS[a]	5 (10.8)
Methicillin-resistant CNS[a]	5 (10.8)
Streptococcus spp.	10 (21.7)
Cutibacterium acnes	4 (8.7)
Gram-negative bacilli	3 (6.6)
Polymicrobial	9 (19.6)
Culture-negative infection	5 (10.8)

[a] *Coagulase-negative Staphylococci.*

in five patients, who nevertheless met the described TKA infection criteria. Concerning patients with a two-stage exchange, a "second look" surgery (spacer exchange) was performed in 5/43 (11.6%) cases before reimplantation. Six patients (13.1%) benefited from at least one plastic surgery procedure for soft tissue losses before reimplantation. A typical x-ray of a patient with a hinged prosthesis used for revision is described in **Figure 1**.

Rotating Hinge Knee Arthroplasty Overall Survival

The 2-year overall survival rate was 89% but dropped to 65% after 7 years of follow-up. A significantly higher risk for implant removal due to septic causes compared to mechanical ones was observed (HR: 6.73; CI: 1.42–31.81; $p = 0.016$) (**Figure 2**). Out of the 10 implants removals, 8 were due to septic failure. Nineteen patients (44.1%) did not undergo any surgery following reimplantation.

Mechanical Complications During the Follow-Up

Fifteen patients (32.6%) experienced at least one complication, detailed in **Table 5**. One patient underwent a one-stage revision of a TKA after mechanical loosening of the femoral component.

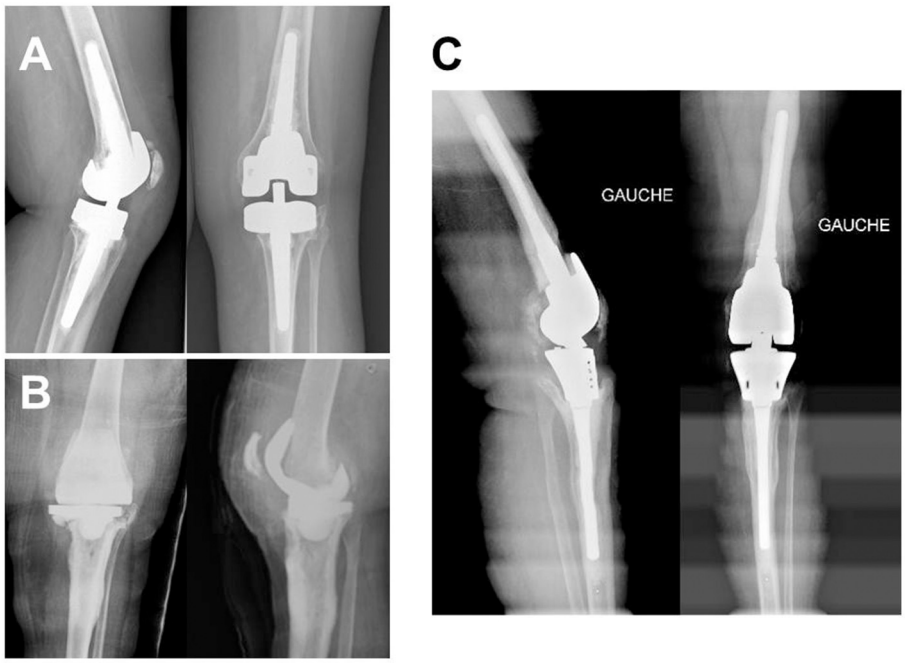

FIGURE 1 | Typical x-ray from a patient with total knee arthroplasty (TKA) infection treated with a two-stage approach with reimplantation of a cemented hinged TKA: An 80-year-old female patient with a history of *Staphylococcus caprae* TKA infection **(A)** from whom explantation was performed (**B**, a gentamicin-cement spacer was used to fill the gap) and for whom reimplantation of a gentamicin-cemented hinged prosthesis because of important femoral and tibial bone loss AORI III **(C)**. The outcome was favorable at 3 years of follow-up.

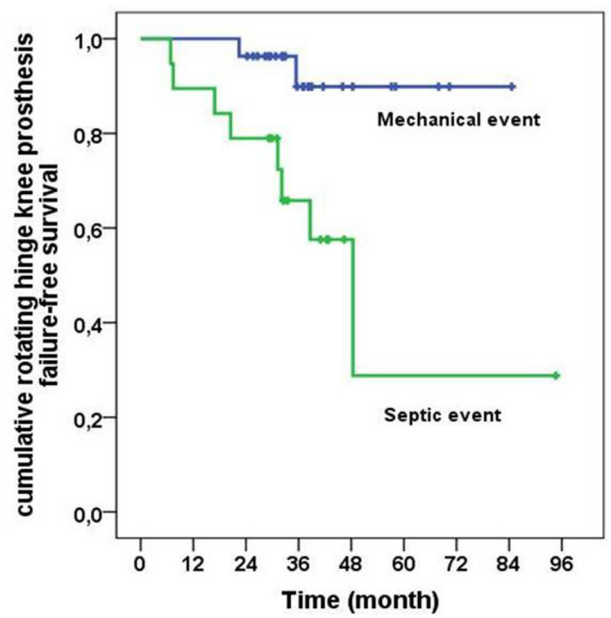

FIGURE 2 | Cumulative probability of survival of rotating hinge knee prosthesis, depending on the cause of the explantation, mechanic vs. septic (log-rank = 0.09).

Another patient underwent trans-femoral amputation for aseptic bipolar loosening. This was a relatively young patient who had already undergone several revision surgeries and requested a definitive solution.

TABLE 5 | Mechanical complications (46 patients followed >2 years).

Complications	*n* (%)
Spacer dislocation	2 (4.2)
Extensor apparatus complications	9 (19.6)
Peri-prosthetic fractures	4 (8.5)
Femur	1 (2.1)
Tibia	2 (4.3)
Patella	1 (2.1)
Neurologic complications (external popliteal sciatic nerve)	2 (4.3)
Aseptic loosening	3 (6.3)
Femur	1 (2.1)
Tibia	0 (0)
Patella	1 (2.1)
Bipolar loosening	1 (2.1)
Major stiffness (flexion < 80°)	5 (10.9)
Algoneurodystrophy	1 (2.1)

Septic Complications During the Follow-Up

Out of the 46 patients with >2 years of follow-up after reimplantation with a hinged TKA, 19 (41.3%) had at least one infectious event in their knee. The mean time of the infectious event was 16.4 months following the definitive surgery.

TABLE 6 | Epidemiology of pathogens involved in septic failures (19 patients).

Pathogens	*n* (%)
Persistent infection	2 (10.5)
Enterobacteriaceae	2 (10.5)
Streptococcus spp.	2 (10.5)
Culture-negative infection	2 (10.5)
Superinfection	15 (78.9)
Streptococci	3 (15.8)
*Enterobacteriaceae**	4 (21.1)
*Staphylococci**	4 (21.1)
P. aeruginosa	2 (10.5)
E. faecalis	1 (5.2)
P. multocida	1 (5.2)

**Including two multidrug-resistant (MDR) isolates.*

TABLE 7 | Univariate Cox analysis revealing risk factors for infectious failure.

	Univariate analysis		
	HR[a]	95% CI[b]	*p*
Age (per 10 years)	0.73	0.48–1.10	0.13
ASA>1	4.93	0.65–37.33	0.12
Immunosuppression	2.61	0.92–7.43	0.07
Peripheral arterial disease	3.28	0.74-14.44	0.12
Acute infection as initial clinical presentation according to Tsukayama	3.02	1.11–8.19	0.03
Acute infection as initial clinical presentation according to Zimmerli	4.11	0.91–18.5	0.07

[a] *Hazard ratio.*
[b] *95% CI.*

Of note, five patients underwent an infectious event in the 3 months following reimplantation (among them, three were superinfections and two were persistent infections). The involved pathogen epidemiology of these infectious events is presented in **Table 6**. Most of them were superinfections (14/19) with new pathogen isolation, and none seemed to be of hematogenous in origin. Among them (i) six were resistant to cefazolin (the usual antimicrobial prophylaxis used at the time of reimplantation), including two multidrug-resistant (MDR) *Enterobacteriaceae*, two *P. aeruginosa*, one MDR *S. epidermidis,* and one *E. faecalis*; (ii) three were resistant to gentamicin (the usual antibiotic in the cement used to fix the hinged prosthesis), and three had a low level of resistance to gentamicin. Among the pathogens responsible for superinfections (i) cefazolin and gentamicin were both active in six of them but failed to prevent the superinfection; (ii) cefazolin and/or gentamicin were not active in eight of them, leading to reconsideration of the systemic and local antimicrobial prophylaxis. Out of these 19 patients (i) 10 were treated with Debridement Antibiotics and Implant Retention (DAIR), including four patients for whom iterative DAIR was performed; (ii) eight were treated with implant removal, among whom two had a new hinged TKA reimplanted, five underwent arthrodesis, and one with no reimplantation proposed (resection arthroplasty); and (iii) one patient had a transfemoral amputation.

Infectious Events Risk Factors

Evaluation of risk factors for septic failure revealed that age did not influence the outcome (**Table 7**). Patients with ASA score>1, immunosuppression, and with peripheral arterial diseases seemed to have a higher risk for septic failure (**Table 7**; **Figures 3A–C**). Patients with acute infection as initial clinical presentation were at higher risk, according to Tsukayama classification, in comparison with other patients (**Table 7**; **Figure 3D**). The variable "acute infection as initial clinical presentation according to Tsukayama classification" remained independently associated with septic failure in three different multivariate Cox models that, respectively, included age, ASA score>1, and peripheral arterial diseases but was not independent with the immunosuppressive status (**Table 8**). Among the nine patients with an acute infection as initial clinical presentation according to Tsukayama, four of them were immunosuppressed (4/9 vs. 3/37, $p = 0.02$ with Fisher test), and five of them experienced a superinfection. All of these cases were treated with a two-stage procedure.

Functional Scores

Four patients could not undergo long leg x-rays because of an inability to fully weight bear. As a result, average "knee" IKS scores were calculated for 32 patients. The average IKS "knee" score was 70.5 points, CI 95% [63.9; 77.1] ($n = 32$ patients). The average IKS "function" score was 46.5 points, CI 95% [36.0; 57.0] ($n = 36$ patients). The average knee flexion was 88.7°, CI 95% [81.0; 96.5].

DISCUSSION

The main finding of this study is that the use of rotating hinged arthroplasty as a revision of a prosthetic-knee infection offers satisfactory functional outcomes following septic revision knee surgery but with a significant reinfection rate. The implications of these findings should encourage research studies toward alternative infection prevention pathways.

Data about rotating hinge knee arthroplasty survival are limited, especially for septic revision knee arthroplasty (5, 28–32) (**Table 9**). Disparities among survival rates could be explained by the heterogeneous distribution of hinged TKAs indications in the literature (9, 18, 33–37). Farid et al. (36) presented survival results for hinged TKAs in septic revisions. The survival rate (78.4%, mean follow-up ∼5 years) was higher than that observed in this study (65%, mean follow-up ∼7 years) in a cohort of 60 patients for whom a two-stage revision was performed. This may be explained by a younger population than ours (59.6 vs. 73.0 years), with significantly less comorbidities. Zahar et al. (38) studied the 10-year results of septic TKA revisions with rotating hinged prosthesis in 70 patients managed with a one-stage exchange. In this study, 93% of their patients were considered cured of

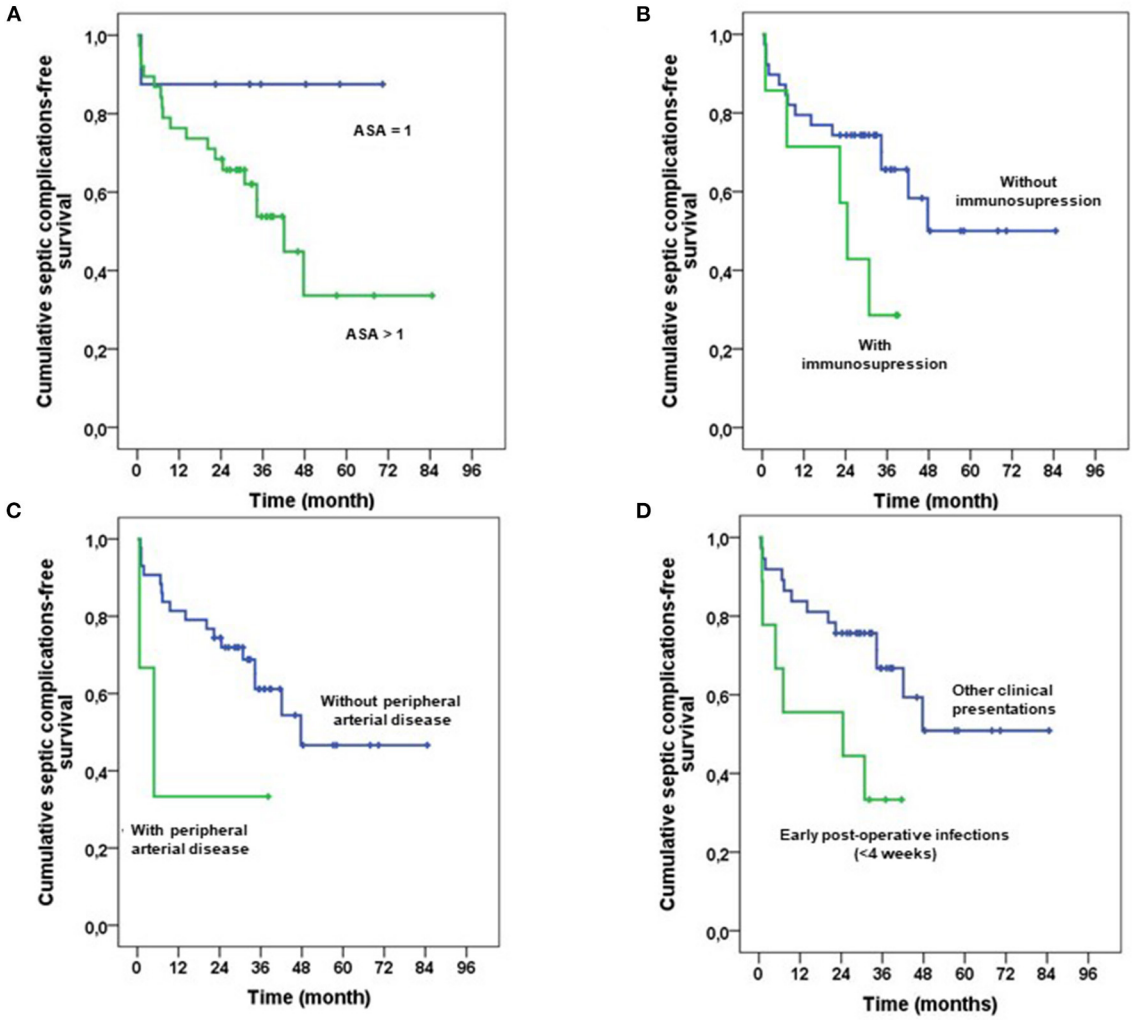

FIGURE 3 | Cumulative probability for the infectious failure of rotating hinge knee prosthesis, depending on the ASA score (**A**; Log-Rank = 0.09), on the immunosuppression status (**B**; log-rank = 0.06), on the presence or not of peripheral arterial disease (**B**; log-rank = 0.10), and on type of infection depending on Tsukayama classification (**D**; log-rank = 0.02).

prosthetic joint infection at 10 years. One explanation could be the wider indication of hinged TKAs in the study of Zahar than in our institution, where hinged implants were used only for severe prosthetic knee infections (5). Furthermore, this study only included patients for whom the pathogen was known before surgery and did not specify the distribution of acute or chronic infections, which may be a crucial element to interpret their results. Finally, the patients who did undergo a new surgical procedure after reimplantation (75% at 10 years, CI95% [60–87%]) were not systematically considered as a failure, and criteria for successful infection control in this study were defined as no clinical signs of infection, no further surgery with the diagnosis of periprosthetic joint infection (PJI), and no further positive cultures after the one-stage septic exchange (38).

Considering functional outcomes, previous studies have not used the same evaluation scores and often with heterogenous indications, making interpretation difficult (**Table 9**). In this study, IKS "knee" scores seemed lower than those found in the literature (33, 37). However, our IKS "function" scores were in line with the literature (13, 14) or slightly more favorable (8, 37). Globally, functional scores are worse in reported series including hinged TKAs used in septic revisions (8, 13, 14, 33, 37) than in cohorts only studying non-septic indications (first-line arthroplasties or mechanical revisions) (33, 34). Nevertheless, the mean range of flexion found in our study was slightly better than that observed in the study of Zahar et al. (32) (respectively, 88.7° vs. 76°).

In our study, patients with chronic knee PJI requiring revision with rotating hinge knee arthroplasty experienced a high rate of septic recurrence. We found that acute infection as initial clinical presentation according to Tsukayama was

TABLE 8 | Multivariate Cox analysis.

	HR[a]	95% CI[b]	*p*
Multivariate Cox model n°1			
Acute infection as initial clinical presentation according to Tsukayama	3.12	1.14–8.58	0.027
Age (per 10 years)	0.70	0.44–1.10	0.120
Multivariate Cox model n°2			
Acute infection as initial clinical presentation according to Tsukayama	2.97	0.10–8.50	0.032
ASA score >1	4.86	0.64–36.81	0.126
Multivariate Cox model n°3			
Acute infection as initial clinical presentation according to Tsukayama	2.46	0.83–7.26	0.104
Immunosuppression	1.895	0.61–5.89	0.269
Multivariate Cox model n°4			
Acute infection as initial clinical presentation according to Tsukayama	2.96	1.08–8.09	0.034
Peripheral arterial diseases	3.11	0.70–13.95	0.138

[a] *Hazard ratio.*
[b] *95% CI.*

a significant risk of septic failure defined by the need for subsequent surgery such as DAIR or implant removal due to clinical signs of infection occurrence. It is unclear why patients with an acute presentation should be more at risk of septic failure, especially as the different mechanisms of persistence such as biofilm are usually developed by the bacteria during chronic infections. It is possible that acute presentation could be potentially associated with a high bacterial inoculum or could be associated with more inflammation among periprosthetic soft tissue that may facilitate bacterial superinfection. Most septic failures were due to bacterial superinfections, probably acquired during reimplantation, despite following the WHO guidelines for the prevention of infection, such as the use of systemic cefazolin and the use of gentamicin-loaded cement for the prosthesis fixation as prevention (23). Checking the antibiogram of each pathogen responsible for superinfection, we found that cefazolin and/or gentamicin were not active in 8 out of the 19 superinfections, leading to reconsider systemic and/or local antimicrobial prophylaxis pathways. We found that patients with ASA score >1, with immunosuppression, or with arterial vascular diseases were at higher risk. Thus, these patients crucially need additional innovative approaches to reduce the rate of superinfection. A more efficient systemic antimicrobial prophylaxis and the use of particular antibiotics-loaded cement for prosthesis fixation could be alternative options. The first option would be using a beta-lactam with a wider spectrum of activity than that of cefazolin. The only one that could target all the involved pathogens in superinfections, except for multi-drug-resistan (MDR) *Staphylococci*, would be imipenem. Unfortunately, it is not possible to use imipenem as systemic prophylaxis, since it is considered as a last resort antibiotic that must be kept for MDR severe infections (39). The second option would be adding systemic gentamicin to cefazolin to increase the spectrum of activity on *Enterobacteriaceae*, *P. aeruginosa,* and *E. faecalis*. Of note, two MDR *Enterobacteriaceae* responsible for superinfection in our study were gentamicin-resistant, and all of our patients received gentamicin as local antimicrobial prophylaxis in the cement used to fix the prosthesis. The final option would be a combination of antimicrobials in the cement used for reimplantation. For that purpose, it is important to use commercial cements that guarantee the mechanical strength of the fixation (39). Manually adding antibiotics into the cement during its preparation is technically feasible for a spacer but is controversial when the cement has only been approved and designed to fix prosthesis (39). Few antibiotic-loaded cements releasing a combination of antimicrobials are available on the market. Gentamicin- and clindamycin-loaded poly-methyl methacrylate (PMMA) cement is available in Europe, but we do not consider it as useful for our patients, even if the dose of gentamicin is higher compared to the one we used, since there is no added value of the clindamycin in terms of the spectrum of activity. An aminoglycoside (tobramycin or gentamicin) could be combined with vancomycin in a PMMA spacer: tobramycin- and vancomycin-cement are available in the US (40), and gentamicin- and vancomycin-cement are available in Europe (41). These cements are interesting as their spectra of activity cover aminoglycoside-sensitive *Enterobacteriaceae, E. faecalis,* and most of the *Staphylococci,* including MDR *staphylococci*. In our study, using this kind of cement during reimplantation would have had an activity on all pathogens responsible for superinfections, except on the two MDR *Enterobacteriaceae* that were also aminoglycoside-resistant. An alternative could have been to use intrawound vancomycin combined with gentamicin PMMA cement. In a recent study that included patients with primary arthroplasty, intrawound vancomycin seems to decrease early periprosthetic joint infection (42). But with this route of application, the local release of vancomycin is probably limited in time, unlike cements that last several days (41). Finally, an additional measure would be to propose *S. aureus* decolonization before reimplantation (43), but only 1 patient out of the 19 developed post-operative *S. aureus* superinfection.

Our study had several limitations. First, there was an obvious selection bias since all patients were managed at the

TABLE 9 | Literature review about septic revision managed with hinged prosthesis.

Study (date)	Number of septic revisions with hinged prosthesis (Number of patients in the cohort)	Surgical strategy	Mean follow-up [min-max]	Type of implant	Survival	Functional outcomes	Post-operative complications
Pradhan et al. (7)	23 (51)	2-stage	4 years [2-6]	Endo-Model®	np	Pre-operative HSS[a] score:32 Post-operative HSS[a] score: 70	- moderate pain: 3/23 - Amputation for septic recurrence: 1/23 - 6 plastic surgeries - persistent pain and stiffness: 1/23
Deehan et al. (33)	11 (72)	2-stage	10 years [3-18]	Howmedica Kinematic rotating hinge	90% at 5 years follow-up, across indications	Across indications: Knee Society Score 28–74	−18% (2/11) of reinfections following septic revision - Across indications: persistent pains (14%), extensor apparatus dysfunction (7%), Infection (7%), Peri-prosthetic fracture (4%)
Molenaers et al. (34)	29 (66)	2 stages	5 years [2-12]	Finn/OSS Biomet	92% at 5 and 10 years, across indications	KSS[b] +27 points KSS[b] pain + 12 points KSS[b] function +20 points	- 1 septic recurrence - Other septic revisions complications unspecified
Smith et al. (35)	46 (111)	Np	Np	- Kinematic 1 Stryker - Kinematic 2 Stryker - Duracon Total Knee System-Modular Rotating Hinge, Stryker - S-ROM Revision Hinge Knee, DePuy - Finn Hinge Knee Rotating Platform System Biomet	77% at 1-year follow-up, 52% at 5 years follow-up, across indications	Np	Across indications: - 63 % complications - 24% infection - 12% soft tissue complications (extensor apparatus and/or scar) - 7% aseptic loosening - 5% peri-prosthetic fracture
Shen et al. (9)	29 (94 hinged prosthesis, 381 non-hinged prosthesis)	Np	6 years [3-10]	Np		- Better functional outcomes of hinged TKA in patients with AORI[d] type II bone loss in septic indication - Improved WOMAC[c] score for hinged TKA in patients with AORI[d] type III bone loss in septic indication	
Farid et al. (36)	60 (142)	2 stages	57 months [24-163]	OSS Biomet	78.4%	Np	- 2-staged revision failure: 21.0.6% - Any cause failure: 26% - Reoperation: 38.5% - Aseptic loosening: 9.2% - Mechanical complications of the hinge: 6.1% - Extensor apparatus complications: 6.1% - Peri-prosthetic fracture 6.1% - Femoral stem fracture: 7.7%

(Continued)

TABLE 9 | Continued

Study (date)	Number of septic revisions with hinged prosthesis (Number of patients in the cohort)	Surgical strategy	Mean follow-up [min-max]	Type of implant	Survival	Functional outcomes	Post-operative complications
Cottino et al. (37)	144 (408)	2 stages	48 months [24-144]	- Howmedica modular rotating hinge - NexGen RH Knee Zimmer - S-ROM Noiles rotating Hinge Depuy - Finn Rotating Hinge Biomet	Across indications: - 84.5% at 5 years follow-up - 71.3% at 10 years follow-up	Across indication: KSS[b]: from 51 to 81 KSS[b] function: from 26 to 36	Across indications: - Infection (11%) - Delayed wound healing (3%) - Stiffness (2.5%) - Aseptic loosening (2.5%) - Superficial infection (1.2%)

HSS[a] score de l'Hospital Special Surgery.
KSS[b] Knee Society Score.
WOMAC[c] score Western Ontario McMaster.
AORI[d] Anderson Orthopaedic Research Institute.
Np, no precisions.

Lyon University hospitals. This also explained most two-stage procedures, which remain the gold standard (44–47). Then, although the number of one-stage-managed patients was low ($n = 3$), this probably heterogenized our study, and we could not establish two comparative groups (one-stage vs. two-stage). In the literature, the meta-analysis of Kunudsor et al. (48) found similar reinfection rates between one- and two-stage exchanges [7.6% CI 95% [3.4–13.1], $p < 0.001$ vs. 8.8% CI 95% [7.2–10.6], $p < 0.001$]. Functional scores were similar between the two groups (IKS score and range of motion). Even if the sample size was low in our study, all patients requiring septic revision were managed in the same way at the stage of rotating hinged prosthesis reimplantation. Last, despite the low sample sizes, we recorded essential signals (high rate of superinfection, particularly in comorbid patients) that must be considered to implement innovative preventive measures in such a population.

CONCLUSIONS

Hinged prostheses in septic revisions of TKAs are a therapeutic alternative with contrasting results. When successful, they offer satisfying functional outcomes and good survival results in the short and medium terms; however, complications are frequent, specifically infectious events. Efforts have to be made in the prevention of superinfections, especially for patients with immunosuppression and peripheral arterial diseases, since the risk of infections after TKA revision with hinged prosthesis is high. These patients require optimal antimicrobial systemic prophylaxis and innovative approaches to reduce the rate of superinfection. More research studies are needed to further evaluate optimal antimicrobial prophylaxis and to identify innovative approaches to reduce the rate of superinfection.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospices Civils de Lyon Ethic Committee. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SL, TF, and FB-S contributed conception and design of the study. FB-S organized the database and wrote the first draft of the manuscript. TF and MC performed the statistical analysis. TF wrote sections of the manuscript. MB-L translated the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

ACKNOWLEDGMENTS

We would like to acknowledge Jobe Shatrov, MD, who helped in the edition of the manuscript.

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