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New threats of antibiotic-resistant bacteria and fungi

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New threats of antibiotic-resistant bacteria and fungi

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Editorial: New threats of antibiotic-resistant bacteria and fungi

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Editorial on the Research Topic
New threats of antibiotic-resistant bacteria and fungi

Today, antimicrobial resistance (AMR) continues to pose a major threat to our community and healthcare systems. It is estimated that AMR will cause 10 million deaths worldwide by 2050, surpassing cancer deaths. Multidrug-resistant (MDR) pathogens which are frequently resistant to almost all antibiotics significantly contributes to the successive reduction of available therapeutic options. The emergence and spread of new forms of resistance continues to raise the alarm for this global crisis, especially resistance shared through genetic mobile elements, as antibiotic resistant microbes can transmit their resistance genes, increasing resistance. Due to the extreme gravity of this issue, it is necessary to investigate and understand the new threats and resistance mechanisms of MDR bacteria or fungi. This Research Topic integrates recent studies on the new threat of MDR infection in clinic, novel characteristics and mechanisms of carbapenem-resistant *Enterobacterales* (CRE), as well as surveillance information and control strategies of resistance to new antibiotics.

Currently MDR pathogens constitute the main challenge in patients admitted to ICU, which is largely due to invasive procedures, impaired protective mechanisms, and the extensive use of antibiotics. In our collection, Odih et al., using whole genome sequencing (WGS) and single nucleotide polymorphism (SNP) analysis, reported the first outbreak of a carbapenem-resistant IC2 *A. baumannii* clone in an ICU in Nigeria. They also highlighted the importance of *A. baumannii* detection and prevention in Nigerian clinical settings. In another study from China, Wang et al. focused on evaluating the risk factors of acquired *S. maltophilia* pneumonia in ICU patients. This study concluded that the prevalence of hospital-acquired *S. maltophilia* pneumonia in ICU patients was high, and severe diseases, undergoing invasive procedures, and recent antibiotic use may be important contributors. A third study targeting MDR in the ICU, by Çaglayan et al.,

proposed a data-driven modeling framework to predict MDR colonization upon ICU admission, and identified the associated socio-demographic and clinical factors. In this large study, MDR colonization was found in 17.59% of 4,670 ICU admissions, and it was associated with long-term care facility stay, underlying diseases, and recent precaution procedures before ICU admission.

Another new MDR threat in our topic have described the emerging infection or coinfection with MDR bacteria and fungi. In a case report, Cui et al. described an acute T lymphoblastic leukemia patient with mixed infections of lethal invasive Mucormycosis and multidrug resistant bacteria. Also, they emphasized the importance of considering malignant hematological conditions and using metagenomic next-generation sequencing (mNGS) to aid in the early and timely diagnosis and treatment of Mucormycosis. Another study conducted by Damas et al. focused on *Chryseobacterium indologenes,* an emerging multidrug resistant nosocomial pathogen, and presented the first detailed molecular characteristics of MDR *C. indologenes* through whole-genome sequencing. The researchers also concluded that their findings could help shape future public health policy and MDR *C. indologenes* infection control. Additionally, insights into the impact of extended-spectrum cephalosporin-resistant (ESC-R) *Enterobacterales* (EB) causing early urinary tract infection (UTI) in kidney transplant recipients, were provided by Aramwittayanukul et al. They determined administration of carbapenem peri-transplant prophylaxis can significantly protect against ESC-R EB UTI early after KT. In addition, Xiang et al. looked into the risk factors and outcomes of postoperative pneumonia. They discovered that postoperative pneumonia was linked to a longer length of hospital stay, a higher ICU occupancy rate, a higher rate of unplanned re-operation, and a higher rate of in-hospital mortality.

Carbapenem-resistant Klebsiella pneumoniae (CRKP), a major threat to global public health, has showed some novel characteristics in our Research Topic. Zeng et al. reports the novel findings on epidemiology and resistance mechanisms in ST 11 carbapenem-resistant *Klebsiella pneumoniae* carry bla_{KPC-2}. Interestingly, the whole-genome sequencing showed that bla_{KPC-2} was found in a genetic context with insertion sequences ISKpn27 upstream and ISKpn6 downstream, all of which were flanked by IS26. Furthermore, the structure of the type IV secretion system (T4SS) aids in the adaptation of bacteria to the environment.

To combat MDR pathogens, effective control strategies need to be explored. Aside from rapid diagnosis and new antibacterial development, appropriate precaution and necessary surveillance are critical. The study by Morel et al. determined the essential elements and requirements of antimicrobial resistance surveillance for new antibiotics. Another work by Amin et al. discussed the practical antibiotic stewardship in outpatient settings in the United States, and they suggested that community prescribers can help move the needle on antibiotic stewardship by keeping in mind the "4 Ds": prescribe an antibiotic for a bacterial infectious Disease, with the appropriate Drug, Dose, and Duration.

In summary, the manuscripts in this Research Topic highlighted new threats and novel mechanisms of multidrug-resistant pathogens broadly. We thank all the editors, authors, reviewers for their contributions to this Research Topic. Looking forward, studies involving the new emergence and mechanisms of MDR fungi are highly encouraged in future collections.

Author contributions

XJ and SH drafted the manuscript. Y-WT and CC revised and edited the Editorial. All authors approved the final version of the manuscript.

Conflict of interest

Author Y-WT is employed by Danaher Diagnostic Platform/Cepheid.

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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Surveillance of Resistance to New Antibiotics in an Era of Limited Treatment Options

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As with any health threat, our ability to respond to the emergence and spread of antimicrobial resistance depends on our ability to understand the scale of the problem, magnitude, geographical spread, and trends over time. This is especially true for resistance emergence to newer antibiotics coming to the market as last-resort treatments. Yet current antibiotic surveillance systems are limited to monitoring resistance to commonly prescribed drugs that have been on the market for a long time. This qualitative study determined the essential elements and requirements of antimicrobial resistance surveillance for new antibiotics based on literature review, interviews and expert consensus. After an extensive mapping exercise, 10 experts participated in a modified Delphi consultation to identify consensus on all elements required for surveillance of resistance to novel antibiotics. The main findings indicate that there is a need for a two-phase system; an early alert system transitioning to routine surveillance, led by the public sector to gather and share essential data on resistance to newer antibiotics in a transparent manner. The system should be decentralized, run largely from national level, but be coordinated by an arm of an existing international public health institution. Priority should be given to monitoring emergence of resistance among already multi-drug resistant pathogens causing infections, over a broader selection of pathogens to maximize clinical impact. In conclusion, we cannot rely on current AMR surveillance systems to monitor resistance emergence to new antibiotics. A new, public system should be set-up, starting with a focus on detecting resistance emergence, but expanding to a more comprehensive surveillance as soon as there is regional spread of resistance to the new antibiotic. This article provides a framework based on expert agreement, which could guide future initiatives.

Keywords: resistance surveillance, antibiotics, antimicrobial resistance, antimicrobial susceptibility, early warning systems

INTRODUCTION

Over the years, antimicrobial resistance (AMR) has been steadily increasing, and new resistance mechanisms have emerged and spread worldwide (1–3). This threatens the effective treatment of patients suffering from community-acquired and healthcare-associated bacterial infections, as well as the success of prophylactic treatment in patients undergoing high-risk surgery. As such, there is a clear need for the development of effective, novel antibiotic treatments (4). While the antibiotic pipeline has been slowly refilling, only one in four new, approved antibiotics represents a novel drug class or mechanism of action, and to-date none of these are active against Gram-negative WHO critical threat pathogens (5). At the same time, the emergence and spread of AMR also requires vigilance to identify changes in the epidemiology of pathogens causing infections—including their resistance to newer antibiotics. Yet, our current surveillance systems focus only on resistance to older drugs. They are not designed to adequately measure resistance to the newer ones used for last-resort treatment.

Current, publicly-led surveillance efforts focus on pathogen resistance within the set of older antibiotics. For example, in Europe, EARS-Net collects data from invasive isolates (blood and cerebrospinal fluid), reporting resistance amongst seven common pathogens[1] to carbapenems, fluoroquinolones, third-generation cephalosporins, aminoglycosides, and penicillins (6). GLASS also collects data on these pathogen-antibiotic combinations, in addition to resistance to a few more, such as tetracyclines, penicillins, sulfonamides plus trimethoprim—all from the older generation of antibiotics (WHO 2). Occasionally some more targeted measures have been taken in response to specific concerns about resistance to new drugs. For example, the European Center for Disease Prevention and Control (ECDC) recommended notifying cases of resistance to the newly marketed broad-spectrum antibiotic ceftazidime-avibactam and exchanging information through existing platforms[2] to enable informed and coordinated action by public health authorities (7). For the most part, AMR surveillance for newer drugs has been initiated by the private sector to support applications for (additional) market approval or to satisfy post-launch requirements to the regulator. Some of the more well-known private sector surveillance initiatives include MYSTIC, ResistNet, and the Alexander Project. However, information from these surveillance activities remain the property of companies who collect it, usually through third parties. A selection of findings from these surveillance activities are published occasionally, but often with limited detail regarding the sampling protocol. This makes it difficult to compare with other data, assess potential for reporting or selection bias, and to assess the level of representativeness and overall usefulness for public health policy. These resistance surveillance findings are certainly never sufficiently detailed or thorough to spur urgent public health action. Asking industry (antibiotic producing pharmaceutical companies and the surveillance companies they contract) to be the *de facto* lead on AMR surveillance of newer antibiotics creates a clear conflict of interest as resistance to their product affects efficacy, and, in turn, decreases future sales. This can engender biases in the data published and ultimately further limit its usefulness. For example, in a recent study, it was shown that the ATLAS data, from Pfizer, consistently reported higher resistance proportions for older antibiotics than did public data from EARS-Net (8).

Given the burden to collect surveillance data and the conflicts of interest involved in depending on industry, the European Medicines Agency (EMA) is anticipating reform to lower post-market surveillance monitoring obligations by companies to the European regulator (9), which will lower the amount of data they collect and publish. Private sector surveillance activities have already slowed down, and altogether ceased within some companies. The public sector will be left to fill the gap if we are to have any insight into emerging resistance—and hence efficacy—of our most valuable new, antibiotic treatments. This study explores the requirements and desired features of a standardized system for monitoring bacterial resistance to newly launched antibiotics within the healthcare setting in order to help advance discussions on how best to enhance and improve AMR surveillance of new antimicrobial agents. The reported results are based on a mapping exercise, combined with a consensus process based on input from international experts.

MATERIALS AND METHODS

Issue Mapping

The first phase of this qualitative study consisted of a mapping exercise to explore key features of a surveillance system for novel antibiotics (those with novel mechanisms of action) through a review of literature and interviews (17–46).

The literature review included studies published between 1998 and 2019 in MEDLINE, using the following search criteria: resistance surveillance, antibiotic/antimicrobial resistance, antibiotic/antimicrobial susceptibility, susceptibility testing. In addition, reference lists of relevant studies were screened, and experts were consulted to identify all relevant studies. This informed the various features of AMR surveillance and helped to evaluate previous and ongoing AMR surveillance systems.

Twenty interviews were then undertaken with a range of stakeholders involved in AMR surveillance, including microbiologists, hospital epidemiologists, infectious disease physicians, private sector companies, and specialists from national and international agencies (e.g., ECDC, CDC, WHO) based in Europe and the United States[3]. Experts were identified from a combination of the authors' professional networks, published literature, and snowball sampling (identifying one led to the identification of another). Interviews were intended to move one step beyond the published literature, to gather key aspects of the surveillance structures that need to be considered

[1] *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter species, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium* (1).

[2] EWRS and EPIS.

[3] Geographic origin was not an established parameter. The inclusion of individuals from Europe and the United States was a result of the selection methodology.

when designing an enhanced, publicly-led AMR surveillance framework that would cover new antibiotics.

Consensus Procedure

Findings from the mapping exercise were used to develop a survey and conduct a modified Delphi consultation. Delphi was chosen amongst numerous other potential expert elicitation methods due to its flexibility and usefulness for very specific questions. The limited number of experts ruled out random participant selection. The inability to repeatedly bring all the experts together for face-to-face moderating ruled out other elicitation forms. Delphi is a structured process that uses a series of rounds to gather information from a heterogeneous panel of experts in order to achieve agreement. Agreement of 70% among responses was used as a threshold to define consensus. Respondents remained anonymous to one another and provided their responses in isolation through structured questioning. The software *Survey Monkey* was used to conduct the survey.

Experts, all external to the study, were selected on the basis of their broad methodological expertise in AMR surveillance: microbiology, infectious disease, epidemiology, and public health. They were identified from their publications in the field and/or their involvement with the main surveillance networks (e.g., EARS-Net, GLASS, Combacte-Magnet, EPI-Net). Overall, 11 experts were identified and invited to participate[4].

Delphi Round 1, which took place in late 2018, consisted of 26 questions covering the key features of a surveillance system: the patient population, the isolate selection process, the sample types, the timing of isolate collection, the institutions that should be reporting to the system, etc. (**Appendix 1** in **Supplementary Materials**). The survey was piloted by three individuals: two microbiologists and one infectious disease specialist. Ten survey responses were received (gender balance: four women, six men) from Europe and North America.

Delphi Round 2, which took place in spring 2019, included the same 10 participants. The survey included the findings from Round 1 followed by 10 additional questions (see **Appendix 2** in **Supplementary Materials**), which sought to gain greater precision on the topics of agreement, to clarify questions or responses from Round 1 that had been deemed ambiguous, and to identify the root of any disagreement. Several of the questions derived from a case study (of a hypothetical "super-penem"), used to understand multidimensional preferences (using scoring 1 to 5). The last question of the questionnaire was posed in an open format to ask participants for any further comments regarding the ideal features of early warning or routine AMR surveillance for new antibiotics. Responses were received from all ten experts who had participated in Round 1.

Expert consultation was terminated when, for each question, there was either broad consensus or consensus was deemed impossible but clarity on the basis for disagreements was achieved, as discussed below.

[4]The 11 individuals identified (10 of whom participated) were all of those deemed to have a very deep understanding of AMR. The individual areas of personal expertise varied amongst several disciplines. It was deemed more important to acquire high quality input than to achieve a greater number of responses.

TABLE 1 | Objectives of surveillance systems [adapted from (13) and (16)].

Establish the prevalence of different forms of resistance, their geographic distribution, and their evolution over time (including outbreaks)
Inform the estimation of burden
Guide empirical therapy
Inform and monitor prevention activities
Provide data to assess the efficacy/effectiveness of interventions
Detect new resistance mechanisms
Evaluate the threat of transmission of an especially worrying resistance mechanism or clone
Bolster capacity-building, standardization and harmonization of antimicrobial susceptibility testing across laboratories
Detect and report abnormal bacteriological events such as low levels of acquired resistance
Suggest transmission of resistance genes between species
Explore the consequences of bacterial resistance over time, such as relationship to patterns in treatment failure, morbidity, mortality, or economic impact

RESULTS AND DISCUSSION

The first part of the issue mapping exercise, based on a literature review, identified nine main surveillance design issues (focus and purpose, timing, governance, antibiotic susceptibility testing (AST) data, AST methods, type of sampling, pathogen, desired patient-level data, desired hospital-level data, data quality, reporting) with 61 sub-issues (**Appendix 3** in **Supplementary Materials**). The second part, based on expert interviews, narrowed the issues down to those requiring expert consensus for prioritization and a more in-depth exploration through the Delphi exercise (**Appendices 1**, **2** in **Supplementary Materials**).

Focus and Purpose of Resistance Surveillance

The objectives of AMR surveillance can differ across systems. Generally, they are intended to achieve the objects listed in **Table 1**.

For AMR surveillance of new antibiotics generally most of the above objectives would qualify as well. However, for novel drugs, since acquired resistance will hopefully be a rare occurrence right after market approval, and laboratory methods for resistance detection might not yet have been developed, it will be more complicated to achieve *all* of the listed objectives.

Timing: Two-Stage Surveillance

As described below, the Delphi exercise clarified that enhanced surveillance for new antibiotics should be conducted in two phases; first an early warning surveillance to detect emergence of resistance, followed by an enhanced routine surveillance to determine frequency, spread, setting and trends.

Phase 1. Early Warning Surveillance to Detect Emergence of Resistance

Immediate early warning surveillance—to be implemented as soon as a newly introduced drug is used locally—was deemed to be particularly relevant for drugs that are critically important,

such as for the treatment of infections with limited treatment options (e.g., multidrug resistant–MDR) and priority AMR threats [e.g., carbapenem-resistant Enterobacterales–CRE, as per (10–12). Such immediate surveillance was considered crucial to act quickly with regard to infection prevention and control measures, and both development and dissemination of diagnostic procedures (including procuring appropriate technologies). Immediate surveillance was considered less important for new members of existing classes that do not substantively add to the existing treatment options. In this study, respondents agreed that the level of use of the new antibiotic was not relevant; while normally all novel antibiotics should have very low levels of use initially, this should not preclude surveillance.

There was some initial disagreement amongst experts regarding the issue of who should be responsible for reporting emergence of resistance to the surveillance governing body. Half of respondents (5/10) in Round 1 felt that, for initial reporting of resistance emergence, we could rely on agreements with existing reference laboratories, while the other half felt we should rely on agreements with existing (largely private) surveillance networks. In Round 2 participants were asked how we should proceed if private sector resistance surveillance activities decrease following the expected EMA regulation reform which greatly decreases the surveillance duties of private companies. Four out of five participants who had initially reported that we should rely on specialized private sector surveillance companies to report resistance emergence ultimately agreed that it would be more prudent to rely on public reference laboratories for this purpose in view of the expected reduction in private sector activity. The one dissenting voice qualified the response, pointing out that many public laboratories in Asia and Africa are inadequate, implying that this may not be a concern in Europe or United States. Acceptance of public reference laboratories for reporting of resistance emergence in Europe and the US was therefore considered unanimous. The reliance on reference laboratories globally may not be currently possible[5].

All hospitals should be expected to send isolates suspected of resistance to the novel drug (including all repeat isolates of that patient) to a reference lab where the resistance can be confirmed and the evolution of the strain can be explored. The role of the reference lab in detecting early resistance was seen to be active—meaning that the reference lab should receive guidelines and have some form of obligation to report to the surveillance body. Many participants felt that the World Health Organization (WHO) early warning system (EAR)—which is a reporting system, not a surveillance system—could be useful for reporting initial resistance emergence, assuming subsequent verification. In Phase 1 the number of resistant samples and patients should be reported initially, soon followed by reporting of resistance proportions in order to have some indication of the relative importance of the problem. Burden of disease measures, like prevalence or incidence were not seen to be essential for early warning surveillance. (See below for further discussion of resistance measures).

[5]In some cases, university laboratories can serve this purpose.

Phase 2. Routine Surveillance

All participants agreed that, as resistance becomes more prominent, routine surveillance should be enhanced, and the selection of institutions for routine surveillance should facilitate as good a coverage as possible. Ideally, the results of AST for new antibiotics should be reported for all samples, including pediatric isolates, by all facilities processing clinical samples. Of course, practically this depends on the level of available resources and infrastructure. For example, if it is possible to connect all laboratory information systems (LIS) to a central database, then all available AST results can be automatically uploaded to the surveillance structure.

The transition between the two phases of surveillance was discussed as part of a hypothetical ("super-penem") case study (see below). In the next paragraphs, we will provide a more detailed description of essential elements of routine AMR surveillance.

Governance: Structure and Funding of the Enhanced Surveillance System

Availability of resources can clearly influence how any resistance surveillance system is designed and assumptions about the availability of resources affects how experts foresee the enhancement of current surveillance practice. Therefore, the resource question was addressed in many of the themes explored below.

In general, international public AMR surveillance systems have been based on networks of networks, where for example national laboratory networks report to an international network—often without any underlying funding—while private AMR surveillance systems have had a more top-down approach supported by funding. Expert consultation suggested that governance of an enhanced surveillance system should mimic public AMR surveillance systems; be run largely from national level, but coordinated by an arm of an existing international public institution (e.g., WHO, ECDC). The enhanced surveillance would require financial support, as this will affect the degree of authority of the governing structure. For example, EARS-Net currently does not offer contributing countries or hospitals any funding, and therefore cannot impose specific sampling schemes, or apply centralized testing. Funding from the public health sector would safeguard transparency.

Governance: Data Dissemination Structure

All Delphi participants agreed that AMR surveillance data should be merged at national/sub-national level first and then fed to the international governing body. It was emphasized that curation of AMR data by national level authorities is preferred prior to sharing it with any supranational entity. In particular, from a quality perspective, the first level of data collection, cleaning, validation, and confirmation should be carried out at the national level. The EARS-Net method for centralizing the data is considered a well-functioning, tried and tested model. However, for the purposes of early warning, direct contact between the laboratories and the surveillance body may be required to avoid delays. Timeliness is indeed crucial, and all efforts must be made to avoid lags in cleaning and verification.

Withholding information in anticipation of publishing must of course be strongly discouraged.

Governance: Hospital Selection

Owing to limitations of resources and the focus on new antibiotics intended for use within the hospital setting for multidrug resistant infections, it was assumed that sentinel surveillance (a small number of selected surveillance sites) would be used over wider, population-based surveillance—even if the former reduces external validity and risks missing the first, emergent strains. At the same time, representativeness of the data provided by the selected centers for similar centers in the country should be an explicit goal. (Representativeness is discussed further in the "Type of sampling" section below).

For surveillance sites, participants agreed that local hospitals (clinical records), not local laboratories (microbiological reports), or reference laboratories should be the site of choice to report data to the governing body. Lab-based often means that clinical data is unavailable, which complicates, or renders impossible, the distinction between contamination, colonization, or infection. Surveillance based on clinical records can be syndrome-driven, has the advantage of availability of clinical outcome data, and can provide metrics to indicate potential detection bias, like "Number of blood cultures conducted per 1,000 patient-days within the contributing hospital" (preferred detection bias metric by respondents).

No consensus could be achieved regarding who should be in charge of selecting hospitals for the sentinel surveillance: six participants thought it should be the surveillance governing body, while 4 argued that it should be left to national authorities. Participants also did not agree on whether a country should have to provide data from a representative set of hospitals/laboratories ($N = 5$ votes) or a convenient sample ($N = 5$ votes) in order to be a full member of the surveillance network. From the free text it became clear that this disagreement was not associated with differences in valuing representativeness, but rather the expectancy that some national governments undervalue AMR surveillance and will not put the effort into collecting representative data, thereby possibly rendering the most important countries ineligible to participate. Indeed, WHO and ECDC currently go through national governments and public health authorities, and as indicated several times during the exploratory phase of this work, obtaining representative data remains a challenge. Comments from participants indicate that, before we can focus on the representativeness of the data, we must first get buy-in from national authorities. Presumably if the increase in AMR is felt more acutely in the years to come, national authorities are likely to agree to more active AMR surveillance, and to work with other countries to make sure the framework is representative. It was also emphasized that, in some countries, the role of private laboratories is so important that their participation will be necessary for representation, but they may be reluctant to provide services without clear financial benefits or other incentives[6].

AST Methods: Phenotypic vs. Genotypic Susceptibility

In most public AMR surveillance systems, phenotypic resistance is the basis of AST reporting, and genotypic susceptibility is optional and limited to a number of common resistance genes that are easy to test for, like extended-spectrum beta-lactamases (ESBL) in Gram-negative pathogens or mecA resistance genes in *Staphylococcus aureus*.

Phenotypic resistance is determined from the concentration of the antibiotic that inhibits bacterial replication *in vitro* and is used to predict whether or not a drug will be capable of inhibiting the bacteria *in vivo*, and thus improve clinical outcome. Genotypic resistance is determined by the presence of specific genes or plasmids that are known to confer decreased drug susceptibility. Presence of resistance elements does not always have direct treatment implications, as the bacterium may not express its resistance, or only express low-level resistance.

While genotypic analysis has been an integral part of some industry-sponsored AMR surveillance systems (e.g., SENTRY and PROTEKT), it should not be considered essential to the basic surveillance framework for antibiotics. The focus of the laboratories reporting AST to participating hospitals should be on phenotypic resistance, which can detect emerging resistance and is important to inform prescribing. However, where possible, new resistant isolates should be retained for genotypic analysis and re-analysis with new analytical techniques to understand the mechanism of action, pattern of spread (stand-alone mutations, clonal spread, or horizontal transfer of resistance genes) and inform diagnostic tests at the local level and for reference laboratories (13–15). At the level of reference laboratories, it is important to use both phenotypic and genotypic tests, as the latter can detect the genetic resistance mechanism (e.g., beta-lactamase production) needed for molecular epidemiology and development of reliable gene-based, diagnostic tests.

Type of Sampling: Representativeness, Population, Isolate Type, and De-Duplication Strategy

In a perfect world, AMR surveillance for a new antibiotic would capture drug-specific AST data of all pathogens causing infections (or even colonization) within a population. Second best is to capture a sample with enough external validity to draw conclusions regarding the general level of resistance to the new antibiotic within the population. However, resources are limited, and sacrifices around representativeness have to be made. This may mean focussing on a patient population in which resistance is most likely to emerge. For example, surveillance efforts could be focused on hospitals where the new drug is being used extensively. Experts agreed that national authorities are best placed to decide when to proceed to more representative sampling by including routine AST data from hospitals that don't use the drug (transition to more routine AST is discussed below).

[6]In some settings, private laboratories are willing to join public networks without financial remuneration in order to receive more training (to support quality assurance), to partake in research, to boost prestige, or simply to support public health.

Participants in this study agreed that isolates from all age groups, including children, should be included. However, a newly approved novel drug is unlikely to have received regulatory approval for use in children, so any resistance emergence in this age group would be associated with transmission or off-label use.

Most respondents felt that all clinical samples should be tested for the novel drug and reported to the surveillance system. If resources are limited, a predetermined number of samples per hospital should be used. However, predetermined numbers need to be based on levels of perceived prevalence, something not possible for a new drug, such that an anticipated level of prevalence would have to be used.

In Delphi 1, the specimen type (i.e., blood, urine, sputum, wound, broncho-alveolar lavage) that should be covered was not yet discussed, as it was assumed that this would be highly dependent on the specific new treatment; pathogen coverage, indication etc. Therefore, this was addressed in the "super-penem" case study in Delphi 2, described below. The same holds true for pathogen type.

In routine surveillance, deduplication is needed in order to avoid biased results due to over-representation of individuals undergoing repeated sampling. Often the first isolate per person, per pathogen, per specimen type, per year is used, but this may not contain the most resistant strain. Opinion suggested that, in order to better capture emerging resistance, it may be useful to include, for each patient, the isolate with the most resistant profile, preferring the isolate with resistance to the new antibiotic.

Data Quality: External Quality Control

To ensure coherent microbiological findings, laboratory services require regular quality control. A customized, surveillance-specific international scheme was considered optimal for new antibiotics.

Desired Patient- or Hospital-Level Data

Efforts should be put toward collecting data on key indicators that can enhance our ability to understand and analyse the measured resistance levels to the new antibiotic. This could include aspects surrounding the patient, treatment, and setting for all resistant isolates. In the case of limited resources, the consensus order of importance, from most to least important, is: laboratory quality, clinical outcome data, individual risk factor data, and infection and prevention indicators (i.e., hand hygiene compliance).

Reporting: From Proportion to Incidence

For routine surveillance, the level of AMR can be expressed in different ways, depending on the denominator used. The most common indicator is the proportion of resistance amongst all samples positive for a specific pathogen, followed by prevalence (denominator = patients) and incidence (denominator = patient-days at risk). The objective of the surveillance system should inform the most appropriate indicator(s). **Table 2** lays out the most important advantages and disadvantages of the different AMR surveillance metrics. All of the reported measures can be influenced by ascertainment bias, especially in settings with very low, routine sampling rates, but the impact will be different. In most cases, low sampling rates are associated with selectively sampling those patients that are most likely to be infected by a drug-resistant pathogen, for example after empirical treatment failure. This means that the number of susceptible infections is more heavily underreported than the number of resistant infections. In this scenario, proportions will overestimate resistance proportions, while prevalence and incidence will underestimate the burden of resistance. This is due to the numerator (slightly underreported) and denominator (more strongly underreported) of proportions being affected by sampling bias, while for prevalence and incidence only the numerator is influenced.

TABLE 2 | Advantages and disadvantages of different expressions of resistance (in routine surveillance).

	Pro	Con
Resistance proportion (lab based)	Relatively easy to measure, only laboratory data is required Useful for local treatment decisions	Changes in the prevalence of the susceptible bacterial population will influence the proportion (e.g., due to antibiotic use) Increasing proportions do not necessarily reflect an increase in the absolute number of resistant isolates Selective sampling can easily result in an overestimate of resistance levels
Prevalence (patient based)	It indicates the absolute size of the problem It can be used to estimate the burden of disease assuming an appropriate level of routine sampling	Infections of short duration will be underrepresented It will only provide a snapshot of the situation at a specific point in time
Incidence (patient-days at risk)	It indicates the risk of acquiring a resistant infection It can be used to estimate the burden of disease assuming an appropriate level of routine sampling Proper picture of disease occurrence over a longer period of time	It requires appropriate de-duplication to prevent double counting Requires combining laboratory and hospital information

For surveillance of resistance emergence to newer drugs, the expert consultation echoed the idea that normalization of the resistance data (using a denominator) may not be necessary. Having the number of isolates and patients is sufficient in Phase 1. However, as surveillance transitions to being more routine in Phase 2, normalization of the data becomes increasingly useful.

Reporting: Frequency

The higher the reporting frequency the better, especially for early warning systems and for new antibiotics needed in case of limited treatment options, but this, of course, depends on the level of

available resources. An automated surveillance network would allow for real-time reporting, which would be ideal in this setting. All participants agreed this would be the way forward, but would not be feasible right now.

Reporting: Destination of Reports

The majority of respondents agreed that the resistance surveillance data should be available to a wide array of users, including the public. The EARS-Net model is thought to be a workable one as anyone in the world can query the aggregated, anonymised data. Detailed, case-based data (without confidential details) are also available for distribution following a request, justification review, and approval/denial process for requests from non-participating countries. Data requests from participating countries, made through participating centers, are always approved. It should be noted that, legally, ECDC is obliged to give out any data that is collected to any EU citizen based on request. When asked if the EARS-Net data access policy should be adapted in any way for a surveillance system for newer antibiotics, participants felt that a policy like the one outlined here would be sufficient.

BOX 1 | Timing of transition from early warning surveillance to routine surveillance along the epidemiological timeline (Stages defined by Grundman and the CNSE Working Group 2010).

- Stage 0: No cases of resistant infections reported
- Stage1: Sporadic occurrence (Single cases, epidemiologically unrelated)
- Stage 2a: Single hospital outbreak (Outbreak defined as two or more epidemiologically related cases in a single institution)
- Stage2b: Sporadic hospital outbreaks (Unrelated hospital outbreaks with independent, i.e., epidemiologically unrelated introduction or different strains, no autochthonous interinstitutional transmission reported)

Transition from Phase 1 (Early warning) to Phase 2 (Routine surveillance)

- Stage 3: Regional spread (More than one epidemiologically related outbreak confined to hospitals that are part of a regional referral network, suggestive of regional autochthonous interinstitutional transmission)
- Stage 4: Inter-regional spread (Multiple epidemiologically related outbreaks occurring in different health districts, suggesting inter-regional autochthonous inter-institutional transmission)
- Stage 5: Endemic situation (Most hospitals in a country are repeatedly seeing cases admitted from autochthonous sources)

Type of Sampling: One Health

Whilst this exercise focussed on human health, it did also explore views on the necessity of extending surveillance of new antibiotics to other sectors. Half of the respondents indicated that a One Health approach is paramount, as resistance will spread in all environments, and treatment of animals with new antibiotics should not be neglected (especially outside Europe and the United States). Although the One Health perspective is important, the ability to extend the surveillance system to animals (pets, food-production) and the environment (production effluent, wastewater) doesn't seem entirely feasible straight away, as it depends on the availability of resources and infrastructure. Therefore, we suggest that the enhanced surveillance framework should first focus on humans and be extended to other sectors only when greater resources become available. Also, as stressed by Grundmann et al. (14), care should be taken not to overburden the capacities of references laboratories in asking them to handle samples from food, water and veterinary sources.

The "Super-Penem" Case Study

In order to explore nuanced preferences on issues with numerous dimensions, the group was asked to consider the case of a fictional new super-penem, which is an antibiotic active against carbapenemase-producing Enterobacteriaceae (CPE), MDR *Pseudomonas* species and MDR *Acinetobacter* species, but is not active against resistant Gram-positive pathogens, and has been approved for complicated urinary tract infection (cUTI) in adults.

Transitioning from Phase 1 (Early warning) to Phase 2 (Routine surveillance). In order to explore the level of resistance at which the transition from Phase 1 and Phase 2 should take place, participants were given the classification from Grundmann and the CNSE Working Group, provided in **box 1**.

Participants agreed ($N = 8/10$) with the suggestion that the transition would be appropriate between Stage 2b and Stage 3. One dissenting voice suggested that such a super-penem would be of such importance in treating the pathogens listed, that the transition should take place earlier, after sporadic detection, i.e., after Stage 1. A second dissenting voice stressed that, in a country with high endemic carbapenem resistance, the transition should be after Stage 1. These responses highlight the importance of availability of alternative treatments in determining when the transition should take place: the fewer the treatment alternatives, the more closely we need to follow resistance. These considerations echo the reasoning of Cornaglia and ESGARS colleagues (16)**: "**If surveillance detects resistance in a dangerous organism, with no or few alternative drugs capable of controlling it, even a very low resistance rate should be considered high risk, and appropriate action should be planned."

In this specific setting, it was expected that urine samples would be the most logical specimen of interest to report AST results to the enhanced surveillance system. Indeed, all participants agreed that urine samples were essential. In addition, they mentioned blood as essential, which is not surprising as urosepsis is an important complication of cUTI. Three out of nine thought that sputum and wound samples were also very important to include, to detect resistance emergence as early as possible. This clearly indicates that, for enhanced surveillance for new antibiotics, there will be no "one size fits all" solution with regards to specimen type.

Isolate type. Participants were asked to rank five suggestions about what isolate type should be collected according to perceived appropriateness, with the assumptions that resources are not a major constraint; that any type of sample is available—BSI, UTI, etc.; and that MDR signifies resistance to two or more antibiotic classes.

The first ranked response (average score 4.3/5) was "*All CPE isolates, regardless of whether the patient was treated with the drug,*" suggesting a strong emphasis on keeping difficult-to-treat infections treatable and keeping track of any emergence or transmission.

The second ranked response was "*Isolates from all patients who are infected with a MDR pathogen and who received the drug*" (score 3.9). This places emphasis on (added) resistance emergence within any already harder-to-treat infection, and co-lateral damage to the microbiome.

The third, "*All MDR Escherichia coli isolates, regardless of whether the patient was treated with the drug*" (score 3.1) suggests an emphasis on (added) resistance emergence within any already harder-to-treat infection. This result also emphasizes the importance of keeping that specific infection treatable and keeping track of emergence and transmission. The greater number of isolates in this case translates into higher probabilities of finding resistance emergence.

Lower ranked was "*All E. coli isolates, regardless of whether the patient was treated with the drug*" (score 2.5), which suggests that casting the figurative net as wide as possible to detect any resistance emergence to the new drug should not be a main priority.

The lowest rank was given to "*Isolates of all bacteria that could be co-presenting in the patient treated with the drug, even those bacteria not causing the primary infection*" (score 1.2), which reflects a lower priority given to potential co-lateral damage.

This questioning explored where priorities laid between two very different strategies: aiming to achieve a high probability of discovering new, emerging resistance through large sample size on one end, and maximizing clinical impact by focussing on the emergence of untreatable MDR infections, based on the indication of the drug, on the other end. The expert group placed the fulcrum on the side of clinical impact maximization.

Limitations

One important limitation in this study is that it included 10 experts, who were all based in high-resource settings, which both narrows the set of possible viewpoints it gathered as well as the external applicability of its findings. While the findings can apply to surveillance systems in any part of the world, this study does not address fundamental challenges in implementing wider resistance surveillance where there are broader systemic challenges and very limited resources. The study also did not address the possible enhancement of surveillance through novel technologies. While we examined the level of priority associated with LIS connectivity and reporting automation (which, for most surveillance systems, would represent a substantial technological advance), we did not explore the potential use of any other new technology likely to have an impact on our ability to collect data and report on antimicrobial resistance [e.g., "smart" antibiograms (47)].

CONCLUSION

AMR surveillance plays an essential role in our ability to preserve effective antibiotics, through early detection of resistance emergence and spread, followed by adequate intervention strategies. The slow pace of novel antibiotic development, combined with emergence of difficult-to-treat infections, makes our understanding of all aspects of emerging resistance to newer antibiotics ever more pressing. Yet current surveillance systems do not collect or report AST data for newer antibiotics, and are not designed to do so, as they would need to match all design issues described in this article. The critical issues that will need to be considered in designing an enhanced surveillance system that includes newer, last resort drugs can be summarized as follows in this call for action above in **Box 2**.

BOX 2 | Call for surveillance for early detection of resistance to new antibiotics

Early resistance surveillance should begin as soon as a new antibiotic is available for treatment, and the system should transition to more routine surveillance once sporadic, unrelated hospital outbreaks have occurred. National authorities should remain in charge of surveillance activities, but with coordination provided by a more centralized authority. As political support for resistance surveillance increases within individual countries, and adequate funding follows, the representativeness and overall quality of data contributed by national health authorities should be held to a higher standard. Financial contribution (from the central governing body) to support data collection activities in individual countries is important to improve the ability of the governing body to make requests to national authorities in the name of greater harmonization and representativeness—which improves our ability to respond on a regional level.

A well-funded surveillance scheme can of course be more ambitious and should include a capacity building element to continuously improve the system internally (increase expertise, improve data quality, increase efficiency) and expand the network geographically and over time. Finally, in extending resistance surveillance to newer antibiotics we should focus our efforts on identifying resistance that occurs in already difficult-to-treat infections, in particular, such that we can respond in time to contain transmission of infections that are completely untreatable. The increasing threat of AMR, combined with the paucity of novel treatment strategies becoming available, requires an adequate public health response, which should include a bespoke AMR surveillance strategy for antibiotics coming to the market today.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

CM and MK reviewed the literature, developed the surveys with close support from SH, and analyzed responses. CM, MK, and SH identified the first potential interviewees. CM conducted the interviews to narrow down the list of crucial issues and identify additional interviewees and wrote the manuscript with close support from MK and SH. Members of the Enhanced surveillance expert consensus group provided input through two survey rounds and provided input into the interpretation of findings and conception of the manuscript. MK developed the table comparing the choice of denominator.

All authors contributed to the article and approved the submitted version.

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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.2021.652638/full#supplementary-material

Appendix 1 | Delphi survey 1.

Appendix 2 | Delphi survey 2.

Appendix 3 | Surveillance design issues identified in the mapping exercise.

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Risk Factors for Acquired *Stenotrophomonas maltophilia* Pneumonia in Intensive Care Unit: A Systematic Review and Meta-Analysis

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Background and Aims: *Stenotrophomonas maltophilia* is increasingly found in critically ill patients, but it is considered a pathogen of limited pathogenicity and therefore it is not often targeted. We systematically evaluated risk factors for *S. maltophilia* pneumonia in ICU patients for better clinical management.

Methods: Prospective and retrospective studies of *S. maltophilia* infection in the ICU from database establishment to August 8, 2021, were searched through PubMed, web of science, Cochrane Library Embase and CNKI. The literature was independently screened and extracted by two authors according to inclusion and exclusion criteria, evaluated for quality by the NOS scale, and meta-analyzed by stata 14.0 software.

Results: A total of eight studies with a sample size of 2,320 cases were included. Meta-analysis showed that APACHE-II score > 20 (OR = 10.98, 95% CI: 5.67 ~ 21.26), COPD (OR = 3.97, 95% CI: 2.39 ~ 6.61), malignant tumor (OR = 2.15, 95% CI: 1.03 ~ 4.50), mechanical ventilation (OR = 8.75, 95% CI: 2.59 ~ 29.58), tracheotomy (OR = 6.12, 95% CI: 2.06 ~ 18.18), endotracheal intubation (OR = 4.25, 95% CI: 2.30 ~ 7.84), β- Lactamase inhibitors (OR = 9.98, 95% CI: 1.51 ~ 65.96), aminoglycosides (OR = 4.01, 95% CI: 2.06 ~ 7.80), carbapenems (OR = 2.82, 95% CI: 1.49 ~ 5.31), and quinolones (OR = 2.17, 95% CI: 1.21 ~ 3.89) were risk factors for ICU-acquired *S. maltophilia* pneumonia.

Conclusion: Many risk factors are associated with *S. maltophilia* pneumonia in ICU patients. Clinical workers should pay more attention to assessing the risk of infection in ICU patients and enhance the prevention and management of high-risk groups, which will help reduce their risk of *S. maltophilia* infection.

Keywords: *Stenotrophomonas maltophilia*, ICU-acquired pneumonia, risk factor, meta-analysis, infection

INTRODUCTION

S. maltophilia is a non-fermentable Gram-negative bacterium that is an opportunistic agent. It is naturally resistant to many commonly used antibiotics, such as carbapenems and aminoglycosides (1, 2). It is due to such characteristics, in the context of drug resistance, that *S. maltophilia* is becoming an important pathogen of hospital infections in the ICU, which can lead to infections in the lungs, bloodstream and many other important parts of the body, even life-threatening (3, 4).

According to the CHINET bacterial resistance surveillance data in 2020, *S. maltophilia* accounted for 2.98% of all strains and ranked 9th, 7th among Gram-negative bacteria, and 3rd among non-fermentative bacteria, after *Bacillus immobilis* and *Pseudomonas aeruginosa*. *S. maltophilia* has the highest resistance to ceftazidime (38.5%), followed by levofloxacin (10.8%), compound sulfamethoxazole (6.7%) and tigecycline (2.7%), and the lowest resistance to minocycline (2.3%) (5). Clinically, it often causes mixed infections with other bacteria, mainly *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, so *S. maltophilia* infections are difficult to be treated and the mortality rate is high. Muder et al. (6) reported a mortality rate of 21% in patients with *S. maltophilia* bacteremia. Paez et al. (7) reported a direct mortality rate of 26.7% due to *S. maltophilia* infection and a mortality rate of 21–69% associated with *S. maltophilia* infection.

The study of risk factors for ICU-acquired *S. maltophilia* pneumonia is of great practical significance for the in-depth study of the hazards of this bacterium and the adoption of appropriate preventive and control measures. Currently, Several studies at home and abroad have investigated the risk factors for the occurrence of *S. maltophilia* infection in ICU patients, but there are drawbacks such as small sample size and incomplete risk factor indicators, and the significance of clinical guidance is limited. This study aims to systematically evaluate the risk factors of hospital-acquired *S. maltophilia* pneumonia by Meta-analysis, and provide a theoretical basis for clinical formulation of prevention and control strategies to reduce the morbidity and mortality of *S. maltophilia* infection.

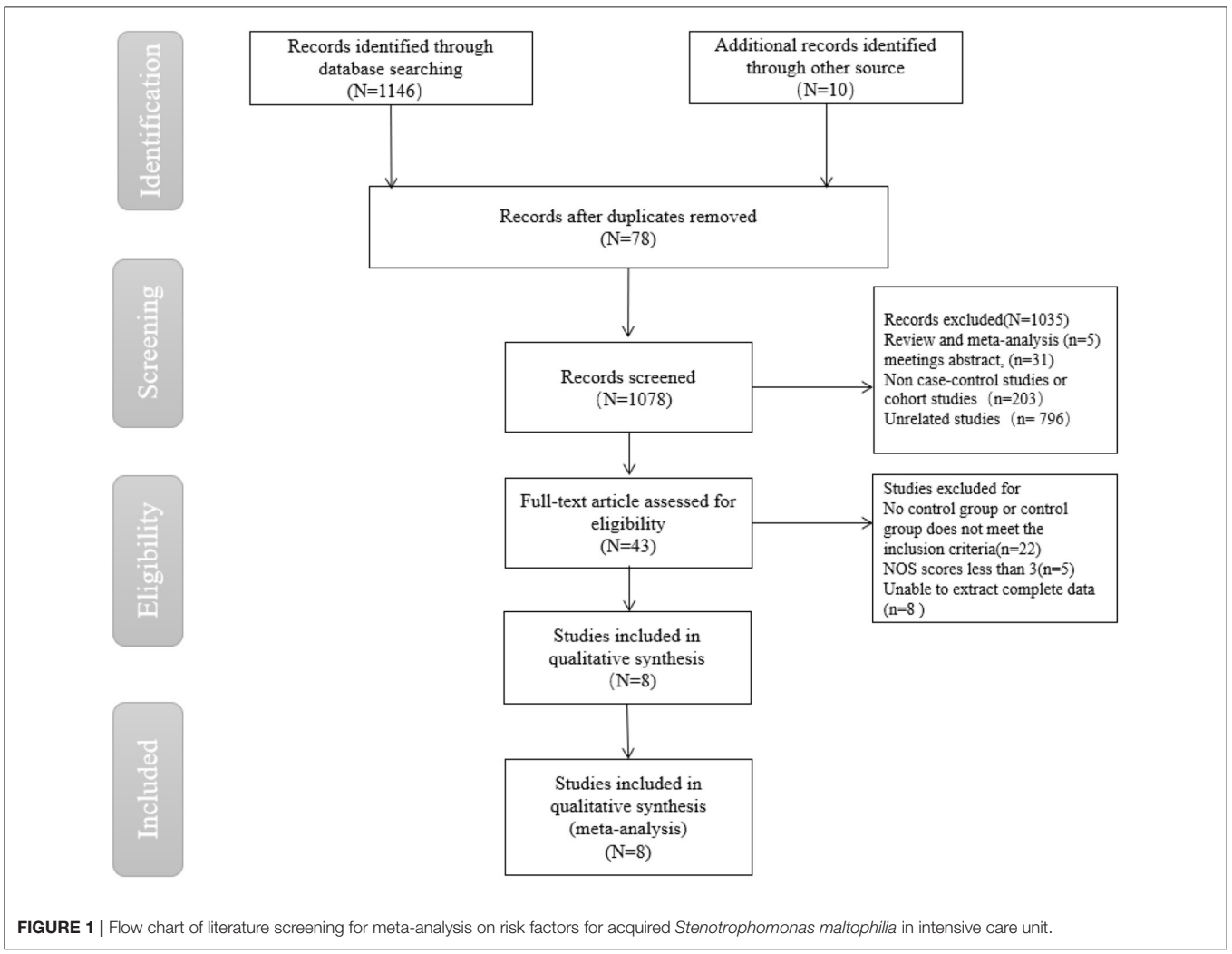

FIGURE 1 | Flow chart of literature screening for meta-analysis on risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

TABLE 1 | Characteristics of the included studies.

Study	Year	Design study	Area	Infection group	Non-infection group	Risk factors
Stang et al. (8)	2002	Case-control	USA	26	137	01.02.03.07.20.21.22.24
Hanes et al. (9)	2006	Cohort	France	30	60	01.02.07.08.09.11.14.15.16.17.18.21.22.23.24.25
Nseir et al. (10)	2011	Case-control	China	35	140	01.02.04.11.14.15.16.19.21.23
Xu et al. (11)	2012	Case-control	Germany	36	28	01.02.03.08.09.10.12.13.14.15.17.18
Guo et al. (12)	2014	Case-control	China	42	84	01.02.04.06.08.09.11.14.15.16.19
Saugel et al. (13)	2016	Case-control	Netherlands	6	15	01.02.03.08.09.10.11.13.14.16
Ibn Saied et al. (14)	2019	Case-control	China	29	58	01.02.03.09.10.11.13.14.17.18.21.22.23.24.25
Lei et al. (15)	2020	Case-control	USA	102	1,492	01.02.05.06.07.11.12.13.20

01, Age, years; 02, Gender; 03, APACHE-II score; 04, APACHE-II score >20; 05, Glasgow score; 06, Glucocorticoid; 07, Length of ICU stay, days; 08, COPD; 09, Diabetes; 10, Malignancy; 11, Cardiovascular disease; 12, kidney dysfunction; 13, Immunosuppression; 14, Mechanical ventilation; 15, Tracheal intubation; 16, Tracheotomy; 17, Central venous catheterization; 18, Urinary catheter; 19, Nasogastric tube; 20, Operation; 21, Carbapenems; 22, β-lactamase inhibitor; 23, Aminoglycosides; 24, Quinolones; 25, Nitroimidazoles.

TABLE 2 | Risk of bias assessment of the included studies according to the Newcastle-Ottawa Scale (NOS).

NOS items/Study ID	Hanes et al.	Xu et al.	Saugel et al.	Guo et al.	Scholte et al.	Shi et al.	Saied et al.
Is the case definition adequate?	*		*	*	*	*	*
Representativeness of the cases	*	*	*	*	*		*
Selection of controls	*	*	*		*	*	*
Definition of controls	*	*	*	*	*	*	*
Compatibility	*	*	*	*	*	*	*
Ascertainment of exposure	*	*	*	*	*	*	*
Same method of ascertainment for cases and control	*	*	*	*			*
Non-response rate	*	*			*	*	*
Total score	8	7	7	6	7	6	8

**Representative studies meet this criteria.*

MATERIALS AND METHODS

Literature Search Strategy

PubMed, Web of Science, Cochrane Library, Embase and CNKI were searched from the time of database establishment to August 8, 2021. A combination of subject terms, free words, and Boolean logical operators was used for the search terms: *Stenotrophomonas maltophilia*, hospital-acquired pneumonia, ventilator-associated pneumonia, intensive care, risk factors, etc. The English databases were searched for the following terms: (*Stenotrophomonas maltophilia* OR *S. maltophilia* OR SMA) AND (nosocomial infection OR hospital infection OR hospital acquired infection OR cross infection OR VAP OR ventilator associated pneumonia OR ventilator-associated pneumonia) AND (ICU OR Intensive Care OR NICU OR PICU OR CCU) AND (risk factor OR factor). A manual search of relevant content reviews and references of included literature was conducted to identify potential studies that met the inclusion criteria.

Literature Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (1) The type of literature was a cohort study or case-control study published nationally and internationally; (2) The study population was divided into two groups based on whether they were infected with *S. maltophilia*, and the diagnosis criteria for *S. maltophilia* pneumonia in this study were described below; (3) Risk factors for *S. maltophilia* pneumonia in ICU patients were present in the literature, such as comorbid underlying diseases, invasive procedures undergone, and use of broad-spectrum antibiotics; (4) Outcome indicators for risk factors for *S. maltophilia* pneumonia in ICU patients could be expressed as odds ratios (OR), and their 95% confidence intervals (CI) were calculated. Studies were excluded when meeting one of the following criteria: (1) duplicate reports, conference reports, and reviews; (2) abnormal or missing data; (3) low quality of literature [Newcastle-Ottawa Scale (NOS) score$\leq$3].

In this study, infection and colonization were considered ICU-acquired if they were diagnosed more than 48 h after ICU admission. Pneumonia was defined as follows: (1) new or progressive pulmonary infiltrates. (2) Temperature $>$ 38°C or $<$36.5°C, leukocyte count $>$12,000 μl^{-1} or $<$4000 μl^{-1}, purulent endotracheal aspirate or sputum. (3) Positive respiratory sample. (4) Decreased oxygenation.

TABLE 3 | Meta-analysis results of risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

Exposure factors	Included studies	Heterogeneity	*p*	Fixed-effect model (FEM)	*p*	Random-effect model (REM)	*p*
General condition							
Age, years	8	0	0.93	−0.76 (−2.62∼1.10)	0.42	−0.76 (−2.62∼1.10)	0.42
Gender	8	0	0.83	0.77 (0.59∼1.02)	0.07	0.77 (0.58∼1.01)	0.06
APACHE-II score	3	0	0.83	2.80 (−0.31∼5.82)	0.08	2.80 (−0.31∼5.82)	0.08
APACHE-II score >20	2	0	0.39	10.98 (5.67∼21.26)	<0.001	11.49 (6.02∼21.92)	<0.001
Glasgow score	2	0	0.66	−0.50 (−1.91∼0.90)	0.49	−0.50 (−1.91∼0.90)	0.49
Glucocorticoid	3	42	0.58	0.91 (0.51∼1.61)	0.74	0.98 (0.42∼2.29)	0.97
Length of ICU stay, days	4	0	0.50	1.65 (0.70∼2.60)	0.001	1.65 (0.70∼2.60)	0.001
Pre-existing medical conditions							
COPD	4	77.8	0.004	3.97 (2.39∼6.61)	<0.001	3.99 (1.19∼13.32)	0.03
Diabetes	5	8.1	0.36	1.50 (0.89∼2.63)	0.13	1.47 (0.82∼2.61)	0.20
Malignancy	3	0	0.99	2.15 (1.03∼4.50)	0.04	2.15 (1.03∼4.50)	0.04
Cardiovascular disease	7	48.2	0.07	0.92 (0.66∼1.29)	0.63	1.0 (0.61∼1.75)	0.92
kidney dysfunction	3	0	0.87	1.20 (0.69∼2.07)	0.52	1.21 (0.70∼2.07)	0.50
Immunosuppression	3	49.6	0.14	1.38 (0.87∼2.21)	0.17	1.70 (0.38∼7.69)	0.49
Invasive procedures							
Mechanical ventilation	5	71.4	0.007	8.22 (4.82∼14.03)	<0.001	8.75 (2.59∼29.58)	<0.001
Tracheal intubation	3	0	0.52	4.25 (2.30∼7.84)	<0.001	4.08 (2.22∼7.51)	<0.001
Tracheotomy	4	67.2	0.03	6.10 (3.54∼10.52)	<0.001	6.12 (2.06∼18.18)	0.001
Central venous catheterization	3	82.7	0.003	3.22 (1.62∼6.42)	0.001	2.30 (0.37∼14.41)	0.37
Urinary catheter	3	0	0.89	2.14 (0.79∼5.84)	0.14	2.10 (0.77∼5.76)	0.15
Nasogastric tube	3	78.3	0.03	3.28 (1.85∼5.83)	<0.001	3.36 (0.95∼11.87)	0.06
Operation	3	0	0.75	0.78 (0.36∼1.70)	0.53	0.80 (0.36∼1.76)	0.57
Antimicrobial agents							
Carbapenems	4	23	0.27	2.82 (1.49∼5.31)	0.001	2.82 (1.30∼6.09)	0.008
β-lactamase inhibitor	3	85.9	0.001	7.88 (4.41∼14.09)	<0.001	9.98 (1.51∼65.96)	0.02
Aminoglycosides	3	27.4	0.25	4.01 (2.06∼7.81)	<0.001	4.12 (1.75∼9.70)	0.001
Quinolones	3	39.1	0.19	2.17 (1.21∼3.89)	0.009	2.25 (1.03∼4.93)	0.04
Nitroimidazoles	2	60.6	0.11	1.63 (0.43∼6.24)	0.48	1.75 (0.14∼22.69)	0.67

Data Extraction and Quality Evaluation

The retrieved literature was screened by two authors independently according to the inclusion and exclusion criteria, and the following data information was extracted: name of the first author, time of publication, source of the literature, basic characteristics of the included cases, and possible risk factors for *S. maltophilia* pneumonia in ICU patients. If the opinions of two reviewing authors do not agree, they discuss. If there was still disagreement after discussion, a third party opinion was sought. The quality of the included literature was also evaluated according to NOS score (8), and the evaluation items include three aspects of population selection, comparability and exposure evaluation, with a score out of 9. A score of 7 and above was considered as high-quality literature, 4–6 as moderate quality literature, and 1–3 as low-quality literature.

Data Analysis

Statistical analysis was performed by stata14.0 software. I^2 was used to determine the heterogeneity of the included literature, and the fixed-effects model was used when $p > 0.1$ and $I^2 < 50\%$; otherwise, the random-effects model was applied. OR and its 95% CI were calculated for count data, while weighted mean difference (WMD) and its 95% CI were calculated for measurement data, and differences were considered statistically significant at $p \leq 0.05$. Sensitivity analysis was performed by calculating the OR and 95% CI for both fixed-effects and random-effects models and comparing the results of the two groups. Sensitivity analysis was performed by changing the data analysis model. If there was no substantial change after the model change (no opposite conclusion was reached after changing the model), the consolidated result was considered to be stable. Begg's test was used to test for publication bias when the number of included papers for individual risk factor analysis was ≥3.

RESULTS

Literature Search

Firstly, 1,156 papers were initially searched in the database through the search strategy, and then 43 papers were selected through title, abstract and keywords, etc. Finally, eight papers

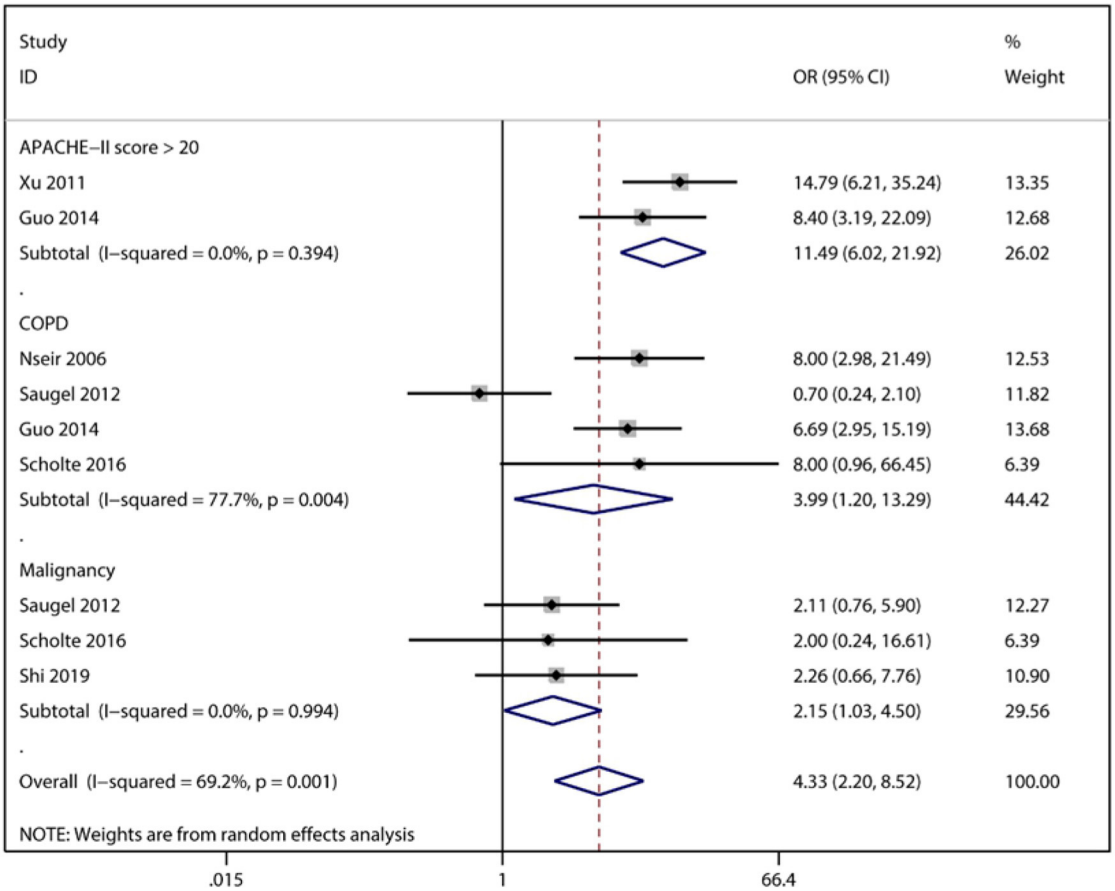

FIGURE 2 | Meta-analysis results of impact of general condition and combined underlying diseases on risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

(9–16) were further screened by reading the full text, including five papers in English and three papers in Chinese (**Figure 1**).

Baseline Characteristics of the Studies

The eight included papers included in the study were published between 2002 and 2020, seven of which were case-control studies and one was a cohort study involving 2320 patients, 306 in the *S. maltophilia*-infected group and 2014 in the non-infected group, and 25 exposure factors for *S. maltophilia* infection were extracted. The quality of the eight papers was evaluated using the NOS scale, including five high-quality papers and tree moderate-quality papers. The basic characteristics of the included literature are shown in **Tables 1**, **2**.

Meta-Analysis of Exposure Factors for *S. maltophilia* Pneumonia

Heterogeneity was tested for exposure factors such as age, gender, APACHE-II score, length of ICU stay, Glasgow score, glucocorticoid use, diabetes, malignancy, cardiovascular disease, renal insufficiency, immunodeficiency disorders, tracheal intubation, indwelling catheters, surgery, and use of carbapenems, quinolones, and aminoglycosides. Heterogeneity was acceptable ($p > 0.10$, $I^2 < 50\%$), and effect sizes were combined using a fixed-effects model. heterogeneity was present for APACHE-II scores >20, COPD, tracheotomy, mechanical ventilation, indwelling nasogastric tube, central venous line, use of β-lactamase inhibitors and nitroimidazole antibiotics ($p < 0.10$, $I^2 > 50\%$), random-effects model combinations of effect sizes were performed.

The meta-analysis showed that risk factors for *S. maltophilia* pneumonia in the ICU included APACHE-II score > 20 (OR = 10.98, 95% CI: 5.67 ∼ 21.26), COPD (OR = 3.97, 95% CI: 2.39 ∼ 6.61), malignant tumor (OR = 2.15, 95% CI: 1.03 ∼ 4.50), mechanical ventilation (OR = 8.75, 95% CI: 2.59 ∼ 29.58), tracheotomy (OR = 6.12, 95% CI: 2.06 ∼ 18.18), endotracheal intubation (OR = 4.25, 95% CI: 2.30 ∼ 7.84), β- Lactamase inhibitors (OR = 9.98, 95% CI: 1.51 ∼ 65.96), aminoglycosides (OR = 4.01, 95% CI: 2.06 ∼ 7.80), carbapenems (OR = 2.82, 95% CI: 1.49 ∼ 5.31), and quinolones (OR = 2.17, 95% CI: 1.21 ∼ 3.89). There was no obvious correlation between risk factors and *S. maltophilia* pneumonia, such as age, gender, APACHE-II score, length of stay in ICU, combined diabetes, combined cardiovascular disease, combined renal insufficiency, combined immunodeficiency disease, indwelling

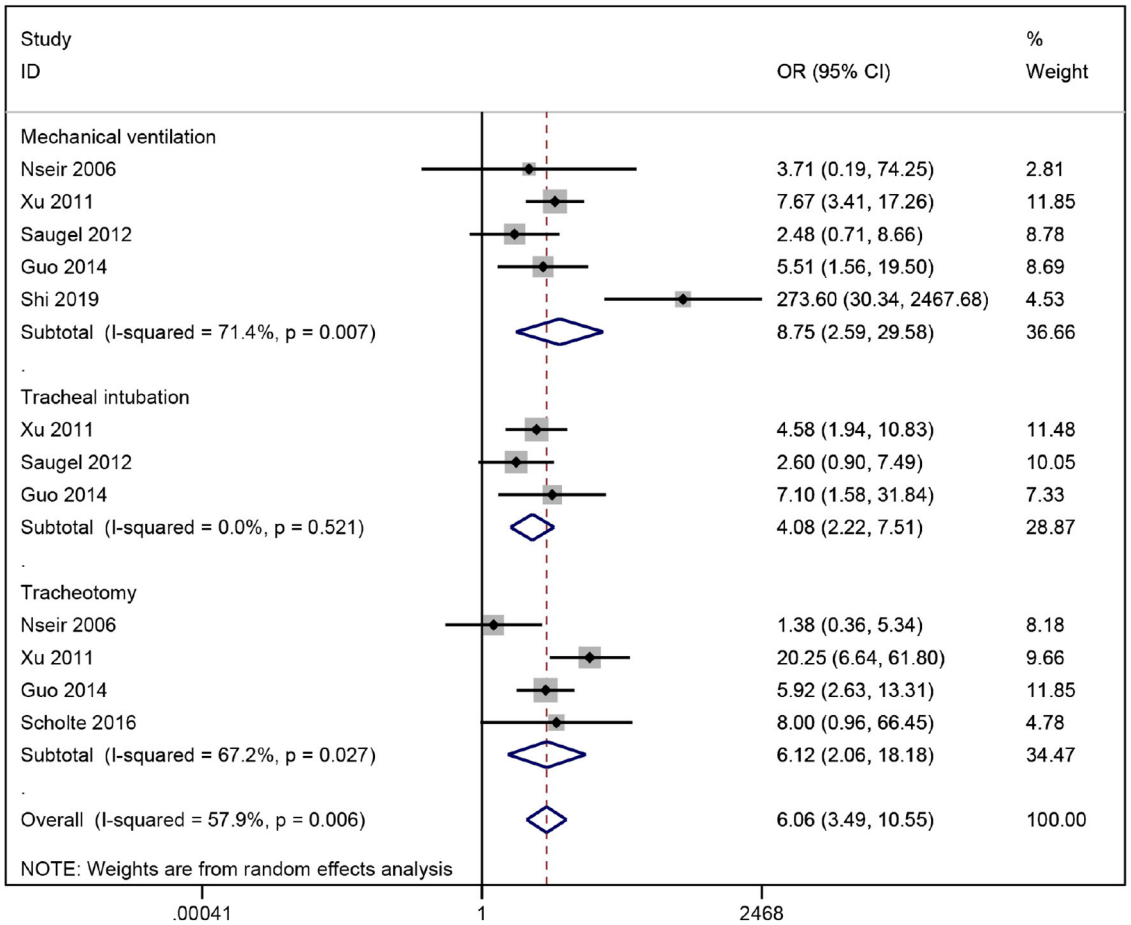

FIGURE 3 | Meta-analysis results of impact of invasive operations on risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

catheter, operation, central venous line, indwelling nasogastric tube, corticosteroids, and nitroimidazole antibiotics.

Sensitivity Analysis and Publication Bias

Sensitivity analysis suggested that the results of the meta-analysis were stable for all outcome indicators except for two exposure factors, central venous placement and indwelling nasal cannula (**Table 3**). Begg's test was used to test for publication bias when the number of included papers for individual risk factor analysis was $\geq$3. The results showed $p > 0.05$, indicating that the publication bias of included papers was not significant.

DISCUSSION

Stenotrophomonas maltophilia is widely distributed in natural environments, such as soil, water, and hospital environments, and can also parasitize the human skin, respiratory and digestive tracts. It is a very common conditional pathogen. The results of studies over the past few years have shown that the detection rate of *S. maltophilia pneumonia* is increasing year by year and has become an important pathogen of ICU infections. ICU-acquired infections associated with *S. maltophilia* are an independent risk factor for mortality in the ICU, and therefore knowledge of the risk factors for *S. maltophilia* pneumonia in the ICU and early targeted empirical treatment are key to reducing mortality from *S. maltophilia* pneumonia. In this study, we conducted a meta-analysis to screen the risk factors for *S. maltophilia* pneumonia in the ICU regarding general condition, co-morbid underlying diseases, invasive procedures, and use of antibiotics.

Correlation of the Patient's General Condition, Co-morbid Underlying Diseases and ICU-Acquired *S. maltophilia* Pneumonia

S. maltophilia pneumonia was associated with the patients' underlying disease status, and among the various underlying diseases, patients with COPD had the highest risk of infection (OR = 3.99), followed by malignant tumor (OR = 2.15). In contrast, underlying diseases such as immunodeficiency disorders, diabetes mellitus, and renal insufficiency were not associated with *S. maltophilia* pneumonia in ICU patients

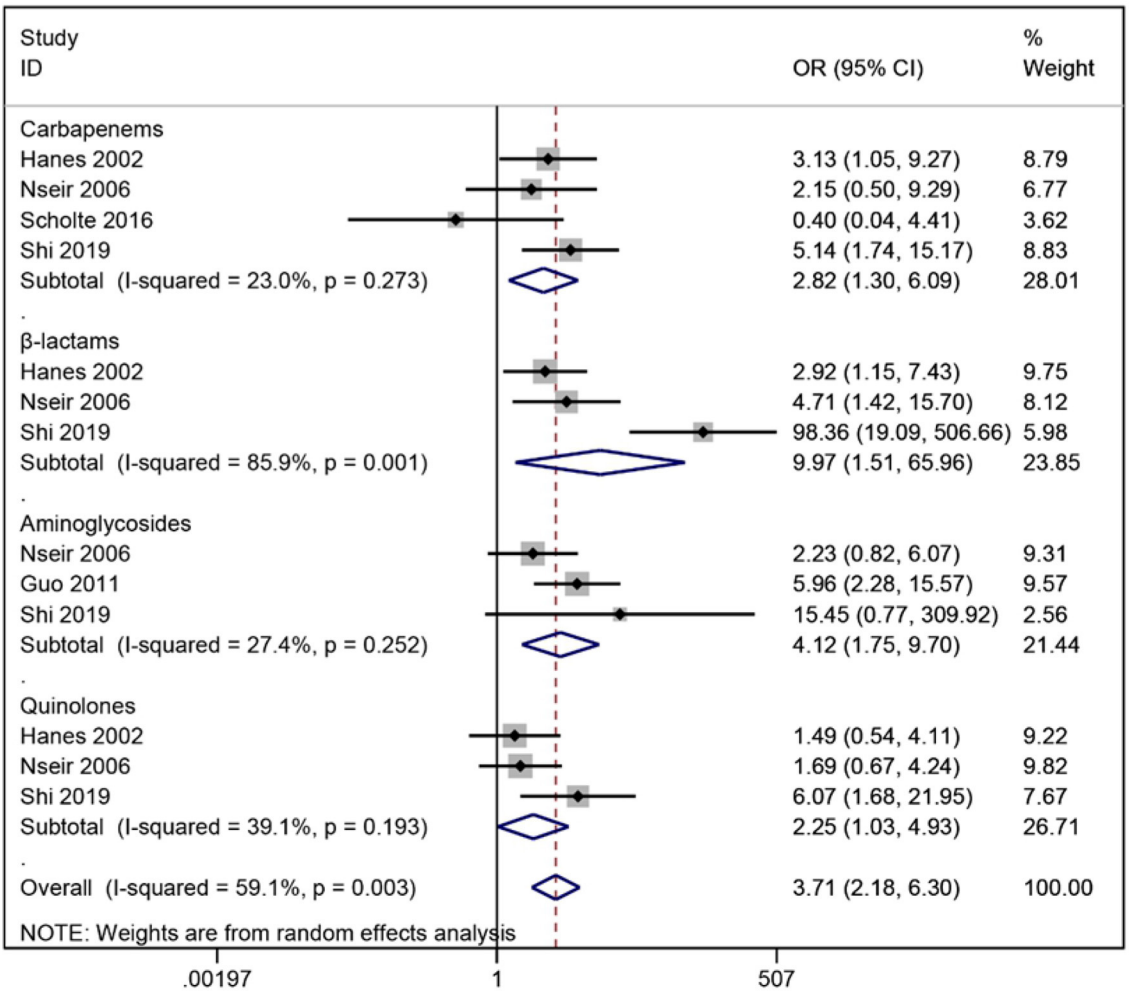

FIGURE 4 | Meta-analysis results of antimicrobial drug impact of on risk factors for acquired *Stenotrophomonas maltophilia* in intensive care unit.

(**Figure 2**). Severity of illness is also an important factor in *S. maltophilia* pneumonia, and APACHE-II score is one of the most widely used tools for critical illness assessment (17). Furthermore, by combining the APACHE-II score of the experimental and control groups, we did not find a statistical difference between the two groups, unlike that reported by a few individual studies (9, 15). However, further analysis informed that there was a hierarchical effect between APACHE-II scores and *S. maltophilia* pneumonia, suggesting that *S. maltophilia* pneumonia is closely related to the severity of the disease. In recent years, the trend of glucocorticoid abuse in clinical practice has become more serious, and related studies have reported that long-term high-dose glucocorticoid use is a high-risk factor for multi-drug-resistant bacteria and fungal infections (18–20). However, we did not find that glucocorticoids increased the incidence of *S. maltophilia* pneumonia in the ICU, there may be bias due to different doses of glucocorticoids, course of treatment and patients' treatment response.

Invasive Procedures and ICU-Acquired *S. maltophilia* Pneumonia

Our meta-analysis indicated that among the risk factors involved in invasive maneuvers, mechanical ventilation was strongly associated with ICU-acquired *S. maltophilia* pneumonia (OR = 8.75), followed by tracheotomy (OR = 6.10) and tracheal intubation (OR = 4.25), demonstrating that invasive procedures such as mechanical ventilation, tracheal intubation and tracheotomy are high risk factors for *S. maltophilia* pneumonia in the ICU (**Figure 3**). Invasive procedures can breach the body's basic defense barriers. *S. maltophilia* colonized in the oral pharynx tends to form bacterial biofilms in the lining of indwelling catheters and tends to enrich at oxygen storage sites, increasing the risk of pulmonary *S. maltophilia* infection (21). In addition, the longer the duration of invasive procedures such as mechanical ventilation, the greater the risk of *S. maltophilia* infection. Guo et al. (12) have reported that the duration of invasive ventilator ventilation (>14 d) is an independent risk factor for ICU-acquired *S. maltophilia*

infection. Therefore, clinical practitioners need to strictly follow the indications for invasive procedures and reduce unnecessary invasive operations, while tracheal intubation should be removed as early as possible when conditions allow to help reduce the risk of *S. maltophilia* infection. In our study, the combination of two exposure factors, central venous cannulation and indwelling nasal cannula, was more heterogeneous and less robust than other invasive procedures, and the combined results should be viewed with caution.

The Association Between Antimicrobial Drug Use and ICU-Acquired Pneumonia With *S. maltophilia*

At present, for the treatment of antibiotic-resistant *S. maltophilia* infection, Chinese experts recommended three combined treatment modes, which are based on compound sulfamethoxazole, combined with ticarcillin clavulanate potassium, cefoperazone sulbactam, fluoroquinolone, minocycline, ceftazidime or polymyxin. Or ceftazidime-based fluoroquinolones, ticarcillin clavulanate potassium or cefoperazone sulbactam regimen; Alternatively, a polymyxin-based regimen combined with ticarcillin clavulanate potassium can be adopted (22). However, the use of antibiotics is a double-edged sword. *S. maltophilia* is naturally resistant to carbapenems, while its AAC(6')-lz acetyltransferase and pumping system make it highly resistant to aminoglycosides, and these characteristics could explain the use of both drugs to passively screen the bacterium for hospital-acquired infections due to the proliferation of dominant bacteria (23). Nseir et al. (10) found that broad-spectrum antibiotics such as fluoroquinolones and cephalosporins can also increase the rate of *S. maltophilia* pneumonia and concluded that broad-spectrum antibiotic use is more significant than carbapenems alone. This study showed that the use of β-lactamase inhibitors had the largest combined OR associated with *S. maltophilia* pneumonia (OR = 7.88), followed by aminoglycosides (OR = 4.01) and carbapenems (OR = 2.82), suggesting that the possibility of *S. maltophilia* pneumonia should be considered when clinical treatment with these three drugs is not effective (**Figure 4**). The long-term heavy use of broad-spectrum antibiotics is an important medical factor for hospital-acquired infections of *S. maltophilia.* Broad-spectrum antibiotics increase the risk of infection by killing other pathogens while screening out dominant species, including *S. maltophilia*. A study by Xu et al. (11) concluded that the use of ≥3 antibiotics for more than 1 week was an independent risk factor for *S. maltophilia* pneumonia in ICU patients, and therefore care should be taken to monitor the risk of infection in ICU patients using multiple antimicrobials simultaneously.

Limitation of this Study

There are a few limitations in this study: (1) Due to the limited number of domestic and international studies on risk factors for *S. maltophilia* pneumonia in ICU and the uneven quality of the literature. Eight papers were screened strictly according to the inclusion and exclusion criteria, including five in English and three in Chinese, which may have a certain degree of publication bias. (2) Due to limited literature, some risk factor indicators in this study were not combined effectively, which may affect the study results. (3) At present, the research on related risk factors in China is not deep enough, and there is a lack of relevant prospective cohort studies. Therefore, more rigorous design, large samples, and multicenter studies are needed to clarify the risk factors for *S. maltophilia* pneumonia in the ICU.

CONCLUSION

In recent years, the disease burden of hospital-acquired *S. maltophilia* pneumonia in ICU patients has been high, and resistance to the organism is increasing. *S. maltophilia* pneumonia occurs in patients with severe disease, comorbid COPD, malignancy, high APACHE-II scores, undergoing invasive procedures, and in ICU patients on broad-spectrum antibiotics due to a combination of host and medical factors. From the host side, these patients are characterized by impaired immune function, severe disease, and the need for prolonged hospitalization, which objectively contributes to the infection of conditional pathogens such as *S. maltophilia* (24). Therefore, strengthening the monitoring, prevention, and control of patients with risk factors of *S. maltophilia* infection is beneficial to reduce the risk of infection and death in ICU patients.

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

NW and CT: screening, data extracting, and writing. NW, CT, and LW: analysis. NW and LW: manuscript revision. LW: manuscript finalization. All authors contributed to the article and approved the submitted version.

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Microbial Characteristics and Genomic Analysis of an ST11 Carbapenem-Resistant *Klebsiella pneumoniae* Strain Carrying bla_{KPC-2} Conjugative Drug-Resistant Plasmid

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Background: The sequence type 11 (ST11) carbapenem-resistant *Klebsiella pneumoniae* (CRKP) carrying bla_{KPC-2} has been widespread all over the world, and it has been reported frequently in China. The bla_{KPC-2} located on the mobile genetic element brings tremendous pressure to control the spread and outbreak of resistant bacteria. Whole-genome sequencing (WGS) technology can comprehensively and in-depth display the molecular characteristics of drug-resistant bacteria, providing a basis for evaluating the genetic diversity within the CRKP genome.

Methods: The ST11 CRKP in this study was collected in the intensive care unit of a major teaching hospital. PCR and Sanger sequencing confirmed the existence of bla_{KPC-2}. The AST-GN card and the microbroth dilution test were used for antimicrobial susceptibility testing. The transferability of plasmid was verified by a conjugation test. The whole genome is sequenced using the Illumina HiSeq short-read and Oxford Nanopore long-read sequencing technology.

Results: The studied strain was named CRKP63, which is a multi-drug resistance bacteria, which carries bla_{KPC-2} and $bla_{SHV-182}$. Its genome consists of a circular chromosome of 5,374,207 bp and an IncFII plasmid named pKPC-063001 of 359,625 bp. In the drug-resistant plasmid pKPC-063001, the key carbapenem resistance gene bla_{KPC-2} was located in the genetic context with insertion sequence IS*Kpn27* upstream and IS*Kpn6* downstream and bracketed by IS*26*. The three copies of the IS*26*–IS*Kpn27*–bla_{KPC-2}–IS*Kpn6*–IS*26* unit were present in tandem. bla_{KPC-2} can be transferred horizontally between other species by conjugation, the complete type IV secretion system (T4SS) structure helps to improve the adaptability of bacteria to the external environment, strengthen the existence of drug-resistant bacteria, and accelerate the spread of drug resistance.

Conclusion: High-throughput sequencing has discovered the different surrounding environments of bla_{KPC-2}, which provides a new idea for further revealing the transmission and inheritance of bla_{KPC-2} at the molecular level. In order to control the further spread and prevalence of drug-resistant bacteria, we should pay close attention to the changes in the genetic environment of bla_{KPC-2} and further study the transcription and expression of T4SS.

Keywords: carbapenem-resistant *Klebsiella pneumoniae*, ST11, whole-genome sequencing, nanopore, KPC-2

BACKGROUND

Carbapenem-resistant *Enterobacteriaceae*, especially *Klebsiella pneumoniae*, have emerged as important causes of morbidity and mortality among hospital-acquired and long-term care-associated infections (1). As of now, carbapenem-resistant *K. pneumoniae* (CRKP) strains have spread worldwide and posed a severe threat to public health. *K. pneumoniae* carbapenemases (*KPC*)-*2*, the most common variant of *KPC* enzymes, is a dominant factor mediating carbapenems resistance in CRKP (2, 3). The most predominant isolates of *KPC*-producing *K. pneumoniae* (*KPC-kpn*) belong to the clonal group 258 (CG258), two representative types of this CG: the ST258 and ST11 strains, have been identified worldwide – ST258 is mostly prevalent in America and Europe, while ST11 is the highly dominant clone in Asia (especially in China) (4).

The bla_{KPC-2} is a typical plasmid-mediated drug resistance gene and mainly carried on plasmids of different incompatibility (Inc) groups, such as IncFII, FIA, I2, A/C, N, X, P, and L/M (4). The bla_{KPC-2} on the plasmid can spread the resistance through different methods, such as gene duplication, transposon elements, or plasmid transfer. The horizontal transmission of drug-resistant plasmids can accelerate the spread of multidrug resistance genes and mediate the production of multidrug-resistant bacteria (MDR). An in-depth understanding of the plasmid structure and its genome characteristics will help to control and prevent the emergence and outbreak of drug-resistant bacteria.

The rapid development of whole-genome sequencing (WGS) technology has gradually matured its application in the field of clinical microbiology (5, 6). WGS has the characteristics of large data information and high resolution, and it plays an important value in the research and detection of MDR. In this study, a whole-genome sequence of a CRKP, which is ST11 type and carrying bla_{KPC-2}in Chongqing, China, was performed and further explored its microbiological and genomic characteristics.

MATERIALS AND METHODS

Bacterial Collection

According to previous research, a total of 51 non-duplicated CRKP samples isolated from the ICU of Yongchuan Affiliated Hospital of Chongqing Medical University (a major teaching hospital in Chongqing, China) were collected from July 2018 to July 2020. Homology analysis based on the result of pulsed-field gel electrophoresis showed that 62.7% of the isolates belonged to the same cluster, indicating that there was a clonal transmission of ST11 carrying bla_{KPC-2} CRKP in the ICU of this hospital. In order to further study the molecular characteristics of CRKP in this ward, we selected one of the strains from the clone group, which carries multiple drug resistance genes and has the ability to conjugate—CRKP63—and conduct in-depth research on it. This bla_{KPC-2}-positive isolate was collected after identification (VITEK-2 automated microbiology analyzer, bioMérieux, France) and routine antimicrobial susceptibility testing by the Microbiology Laboratory in April 2020. The strain was identified as bla_{KPC-2} producing carbapenem-resistant *K. pneumoniae* by PCR detection and drug sensitivity (carbapenems) review. The isolate was stored at −80°C for further study.

Antimicrobial Susceptibility Testing

The VITEK-2 Compact automatic microbiological analyzer Antimicrobial Susceptibility Testing-Gram-Negtive (AST-GN) card (bioMérieux, France) was used for routine antimicrobial susceptibility testing. Minimum inhibitory concentration (MIC) is defined as the lowest compound concentration (μg/ml) required to stop bacterial growth was determined by using the microbroth dilution method. Imipenem (IPM), meropenem (MEM), amikacin (AMK), levofloxacin (LEV), tigecycline (TIG), polymyxin B (PLB), and ceftazidime-avibactam (CAZ-AVI) were used to determine the MIC by the microbroth dilution method. ATCC 25922, ATCC 700603, and BAA-1705 were used as quality control strains. Three parallel assays were performed for each sample. The IPM, MEM, AMK, LEV, PLB, and CAZ-AVI results were interpreted based on the Clinical and Laboratory Standards Institute (CLSI) criteria (7), whereas the TIG results were interpreted based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (8) breakpoint recommendations.

Conjugation Experiment

The conjugation experiment was carried out using a membrane bonding experiment as previously described (9). Both the donor (CRKP) and the recipient strains (*E. coli EC600*) were mixed in Luria-Bertani broth at a ratio of 1:3, and the mixtures were placed on a membrane and incubated for 24 h at 35°C. Transconjugants were selected on Mueller-Hinton agar II (MHA) plates supplemented with rifampicin (600 μg/ml) and MEM (1 μg/ml). Colonies that grew on the selective medium were identified by the VITEK-2 Compact system and *16S rRNA* sequence. A strain that harbored carbapenemase and exhibited

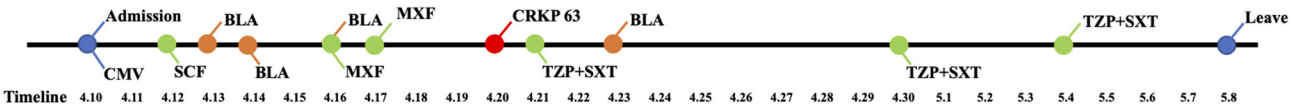

FIGURE 1 | The main process of the patient during hospitalization. CMV, mechanical ventilation; BLA, bronchoalveolar lavage; SCF, cefoperazone/sulbactam; MXF, moxifloxacin; TZP, piperacillin tazobactam; SXT, sulfamethoxazole.

TABLE 1 | Susceptibility results of various antibiotics (μg/ml).

Isolate	Resistance genes	Susceptibility results of various antibiotics (μg/ml)										
CRKP isolate												
CRKP 63	*KPC-2 SHV-182*	AMK	AMP	SAM	ATM	CZO	FEP	CTT	CAZ	CRO	CXM	CIP
		≤1/2	>16	>16	>32	>32	>32	>32	>32	>32	6	>2
		GEN	IPM	LVX	NIT	TZP	TOB	SXT	MEM	TIG	PB	CAZ-AVI
		≤1	256	32	256	>64	≤1	≤1	256	1	2	4, 4
***E. col* transconjugant strain**												
CRKP*J63*	*KPC-2*	AMK	AMP	SAM	ATM	CZO	FEP	CTT	CAZ	CRO	CXM	CIP
		≤1/2	>16	>16	>32	16	8	32	16	>32	4	≤1/4
		GEN	IPM	LVX	NIT	TZP	TOB	SXT	MEM	TIG	PB	CAZ-AVI
		≤1	4	1	≤16	>64	≤1	≤1	4	1	≤1/2	1/2, 4

AMK, amikacin; AMP, ampicillin; SAM, ampicillin/sulbactam; ATM, aztreonam; CZO, cefazolin; FEP, cefepime; CTT, cefotetan; CAZ, ceftazidime; CRO, ceftriaxone; CXM, cefuroxime; CIP, ciprofloxacin; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; NIT, nitrofurantoin; TZP, piperacillin/sulbactam; TOB, tobramycin; SXT, sulfamethoxazole; MEM, meropenem; TIG, tigecycline; PB, polymyxin B; CAZ-AVI, ceftazidime/avibactam.

higher MICs of resistance to carbapenems than *EC600* was defined as the transconjugants and the presence of resistance determinants was confirmed by PCR.

WGS and Data Analysis

Genomic DNA was isolated using the MagAttract HMW DNA Kit (Qiagen, Hilden, Germany) and submitted to next-generation high-throughput sequencing (NGS) on a HiSeq 2000^{TM} platform (Illumina Inc., San Diego, CA, USA) with 2 × 100-bp paired-end reads and to long-read high-throughput sequencing (LRS) on a MinION platform (Oxford Nanopore Technologies, Oxford, UK). The long reads generated by MinION were assembled using Canu v. 1.6 (10) and polished with the short reads generated by HiSeq using Pilon v1.22 (11) to obtain the whole genome and complete plasmid sequences. The chromosome and plasmid sequences were annotated using the prokaryotic gene prediction tool Prokka (12). The plasmid incompatibility type was searched using the online tool PlasmidFinder (https://cge.cbs.dtu. dk//services/PlasmidFinder/) (13). Antibiotic resistance genes were identified using both the Comprehensive Antibiotic Resistance Database (CARD) (14) and ResFinder database (https://cge.cbs.dtu.dk/services/ResFinder/) (15). Meanwhile, virulence-associated genes were identified using VirulenceFinder (https://cge.cbs.dtu.dk/services/VirulenceFinder/) (16). Transposon and insertion sequence (IS) elements were scanned using the ISfinder database (https://www-is.biotoul.fr/) (17). Comparative plasmid illustration was implemented by BRIG (http://brig.sourceforge.net) (18) or Easyfig tools (https://github.com/mjsull/Easyfig) (19). BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (20) was used for comparative analysis through the coverages and identities.

Nucleotide Sequence Accession Numbers

The complete sequences were submitted to GeneBank under accession numbers.

RESULT

Clinical Character

The bla_{KPC-2}-positive CRKP, CRKP63, collected in this study was derived from a 90-year-old female patient in the ICU ward. Concurrently, this strain also carried $bla_{SHV-182}$. The patient was admitted to the hospital due to acute exacerbation of chronic obstructive pulmonary disease on April 10, 2020, on the day of admission, the patient underwent mechanical ventilation. The patient underwent bronchoalveolar lavage 4 times during the hospitalization, and 10 days after admission, *K. pneumoniae* was detected in the bronchoalveolar lavage fluid, which was identified as a carbapenemase-producing CRKP. During the patient's hospitalization, infectious symptoms were repeatedly realized and anti-infective treatment continued. Twenty-eight days after admission, the condition of the patient did not improve, and finally, the patient and the patient's family gave up treatment and was discharged voluntarily. The main process of the patient during hospitalization is shown in **Figure 1**.

Result of Antimicrobial Susceptibility

Carbapenem-resistant *Klebsiella pneumoniae*63 shows resistance to more than three antibiotics and can be defined as MDR (21). The MIC value of IPM and MEM was as high as 256 μg/ml. However, it was sensitive to tobramycin (TOB), sulfamethoxazole (SXT), TIG, and CAZ-AVI. The specific information of various antibiotics is shown in **Table 1**.

Conjugation Experiment

After the conjugation experiment was successful, the transconjugant CRKP*J63* was obtained. Compared with the original donor, CRKP*J63* still carried bla_{KPC-2}, but not carried $bla_{SHV-182}$. Obviously, the MIC value of transconjugant for carbapenems (IMP and MEM) was significantly decreased. The variation on donor and transconjugant susceptibility profiles is shown in **Table 1**.

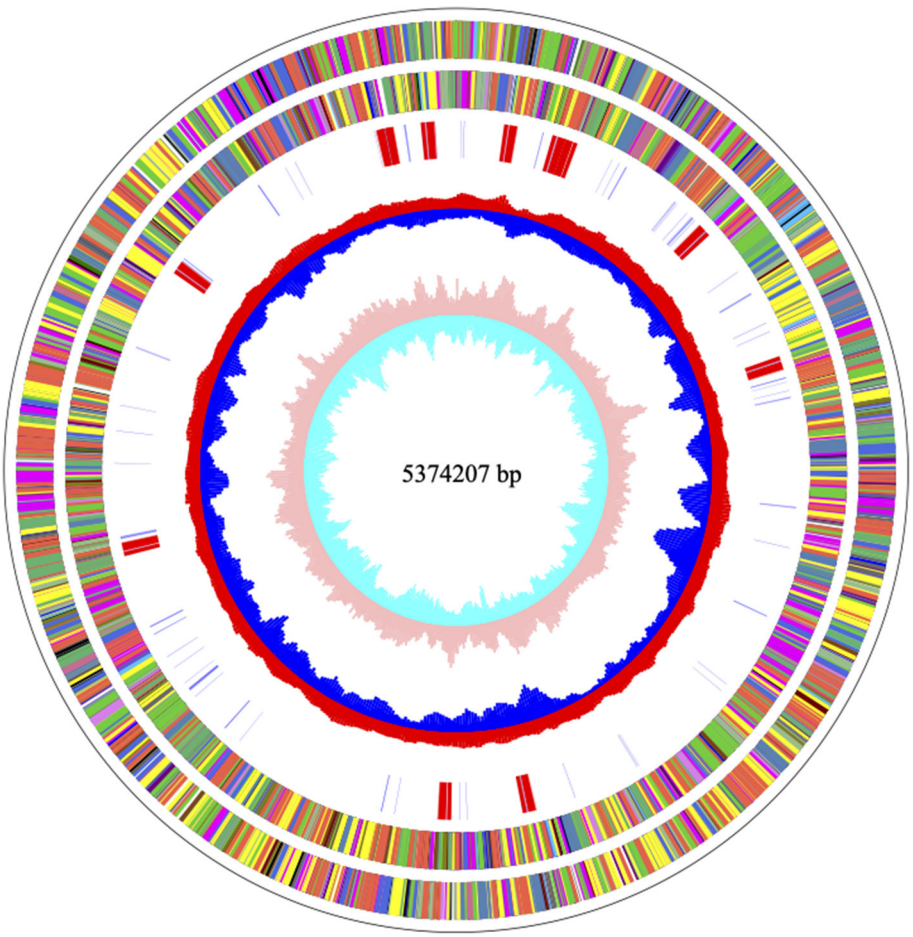

FIGURE 2 | The visual circle map of the chromosome. From outside to inside, the first and second circles are CDS on the positive and negative strands, the third circle is rRNA and tRNA; the fourth circle is the GC content, and the outward red part indicates that the GC content of this region is higher than the average GC content of the whole genome. The higher the peak, the greater the difference from the average GC content, the inward blue part indicates that the GC content of the region is lower than the average GC content of the whole genome, the higher the peak, the greater the difference from the average GC content; the innermost circle (fifth) is the GC skew value, the specific algorithm is G – C/G + C, when the value is positive in the biological sense, the positive chain is more inclined to transcribe CDS, which is a negative value (22, 23).

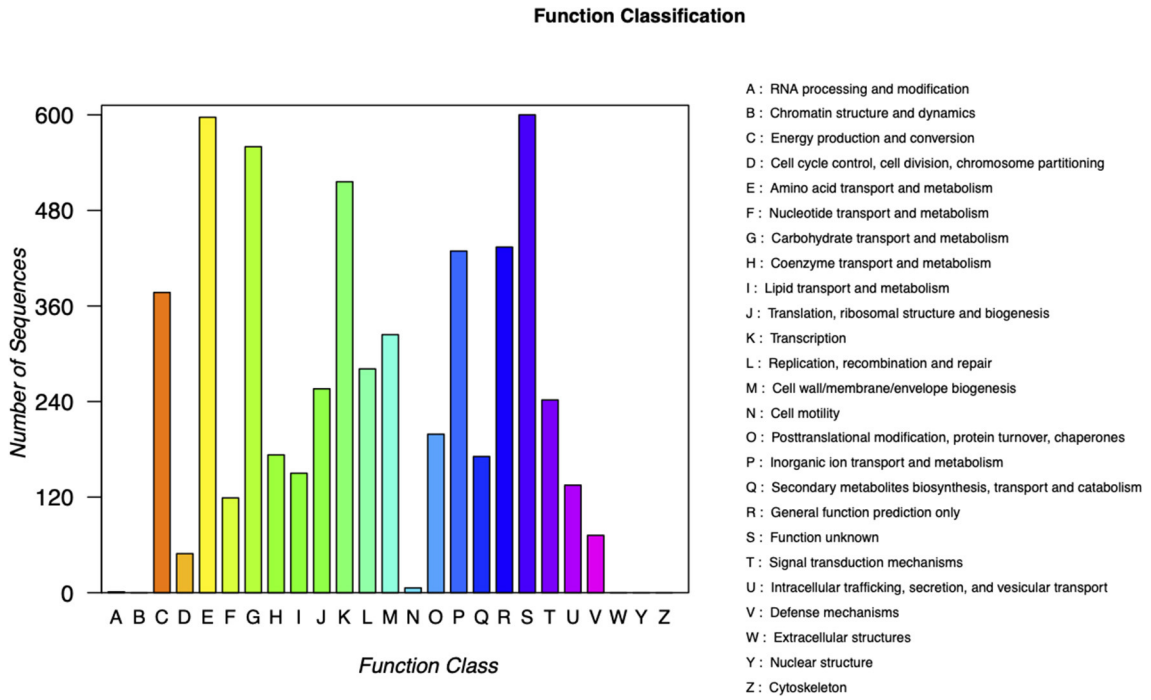

FIGURE 3 | Classification of COG functions of chromosome in DNA libraries. COG, Cluster of Orthologous Groups.

Results of WGS

Carbapenem-resistant *Klebsiella pneumoniae*63 carries two drug resistance genes at the same time, bla_{KPC-2} and $bla_{SHV-182}$. The extended-spectrum β-lactamase (ESBL) gene $bla_{SHV-182}$ is located on the chromosome (position in contig 1,037,173–1,038,033). Yet, the key carbapenem resistance gene bla_{KPC-2} is located on the drug resistance plasmid pKPC-063001, which is of type IncFII. Therefore, the whole genome of CRKP63 consists of a circular chromosome of 5,374,207 bp and a drug-resistant plasmid (named pKPC-063001) of 359,625 bp. For chromosome, the final draft genome showed a G + C content of 60.4%, with a total of 5,165 annotated protein-coding sequences (CDSs). The visual circle map is shown in **Figure 2**. The Cluster of Orthologous Groups [(COGs) (of proteins)] database was used to annotate its genome, and it was found that genes related to metabolism and genes related to genetic information processing accounted for a relatively large proportion. In addition, there are also functional proteins related to gene processing and material conversion. The class of protein function and its number are shown in **Figure 3**.

pKPC-063001 is a 359,625 bp circular molecule with an average G + C content of 58.19% and was predicted to encode a total of 409 CDSs. In addition to the bla_{KPC-2}, it also contains virulence factors *iucA*, *iucB*, *iucC*, and *iucD*, plasmid replication protein (*repA*), plasmid stabilization protein (*parA*), and the type IV secretion system (T4SS) proteins *traA*, *traB*, *traD*, *traM*, and *traK* that mediate the conjugation and transfer of plasmids. The visual circle map is shown in **Figure 4**. After a detailed analysis of the surrounding structure of the key gene bla_{KPC-2}, it was found that bla_{KPC-2} is located in a gene fragment with IS*26* repeat inserts at both ends. This gene fragment has IS*26* repeats at both ends, and IS*kpn27* and IS*kpn6* in the middle, and there is also a *Tn3-tnpR* structure between IS*kpn27* and IS*26* (**Figure 5**).

Accession Numbers

The result obtained by sequencing in this study has been uploaded to the Genebank website (www.ncbi.nlm.nih.gov). The accession number of the chromosome is CP077763 (GenBank: CP077763.1), and the accession number of the plasmid is MZ156798 (GenBank: MZ156798.1).

DISCUSSION

The bla_{KPC} gene still plays a key role in high-level carbapenem resistance (24). One of the key factors contributing to the rapid and wide dissemination of bla_{KPC-2} is its location on a transposable element (25, 26). In Europe, bla_{KPC-2} is mainly located on the conserved Tn*3* family transposon Tn*4401* (25) and is considered the origin of the acquisition and dissemination of this marker (27). Tn*4401* is approximately 10 kb in size and delimited by two 39 bp imperfect inverted repeat sequences. It harbors two ISs flanking bla_{KPC}, IS*Kpn6*, and IS*Kpn7*, in addition to transposase (*tnpA*) and resolvase (*tnpR*) genes. However, Tn*1721*-like transposons among ST11 *K. pneumoniae* are mainly responsible for the effective spread of the bla_{KPC-2} gene in China (28). There are three inverted repeats, *IRR*, *IRL1,* and *IRL2*, in the Tn*1721a* transposon. *tnpA* and *tnpR* are located between *IRR* and *IRL1*, while the 5′ end of bla_{KPC-2} has a

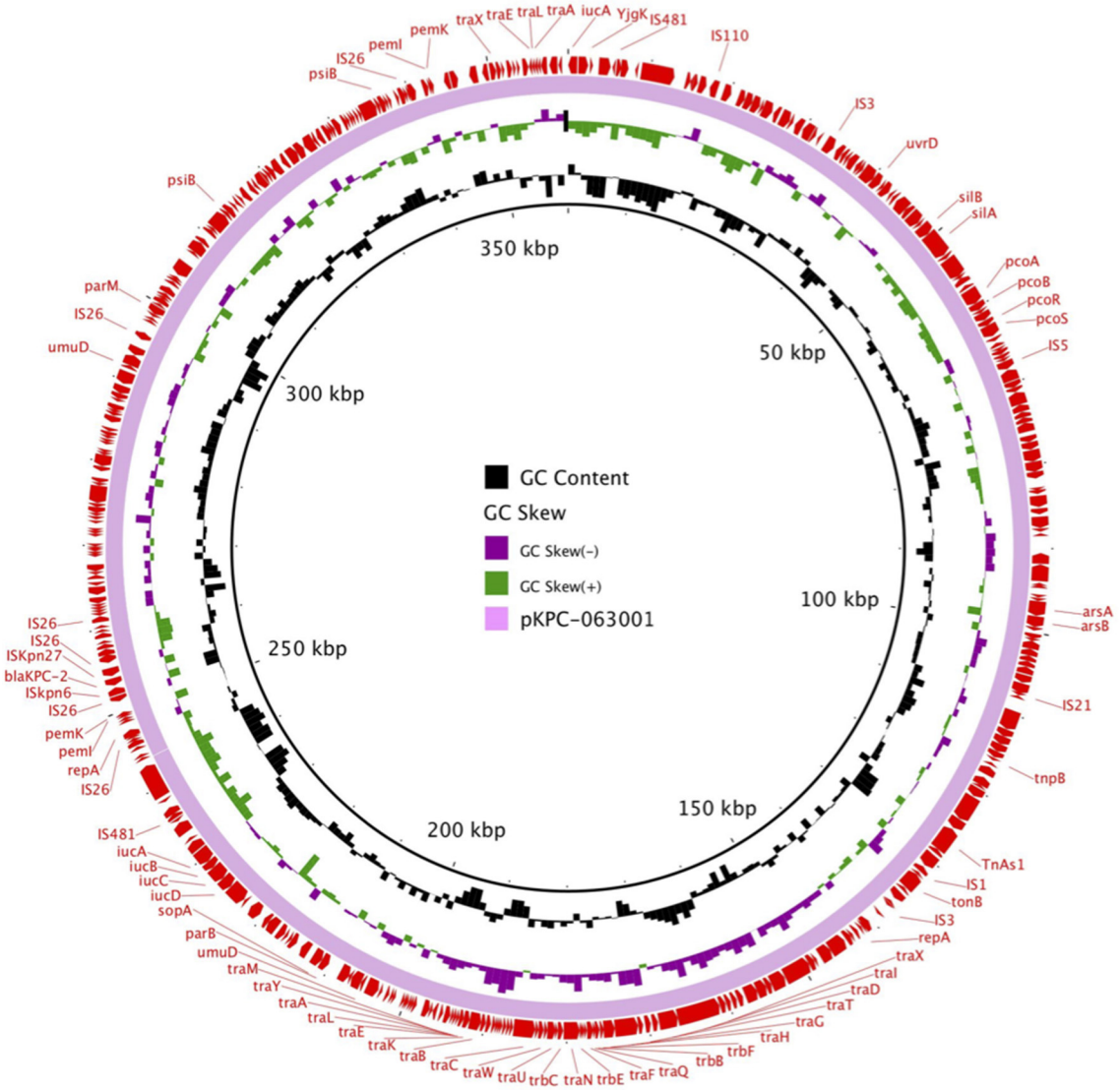

FIGURE 4 | The visual circle map of plasmid pKPC-063001, where bla_{KPC-2} is located. GC content, GC skew+, and GC skew– are, respectively, indicated in black, green, and purple.

complete Tn*3* located between *IRL1* and *IRL2*. When Tn*3*-tnp R is interrupted by IS*26*, Tn*3*-tnp A and *IRL2* are lost, and a new structure, such as Tn*1721*-bla_{KPC-2}-ΔTn*3*-IS*26* (Tn*1721b*), is formed. The Tn*1722*-based unit transposons with the IS*Kpn27*-bla_{KPC-2}-ΔIS*Kpn6* core structure (IS*Kpn27* is initially named in the ISfinder database) has been also reported in China, such as pKP048 (29), p628-KPC (30), and pHS102707.

In this study, the surrounding environment of the bla_{KPC-2} is different from the epidemic structure of domestic and international, we call it "composite transposon-based on IS*26*". The original 1319bp IS*Kpn6* was truncated by IS*26* and became 881bp ΔIS*Kpn6*. IS*Kpn27* is quite different from other inserted sequences, and there is a "recombinase" gene between IS*Kpn27* and IS*26* repeats (**Figure 5**). Notably, different lengths of truncated IS*Kpn6* can be observed for these IS*26*-based transposons from different plasmids. This structure has also been reported by others (31), such as pC2660-3-KPC (32). This difference in transposons indicates that there is a certain variability and diversity surrounding the environment of the bla_{KPC-2} gene, which may also be one of the factors causing the rapid spread of bla_{KPC-2} strain and the different epidemic status in different regions. Loftie et al. (33) found that Tn*6231* can significantly improve the persistence of resistant plasmid pMS0506 and broaden the host range of plasmid, which shows that the evolutionary behavior of plasmid (such as transposon) can affect the spread of resistance gene and improve the selection of plasmid host. Mutations in the genetic environment help to improve the durability of antibiotic resistance, so that the host adapts to changes in the external environment and then affects the spread of bacterial resistance. Therefore, the bla_{KPC-2}gene

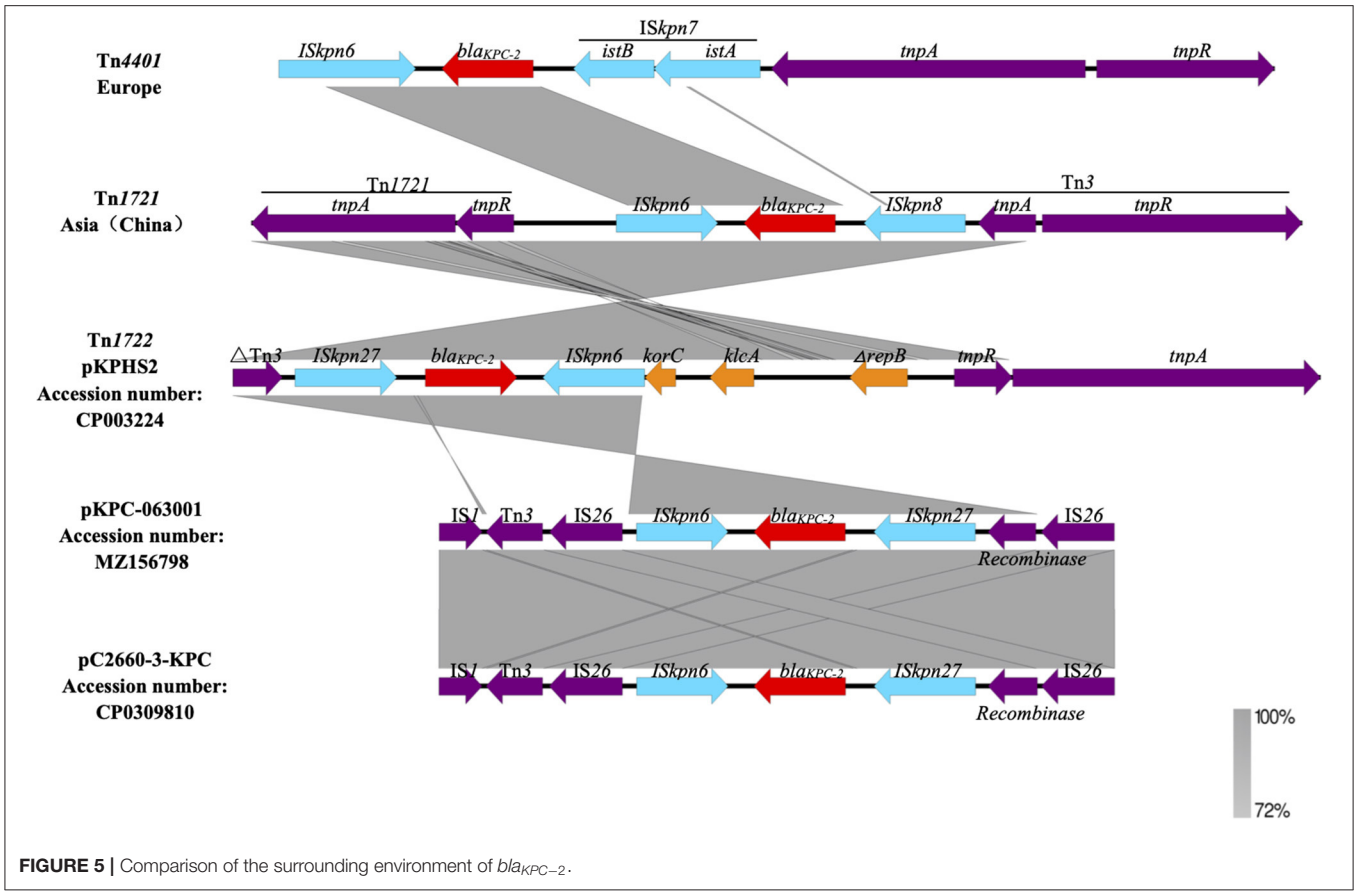

FIGURE 5 | Comparison of the surrounding environment of bla_{KPC-2}.

environment is constantly changing, and its horizontal transfer with transposable elements shows greater flexibility than plasmid transmission. In this way, the clinical harm will be greater.

As the key carbapenemase, bla_{KPC-2} can exist on many different types of plasmids. The bla_{KPC} found in this study is located on the IncFII type plasmid. The success of the conjugation experiment also confirmed the conjugability of the plasmid. It can be seen that the pKPC-063001 has a complete functional skeleton structure, i.e., plasmid replication structure, plasmid stabilization structure, and plasmid conjugation transfer structure. These structures provide a structural basis for the widespread of bla_{KPC-2}. Among them, theT4SS plays an important role in conjugative transfer (34, 35). The classic T4SS system was originated from *Agrobacterium rhizogenes*, it was generally composed of 12 proteins (i.e., 11 *VirB* proteins and 1 *VirD* coupling protein (*VirD4*)) (36, 37), coupling protein–relaxosome contact could lead to DNA unwinding, generating a single strand of DNA that is then transferred to the recipient in a 5′ to 3′ direction (38), so that the genetic information is transferred. Then, T4SS was also found in Gram-negative bacteria (39). Among the two major phylogenetic groups of gram-negative bacteria T4SSs, type IVA (the conjugation systems of the IncF and IncP plasmids) is more common (40, 41). Lawley et al. (34) reported that T4SSs, also known as the mating pair formation (Mpf) apparatus, are central to the dissemination of numerous genetic determinants between bacteria, as highlighted by the spread of antibiotic resistance among pathogens. After studying the gene deletion of the T4SS regulatory region of the pCTX-M3 plasmid of the IncM group, Dmowski et al. found that (42) the knockout of the conjunction structural gene will result in no transfer of the resistance gene or low conjunction rate. However, when *orf35* and *orf36* were knocked out, the plasmid conjugation rate could be improved, this is because two genes are involved in suppressing the transcriptional regulation of the T4SS gene according to transcription analysis. This shows that the existence of T4SS undoubtedly provides strong support for the global popularity of bla_{KPC-2}. The complete T4SS structure helps to improve the adaptability of bacteria to the external environment, thereby enhancing the existence of drug-resistant bacteria and accelerating the spread of drug-resistant bacteria. An in-depth study of the function and transcriptional expression of T4SS will help prevent the further spread of bla_{KPC-2}.

In addition, it should be noted that $bla_{SHV-182}$ located on the chromosome did not transfer with bla_{KPC-2}, simultaneously, the results of conjugation showed that the MIC value of conjugants for carbapenems decreased, indicating that other causes of drug resistance, such as membrane protein, or efflux pump, for example, did not transfer with the plasmid. This also suggests that the resistance of CRKP63 is caused by a variety of factors, not simply caused by pKPC-063001.

Combined with previous research (43), we know that CRKP63 was collected in the ICU ward of a hospital in Chongqing, China. There had been an outbreak of ST11 CRKP carrying bla_{KPC-2} in this ward. This outbreak was closely related to the horizontal transfer of bla_{KPC-2}. Similarly, over the past period, IncFII plasmids carrying bla_{KPC-2} often have been reported in *K. pneumoniae* in China, especially the ST11 (44), the prevalence and dissemination of InFII plasmid carrying *KPC-2* in China has become a fact. However, unlike the dominant Tn*1721* in China, the presence of "IS*26*-based composite transposon" structure represented by pKPC-063001 implies the variability and complexity of bla_{KPC-2}. In the future, "IS*26*-based composite transposon" is likely to become the dominant clone group in China and even the world with amazing speed and adaptability, thence, continuous monitoring will be necessary to prevent further dissemination of pKPC-063001 type plasmid.

In conclusion, this study reported the microbial and genomic characteristics of an ST11 CRKP carrying bla_{KPC-2} in Chongqing, China. Through WGS, we found the different surrounding environments of bla_{KPC-2}, which provides a new research idea for further revealing the transmission and inheritance of bla_{KPC-2} at the molecular level. The differences in the surrounding environment of bla_{KPC-2} create convenience for its dissemination and popularity, and the complete T4SS structure provides a solid guarantee for it. Therefore, in order to control the further spread and prevalence of drug-resistant bacteria, we should also pay close attention to the genetic environment of bla_{KPC-2}, and further study the transcription and expression of T4SS.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, CP077763 and https://www.ncbi.nlm.nih.gov/genbank/, MZ156798.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of Yongchuan Hospital of ChongQing Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XZ conceived and designed the study. LZ and JZ wrote this paper and contributed equally to this work. LZ, JW, CY, and WH performed the experiments. JL, KH, LY, and LZ analyzed the data. All authors contributed to the article and approved the submitted version.

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Rectal Colonization and Nosocomial Transmission of Carbapenem-Resistant *Acinetobacter baumannii* in an Intensive Care Unit, Southwest Nigeria

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Background: *Acinetobacter baumannii* are of major human health importance because they cause life-threatening nosocomial infections and often are highly resistant to antimicrobials. Specific multidrug-resistant *A. baumannii* lineages are implicated in hospital outbreaks globally. We retrospectively investigated a suspected outbreak of carbapenem-resistant *A. baumannii* (CRAB) colonizing patients in an intensive care unit (ICU) of a tertiary hospital in Southwest Nigeria where genomic surveillance of *Acinetobacter* has hitherto not been conducted.

Methods: A prospective observational study was conducted among all patients admitted to the ICU between August 2017 and June 2018. *Acinetobacter* species were isolated from rectal swabs and verified phenotypically with the Biomerieux Vitek 2 system. Whole genome sequencing (WGS) was performed on the Illumina platform to characterize isolates from a suspected outbreak during the study period. Phylogenetic analysis, multilocus sequence typing, and antimicrobial resistance gene prediction were carried out *in silico*.

Results: *Acinetobacter* isolates belonging to the *A. baumannii* complex were recovered from 20 (18.5%) ICU patients. Single nucleotide polymorphism (SNP) analysis and epidemiological information revealed a putative outbreak clone comprising seven CRAB strains belonging to the globally disseminated international clone (IC) 2. These isolates

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had ≤2 SNP differences, identical antimicrobial resistance and virulence genes, and were all ST1114/1841.

Conclusion: We report a carbapenem-resistant IC2 *A. baumannii* clone causing an outbreak in an ICU in Nigeria. The study findings underscore the need to strengthen the capacity to detect *A. baumannii* in human clinical samples in Nigeria and assess which interventions can effectively mitigate CRAB transmission in Nigerian hospital settings.

Keywords: *Acinetobacter baumannii*, rectal colonization, nosocomial, hospital-acquired infections, carbapenem-resistant *Acinetobacter baumannii*, antimicrobial resistance

INTRODUCTION

Acinetobacter baumannii are opportunistic pathogens of increasing global public health concern. These Gram-negative organisms are widely implicated in life-threatening drug-resistant infections in hospitalized patients. *A. baumannii* are often introduced in patients through contaminated medical devices, and infections include pneumonia, urinary tract infection, bloodstream infection, endocarditis, meningitis and wound infection (1). More so, these infections are often prone to epidemic spread within hospital settings, facilitated by the excellent ability of *A. baumannii* to survive in harsh hospital environments (2).

High resistance to desiccation, efficient biofilm-forming ability and the frequent carriage of resistance determinants to both antimicrobials and commonly used disinfectants mean that *A. baumannii* are especially well suited for survival in the hospital environment, including hospital surfaces, utensils and equipment, invasive medical devices, as well as hospital personnel (3–6). Prolonged survival allows *A. baumannii* to excel as nosocomial pathogens and makes their transmission difficult to control within hospitals. Healthcare personnel play important roles in this cross-transmission within the hospital as they get frequently contaminated *via* contact with infected or colonized patients as well as contaminated abiotic surfaces (4). Nosocomial *A. baumannii* infections are associated with high mortality, lengthened hospital stay and increased hospital costs (7).

The clinical burden of *A. baumannii* is further worsened by their frequent carriage of multiple resistance determinants, limiting treatment options and worsening outcomes. Their highly plastic genomes facilitate the acquisition and maintenance of genes conferring resistance to different antimicrobials, including the carbapenems which are last-line antimicrobials for treatment of these infections (1, 8). The limited treatment options available for carbapenem-resistant *A. baumannii* (CRAB) infections caused the World Health Organization to prioritize CRAB as number one on the priority list of pathogens for which new antimicrobials are urgently needed (9). Mechanisms of resistance to carbapenems in *A. baumannii* include overexpression of efflux pumps, membrane porin modification and, most prominently, possession of resistance genes, particularly the OXA- and NDM-type carbapenemases, which are borne on either plasmids or chromosomes (2, 10, 11). Resistance to carbapenems is associated with the successful and globally disseminated major *A. baumannii* clones, international clones (ICs) 1–3, with very high resistance rates reported (12). These clones, with IC2 being the most successful and widely described, are extensively drug-resistant and are the most frequently reported cause of CRAB outbreaks in hospital settings worldwide (2). Nevertheless, high carbapenem resistance rates have been reported in non-IC *A. baumannii* clones endemic in several countries (11, 13, 14).

Little is known about the molecular epidemiology and antimicrobial resistance profiles of *A. baumannii* infections in Nigeria, largely due to limited capacity for the isolation, identification and antimicrobial susceptibility testing of *A. baumannii* in routine clinical laboratories, as well as poor access to molecular characterization techniques. However, a few reports exist describing CRAB causing clinical infections in hospitals in Nigeria. These studies, one conducted almost 10 years ago in 2012 (15), and another in 2018 (16), reported the detection of *blaOXA*-23 and *blaNDM*-1 carbapenem resistance genes in clinical CRAB isolates mostly from southwestern Nigeria hospitals. Surveillance and understanding of the risk factors for transmission of *A. baumannii* infections within hospital intensive care units (ICUs) is critical for establishing effective infection control and prevention measures to curtail further spread between high-risk immunosuppressed patients. Evidence suggests that even the seemingly harmless colonization of body sites, including the axilla, pharynx and gastrointestinal tract, by *A. baumannii* within ICUs can precede subsequent infection (17). We retrospectively investigated a suspected outbreak of *A. baumannii* in the intensive care unit (ICU) of a tertiary hospital in Southwest Nigeria.

MATERIALS AND METHODS

Ethical Considerations

Ethical approval for the study was granted by the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex, Ife, Nigeria with protocol number ERC/2017/06/13. Participants provided written informed consent before voluntarily participating in the study. Patient confidentiality and anonymity were maintained. Only

Abbreviations: AMR, Antimicrobial resistance; CRAB, Carbapenem-resistant Acinetobacter baumannii; GHRU, Global Health Research Unit; IC, International clone; ICU, Intensive care unit; MLST, Multi-locus sequence typing; OAUTHC, Obafemi Awolowo University Teaching Hospitals Complex; OR, Odds ratio; SNP, Single nucleotide polymorphism; UCH, University College Hospital; VFDB, Virulence factor database; WGS, Whole genome sequencing.

de-identified patient metadata with no traceability to patients was collected and analyzed.

Sample Collection and Bacterial Identification

A prospective observational study was conducted to determine the colonization and transmission of CRAB among all new patients admitted into the ICU of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ife, Osun State, Nigeria between August 2017 and June 2018. A total of 108 patients were recruited. Rectal swabs were collected from each patient within 48 h of ICU admission and, thereafter, weekly until exit using the protocol for active surveillance cultures by the US Centres for Disease Control and Prevention (18). Acquisition rate was defined as the percentage of patients who acquired CRAB that was absent on admission. CRAB was isolated from the rectal swab samples and preliminarily identified using standard microbiological procedures, and then cryopreserved at −80°C. We also verified all *A. baumannii* isolates from human specimens and associated metadata retrospectively submitted to the then-new Nigerian antimicrobial resistance (AMR) surveillance system by OAUTHC, Ife, Osun State and from the University College Hospital (UCH), Ibadan, located in the adjacent Oyo State. The identities of all presumptive *Acinetobacter* species were verified by culture on CHROMagar™ Acinetobacter media with CHROMagar MDR Supplement CR102 (CHROMagar, Paris, France) and identification on a VITEK 2 automated system (bioMérieux, Inc., Marcy-l'Étoile, France) following manufacturer instructions using GN ID (reference number: 21341) cards. Antimicrobial susceptibility testing was also carried out on the Vitek 2 automated system using the AST N281 (reference number: 414531) cards. Antibiotics tested were ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefoperazone/sulbactam, cefepime, doripenem, imipenem, meropenem, gentamicin, ciprofloxacin, levofloxacin, minocycline, tigecycline and trimethoprim/sulfamethoxazole. Minimum inhibitory concentration values were interpreted according to the Clinical Laboratory Standards Institute guidelines (19).

DNA Extraction, Library Preparation, and Sequencing

Genomic DNA extraction was carried out using the FastDNA Spin Kit for Soil (MP Biomedicals, Irvine, CA, United States) with protocols modified for bacterial genomic DNA extraction. The *Acinetobacter* isolates were grown overnight in Tryptone Soy Broth (Oxoid, Basingstoke, United Kingdom) and centrifuged to obtain the cell pellets at 6,000 revolutions per minute for 5 min. The pellet was then used for extracting the DNA following the manufacturer's instructions. Quantification of extracted DNA was done using the Qubit™ dsDNA BR Assay Kit (Invitrogen, Waltham, MA, United States). Library preparation was done using the Covaris LC220 for fragmentation, and the NEBNext Ultra II FS DNA library kit for Illumina with 384-unique indexes (New England Biolabs, Ipswich, MA, United States). The double-stranded DNA libraries (avg. 500 bp) were sequenced using the HiSeq X10 with 150 bp paired-end chemistry (Illumina, San Diego, CA, United States).

Whole Genome Sequence Analysis

All sequence analyses, except where otherwise stated, were carried out as described in the Global Health Research Unit (GHRU) protocol[1] using publicly available Nextflow pipelines. *De novo* assembly, species identification, and quality control were carried out using the *De novo* assembly pipeline with default parameters. Quality checks included total bases between 3,340,530–4,776,219 megabase pairs (Mbp), contamination levels <5%, N50 scores >25000, and number of contigs <300; assemblies that "failed" any of these checks were excluded from the downstream analyses.

To determine evolutionary relationships among the isolates, single nucleotide polymorphism (SNP) phylogeny analysis was conducted using the SNP phylogeny pipeline with default parameters. Briefly, Bactinspector[2] was used to select the closest reference to the *A. baumannii* sequences (Genbank accession: GCA_000830055.1). Reads were mapped to the reference, and variants were called, filtered and concatenated into pseudogenomes. Afterward, the pseudogenomes were aligned and a maximum likelihood tree was constructed from the aligned pseudogenomes with the GTR + G model and 1,000 bootstraps. To determine the clonality of isolates within suspected outbreak clades, we re-selected a reference sequence (NZ_CP016298.1) more closely related to the strains of interest, computed pseudogenome alignments as previously described and calculated the pair-wise SNP distances between the strains based on the aligned pseudogenomes using FastaDist[3].

Multi-locus sequence types (MLST) of the strains were determined *in silico* as described in the GHRU protocol based on the Oxford and Pasteur MLST schemes (20, 21). The goeBURST software[4] was used to assign the predicted sequence types (STs) to IC groups based on locus similarities to known international clones (22). The acquired antimicrobial resistance determinants harbored by each of the isolates were also predicted *in silico* as described in the aforementioned GHRU protocol using the Ariba software and the National Center for Biotechnology Information's (NCBI) Bacterial Antimicrobial Resistance Reference Gene Database[5]. Only predicted genes tagged by the ariba software as "yes" or "yes_nonunique" were regarded as present in the genome.

Statistical Analysis

Patient clinical data was entered into WHONET 5.6[6] and cleaned on Microsoft Excel® (Microsoft Corporation, Redmond, WA, United States) spreadsheet. Data was then analyzed using Statistical Package for the Social Science (SPSS®, IBM Corp.,

[1]https://www.protocols.io/view/ghru-genomic-surveillance-of-antimicrobial-resista-bpn6mmhe

[2]https://gitlab.com/antunderwood/bactinspector

[3]https://gitlab.com/antunderwood/fastadist

[4]http://www.phyloviz.net/goeburst/

[5]https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047

[6]http://www.who.int/drugresistance/whonetsoftware/en/

Armonk, NY, United States) version 20. A descriptive statistical analysis was carried out to summarize the demographic data and results were presented as frequency distribution, percentages, mean, and standard deviation. Risk factors for CRAB-colonization/infection were assessed using bivariate analysis with Chi-square and multivariate analysis with logistic regression. *P*-values <0.05 were considered statistically significant.

RESULTS

Study Population and Species Distribution

Rectal swab samples were obtained from 108 patients admitted to the ICU at OAUTHC between August 2017 and June 2018. Carbapenem-resistant *A. baumannii* (CRAB) was recovered from 20 (18.5%) patients. The acquisition rate was 8.3% (8/96), while 12 (11.1%) patients were positive for CRAB within 48 h of admission. Patients that acquired CRAB had seven times the odds of subsequent bloodstream infection (OR = 7.41; 95% CI 2.39–22.92). The mortality rate among CRAB-colonized patients was 50% and the odds of death was almost two times higher among the CRAB-colonized patients compared to patients with no CRAB colonization (OR = 1.84, 95%; CI = 0.69–4.90).

Rectal Colonization Outbreak in Obafemi Awolowo University Teaching Hospitals Complex Intensive Care Unit Ward Identified by Whole Genome Sequencing

A total of 36 bacterial genomes were analyzed; 20 were isolated from the ICU at OAUTHC between 2017 and 2018 and were part of the suspected rectal colonization outbreak, while the remaining 16 were retrospective isolates submitted to Nigeria's AMR surveillance system (2017 – 2019) by OAUTHC and UCH. The isolates were identified as *A. baumannii* (34), *A. nosocomialis* (one), and *A. pittii* (one) based on their whole-genome sequences.

The 34 *A. baumannii* strains segregated into clear evolutionarily distinct lineages, with isolates in each lineage belonging to identical STs. Half (17/34; 50.0%) of the isolates belonged to the two major clades observed (clade 1 and clade 2) and three different STs: ST1114 and ST1841 (which we later found to be the same ST but were artifactually different due to a duplicated *gdhB* gene in the strains) in clade 1, and ST1089 in clade 2 (**Figure 1**).

All nine clade 1 (IC2) strains and five of the eight clade 2 strains were from the OAUTHC ICU. Based on phylogenetic relatedness, timeline of isolation and other epidemiological data, we hypothesize that the clade 1 isolates were part of an outbreak in the ICU, while the others, including those in clade 2, were endemic circulating strains isolated during the same period. After the isolation of the first clade 1 strain on 2nd January 2018, seven more identical strains belonging to this clade were isolated within 3 weeks (between 17th March 2018 and 9th April 2018) of each other from patients in the same ICU. Conversely, and further supporting our hypothesis of repeated introduction, the five clade 2 strains from the ICU were isolated over 42 weeks. The intra-clade SNP distances between the isolates ranged from 0–213 SNPs. Also, one of the clade 2 strains was isolated at another tertiary facility in a different state in southwest Nigeria; the date of isolation was not available for this as well as most of the other non-ICU isolates.

Clade 1 isolates all had an identical AMR and virulence gene composition and resolved into two sub-clades – clade 1A (two isolates) and clade 1B (seven isolates). Each sub-clade had a maximum within-clone distance of two SNPs. The SNP distances between the two sub-clades were ∼48 SNPs. A previous study reported a threshold of ∼2.5 core-genome SNPs for distinguishing outbreak and non-outbreak *A. baumannii* strains (23). We conclude that clade 1B isolates were part of a definite outbreak; however, as there were only 2 clade 1A isolates, it could not be determined if these isolates were also part of an independent and concurrent outbreak in the ICU during the study period.

Whole Genome Sequencing Resolution Allowed the Identification of Outbreak Strains

As the suspected outbreak isolates within clade 1 were close to identical (≤2 SNPs), we further investigated the reason for the different ST assignments of the isolates within this clade. Based on the Oxford MLST scheme, ST1114 and ST1841 strains are single locus variants with allelic differences in the *gdhB gene*. We retrieved the sequences of these two alleles from the pubMLST database and searched them against the contigs of all clade 1 isolates using BLAST. This revealed two copies of the *gdhB* gene within all nine clade 1 isolates, with each copy sharing 100% identity with either allele 3 or 189. This demonstrated that all clade 1 strains belonged to the same ST and supported our outbreak hypothesis. With repeated runs, however, our MLST pipeline consistently detected allele 189 among ST1841 isolates and allele 3 among the ST1114 isolates.

Local Epidemiology of *Acinetobacter baumannii* Infections

We characterized the strains based on MLST data to identify the lineages causing *A. baumannii* infections in Nigeria. Nine of the 34 (20.6%) *A. baumannii* isolates, and the single *A. pittii* isolate, had novel MLST allelic profiles (**Figure 2**). Upon submission of these isolates and profiles to the PubMLST database, we found that two of the STs had also been detected in other studies – one in China (ST2417) and the other in Ghana (ST2146) – around the same period and submitted to the database. The other eight novel ST profiles were submitted to the PubMLST database with submission ID BIGSdb_20210908105012_023242_52326 and have been assigned STs (**Supplementary Table 1**). The nine outbreak *A. baumannii* isolates (ST1114 and ST1841), as well as one of the novel ST strains (G20500013; ST2456), belonged to IC2, while three of the strains belonged to IC1 (ST231, ST441, and the novel ST2451 strain). Other international clones of *A. baumannii* were also present among our strains. However, the majority (19/34; 55.9%) of the *A. baumannii* isolates were singletons, non-major international clones or novel.

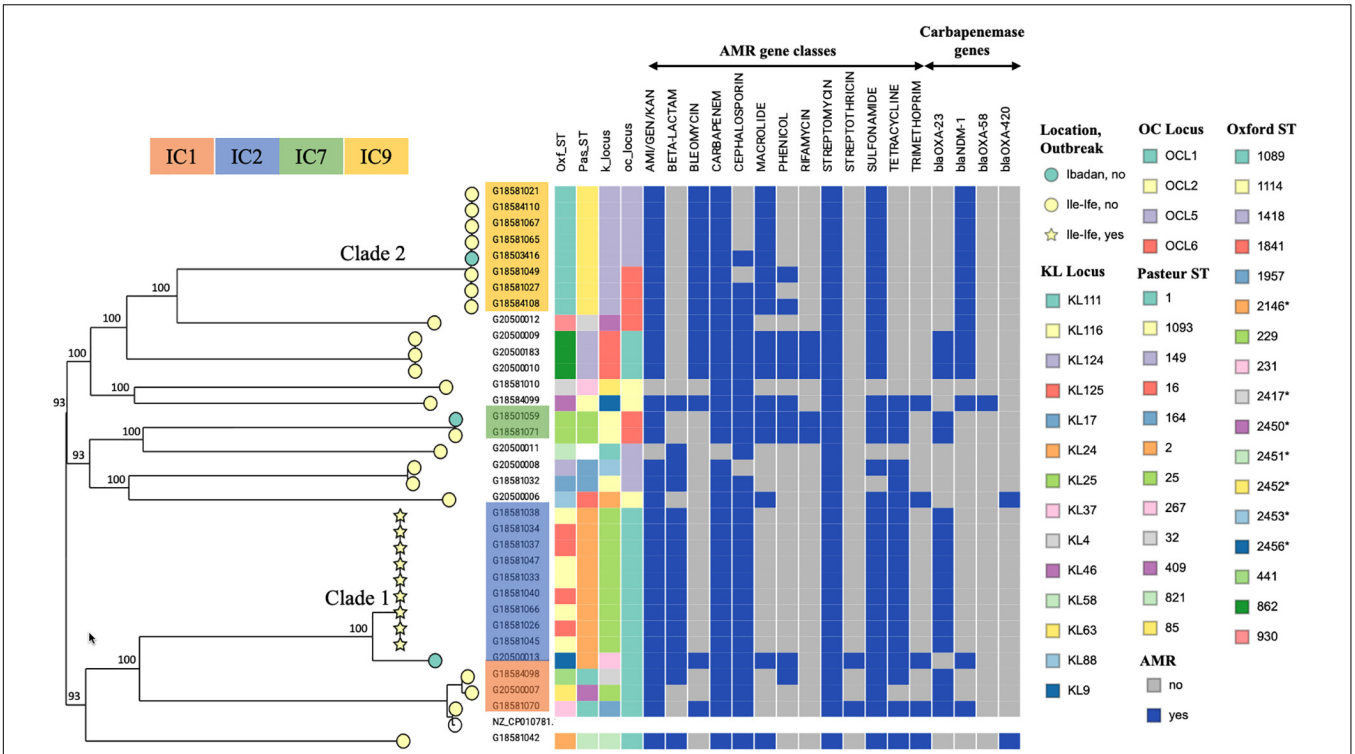

FIGURE 1 | Maximum likelihood phylogeny of *A. baumannii* isolates from OAUTHC, Ife, Osun State and UCH, Ibadan, Oyo State, Nigeria between 2017 and 2019. Sequence types, KL and OC types, classes of antimicrobials to which resistance is conferred by harbored genes, and carbapenem resistance genes are shown. Tree node colors represent isolate collection location; nodes in star shape represent the confirmed outbreak strains. Internal node labels show percentage bootstrap support for all bifurcations. https://microreact.org/project/eWkGUYTxYUJ6Ctv79Lyxj9-acinetobacter-2021-10-03.

The eight ST1089 strains clustered with other IC9 sequence types in the PubMLST database, while the two ST229 strains clustered with other IC7 sequence types. Both ST229 isolates were phylogenetically identical and had identical AMR and virulence genes but were isolated from different locations, one from UCH and the other from OAUTHC.

Antimicrobial Resistance Determinants

All 34 *A. baumannii* isolates harbored variants of the intrinsically encoded *blaOXA-51*-family carbapenemase gene (**Figure 3** and **Supplementary Table 2**). In addition to these intrinsic *blaOXA-51* family genes, all but one of the *A. baumannii* isolates carried other carbapenem resistance genes. Seventeen (50.0%) isolates, including all IC1, IC2 (clade 1) and IC7 strains harbored variants of the potent *blaOXA-23*-like carbapenemase gene; four of these strains also harbored the *blaNDM-1* gene. *blaNDM-1* was present in 15 (44.1%) strains, including all eight ST1089 (clade 2) strains and two of the novel ST strains (ST2450 and ST2456). This ST2450 strain was highly resistant and was the only strain that carried the *blaOXA-58* carbapenemase gene. Two other strains carried the *blaOXA*-420 variant of the *blaOXA*-58 family. Phenotypically, thirty-one (91.2%) strains were resistant to at least one of the carbapenem antibiotics tested; 31 strains were resistant to meropenem, 30 were resistant to doripenem, and 28 were resistant to imipenem (**Figure 4** and **Supplementary Table 3**).

Other beta-lactam resistance genes were found in most of the isolates. Several variants of the AmpC gene, *blaADC*, which is an intrinsic chromosomally encoded gene that confers cephalosporin resistance, were detected in 28 (82.4%) of the isolates. The remaining six isolates carried copies of the gene that were flagged by the ariba prediction software as being either "fragmented" or "interrupted," indicating either a non-coding variant or a gene structure significantly different from that of the gene in the NCBI AMR database, respectively. Furthermore, all ten IC2 isolates, as well as the ST441 strain, harbored the broad-spectrum beta-lactamase gene, *blaTEM-84*. All *Acinetobacter* isolates harbored genes conferring streptomycin resistance. Amikacin, gentamicin and kanamycin resistance genes were also detected in 32 (94.1%) of the *A. baumannii* isolates. Thirty-one (91.2%) *A. baumannii* isolates also harbored at least one sulfonamide resistance gene; 15 isolates carried both *sul1 and sul2*. At least one of the tetracycline efflux transporter genes, *tetA*, *tetB,* and *tet39* was detected in 19 (55.9%) of the *A. baumannii* isolates. The resistance rate among *A. baumannii* strains in this study was very high, with >88% of the strains being resistant or intermediate to 10 of the 14 antibiotics tested, including the carbapenems.

The outbreak strains in clade 1 all harbored multiple resistance genes. All of them harbored the *blaOXA-23* gene and were all phenotypically resistant to doripenem, imipenem, and meropenem. They also harbored genes mediating resistance

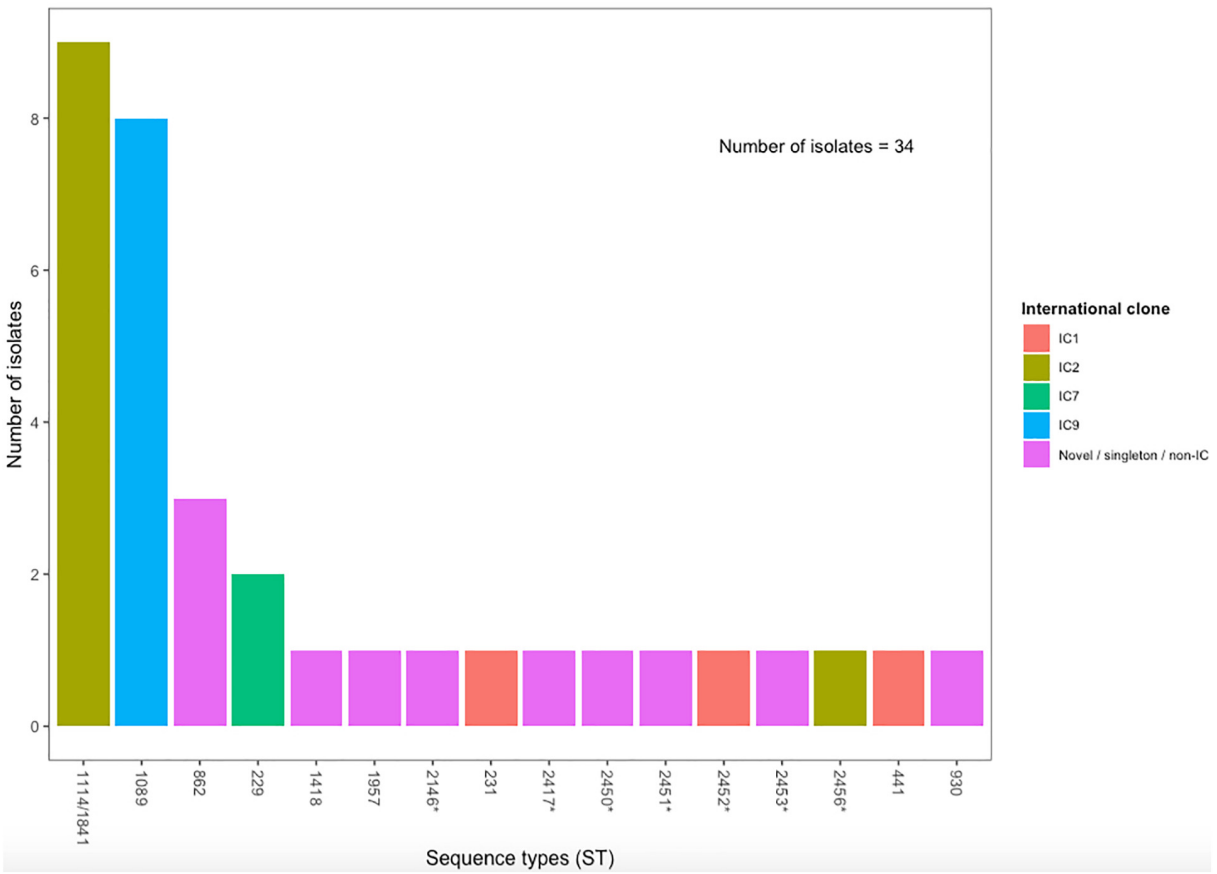

FIGURE 2 | MLST distribution of *A. baumannii* isolates from OAUTHC, Ife, Osun State and UCH, Ibadan, Oyo State, Nigeria between 2017 and 2019. Bars are colored according to the international clone assignment (20, 34). STs with asterisks are novel STs detected in this study.

to streptomycin, beta-lactams/cephalosporins, sulfonamides, tetracycline, and other aminoglycosides, including amikacin, gentamicin and kanamycin, and were only sensitive to tigecycline amongst all the antibiotics tested.

DISCUSSION

Carbapenem-resistant *A. baumannii* are critically important nosocomial pathogens with limited treatment options. In this study, we confirmed an outbreak of CRAB belonging to IC2 and carrying multiple resistance determinants. We also demonstrated the preponderance of relatively unknown and previously undescribed CRAB lineages in our study area in South-west Nigeria.

Nearly a tenth of the patients acquired CRAB infections while admitted in the ICU during the study period, and CRAB acquisition was associated with higher odds of a subsequent bacterial bloodstream infection. As no CRAB was detected in swab samples collected from the ICU environment during the study period, we hypothesize that CRAB transmission may have occurred *via* health care workers. It is noteworthy that before this study, *A. baumannii* detection in the ICU of this hospital was almost non-existent due to limited diagnostic capacity and the difficulty in identifying *A. baumannii* using the conventional microbiological techniques in use. This was also exemplified in the number of *A. baumannii* isolates received from the AMR surveillance sentinel hospitals across Nigeria; only 16 *Acinetobacter* isolates were submitted to the AMR reference laboratory by the sentinel surveillance sites as part of their retrospective (2017–2019) collection. One limitation of the study was our inability to assess the differences in clonality and/or antimicrobial resistance phenotype or genotype between the community-acquired and hospital-acquired strains as information needed to robustly categorize the isolates was not available to us.

Our phylogenetic analyses revealed an *A. baumannii* outbreak in the ICU ward of OAUTHC. Six of the outbreak strains identified were isolated within 2 weeks of each other from six different patients within the same ICU unit. These strains belong to the IC2 lineage previously thought to be endemic in Europe and the United States (24) but increasingly reported as causes of nosocomial infections globally (12, 25–29). Strains in this lineage are highly adapted to the hospital setting, can spread rapidly within hospitals, and are highly resistant to antimicrobials, including carbapenems (12, 30).

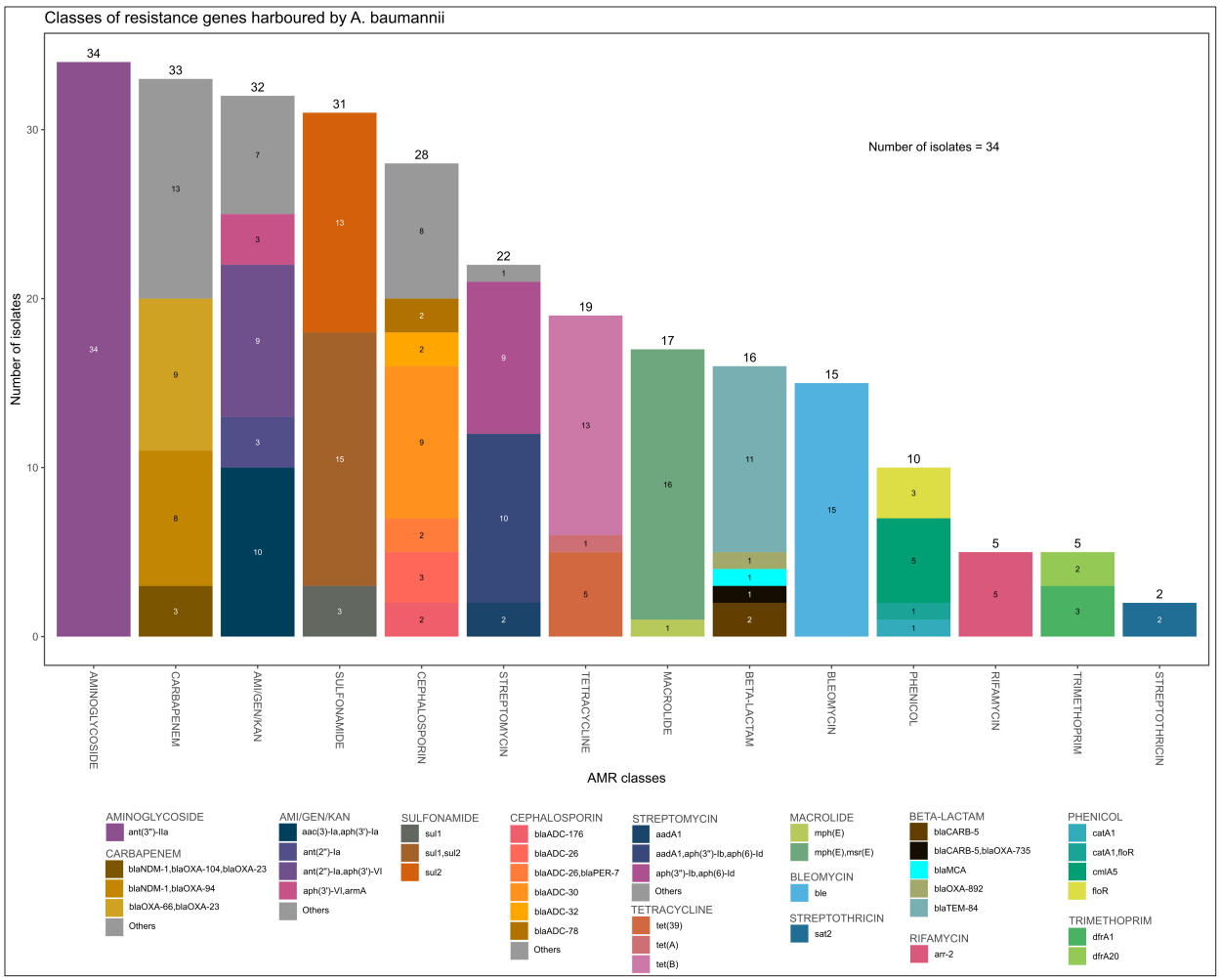

FIGURE 3 | Resistance genes detected in *A. baumannii* isolates from OAUTCH, Ife, Osun State, and UCH, Ibadan, Oyo State, Nigeria, between 2017 and 2019. AMI, Amikacin; GEN, Gentamicin; KAN, Kanamycin.

One interesting observation was that the phylogenetically identical outbreak strains resolved into two different Oxford STs; ST1114 and ST1841 due to the presence of two *ghdB* loci in each of the strains. The *gdhB* gene has been described to be prone to duplication in *A. baumannii*, and indeed a previous study found that several artifactual and unreal *A. baumannii* STs exist in the Oxford PubMLST database due primarily to typing based on a second *gdhB* locus (31). As the replacement of the *gdhB* locus in the Oxford MLST scheme may not be feasible, exploiting long read or paired-end short-read data to identify the two *gdhB* loci during genome-based MLST is recommended. Although core genome MLST and core genome or whole genome SNP-based phylogenetic analyses are sufficient to resolve *A. baumannii* population structures, the Oxford MLST scheme remains important in the description of *A. baumannii* lineages due to its high discriminatory power. Cleaning artifactual STs from the database should be possible as has been described (31), and resolving this simple problem will streamline and facilitate MLST-based surveillance where whole genome sequencing resources are unavailable.

The IC2 strains identified as part of the outbreak made up a quarter of the *A. baumannii* isolates characterized in this study. Nevertheless, this prevalence may have been an overrepresentation caused by the outbreak. The ST1089 (IC9) strains were the second most common lineage in this study. These strains have been isolated from clinical samples and hospital environments, particularly from studies in Northern Africa and in the Middle East, and are frequently reported to carry *blaNDM-1* and *blaOXA-94* carbapenem resistance genes (32, 33–39). An ST1089 strain carrying *blaNDM-1* was also isolated from raw milk in a dairy farm in Algeria (40). All eight ST1089 strains in our study carried the *blaNDM-1* gene, as well as the *blaOXA-94* variant of the *blaOXA-51*-family carbapenemase genes. Although five of these strains were isolated in the ICU, these five strains were isolated over a 42-week period and shared between 6 and 207 SNP differences between them. The remaining three strains were isolated from OAUTHC, Ife, and UCH, Ibadan,

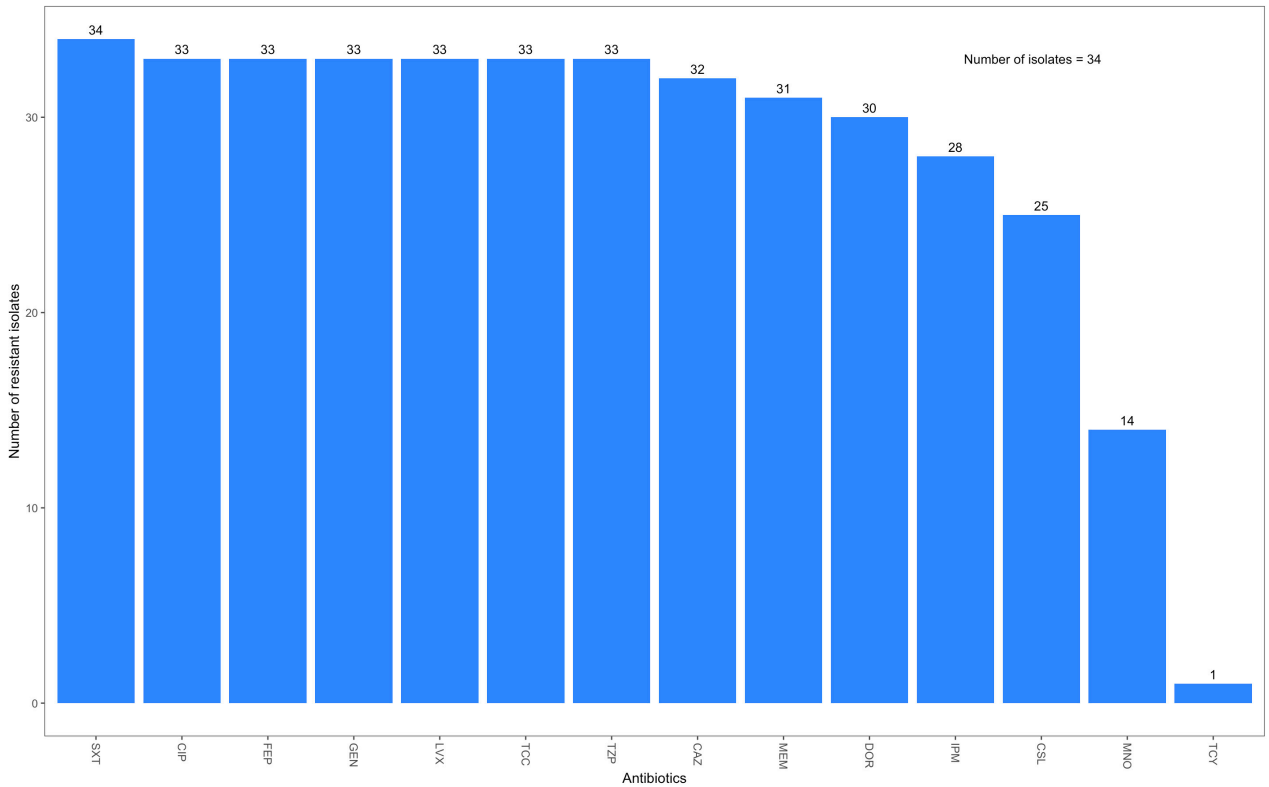

FIGURE 4 | Phenotypic resistance of *A. baumannii* isolates from OAUTCH, Ife, Osun State, and UCH, Ibadan, Oyo State, Nigeria, between 2017 and 2019. SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; FEP, cefepime; GEN, gentamicin; LVX, levofloxacin; TCC, ticarcillin/clavulanic acid; TZP, piperacillin/tazobactam; CAZ, ceftazidime; MEM, meropenem; DOR, doripenem; IPM, imipenem; CSL, cefoperazone/sulbactam; MNO, minocycline; TCY, tigecycline.

and all eight strains differed by up to 213 SNPs, indicating that this clone may be endemic and circulating in Nigeria. About a third of the *A. baumannii* lineages in the hospital setting in this study were novel. A retrospective study that examined the *A. baumannii* lineages circulating and causing infections in Chile between 1990 and 2015 had similar findings, demonstrating that endemic clones different from those that had been described globally were predominant in Chilean settings but that these lineages were similar to those described elsewhere in South America (11). Our retrospective dataset is small, unrepresentative and insufficient to describe the epidemiology of *A. baumannii* lineages in Nigeria, but the available data does suggest that previously undescribed *A. baumannii* lineages may predominate in our setting.

In silico prediction of AMR determinants revealed most of the strains to be highly resistant, possessing resistance determinants to multiple antimicrobial classes. There was no difference in the number of resistance genes harbored between the outbreak strains and the other *A. baumannii* strains. Expectedly, all the *A. baumannii* isolates harbored variants of the intrinsic chromosomally encoded *blaOXA-51*-like gene, some of which may confer carbapenem resistance under certain conditions such as overexpression caused by the presence of an ISAba1 upstream of the gene (41). Carbapenem resistance in *A. baumannii* is most often mediated by acquired carbapenemase genes, particularly of the oxacillinase type, with *blaOXA-23*-like, *blaOXA-24*-like, *blaOXA-58*-like, *blaOXA-143*-like, and *blaOXA-235*-like genes being the most notable (2). Only *blaOXA-58*-like genes and *blaOXA-23*, which is the most widespread of the carbapenemase genes reported in clinical *A. baumannii* globally (42–46), were detected in the isolates in this study. *blaOXA*-58 had not previously been reported in Nigeria, and *blaOXA*-23 has been reported in only two Nigerian studies (15, 16). The *blaOXA-420* variant of the *blaOXA-58* family detected in one isolate has been reported in *A. baumannii* in only a handful of studies, including two conducted in neighboring Ghana (47–49). *blaNDM-1*, which is a less commonly described but highly potent carbapenem resistance gene in *A. baumannii,* was present in over a third of the isolates, four of which also co-carried *blaOXA*-23 (50). Carbapenem resistance in *A. baumannii* always translates to multidrug resistance, and often to extensive drug resistance (51), as evidenced by the high proportion of CRAB isolates carrying genes conferring resistance to other antibiotic classes. Thus, the high prevalence of mobilizable carbapenem resistance genes in this setting is worrying, particularly as the remaining treatment options (51) outside carbapenems are hugely limited in most hospitals in Nigeria as well as in other African countries. Furthermore, the true picture of *A. baumannii* prevalence and

modes of spread in hospital settings in Nigeria remains unknown. As *A. baumannii* are known to spread primarily through clonal dissemination, we urgently need to identify contributors to their increasing spread in hospital settings in Nigeria. This would entail a holistic description of the endemic and circulating lineages, an identification of environmental reservoirs, if any, and a description of the infection prevention and control gaps that facilitate their spread in hospitals in our setting.

CONCLUSION

We report the first description of IC2 *A. baumannii* strains causing an outbreak in an ICU in Nigeria. This study underscores the need to improve the capacity for the recovery and detection of *A. baumannii* in clinical samples in Nigeria and for intervention studies to mitigate CRAB transmission in Nigerian hospital settings.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/ena, ERR4783259, ERR4783518, ERR4783200, ERR4783180, ERR4783258, ERR4783419, ERR4783264, ERR4783252, ERR4783188, ERR4783391, ERR4783231, ERR4783186, ERR4783194, ERR4783223, ERR4783203, ERR4783222, ERR4783387, ERR4783195, ERR4783401, ERR4783244, ERR4783250, ERR4783214, ERR4783172, ERR4783228, ERR4783208, ERR6938084, ERR6938111, ERR6938087, ERR6938090, ERR6938114, ERR6938093, ERR6938096, ERR6938099, ERR6938061, ERR4783267, and ERR4783236.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex, Ife, Nigeria (protocol number: ERC/2017/06/13). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EEO, EOI, INO, and AOAb conceptualized the study. EEO, EOI, AOO, AOAf, AU, SA, INO, and AOAb designed the analytical methods. EEO, EOI, TOO, AOO, ATA, and VOO contributed to the data collection and processing. EEO, EOI, and AOAf analyzed and interpreted the data. AOAf, AU, SA, AD, DMA, INO, and AOAb supervised the study. AD, DMA, INO, AOAb, EOI, TOO, ATA, and VOO provided resources or materials for the research. EEO drafted the manuscript. AD, AU, and INO critically reviewed the manuscript. All authors have read and approved the final version of the manuscript.

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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.2022.846051/full#supplementary-material

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A Data-Driven Framework for Identifying Intensive Care Unit Admissions Colonized With Multidrug-Resistant Organisms

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Background: The rising prevalence of multi-drug resistant organisms (MDROs), such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococci* (VRE), and Carbapenem-resistant *Enterobacteriaceae* (CRE), is an increasing concern in healthcare settings.

Materials and Methods: Leveraging data from electronic healthcare records and a unique MDRO universal screening program, we developed a data-driven modeling framework to predict MRSA, VRE, and CRE colonization upon intensive care unit (ICU) admission, and identified the associated socio-demographic and clinical factors using logistic regression (LR), random forest (RF), and XGBoost algorithms. We performed threshold optimization for converting predicted probabilities into binary predictions and identified the cut-off maximizing the sum of sensitivity and specificity.

Results: Four thousand six hundred seventy ICU admissions (3,958 patients) were examined. MDRO colonization rate was 17.59% (13.03% VRE, 1.45% CRE, and 7.47% MRSA). Our study achieved the following sensitivity and specificity values with the best performing models, respectively: 80% and 66% for VRE with LR, 73% and 77% for CRE with XGBoost, 76% and 59% for MRSA with RF, and 82% and 83% for MDRO (i.e., VRE or CRE or MRSA) with RF. Further, we identified several predictors of MDRO colonization, including long-term care facility stay, current diagnosis of skin/subcutaneous tissue or infectious/parasitic disease, and recent isolation precaution procedures before ICU admission.

Conclusion: Our data-driven modeling framework can be used as a clinical decision support tool for timely predictions, characterization and identification of high-risk patients, and selective and timely use of infection control measures in ICUs.

Keywords: multidrug-resistant organisms (MDROs), carbapenem-resistant *Enterobacteriaceae* (CRE), vancomycin-resistant enterococci (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA), healthcare-associated infections (HAIs), machine learning (ML), data-centric analytics, predictive analytics

INTRODUCTION

The increasing prevalence of multidrug resistant organisms (MDROs), bacteria that are resistant to one or more classes of antibiotics, is an increasingly concerning issue in the community, and in particular, in healthcare settings, where admitted patients are especially susceptible to developing an infection (1–3). These organisms (also known as multidrug-resistant bacteria) pose a significant threat to patient safety in the form of healthcare-associated (i.e., nosocomial) infections (HAIs) (4), which are associated with considerable morbidity, mortality, and healthcare costs (5), and have the potential to spread within the community (6, 7).

Two MDROs that are the most prevalent causes of HAIs are Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) (8, 9), which are currently classified as serious threats by the U.S. Centers for Disease Control and Prevention (CDC) (10). MRSA is reported to cause an estimate of 80,461 infections and 11,285 deaths per year, and VRE is estimated to cause 20,000 infections and 11,300 deaths per year (1), with both MDROs being associated with poor treatment outcomes following infections (11, 12), longer length of hospitalization, and higher healthcare costs (13–15).

In recent years, Carbapenem-resistant *Enterobacteriaceae* (CRE), an MDRO class that is highly resistant to carbapenems and other antibiotics reserved for treatment of severe infections, have reached concerning levels in healthcare facilities in the U.S. (16), and around the world (17). This trend has prompted the CDC to classify CRE as an urgent threat to public health, its highest risk category (1). CRE is currently less prevalent than MRSA and VRE, estimated to cause 9,000 infections and 600 deaths per year (1), but is an immediate public health threat because infections caused by CRE (e.g., pneumonia, urinary tract infections, bloodstream infections and wound infections) are very difficult to treat (18, 19) and have been associated with poor treatment outcomes (20–23), and high costs (24).

Besides the high morbidity and mortality rates, multidrug-resistant pathogens can also place a heavy economic burden on individual healthcare facilities, as well as on the entire U.S. healthcare system. Among other factors, MDRO-related costs are increased due to prolonged hospital stay, additional treatments, post-discharge complications, and implemented infection control measures including the set-up of isolation wards and cleaning or replacement of contaminated materials (25). In particular, earlier studies reported average additional hospital costs attributable to each VRE infection as high as $77,558, whereas the lower bound estimate was around $10,000 (in 2003) (14, 26). Despite its lower prevalence, a single CRE infection was also estimated to be costly for hospitals ($22,484–$66,031), and third-party payers ($10,440–$31,621). Further, including out-of-pocket costs and labor and productivity losses, CRE was estimated to cost society $37,778–$83,512 per infection (24). Finally, averaging around $60,000–$70,000 per infected patient, total healthcare spending for MRSA was estimated to be around $10 billion per year in the U.S. (27). These estimates not only show the heavy financial burden of MDROs at an individual and a population level, but also demonstrate the value of prevention, early detection, and early intervention. If MDRO colonization are detected and intervened upon before they harm patients and drive up costs, then the valuable resources spent for MDRO treatments (28) could be allocated to other pressing public health problems for the greater good of the U.S. society.

Colonized patients carry an MDRO at a detectable level, meaning that a cultured swab sample would test positive, but the patient would not show clinical indications (i.e., signs or symptoms) of illness caused by an MDRO. Harboring MDROs, these patients are at a risk for subsequent infection, as a significant fraction of MDRO colonization will eventually cause clinically apparent infections that are difficult and costly to treat (28–30). They also pose a threat to other patients, as healthcare workers who interact with these patients can become contaminated with the organism and transmit it to other patients. As a result, it is important to rapidly identify and then monitor colonized patients to reduce the risk of disease transmission and subsequent infections (31).

The importation of MDROs into hospitals and other healthcare settings is a major determinant for (the rate and magnitude of) transmission and outbreak (32–34). Among hospital departments, intensive care units (ICUs) are the wards where the prevalence of MDROs has reported to be higher (35, 36). Further, patients admitted to the ICUs are more vulnerable to develop infections from these organisms (37, 38). Accordingly, ICUs have become a central point of focus for the control and prevention of MDRO colonization and infection within hospitals (39).

A variety of interventions have been proposed and implemented in order to prevent the transmission of MDROs in ICUs. Effective and commonly utilized interventions include (i) hand hygiene, especially when healthcare workers contact colonized or infected patients (40), (ii) contact precautions (e.g., wearing gloves and gowns) when caring for colonized or infected patients (41), and (iii) isolation or cohorting of colonized or infected patients (42). Despite their effectiveness, however, these preventive measures are often not applied in a timely manner due to imperfect compliance and the delay (or even failure) to detect patients colonized with an MDRO (9).

Surveillance for MDRO colonization is an instrumental practice for detecting patients who may require an intervention (43, 44). Yet, the implementation and cost-effectiveness of universal (i.e., active) surveillance and testing strategies, such as screening of all newly admitted ICU patients, has been a controversial topic (45). Some critics argue that the costs associated with universal screening, including the opportunity costs of the human and physical resources being utilized, are likely to outweigh the benefits of active surveillance (46). Accordingly, universal surveillance of all patients may not be feasible to implement in many healthcare facilities due to resource constraints (47–49). Instead, targeted surveillance strategies, which offer a cost-effective compromise for detecting asymptomatic colonization, have been advocated by national guidelines (50–52) when a sufficiently accurate method for identifying high-risk individuals is available. Accordingly, rapid and accurate identification of patients who are at high risk for MDRO colonization is critical for timely and targeted

implementation of screening protocols and other preventive measures, as well as administration of appropriate treatments (e.g., avoiding the misuse of antibiotics).

Given the aforementioned challenges, a system that facilitates timely and reliable identification of newly admitted patients who are likely to be colonized with an MDRO would be quite useful to improve patient safety and effective utilization of critical hospital resources (53). By accurately identifying significant risk factors, this system can help define high-risk subpopulations and hence, could enable the implementation of a cost-effective targeted screening program. Moreover, if highly predictive, it can further be used to immediately initiate clinical interventions, such as contact precautions, as soon as a high-risk individual is admitted to the ICU. Such a real-time system would be particularly useful in ICUs because, currently, identification of colonized patients relies on costly and labor intensive clinical laboratory results that usually require at least 1–2 days to process and hence, delay subsequent necessary actions to prevent and control the spread of MDROs.

A particular challenge for the design of a reliable prediction framework is the class imbalance problem that is commonly observed in clinical datasets. Clinical datasets are often not balanced in their class labels, where the predictors and/or prediction outcomes do not make up an equal portion of the data. The imbalance can be particularly large when the prediction outcomes are MDROs, as their prevalence is usually $< 15\%$ and can be as low as $< 2\%$ as observed in our data. Given that ignoring the class imbalance, especially when it is large, yields poor predictions, it is necessary to consider and address this challenge up front while developing a prediction framework for accurate and reliable results.

In this study, we developed a data-driven framework to identify patients who are likely to be colonized with VRE, CRE, or MRSA upon ICU admission, leveraging 2 years of electronic health record (EHR) data from a large academic medical center. The objective of our study was to develop a modeling framework that can cope with significant class imbalance, commonly observed in clinical datasets, and can be used (1) to generate timely and accurate predictions for newly admitted ICU patients, and (2) to identify the key socio-demographic and clinical factors affecting the incidence of MDRO colonization. The developed framework relied on three supervised machine learning algorithms (namely, regularized logistic regression, random forest, and XGBoost), which were trained on the EHR data to make timely and accurate predictions for the patients newly admitted to the ICU.

Our study achieved the following results for the primary MDRO colonization outcomes: 80% sensitivity and 66% specificity for VRE, 73% and 77% for CRE, 76% and 59% for MRSA, and 82% and 83% for colonization with any MDRO (i.e., VRE, CRE, or MRSA). Moreover, our modeling approach identified long-term care facility stay, current diagnosis of skin/subcutaneous tissue conditions or infectious/parasitic disease, and recent isolation precaution procedures before ICU admission as key predictors. The proposed modeling framework was able to detect over 80% of positive MDRO cases upon ICU admission with less than a 20% false-positive rate, which would enable timely and targeted implementation of preventive measures for infection control in ICUs.

Currently most hospitals lack (or choose not implement) universal screening programs for MDROs. The practical utility and impact of this study was to translate EHR data into insights and real-time predictions to effectively guide VRE, CRE, and MRSA-related infection control decisions in ICUs. The means to achieve this impact was to build a robust predictive analytics framework that produces reliable and evidence-based predictions with high sensitivity, ensuring timely detection of MDRO colonization, and high specificity, preventing inefficient use of limited resources. This was the primary objective of our study. Once thoroughly and externally validated, this modeling framework would allow hospitals to implement a clinical decision support system that could serve as a cost-effective universal MDRO screening tool at ICU admission without using any hospital resources except for EHR data.

The remainder of this article is organized as follows: In Section Materials and Methods, we present our data and describe our methodology. In particular, in Section Data Description, we introduce our data and describe the clinical and socio-demographic predictors included in our models. Then, in Section Prediction Models, Model Training and Validation, and Threshold Optimization, we introduce the predictive models and describe the techniques we utilize to improve prediction accuracy and address class imbalance. In Section Results, we present our prediction results and report the key predictors for MDRO colonization in our data set. In Section Discussions, we summarize our results, and discuss the policy implications of our approach and findings. Finally, in Section Conclusion and Future Work, we propose directions for future research, and conclude our study.

MATERIALS AND METHODS

In this section, we first describe our data source, in Section Data Description, and present the variables and prediction outcomes in our dataset. Then, in Section Prediction Models, Model Training and Validation, and Threshold Optimization, we introduce our modeling framework and describe our methods. In particular, first, we introduce the prediction models we used, and then, discuss our model specification (training) and performance evaluation (testing) stages, describing how we performed hyperparameter tuning, stratified cross-validation, threshold optimization, and finally, out-of-sample evaluations.

Data Description

In this study, we used electronic healthcare record (EHR) data from the University of Maryland Medical Center (UMMC), an academic teaching hospital located in Baltimore, Maryland. Our dataset contained records for 3,958 patients admitted to a surgical or medical ICU in 2017 or 2018. In total, we observed 4,670 individual admissions. Our dataset included the following variables: (1) hospital admission source and type, (2) age, (3) sex, (4) race and ethnicity, (5) region/state of residency, (6) total time of prior ICU stays and hospital inpatient stays within the previous year, (7) prior antibiotic prescriptions,

(8) diagnoses for prior hospital and/or ICU stays within the previous year, (9) diagnoses for current hospital stay before ICU admission, (10) surgical and medical procedures conducted during prior hospital and/or ICU stays within the previous year, and (11) recent procedures conducted for current hospital stay prior to ICU admission. We treated all predictors utilized in the models as categorical. Descriptive statistics regarding these variables and their categories can be found in the **Supplementary Material (Appendix A)**.

The prediction outcomes were colonization with VRE, CRE, or MRSA upon ICU admission, both separately and as an aggregate (union) outcome. Conducting active surveillance in the ICUs, UMMC screened newly admitted patients for colonization upon admission and periodically during their stay. At UMMC, active surveillance involves taking routine peri-rectal cultures for VRE and nasal cultures for MRSA on all patients admitted to an ICU at the time of admission, weekly, and upon discharge. CRE detection was also primarily done *via* perirectal swabs and also included clinical cultures (e.g., blood, urine, wound cultures). We identified the positive (i.e., colonized) and negative (i.e., uncolonized) results based on the laboratory tests conducted within 2 days (i.e., both before and after) of ICU admissions. We limited the time window for the screening results within 2 days (54, 55) in an attempt to avoid inclusion of acquisition cases, for which initially susceptible (i.e., colonization-free) patients acquire an MDRO during their ICU stay. Screening outcomes were not available for all 4,670 ICU admissions. The total number of screening results available was 3,860 for VRE, 3,661 for CRE, 4,446 for MRSA, and 4,503 for MDRO. In the dataset, 503 (13.03%) of ICU admissions tested positive for VRE, 53 (1.45%) for CRE, 332 (7.47%) for MRSA, and 792 (17.59%) for any one of these MDROs.

In the UMMC dataset, all prior and current diagnoses were coded using the International Statistical Classification of Diseases and Related Health Problems (ICD)-10 codification. We used the Agency for Healthcare Research and Quality's Clinical Classifications Software (CCS) to further categorize the prior and current diagnoses that were present on admission (PoA). The CCS is a diagnosis and procedure categorization catalog (*https://www.hcup-us.ahrq.gov/toolssoftware/ccs10/ccs10.jsp*), mapping the ICD-10 diagnosis codes into 18 categories: (1) Infectious and parasitic diseases, (2) Neoplasms, (3) Endocrine, nutritional, and metabolic diseases and immunity disorders, (4) Diseases of the blood and blood-forming organs, (5) Mental illness, (6) Diseases of the nervous system and sense organs, (7) Diseases of the circulatory system, (8) Diseases of the respiratory system, (9) Diseases of the digestive system, (10) Diseases of the genitourinary system, (11) Complications of pregnancy, childbirth, and the puerperium, (12) Diseases of the skin and subcutaneous tissue, (13) Diseases of the musculoskeletal system and connective tissue, (14) Congenital anomalies, (15) Certain conditions originating in the perinatal period, (16) Injury and poisoning, (17) Symptoms, signs, and ill-defined conditions and factors influencing health status, and (18) Residual or unclassified codes.

We labeled a procedure as recent if it was performed during the current hospital stay. We recorded all recent procedures performed in the hospital inpatient settings prior to the current ICU admission with respect to the ICD-10 Procedure Coding System (PCS), for which each character has a categorical indication. Using the first character of the ICD-10 PCS codes, we classify the recent procedures into eight categories as follows: (i) Medical and Surgical ("0"), (ii) Placement ("2"), (iii) Administration ("3"), (iv) Measurement and Monitoring ("4"), (v) Extracorporeal or Systemic Procedures ("5" and "6"), (vi) Other Procedures ("8"), (vii) Imaging ("B"), and (viii) Other/Miscellaneous ("1", "7", "9", "C", "D", "F", "G", and "X"). Further, using the first two characters of the ICD-10 PCS codes, we also map the recent procedures into 44 categories (see **Supplementary Material Appendix A**). In our analysis, we include both the single- and double-character based categorizations so that our algorithms can learn which specifications are more important for predicting our MDRO outcomes. We classified prior hospital procedures having the ICD-10 PCS codes in a similar manner as the recent procedures.

Prior outpatient procedures were recorded using the Current Procedural Terminology (CPT) system (*https://www.ama-assn.org/amaone/cpt-current-procedural-terminology)*, which we classified into 6 categories: (i) Evaluation and Management, (ii) Anesthesia (iii) Medicine (iv) Radiology (v) Pathology and Laboratory, and (vi) Surgery. The CPT codes for surgery include 18 sub-types, enabling us to construct a more detailed categorization with 23 classes. We used both the 6-class and 23-class CPT codes as predictors for our descriptive and predictive analyses.

Prediction Models, Model Training and Validation, and Threshold Optimization

A variety of techniques have been utilized to analyze complex disease dynamics and quantify its parameters (e.g., the estimation of transmission rate), identify risk factors, and assess the impact of infection control strategies (56). These approaches include prediction modeling, computational simulation, and analytic-formula based models such as decision trees (57), artificial neural network (58), agent-based simulation for a hospital ward (59, 60) or healthcare system (61), dynamic patient and healthcare worker networks (62–64), compartmental systems dynamics models (based on ordinary differential equations) (65, 66), (approximate) Bayesian (computation) techniques (67), and Markov chain based approaches (68, 69). Among these techniques, data-driven prediction models, such as the ones we used in this study, are particularly valuable tools for generating real-time predictions, identifying the significant risk factors, and quantifying their impact on the outcomes of interest (70). In addition to these modeling-based approaches, there is also rich clinical literature studying MDRO colonization. See the **Supplementary Material (Appendix B)** for a summary of the clinical studies that assessed the risk factors associated with MDRO colonization, and developed simple clinical prediction rules based on the identified predictors.

We utilized three supervised machine learning (ML) algorithms to predict colonized patients upon ICU admission and to identify significant clinical and socio-demographic factors

Let $X \in \{0,1\}^n$ be the vector of predictions for n data points, $P \in [0,1]^n$ be the vector of predicted probabilities, and $Y \in \{0,1\}^n$ be the vector of true values of the prediction outcome. Let $\tau \in [0,1]$ be a threshold value converting (i.e., mapping) P to X such that $X_i = 0$ if $P_i \leq \tau$ and $X_i = 1$ when $P_i > \tau$ for every data point $i = 1,2,\ldots,n$. Then, the threshold optimization problem that identifies the optimal threshold value τ^* for maximizing Youden's index can be formulated as follows:

$$\underset{\tau \in [0,1]}{\text{maximize}} \quad \text{sensitivity}(X,Y) + \text{specificity}(X,Y) - 1$$

such that

$$P_i - \tau < X_i \qquad i = 1,\ldots,n$$
$$\tau - P_i > X_i \qquad i = 1,\ldots,n$$
$$P \in [0,1]^n \qquad X \in \{0,1\}^n \qquad Y \in \{0,1\}^n$$

FIGURE 1 | Threshold optimization formulation.

TABLE 1 | Model parameters for best performing logistic regression models.

Best models-logistic regression				
Parameter	**VRE**	**CRE**	**MRSA**	**MDRO**
Cs	100	100	100	100
class_weight	None	None	None	None
cv=StratifiedKFold	n_splits=10	n_splits=10	n_splits=10	n_splits=10
dual	False	False	False	False
fit_intercept	True	True	True	True
intercept_scaling	1	1	1	1
max_iter	100	100	100	100
multi_class	'ovr'	'ovr'	'ovr'	'ovr'
n_jobs	1	1	1	1
penalty	L1	L1	L1	L1
random_state	None	None	None	None
refit	True	True	True	True
scoring	roc_auc	roc_auc	roc_auc	roc_auc
solver	liblinear	liblinear	liblinear	liblinear
tol	0.0001	0.0001	0.0001	0.0001
verbose	0	0	0	0
Threshold Bound	0.15	0.025	0.20	0.50

associated with the outcomes of interest: (1) logistic regression (LR) (71, 72), (2) random forest (RF) (73), and (3) extreme gradient boosting (XGBoost) (74). To perform regularization and feature selection for our logistic regression models, we used least absolute shrinkage and selection operator (LASSO), which was originally developed for linear regression (75) and then applied to other algorithms including LR (76).

For each model, we split the data into an 80% subset for model training and cross-validation and a 20% subset for out-of-sample evaluation. We used a 10-fold stratified cross-validation scheme both for hyperparameters tuning for the algorithms and threshold optimization for the conversion of predicted colonization risks into binary predictions (see **Figure 1**). We selected the 10-fold due to the relatively small sample size of our data, in an effort to preserve as much data as possible for model development and training. We selected the stratified scheme to account for the class imbalance in our data, which preserves a proportion of the positive outcome for each fold similar to the complete dataset.

We defined a grid search for a core set of hyperparameters for each algorithm, and used the area under the receiver operating characteristic curve (AUC) as the objective function to maximize (out-of-sample) model performance. We selected the hyperparameters achieving the highest mean AUC across the 10 folds for model training. In particular, the hyperparameters were optimized and fine-tuned by the function "*LogisticRegressionCV*" for LR, and "*GridSearchCV*" for RF and XGBoost. For each machine learning algorithm, we summarize the hyperparameters and model parameters corresponding to the best performing machine learning models of our study in **Tables 1–3**. The programming code samples of the supervised ML algorithms utilized in this study are also provided in the **Supplementary Material (Appendix D)**.

After choosing the hyperparameters, the next step of the model specification was to identify the ideal cut-off (i.e., optimal threshold) value for converting predicting probabilities into binary predictions. As an initial output, the ML algorithms generate predicted probabilities for the training instances, indicating how likely each patient to be colonized with an MDRO. These predicted probabilities are then translated into binary prediction outcomes using a threshold value. Specifically, observations for which the predicted probabilities are greater than this threshold, denoted as τ, are classified as positive (i.e., colonized), and otherwise, the patient is assigned to the negative (i.e., susceptible) class. Given the class imbalance observed in our dataset, the default threshold value of 0.5 was unlikely to be effective for our study (see **Figure 2**). Consequently, we performed an optimization (77) to search for the best threshold that classifies the predicted probabilities while maximizing the Youden Index (i.e., sensitivity + specificity - 1) for out-of-sample predictions (78).

We performed the threshold optimization using the same 10-fold stratified cross validation scheme used for the hyperparameter tuning. The optimal threshold was determined for each fold using the in-sample predicted probabilities from the 90% subset of training data. Then, we evaluated the performance (i.e., Youden's index) of this threshold over the 10% subset. We repeated this process for each fold, and selected the mean of these 10 optimal thresholds as the final cut-off value. We used a bounded numerical search algorithm to solve the optimization problem (79), using a lower bound of zero and varying the upper bound for each algorithm to ensure an effective threshold is found. It is noteworthy to emphasize that the upper bound values we considered for each specific outcome were different because

TABLE 2 | Model parameters for best performing random forest models.

Best models-random forest				
Parameter	**VRE**	**CRE**	**MRSA**	**MDRO**
cv=StratifiedKFold	n_splits=10	n_splits=10	n_splits=10	n_splits=10
estimator=RandomForestClassifier	Yes	Yes	Yes	Yes
bootstrap	True	True	True	True
max_depth	None	None	None	None
max_leaf_nodes	None	None	None	None
min_impurity_decrease	0	0	0	0
init_min_samples_leaf	1	1	1	1
init_min_samples_split	2	2	2	2
n_estimators	200	200	200	200
n_jobs	4	4	4	4
param_grid={'min_samples_leaf'}	[5, 10,..., 250]	[5, 10,..., 250]	[5, 10,..., 250]	[5, 10,..., 250]
param_grid={pre_dispatch}	2*n_jobs	2*n_jobs	2*n_jobs	2*n_jobs
param_grid={scoring}	roc_auc	roc_auc	roc_auc	roc_auc
optimal_min_samples_leaf	5	30	10	5
Threshold Bound	0.20	0.05	0.30	0.40

TABLE 3 | Model parameters for best performing XGBoost models.

Best models-XGBoost				
Parameter	**VRE**	**CRE**	**MRSA**	**MDRO**
colsample_bytree	0.8	0.8	0.8	0.8
gamma	0	0	0	0
learning_rate	0.05	0.05	0.05	0.05
max_depth	5	5	5	5
min_child_weight	1	1	1	1
n_estimators	200	200	200	200
nthread	4	4	4	4
objective	binary:logistic	binary:logistic	binary:logistic	binary:logistic
seed	1337	1337	1337	1337
subsample	0.8	0.8	0.8	0.8
Threshold Bound	0.15	0.015	0.10	0.30

the prevalence of the colonized (i.e., positive) instances among VRE, CRE, MRSA, and MDRO were different, which directly affected the outcome of the threshold optimization procedure.

Model specification was completed when we determined the hyperparameters, chose the threshold value (for each model), and re-trained the models on the full (80%) training set. Next, we evaluated the (out-of-sample) performance of the trained models on the (20%) test sets, reporting the AUC, sensitivity, and specificity values obtained. For each MDRO, we conducted a systematic numerical experiment with a range of upper bound values for threshold optimization, and obtained predictions with varying sensitivity and specificity values for VRE, CRE, MRSA, and MDRO (the aggregate prediction outcome). We provide these results in Section Discussions for each outcome (e.g., VRE) and algorithm (e.g., XGBoost), and separately, discuss the best performing models for each MDRO.

We also used our modeling framework to identify the key socio-demographic and clinical factors for predicting colonization with VRE, CRE, and MRSA separately and in aggregate. For the LR models, we used odds ratios (ORs), which quantify the associated increase (for values >1) or decrease (for values <1) in the likelihood of colonization. For the tree-based models (i.e., RF and XGBoost), we used feature importance (FI), which quantifies the relative frequency that each factor is used to construct the ensemble. Using these two metrics (i.e., OR and FI), we ordered the identified predictors for each MDRO and report the top five key predictors that are highly ranked across all of the best performing ML models, calculated by the average ranking across the best models.

RESULTS

In a total of 4,670 ICU admissions corresponding to 3,958 patients examined, the rate of colonization was 17.59% for MDRO (13.03% VRE, 1.45% CRE, and 7.47% MRSA). This study separately predicted VRE, CRE, and MRSA colonization upon ICU admission. In addition, combining these three antibiotic-resistant bacteria, the models we developed also predicted colonization with any of these MDROs (i.e., VRE, CRE, or MRSA) upon ICU admission without specifying the particular organism. As a result, our modeling framework generated separate predictions for four cases (namely, VRE, CRE, MRSA, and MDRO) using logistic regression (with LASSO regularization), random forest, and XGBoost algorithms. In **Table 4**, we summarize the model results for these four outcomes under different upper bound values corresponding to the threshold optimization process.

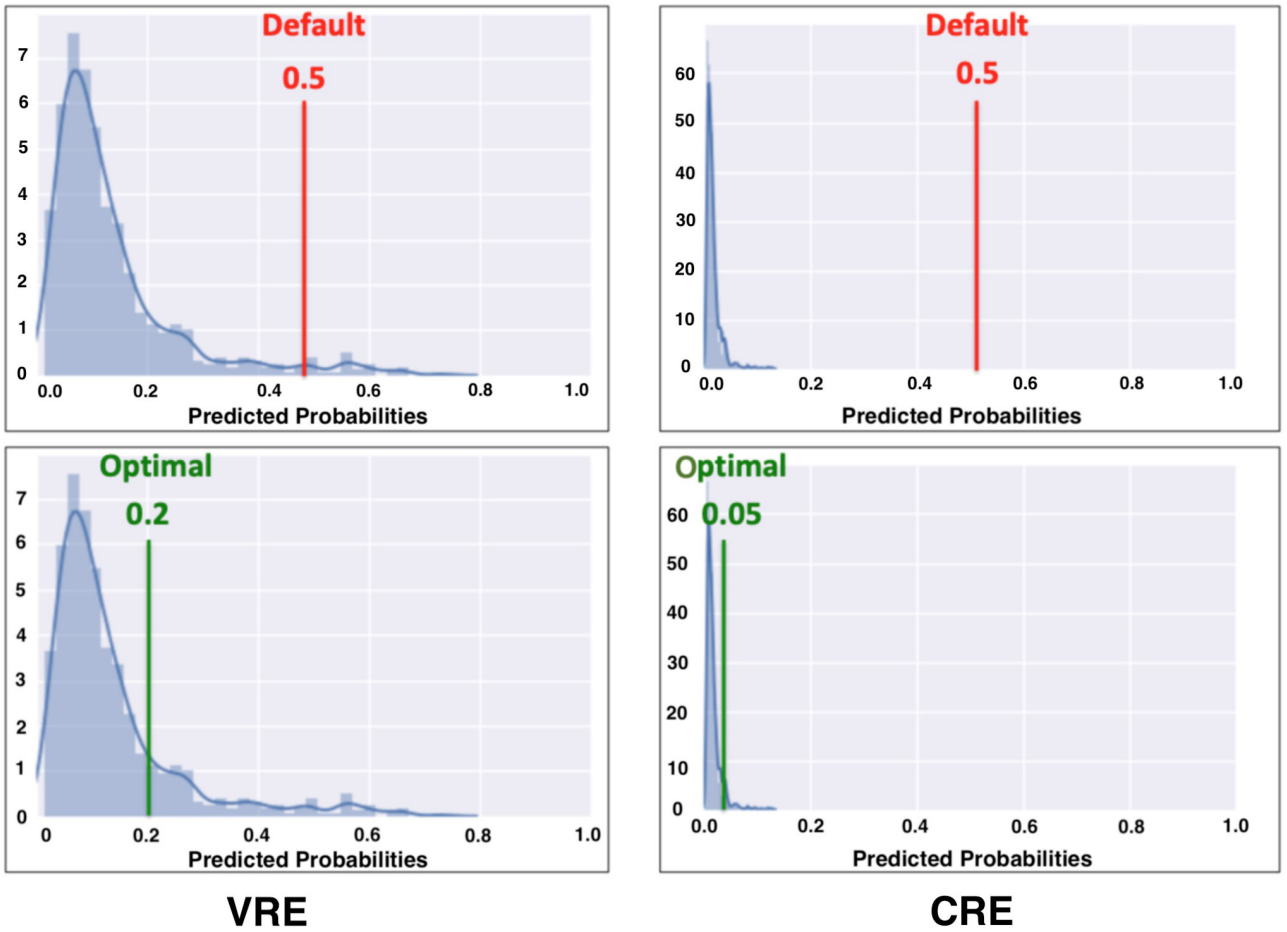

FIGURE 2 | Threshold value for converting predicted probabilities to binary predictions.

After considering all of the models that we trained for each outcome, we selected the ones with the highest (out-of-sample) Youden index, which we summarize in **Table 5**. For VRE, the best performing model generated a Youden index of 0.46, achieved *via* the LR model. By comparison, the RF and XGBoost models generated Youden index values of 0.41 and 0.39, respectively. For CRE, the XGBoost algorithm generate the highest Youden index (0.50), followed by LR (0.45) and RF (0.42). The performance for MRSA was noticeably lower than the other outcomes, for which RF achieved the highest Youden index (0.34). Finally, the prediction models for the aggregate MDRO outcome produced the highest Youden index values when compared to the individual MDRO outcomes, with the RF model (0.65) outperforming the XGBoost (0.57) and LR models (0.30). We note here that the tree-based models performed significantly better than the linear LR model for this aggregated outcome, which was likely due to the former's natural ability to capture nonlinear and complex interactions. In an effort to provide support for this hypothesis, we also tested the performance of a single classification tree (80) (0.54), which also performed significantly better than the LR model for this particular outcome. On the other hand, for separate VRE, CRE, and MRSA predictions, the single tree models were always dominated by (at least one of) the other algorithms, and hence, not presented in **Table 4**.

For each model presented in **Table 5**, the difference between the (out-of-sample) AUC for the (cross-validated) training and testing sets were typically small, suggesting well-trained models without significant overfitting. The LR and RF models for CRE demonstrated larger gaps, suggesting that these models might be slightly less robust than others; however, this volatility is likely explained by the extremely low prevalence of positive cases on which to train the models. The best predictions for VRE colonization upon ICU admission were generated by the LR model, which achieved 80% sensitivity and 66% specificity. For CRE, XGBoost produced the best model, having 73% sensitivity and 77% specificity. For MRSA, the RF model performed best, yielding 76% sensitivity and 59% specificity. Finally, the most effective model for the aggregate MDRO outcome was a random forest model, which

TABLE 4 | Performance summary of the machine learning models for VRE, CRE, MRSA, and MDRO colonization predictions.

Threshold opt. upper bound = 0.05	**VRE (503/3860 = 13.03%)**			**Threshold opt. upper bound = 0.010**	**CRE (53/3661 = 1.45%)**			**Threshold opt. upper bound = 0.03**	**MRSA (332/4446 = 7.47%)**			**Threshold opt. upper bound = 0.1**	**MDRO (792/4503 = 17.59%)**			
	Log. Reg.	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**	**Dec. Tree**
Training AUC	0.76	0.77	0.77	Training AUC	0.70	0.76	0.78	Training AUC	0.65	0.66	0.66	Training AUC	0.72	0.86	0.88	0.75
Testing AUC	0.80	0.77	0.77	Testing AUC	0.78	0.72	0.71	Testing AUC	0.66	0.66	0.69	Testing AUC	0.70	0.87	0.89	0.76
Testing sensitivity	0.99	0.97	1.00	Testing sensitivity	1.00	0.73	0.82	Testing sensitivity	1.00	0.88	1.00	Testing sensitivity	0.92	0.97	1.00	0.93
Testing specificity	0.09	0.33	0.15	Testing specificity	0.31	0.68	0.37	Testing specificity	0.02	0.22	0.00	Testing specificity	0.30	0.39	0.21	0.51
Threshold opt. upper bound = 0.1	**VRE (503/3860 = 13.03%)**			**Threshold opt. upper bound = 0.015**	**CRE (53/3661 = 1.45%)**			**Threshold opt. upper bound = 0.075**	**MRSA (332/4446 = 7.47%)**			**Threshold opt. upper bound = 0.2**	**MDRO (792/4503 = 17.59%)**			
	Log. Reg.	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**	**Dec. Tree**
Training AUC	0.76	0.77	0.76	Training AUC	0.70	0.76	0.80	Training AUC	0.65	0.66	0.67	Training AUC	0.72	0.86	0.88	0.76
Testing AUC	0.80	0.77	0.77	Testing AUC	0.78	0.72	0.71	Testing AUC	0.66	0.66	0.71	Testing AUC	0.70	0.87	0.89	0.81
Testing sensitivity	0.89	0.79	0.88	Testing sensitivity	0.82	0.73	0.73	Testing sensitivity	0.76	0.71	0.82	Testing sensitivity	0.68	0.82	0.90	0.93
Testing specificity	0.49	0.57	0.48	Testing specificity	0.57	0.77	0.51	Testing specificity	0.45	0.53	0.45	Testing specificity	0.59	0.74	0.69	0.58
Threshold opt. upper bound = 0.15	**VRE (503/3860 = 13.03%)**			**Threshold opt. upper bound = 0.020**	**CRE (53/3661 = 1.45%)**			**Threshold opt. upper bound = 0.1**	**MRSA (332/4446 = 7.47%)**			**Threshold opt. upper bound = 0.3**	**MDRO (792/4503 = 17.59%)**			
	Log. Reg.	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**	**Dec. Tree**
Training AUC	0.76	0.77	0.76	Training AUC	0.70	0.76	0.80	Training AUC	0.65	0.66	0.66	Training AUC	0.72	0.86	0.87	0.76
Testing AUC	0.80	0.77	0.78	Testing AUC	0.78	0.72	0.73	Testing AUC	0.66	0.66	0.68	Testing AUC	0.70	0.87	0.89	0.81
Testing sensitivity	0.80	0.73	0.78	Testing sensitivity	0.73	0.55	0.73	Testing sensitivity	0.64	0.67	0.73	Testing sensitivity	0.65	0.75	0.85	0.89
Testing specificity	0.66	0.65	0.59	Testing specificity	0.69	0.83	0.63	Testing specificity	0.60	0.60	0.57	Testing specificity	0.63	0.82	0.79	0.65
Threshold opt. upper bound = 0.2	**VRE (503/3860 = 13.03%)**			**Threshold opt. upper bound = 0.025**	**CRE (53/3661 = 1.45%)**			**Threshold opt. upper bound = 0.15**	**MRSA (332/4446 = 7.47%)**			**Threshold opt. upper bound = 0.4**	**MDRO (792/4503 = 17.59%)**			
	Log. Reg.	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**	**Dec. Tree**
Training AUC	0.76	0.77	0.77	Training AUC	0.70	0.76	0.79	Training AUC	0.65	0.66	0.67	Training AUC	0.72	0.86	0.87	0.76
Testing AUC	0.80	0.77	0.77	Testing AUC	0.78	0.72	0.71	Testing AUC	0.66	0.66	0.69	Testing AUC	0.70	0.87	0.89	0.79
Testing sensitivity	0.66	0.63	0.75	Testing sensitivity	0.73	0.36	0.64	Testing sensitivity	0.48	0.56	0.71	Testing sensitivity	0.57	0.69	0.82	0.79
Testing specificity	0.76	0.75	0.66	Testing specificity	0.73	0.89	0.67	Testing specificity	0.75	0.71	0.58	Testing specificity	0.73	0.86	0.83	0.64
Threshold opt. upper bound = 0.3	**VRE (503/3860 = 13.03%)**			**Threshold opt. upper bound = 0.030**	**CRE (53/3661 = 1.45%)**			**Threshold opt. upper bound = 0.2**	**MRSA (332/4446 = 7.47%)**			**Threshold opt. upper bound = 0.5**	**MDRO (792/4503 = 17.59%)**			
	Log. Reg.	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**	**Dec. Tree**
Training AUC	0.76	0.77	0.77	Training AUC	0.70	0.76	0.79	Training AUC	0.65	0.66	0.66	Training AUC	0.72	0.86	0.88	0.76
Testing AUC	0.80	0.77	0.77	Testing AUC	0.78	0.72	0.71	Testing AUC	0.66	0.66	0.69	Testing AUC	0.70	0.87	0.89	0.80
Testing sensitivity	0.66	0.65	0.61	Testing sensitivity	0.64	0.36	0.64	Testing sensitivity	0.70	0.45	0.67	Testing sensitivity	0.56	0.70	0.85	0.77
Testing specificity	0.78	0.72	0.74	Testing specificity	0.78	0.91	0.74	Testing specificity	0.55	0.78	0.59	Testing specificity	0.75	0.84	0.79	0.70
Threshold opt. upper bound = 0.5	**VRE (503/3860 = 13.03%)**			**Threshold opt. upper bound = 0.050**	**CRE (53/3661 = 1.45%)**			**Threshold opt. upper bound = 0.3**	**MRSA (332/4446 = 7.47%)**			**Threshold opt. upper bound = 0.6**	**MDRO (792/4503 = 17.59%)**			
	Log. Reg.	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**	**Dec. Tree**
Training AUC	0.76	0.77	0.77	Training AUC	0.70	0.76	0.79	Training AUC	0.65	0.65	0.66	Training AUC	0.72	0.86	0.88	0.77
Testing AUC	0.80	0.77	0.78	Testing AUC	0.78	0.72	0.72	Testing AUC	0.66	0.66	0.70	Testing AUC	0.70	0.87	0.89	0.80
Testing sensitivity	0.61	0.59	0.63	Testing sensitivity	0.55	0.27	0.64	Testing sensitivity	0.48	0.24	0.76	Testing sensitivity	0.58	0.70	0.84	0.89
Testing specificity	0.82	0.77	0.74	Testing specificity	0.82	0.94	0.79	Testing specificity	0.75	0.89	0.59	Testing specificity	0.72	0.85	0.79	0.62

TABLE 5 | Performance summary of the supervised machine learning models with the highest Youden's index.

Models with the best Youden index	**VRE (503/3860 = 13.03%)**			**Models with the best Youden index**	**MRSA (332/4446 = 7.47%)**		
	Log. Reg.	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**
Training AUC	0.76	0.77	0.77	Training AUC	0.65	0.66	0.66
Testing AUC	0.80	0.77	0.77	Testing AUC	0.66	0.66	0.70
Testing sensitivity	0.80	0.73	0.75	Testing sensitivity	0.70	0.67	0.76
Testing specificity	0.66	0.65	0.66	Testing specificity	0.55	0.60	0.59
Youden index	0.46	0.39	0.41	Youden index	0.24	0.27	0.34
Threshold opt. bound	0.15	0.15	0.20	Threshold opt. bound	0.20	0.10	0.30
Models with the best Youden index	**CRE (53/3661 = 1.45%)**			**Models with the best Youden index**	**MDRO (792/4503 = 17.59%)**		
	Log. Reg.	**XGBoost**	**Rand. Forest**		**Log. Reg.**	**XGBoost**	**Rand. Forest**	**Dec. Tree**
Training AUC	0.70	0.76	0.79	Training AUC	0.72	0.86	0.87	0.76
Testing AUC	0.78	0.72	0.72	Testing AUC	0.70	0.87	0.89	0.81
Testing sensitivity	0.73	0.73	0.64	Testing sensitivity	0.56	0.75	0.82	0.89
Testing specificity	0.73	0.77	0.79	Testing specificity	0.75	0.82	0.83	0.65
Youden index	0.45	0.50	0.42	Youden index	0.30	0.57	0.65	0.54
Threshold opt. bound	0.025	0.015	0.05	Threshold opt. bound	0.50	0.30	0.40	0.30

was capable of detecting 82% of colonized patients with 83% specificity.

In addition to generating predictions, we also used our modeling framework to identify the key predictors for separate and aggregate VRE, CRE, and MRSA colonization. In **Table 6**, we summarize the top five predictors for the models reported in **Table 2**, and provide their ranking in the corresponding models as indicated by OR and FI. See the **Supplementary Material (Appendix C)** for the OR and FI values of the factors presented in **Table 6**.

Among the recent ICD-10 procedures that were performed during the current hospital stay before ICU admission, the procedures categorized as "Other Procedures" in the ICD-10 PCS were among the top five predictors for VRE, CRE, MRSA, and MDRO. In our dataset, a significant proportion of these procedures were "8E0ZXY6", an ICD-10 code designated for isolation precautions. The patients having a history of a prior colonization or infection for a given MDRO (or are at risk for another indication) were flagged with this code upon admission to the hospital so that they were closely monitored (and if needed, isolated) during their hospital stay. Our results presented in **Table 6** show that these patients were at a higher risk for being colonized with an MDRO at ICU admission regardless of the specific indication for which the close monitoring and isolation precautions were put in place.

Another key predictor for VRE, CRE, MRSA, and MDRO colonization is the CCS-based diagnosis category "skin and subcutaneous tissue disease" that was PoA (**Table 6**). The diagnoses that fall under this CCS category were determined for the current hospital admission and included rash, cellulitis, cutaneous abscess, pressure ulcer, non-pressure chronic ulcer, and other skin conditions. Our finding resonates with the clinical literature and practice, as skin and soft tissue infections are amongst the most common bacterial infections, are mostly treated with antibiotics that might cause antimicrobial resistance (81). Further, skin and soft tissue infections are the most frequently reported clinical manifestations of community-acquired MRSA (82).

For MDRO and in particular MRSA, the CCS-based current diagnosis category "infectious and parasitic diseases" was one of the critical factors that increase the risk of colonization. This category included diseases such as chronic viral hepatitis C, bacteremia, human immunodeficiency virus (HIV), and sepsis. Patients with these diseases might be at higher risk for MDRO, and in particular MRSA, colonization due to a compromised immune system.

For VRE and CRE, having a prior long-term care facility (LTCF) stay was one of the key predictors for colonization upon ICU admission. This association between VRE or CRE colonization and a previous LTCF stay has been reported by other studies (83, 84) (also see the **Supplementary Material Appendix B**). High rates of MDRO colonization, debilitating diseases, and the receipt of multiple antibiotics among LTCF residents are likely to be the primary causes of this association both for VRE and CRE colonization (85).

Other key predictors for VRE were recent procedures "administration circulatory" (ICD-10-PCS '30'), such as transfusion, and "medical and surgical anatomical regions, general" ('0W'), such as drainage, insertion, removal, and transplantation procedures. For CRE, a prior ICU stay longer than 20 days and a total number of diagnoses PoA (i.e., current

TABLE 6 | Top five common predictors for VRE, CRE, MRSA, and MDRO colonization identified by the machine learning models.

Top five common predictors for VRE colonization upon ICU admission

VRE colonization upon ICU admission		Relative ranking		
Factors	**Features**	**Log. Reg.**	**XGBoost**	**Rand. Forest**
Long-term care facility stay	Yes	1	3	1
Recent 1-digit ICD10 procedure	Other procedures	2	1	2
Current diagnosis CCS class	Skin and subcutaneous tissue	3	2	3
Recent 2-digit ICD10 procedure	Medical/surgical anatomical	6	5	8
Recent 2-digit ICD10 procedure	Administration circulatory	8	4	6

Top five common predictors for CRE colonization upon ICU admission

CRE Colonization upon ICU Admission		Relative Ranking		
Factors	**Features**	**Log. Reg.**	**XGBoost**	**Rand. Forest**
Current diagnosis CCS class	Skin and subcutaneous tissue	2	2	1
Recent 1-digit ICD10 procedure	Other procedures	3	3	2
Prior ICU stay	$>$ 20 days	4	6	5
Long-term care facility stay	Yes	5	6	6
Number of current diagnosis PoA	$>$ 30 and $\leq$ 50	6	8	3

Top five common predictors for MRSA colonization upon ICU admission

MRSA colonization upon ICU admission		Relative ranking		
Factors	**Features**	**Log. Reg.**	**XGBoost**	**Rand. Forest**
Recent 1-digit ICD10 procedure	Other procedures	1	2	1
Current diagnosis CCS class	Skin and subcutaneous tissue	2	9	2
Current diagnosis CCS class	Injury and poisoning	7	1	8
Current diagnosis CCS class	Infectious and parasitic	9	8	5
Recent 1-digit ICD10 procedure	Administration	−3	3	14

Top five common predictors for MDRO colonization upon ICU admission

MDRO colonization upon ICU admission		Relative ranking			
Factors	**Features**	**Log. Reg.**	**XGBoost**	**Rand. Forest**	**Dec. Tree**
Recent 1-digit ICD10 procedure	Other procedures	7	1	1	2
Current diagnosis CCS class	Skin and subcutaneous tissue	16	2	2	14
Current diagnosis CCS class	Mental illness	35	6	3	12
Current diagnosis CCS class	Infectious and parasitic	57	12	4	16
Sex	Female	89	3	5	9

diagnoses) >30 were two critical factors increasing the risk of colonization. For MRSA, the current diagnosis for "injury and poisoning", mostly consisting of procedural injuries such as accidental puncture or dural laceration during a procedure, is associated with an increased colonization risk. On the contrary, the recent procedure code for "administration" (i.e., ICD-10 PCS codes with first character "3") was found to lower the risk of colonization. Finally, female sex and the "mental illness" category for current diagnosis, including diagnosis for cocaine abuse, opioid abuse, poisoning by heroin and psychological disorders, were two other key factors associated with an increased risk for MDRO colonization. Patients in this category (i.e., the "mental illness") are at higher risk for using injections and causing damage to their skin, which might explain the increased risk for MDRO colonization.

DISCUSSIONS

Leveraging a rich dataset and supervised ML algorithms, we developed an accurate and interpretable framework for predicting VRE, CRE, and MRSA colonization upon ICU admission. The developed predictive analytics framework achieved the following sensitivity and specificity values for VRE, CRE, and MRSA colonization: 80% and 66% for VRE with LR, 73% and 77% for CRE with XGBoost, and 76% and 59% for MRSA with RF. Further, we predicted MDRO (i.e., VRE, CRE, or MRSA) colonization as an aggregate outcome with 82% sensitivity and 83% specificity for MDRO using RF.

These results indicate that predicting MDRO colonization in aggregate, rather than separately predicting VRE, CRE, and MRSA, achieved the highest prediction accuracy in terms of both AUC and Youden's index. On the one hand, predicting a specific MDRO would be preferable, as it would enable more customized interventions such as tailored antibiotic therapy. On the other hand, accurately predicting MDRO colonization without specifying whether it is VRE, CRE, or MRSA is still quite important for clinical practice. This is because the key interventions for these MDROs are the same or similar, such as contact precautions and enhanced environmental cleaning, and can later be followed up by more specific testing protocols to identify the underlying organism. Accordingly, many infection control measures can be implemented rapidly upon ICU admission for the patients who are suspected to be colonized, and treatment strategies and more advanced interventions can be tailored later as more information becomes available.

In addition to producing timely predictions for newly admitted ICU patients, our ML-based modeling framework can also be utilized to identify the key predictors for VRE, CRE, and MRSA colonization upon ICU admission. We identified several important predictors of MDRO colonization, including long-term care facility exposure, a current diagnosis of skin/subcutaneous tissue or infectious/parasitic disease, and a recent ICD-10 procedure "Other Procedures", including isolation precaution procedures, as the key predictors for MDRO colonization upon ICU admission. These predictors can help characterize and identify ICU patients at high-risk for MDRO colonization and hence, facilitate timely implementation of infection control measures such as selective use of contact precautions, targeted surveillance, and tailored antibiotic therapy.

The primary limitation of our study was that we did not utilize any data on patient medical history outside of UMMC. For example, we did not take into account antibiotic consumption outside of UMMC or during outpatient visits. Similarly, we did not have information about patients who could have been admitted elsewhere, thus censoring any information about whether they received or underwent additional treatments and procedures in other healthcare facilities. As we utilized administrative data for procedures and diagnoses, which are primarily used for billing, we did not have full access to exact clinical conditions and we did not know the exact reason why a specific procedure was performed or diagnosis was established. Our discussions with clinicians shed some light on these uncertainties but we could not determine the exact details for each individual patient other than what the data conveys. Finally, our data were derived from a single source and we were only able to observe the performance of our modeling framework on an out-of-sample subset from the same facility.

The machine learning algorithms we used in this study had additional limitations. Specifically, logistic regression models assume predictors to have a linear relationship with the log odds (i.e., the logit form) of the prediction variable and may have difficulty in capturing complex non-linear relations. Furthermore, in their standard forms, logistic regression models require minimal or no multicollinearity between independent variables, and hence, the presence of highly correlated predictors might be problematic. Overfitting might also be a significant issue for the logistic regression algorithm but this can be avoided by the use of a regularization technique. XGBoost (i.e., eXtreme Gradient Boosting) can also easily overfit if its parameters are not tuned properly. Further, like any other boosting method, XGBoost models are quite sensitive to outliers since the XGBoost method relies on the sequential ensemble of decision trees and every decision tree classifier attempts to fix the errors of its predecessor learners. Finally, assuming no formal parametric structure or distribution and relying on the parallel ensemble of decision trees, random forest models can cope with skewed data and can capture complex non-linear relationship. Yet, using a random forest algorithm with the default values can also generate suboptimal results (86), and hence, parameter and hyperparameters tuning should be performed to increase model performance. Moreover, generated feature importance scores, demonstrating the relevant importance of each feature for prediction, are not sufficient to capture all forms of dependencies between predictors and prediction outcome. Partial dependence plots have been recommended to be used to address this shortcoming (86). Last but not least, random forest models are biased in favor of categorical predictors having noticeably more levels and hence, general conclusions solely based on feature importance scores might not always be reliable.

It is noteworthy to emphasize that our study, which focused on predicting MDRO colonization for newly admitted ICU patients,

would not prevent the importation of VRE, CRE, and MRSA into the ICU setting. However, by producing reliable predictions and identifying key risk factors for colonization, our approach could enable early detection of colonized patients and facilitate timely and targeted implementation of preventive measures on asymptomatic MDRO carriers. That is, once implemented as a clinical decision support system, our predictive analytics framework could alert healthcare providers in real-time when a high-risk patient, characterized by the predictors identified by this study, is admitted to the ICU so that the medical team can apply the necessary precautions, such as contact precautions, in a timely manner to prevent potential transmissions. This approach could help reduce transmission of these so-called "superbugs" in ICUs, and would particularly be useful for healthcare settings where active surveillance is not performed. In future efforts, we plan to examine the practical utility of our modeling framework *via* a comprehensive computational simulation study that investigates and quantifies the estimated value of early detections flagged by our model both in hospital and region settings by separately using agent-based and network-based simulation models (87).

Several recent studies also proposed or assessed a predictive modeling approach for MDROs. Studying MDRO infections in emergency department settings, González del Castillo et al. (88) proposed a prediction model, developed by using backward logistic regression. The model achieved an AUC of 0.76 and 0.72 in the model training and testing sets, respectively. Splitting patients into six risk categories, the authors also examined different cut-off values for the risk scores. The model with the optimal cut-off value achieved 59% sensitivity and 74% specificity. Faine et al. (89) performed an external validation study to test the performance of the predictive clinical decision rule they previously developed *via* logistic regression to identify multidrug-resistant urinary pathogens in the emergency department. The model yielded a sensitivity of 56% and specificity of 66% in the validation cohort. Tseng et al. (90) utilized a multivariate logistic regression to develop a statistical model for predicting multidrug-resistant gram-negative bacteria colonization and infections at the time of hospital admission. The AUC values of their model were 0.75 and 0.80 in the model development and validation sets, respectively. The authors also identified the best threshold value maximizing the Youden index with 57% sensitivity and 85% specificity. Goodman et al. (91) derived and compared a ML-based decision tree (i.e., classification and regression tree) with a logistic regression-derived risk score for *extended-spectrum beta-lactamase* (ESBL) bacterial infections. The sensitivity and specificity values of the classification and regression tree (CART) were 51.0 and 99.1%, respectively. The AUC was 0.77 for the CART model, 0.87 for the multivariable LR model, and 0.87 (and 0.89 following cross-validation) for the LR-based risk score. The risk score achieved a sensitivity of 49.5% and a specificity of 99.5% with the cutoff value that maximizes the overall ESBL classification accuracy. Sullivan et al. (92) developed a regression model to predict carbapenem resistance among patients with *Klebsiella pneumoniae bacteremia*. The mean AUC of the model was 0.73, which achieved 73% sensitivity and 59% specificity in the testing set. Lee et al. (93) assessed the performance of an artificial neural network (ANN)-based prediction model for predicting bacteremia in comparison with naïve Bayesian, support vector machine (SVM), and RF models. Among the compared models, the multi-layer perceptron, a feedforward ANN model, the authors developed exhibited the highest sensitivity (81%) and had a specificity rate 59% with an AUC 0.73. Finally, Lewin-Epstein et al. (94) applied several ML algorithms, consisting of LR with LASSO, neural networks, gradient boosted trees, and an ensemble of these three ML algorithms, to predict antibiotic resistance profiles of bacterial infections among hospitalized patients. The ensemble model achieved AUC values ranging from 0.73 and 0.79 for different types of antibiotics, which were improved to 0.80–0.88 if the infecting bacterial species was assumed to be known. As a comparison with these studies, the best performing model in our study (RF for MDRO prediction) achieved 0.87 and 0.89 AUC in training and testing sets, respectively, and yielded 82% sensitivity and 83% specificity in the validation/testing cohort. In general, the use of tree-based ensemble algorithms, such as XGBoost and random forest, played an important role in achieving higher predictive accuracy in our study.

Prediction models have been previously reported to perform worse when they are implemented in clinical practice and applied to new individuals that are different than the original study population that the model was derived (95). Therefore, before being integrated into practice for clinical decision support, the robustness of the proposed approach must be thoroughly examined and externally validated in different populations. To address this critical concern, we are currently studying the transportability, generalizability, and external validation of our ML models and predictive analytics framework by leveraging retrospective EHR data from another academic teaching hospital, located in Baltimore, Maryland, USA. We plan to publish the findings of this ongoing study in a separate article.

Traditionally, many prediction rules, developed as a decision support tool for clinicians, are designed to be very simple, relying on only a small number of variables, for practicality. Yet, with the increasing availability of electronic healthcare record data and the expansion of modern database and software systems, the use of data-driven prediction models and other analytical and computational methods for the identification, control, and prevention of MDROs and other HAIs has been increasing (56). As a result, a growing number of healthcare facilities are capable of generating more complex prediction models in an automated fashion. Accordingly, taking advantage of the advances in computational and data recording technologies, many healthcare organizations can use our data-driven prediction framework to produce real-time predictions and identify the high-risk patients for MDRO colonization.

Finally, we touch upon the topic of the general trade-off between the predictive power of ML algorithms and the interpretability of ML models and their results. This trade-off derives from the fact that the best performing algorithms are often the most complex ones. That is, while simpler models such as regressions and decision trees, are transparent and explainable by design, more advanced models that can capture and cope

TABLE 7 | Predictors and coefficients (i.e., odds ratios) of the best performing logistic regression models.

Predictor/factor	Categorical level	CRE	VRE	MRSA
Prior diagnosis CCS class	Neoplasms	2.00	-	-
	Blood and blood-forming organs	-	1.36	-
	Infectious and parasitic	-	1.18	-
	Mental illness	0.84	-	-
	Symptoms, signs, ill-defined conditions	0.79	-	-
	Circulatory system	-	-	0.81
Current diagnosis CCS class	Skin and subcutaneous tissue	1.92	1.55	1.52
	Nervous system and sense organs			1.26
	Respiratory system			1.25
	Injury and poisoning			1.15
	Infectious and parasitic			1.07
	Genitourinary system	-	1.18	-
	Mental illness		-	1.02
	Circulatory system	-	-	0.85
	Endocrine, nutritional, metabolic, immunity	0.88	-	0.65
	Neoplasms	0.75	-	0.83
Recent 1-digit ICD 10 procedure	Other procedures	1.76	1.80	1.76
	Extracorporeal/systemic	1.30	-	-
	Administration	-	-	0.68
	Medical and surgical	-	-	0.90
Prior ICU stay	$>$ 0 Days and $<$ 5 Days	1.20	1.02	-
	10–20 Days	-	1.39	-
	$>$ 20 Days	1.73	-	-
Prior 2-digit ICD 10 procedure	Medical/surgical gastrointestinal	1.35	-	1.05
	Medical/surgical upper veins	-	1.03	-
	Medical/surgical respiratory	-	-	1.33
Recent 2-digit ICD 10 procedure	Medical/surgical gastrointestinal	-	-	1.32
	Medical/surgical anatomical	-	1.33	-
	Medical/surgical heart and vessels	1.21	-	-
	Administration circulatory	1.27	1.28	-
	Medical/surgical hepatobiliary	-	1.13	-
Prior antibiotics use	Yes	1.25	-	-
	Prior antibiotics Fluoro use	1.28	-	-
	Prior antibiotics Ceph use	-	1.09	-
	Number of different types used = 3	1.19	-	-
Number of recent procedures	$\leq$ 2	0.92	-	0.87
	$>$ 2 and $\leq$ 5	-	0.91	-
	$>$ 5 and $\leq$ 10	-	0.89	0.96
Number of prior diagnosis	$\leq$ 10	-	-	0.91
	$>$ 10 and $\leq$ 20	-	1.01	-
	$>$ 50 and $\leq$ 100	-	1.04	-
Number of prior procedures	$>$ 20	-	1.31	-
Number of current diagnosis	$\leq$ 10	-	0.72	0.67
	$>$ 10 and $\leq$ 20	0.74	0.89	-
	$>$ 30 and $\leq$ 50	1.38	1.03	-
Admission type or source	Elective	0.96	0.78	-
	Home or self referral	0.73	0.83	-
	Physician referral	0.65	0.70	-
Race/ethinicity	Black	0.91	0.94	-
Sex	Female	-	-	0.89
Age group	Age 30–40	-	0.97	-
	Age 40–50	0.93	-	-
Long-term care facility stay	Yes	1.69	1.95	-

with higher levels of complexities (e.g., neural network, random forest, XGBoost) are typically more complex and of "black-box" nature (96). Clinicians are more accustomed to simpler traditional models (e.g., logistic regression), as these models usually provide better understanding for the reasoning chain behind the predictions made. Therefore, we summarize the odds ratios of the best performing LR models in **Table 7**, separately for CRE, VRE, and MRSA colonization. As known, an odds ratio value > 1 indicates positive correlation whereas an odds ratio value < 1 means that the presence of the corresponding feature reduces the risk of colonization. We note that the best performing LR models are not necessarily the best performing ML models but their outputs (i.e., the odds ratios for each feature) offer an easier interpretation of the results.

There are several other analyses that can be performed to improve the interpretability of the models and better communicate results with clinicians. One approach is to utilize the significant predictors and predicted probabilities identified and estimated by the best performing ML model and to link them with a linear regression. That is, after the predictive analytics study is performed, the modeler can fit a linear regression model to the significant predictors (i.e., the features with non-zero coefficients) to explain the predicted probabilities (i.e., MDRO colonization risks that the ML model predicts for each patient) and as a result, can provide a direct means to quantify the impact of each predictor on MDRO colonization risk. If desired, this approach can be taken a step further by developing a simple clinical decision rule based on the weights the linear regression model provides for each significant predictor (though, usually, at the expense of predictive power). Alternatively, another approach that can facilitate the interpretability of the results is to conduct a univariate sensitivity analysis, again, on the significant predictors and predicted probabilities of the best performing ML model. By taking this approach, the modeler can set the value of a single feature equal to zero (or equivalently, momentarily exclude it from the analysis) and then calculate the predicted probabilities by using the already trained ML model and all other significant predictors. The average decrease in the predicted probabilities (due to the absence of the feature of interest) can, then, be used to quantify the impact of (missing) feature on MDRO colonization risk. By doing this univariate sensitivity analysis on each and every significant feature, the modeler can again provide a numeric value quantifying the strength of the association between each predictor and the (predicted) MDRO colonization risk.

CONCLUSION AND FUTURE WORK

Timely detection of MDRO colonization, prevention of MDRO infections, and early implementation of counter-measures are of utmost importance to alleviate the harms and minimize the costs associated with MDROs at patient, hospital, and national levels. Following the advances in database management technologies, increased computational power of computers, and the availability of user-friendly software packages, descriptive and predictive analytics methods can now play a pivotal role for the analysis of patient data and the identification of patients with MDRO colonization. This was the primary objective of our study in this paper, which showcased the use and the practical utility of such data-driven methods to correctly predict the presence of VRE, CRE, and MRSA colonization at the time of ICU admission.

In this paper, we proposed a data-centric modeling framework to predict VRE, CRE, and MRSA colonization upon ICU admission and identify the associated risk factors. Our study achieved the highest prediction accuracy, measured by Youden's index, when VRE, CRE, and MRSA colonization were combined and predicted as an aggregate outcome. Capable of coping with significant class imbalance, a feature commonly observed in clinical datasets, the framework described in this study can be used as a clinical decision support tool to provide accurate on-time predictions especially if it is regularly updated and trained off-line as additional (i.e., more recent) data become available. This predictive analytics approach can further be used to identify the key risk factors and define high-risk populations, for which targeted interventions can be implemented rapidly to reduce transmission of MDROs in ICUs.

There are three research directions that we plan to pursue in near future: First, we will study the acquisition outcomes, where we focus on the ICU patients who were initially colonization-free but acquired VRE, CRE, or MRSA colonization during their ICU stay. Second, we will develop a comprehensive agent-based simulation model to analyze MDRO colonization and infection in ICUs and assess the impact of commonly utilized prevention and control measures on MDRO transmission. Finally, we are in the process of acquiring more data from another major healthcare facility to conduct a similar study by leveraging this additional dataset. This will not only enable us to enlarge the size our dataset, leading to more accurate predictions, but will also give us an opportunity to assess the generalizability of our findings and help us develop more robust predictions.

DATA AVAILABILITY STATEMENT

Data cannot be shared publicly because of private ownership. Data were obtained *via* electronic healthcare records from the University of Maryland Medical Center (UMMC), an academic teaching hospital located in Baltimore, Maryland, United States of America. Requests to access the datasets should be directed to LP, lpineles@som.umaryland.edu.

AUTHOR CONTRIBUTIONS

ÇÇ and SB designed the analytical modeling framework and performed verification and validation analysis. ÇÇ performed data and statistical analyses, developed the machine learning models, conducted the predictive analytics study, and generated the numerical results under the supervision of SB and wrote the first draft of the manuscript. LP coordinated the data retrieval efforts from the University of Maryland Medical Center

(UMMC). EK managed the overall project. EK, LP, and AH served as subject-matter experts. SB, EK, and AH supervised the project and provided mentorship. EK, SB, LP, and AH wrote the grant proposal for funding. All authors contributed to conception, design of the study, performed major edits on the manuscript, contributed to manuscript revision, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.853757/full#supplementary-material

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CASE REPORT
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Case Report: A Case of Acute T Lymphoblastic Leukemia With Mixed Infection of Lethal Invasive Mucormycosis and Multi-Drug Resistant Bacteria

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Pulmonary mucormycosis (PM) is a rare and life-threatening fungal infection. Here, we report a case of an acute T lymphoblastic leukemia patient with mixed infections of lethal invasive Mucormycosis and multi-drug resistant (MDR) bacteria. After receiving anti-infection drugs to control the patient's fever, he was treated with induction chemotherapy. However, the malignant hematological disease was poorly controlled by the chemotherapy and the patient developed more symptoms of infection. Although the results of multiple β-D-Glucan (G) and Galactomannan (GM) tests remained negative, several pathogens were detected using metagenomic next-generation sequencing (mNGS). In particular, mNGS identified *Malassezia pachydermum*, *Mucor racemosus*, and *Lauteria mirabilis* in the peripheral blood and local secretion samples. The Mucor and bacterial infections were further confirmed via multi-site and repeated fungal and bacterial cultures, respectively. Despite adjusting the anti-infection therapy according to the diagnostic results, the patient's blood disease and symptoms of infection were not alleviated. Additionally, the MDR *Acinetobacter baumannii* infection/colonization was not confirmed until the seventh culture of the peripheral venous catheter tip. Due to the patient's deteriorating conditions, his family decided to withdraw him from further treatment. Overall, mNGS can facilitate a diagnosis of Mucormycosis by providing clinical and therapeutic information to support conventional diagnostic approaches. For the early and timely diagnosis and treatment of PM, it is also necessary to consider the malignant hematological conditions and repeated tests through multiple detection methods.

Keywords: pulmonary mucormycosis, multi-drug resistant bacteria, Mucor infection, metagenomic next-generation sequencing, acute T lymphoblastic leukemia

INTRODUCTION

Pulmonary mucormycosis (PM) is caused by uncommon fungal infections and its mortality rate is between 30 and 65%, with an average survival time of only 27 days (1, 2). Despite the importance of the early diagnosis and treatment of PM, the detection of PM is challenging due to the lack of specific clinical manifestations. In particular, PM can occur with either the

presence or absence of suppurative inflammation, and some common pathological manifestations of PM include the invasion of fungi in bronchus and lung tissue, the coagulative necrosis of the lungs, pulmonary hemorrhage, vascular invasion-induced thrombosis, hemorrhagic pulmonary infarction, and hematogenous dissemination (3). The imaging manifestations and the dynamic changes during PM are similar to invasive pulmonary aspergillosis (IPA), and are relatively complex, thus, further complicating diagnosis (4, 5).

Voriconazole is generally ineffective as a preventive treatment for PM, and PM patients generally experience massive hemoptysis and tend to be negative in β-D-Glucan and Galactomannan (G and GM) tests (6, 7). PM patients are likely to exhibit additional clinical features, including pulmonary lesions with sinusitis (e.g., bone destruction), halo sign, multifocal pneumonia, and pleural effusion (8).

In this study, we report a case of an acute T-cell lymphoblastic leukemia patient who experienced mixed infections of Mucormycosis and multi-drug resistant (MDR) bacteria. The case provides potential insights into the diagnosis and treatment of PM patients with complex disease conditions.

CASE DESCRIPTION AND DIAGNOSTIC ASSESSMENT

A male patient was admitted to hospital on November 21, 2018 because of pain in the right rib that had lasted for 1 week, and fever that had been present for 1 day. The patient was working in a tea stir-frying company and had no previous medical, family, or psychosocial history. The patient's routine blood examination revealed a hemoglobin content (Hb) of 84 g/L, white blood cell (WBC) count of 64.18 × 10^9/L, a neutrophil count of 0.3 × 10^9/L, and a platelet count (PLT) of 75 × 10^9/L. The patient's bone marrow morphology and immunology results suggested that he had acute T-cell lymphoblastic leukemia. Specifically, a chest computed tomography (CT) scan revealed a soft tissue shadow at the anterior superior mediastinum, as well as lymph node enlargement and splenomegaly. Bone marrow cell morphology revealed significantly active hyperplasia, 91% of which was due to primitive and immature cells. By analyzing 92.3% of the immature cell population, bone marrow immunostaining was positive for CD7, CD34, CD10, CD56, cCD3, and CD99, and weakly-positive for CD13, CD38, and T lymphocyte expression, which was consistent with the immunostaining data for early T-cell progenitors (ETPs). According to genetic analyses, the karyotype of the patient was 46,XY,del(11)(p11),del(17)(p11)[6]/46,idem,der(1)[4]. Upon examining 43 leukemia-related fusion genes by multiplex PCR, no fusion transcripts were detected. Additionally, the copy number of *WT1* was 1,322 copies/10,000 copies of *ABL*. However, mutations in *SF3B1*, *NOTCH1*, *PHF6*, *SUZ12*, *SUZ12p*, *GATA3*, and *CTCF* were detected by next-generation sequencing (NGS).

Based on the patient's neutrophil counts, which suggested agranulocytosis, anti-infection treatment with meropenem and caspofungin was administered. VP (Vincristine + Prednisone) combined with decitabine (DAC; 20 mg/m^2, days 1–5) plus HAAG regimen-based chemotherapy (homoharringtonine (H) 1 mg/d, days 3–16; cytarabine (A) 10 mg/m^2, injected subcutaneously every 12 h, days 3–16; aclarubicin (A) 10 mg/d, days 3–10; granulocyte colony stimulating factor (G-CSF) 50-600 μg/day, days 2–9 unless WBC count was higher than 20 × 10^9/L) was administered after controlling the fever (**Figure 1**). By re-examining the bone marrow cell morphology on the 1st day of chemotherapy, the result revealed low bone marrow hyperplasia, 91% of which were due to primitive and immature cells. In addition, the patient experienced pain in the right nasal cavity and upper palate, together with a low fever and swelling of the right side of the face. The patient's antibiotic therapy was therefore adjusted to imipenem/cilastatin, vancomycin hydrochloride, and intravenous voriconazole. Twenty-four hours later, the patient's skin on the right nasal wing was cyanotic with numbness, his swelling and the upper gum pain became more severe, and he developed a continuously high fever. The patient was negative for procalcitonin and the results of the GM and G test remained negative after multiple trials. The patient's cranial CT revealed foreign bodies in the nasal cavity and maxillary sinusitis, while endoscopic tests revealed nasal suppurative infection. The therapy was then immediately adjusted to imipenem/cilastatin, daptomycin, amphotericin B liposome, and local douche (**Figure 1**), and peripheral blood and local secretion were collected for metagenomic next-generation sequencing (mNGS). On the 3rd day, the chest CT revealed nodular lesions (**Figures 2A–C**), while the patient continued to suffer from continuous high fever. The numbness of the skin on the right nasal wing remained and the right nasal cyanosis became enlarged and darker (**Figures 2D,E**). The upper gums exhibited obvious swelling, pain, and ulceration, and the purple area of the maxillary mucosa expanded, which was accompanied by ulceration.

Based on the mNGS results, the patient was positive for the presence of *Malassezia pachydermum*, *Mucor racemosus*, and *Lauteria mirabilis*. The antibiotic therapy was then adjusted to amphotericin B liposome, imipenem/cilastatin, daptomycin, piperacillin/tazobactam, and posaconazole (**Figure 1**). On the 4th day, we infused the maternal neutrophils for 3 days (**Figure 1**). On the 5th day, the skin of the nose was ulcerated and sunken, which was not alleviated during the course of the treatment (**Figures 2F,G**). Additionally, the ulcer on the upper palate was aggravated, and the nasal cavity displayed black secretions. The chest CT consistently indicated the progression of the lung infection. Bacterial cultures of the nasal secretions indicated the presence of *Staphylococcus haemolyticus*, MDR *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* (**Figures 2H,I**), while fungal cultures suggested Mucor infection after multiple cultures (**Figures 2J,K**). The results of the first six catheter cultures of the peripheral blood were all negative, and it was not until the 7th culture (on the 5th day) that MDR *Acinetobacter baumannii* was detected. The antibiotic therapy was then adjusted to imipenem/cilastatin, datamycin, piperacillin/tazobactam, amphotericin B liposome, posaconazole, and caspofungin (**Figure 1**). Nevertheless, the patient experienced a sustained high fever, hemodynamic

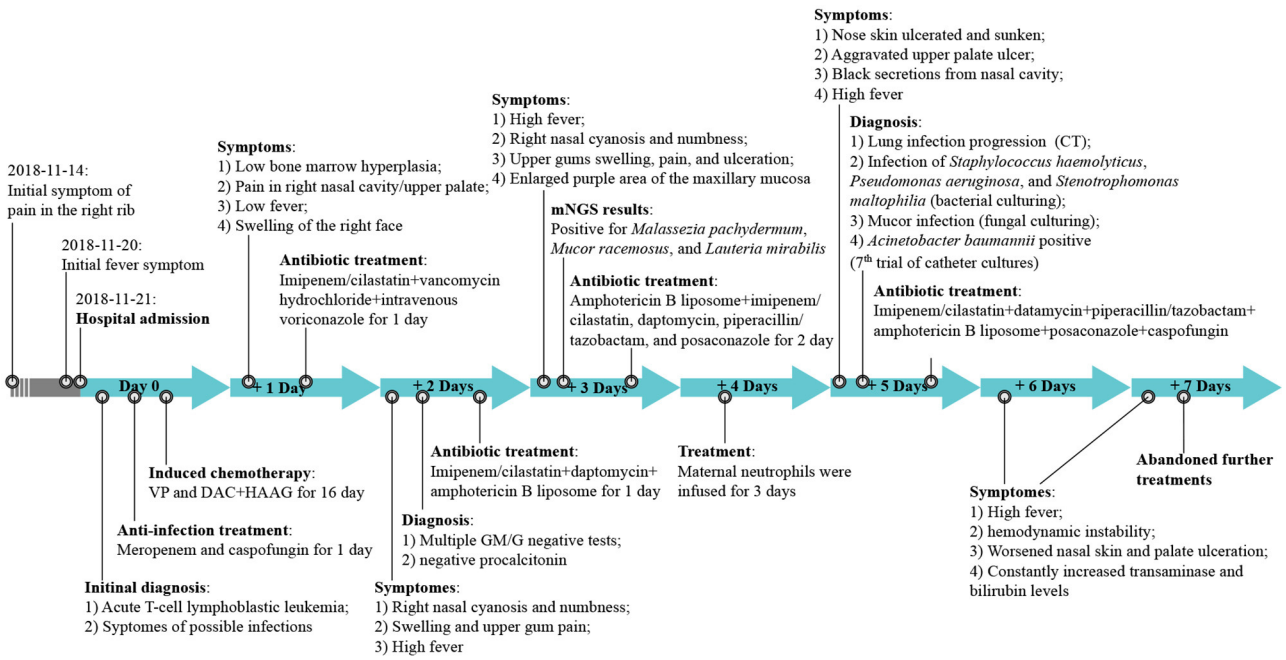

FIGURE 1 | The treatment timeline. VP, Vincristine + Prednisone; DAC, decitabine; HAAG, homoharringtonine (H), cytarabine (A), aclarubicin (A), granulocyte colony stimulating factor (G-CSF); CT, computed tomography; GM/M, β-D-Glucan (G); Galactomannan (GM) tests; mNGS, metagenomic next-generation sequencing.

instability, continuously worsening nasal skin and palate ulcerations, and exhibited increasing transaminase and bilirubin levels. Due to the deteriorating conditions, the patient's family decided to withdraw him from further treatment. The patient failed to achieve remission after 16 days of induction chemotherapy, and experienced serious infections and agranulocytosis after chemotherapy. The patient died the day after discharge, on day 16.

DISCUSSION

PM is a severe and deadly disease. According to Chamilos et al., lung disease patients who had sinusitis and did not responded to preventive treatment with voriconazole were more likely to have PM (9). Additionally, multiple lesions (≥10 nodules) and pleural effusion were the two major independent predictors of PM (9). In our case, the patient was positive for ETP with a poor prognosis, and induction chemotherapy did not alleviate the symptoms. The patient also had nasal sinus infections, multiple negative GM and G results, and an insignificant increase of procalcitonin (PCT) levels, which was consistent with the criteria of PM put forth by Chamilos et al. The culture results of the local secretion confirmed the presence of mucormycosis infection in the nasal sinus, which implied that the pulmonary infection might have resulted from the pathogen spreading via the airway.

Although we adjusted the antifungal therapy (i.e., amphotericin B liposome, posaconazole and caspofungin, and donor neutrophil infusion), the patient's blood disease was not alleviated. Furthermore, he continued to suffer from persistent severe agranulocytosis, uncontrolled local infection, and the spread of pulmonary lesions. The results of the 7th blood culture revealed the presence of MDR *Acinetobacter baumannii*, thus, indicating a worse and more fatal disease condition than previously expected.

Several clinical tests are needed to confirm Mucormycosis infections, including histopathology, direct examinations, tissue culture, and the testing of respiratory secretions and bronchoalveolar lavage fluid. Conventional evaluation techniques typically have limited sensitivity and specificity, and bacterial/fungal cultures often give negative results, despite positive microscopic examinations (7). Indeed, cultures can only detect ∼50% of cases of Mucormycosis infection (10). Recent advancements in polymerase chain reaction (PCR) technology have contributed to the development of rapid, accurate, and sensitive methods for pathogen detection. However, some PCR assays cannot provide such information due to their limit of detection, sensitivity, specificity, and cross-reactivity. Given that many such PCR assays lack clinical validation and internal evaluation, their applications are mainly restricted to research purposes (11).

mNGS is a high-throughput technology that provides direct information about the type of infection, without relying on microbial cultures (12). Considering the rapid speed and high sensitivity of mNGS, this technology may facilitate the timely diagnosis of disease, especially in life-threatening scenarios. In the present case, mNGS detected *Mucor racemosus* infection on day 3, whereas it was not until day 5 that fungal culture confirmed the Mucor infection. As a result, mNGS demonstrated its substantial clinical potential in facilitating

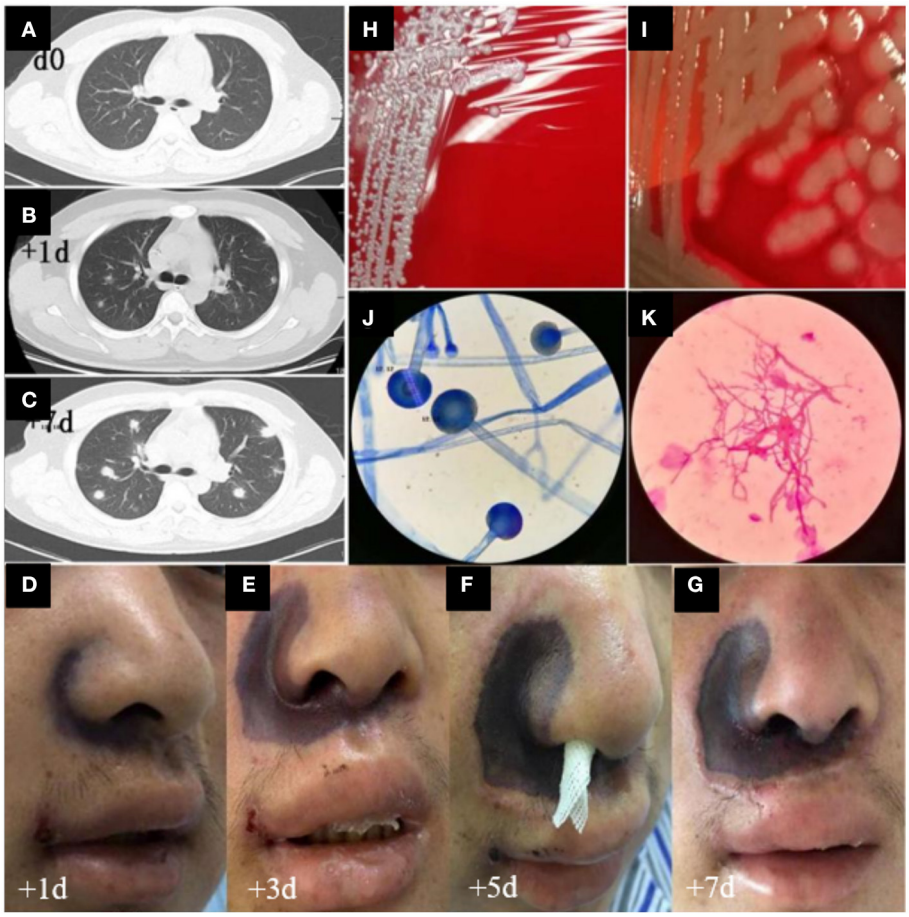

FIGURE 2 | CT images, advancement of skin damage, and morphologic features of microbiological cultures in this case. **(A–C)** Day 0, day 1, and day 7 of the chest CT images, respectively. **(D–G)** Day 1, 3, 5, and 7 images of the nasal skin, respectively. **(H,I)** Bacterial cultures of *Staphylococcus haemolyticus* and MDR *Pseudomonas aeruginosa*, respectively. **(J,K)** The morphologic features of Mucor.

diagnosis. However, as some pathogens identified by mNGS are opportunistic and rarely lead to infection, it remains necessary to verify the infectious agent via culture tests. Thus, mNGS can be used to guide clinical laboratories to adjust the culture conditions for fastidious or specific microorganisms, which may increase diagnostic and prognostic accuracy, and improve treatment efficacy.

The unfavorable outcomes of the current PM case revealed several clinical implications: (1) One of the major reasons for the failure of the anti-PM therapy was due to the uncontrolled malignant hematological disease. (2) The patient's occupational environment imparted potential risks for the long-term exposure to molds, which might have led to the Mucor infection; however, we did not sufficiently consider the link between his disease and his working environment. (3) To determine the etiological basis for PM treatment, multi-site fungal and bacterial cultures, as well as mNGS may be necessary in patients with nasopharyngeal infections. (4) In cases of poorly-controlled nasopharyngeal infections after chemotherapy, the PM treatment should be adjusted in a timely manner. (5) Particular focus should be given to PM patients with severe agranulocytosis, as their disease could progress much faster than patients with non-malignant hematological diseases.

In conclusion, we reported a case of an acute T lymphoblastic leukemia patient with mixed infections of lethal invasive Mucormycosis and MDR bacteria. The patient experienced a poor clinical outcome, which indicated the importance of considering the malignant hematological disease conditions and patients' working/living environments. This case also highlighted the need to employ multiple diagnostic assays in cases of PM. mNGS may also facilitate the diagnosis of Mucormycosis by providing additional details in support of conventional diagnostic approaches.

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

The studies involving human participants were reviewed and approved by the First Hospital Affiliated to Soochow University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the patient's family for the publication of this study.

AUTHOR CONTRIBUTIONS

XT and JX conceived and supervised the study. QC, HD, DW, JH, XT, and JX acquired the clinical data and performed patient follow-up. QC, HD, DW, JH, and YX acquired patient samples and performed the clinical testing. All authors participated in data analysis and interpretation, and were involved in manuscript preparation. All authors approved the final manuscript.

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Conflict of Interest: YX is the employee of Dinfectome Inc.

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Impact of Carbapenem Peri-Transplant Prophylaxis and Risk of Extended-Spectrum Cephalosporin-Resistant Enterobacterales Early Urinary Tract Infection in Kidney Transplant Recipients: A Propensity Score-Matched Analysis

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Background: Urinary tract infection (UTI) is the most common bacterial infection after kidney transplantation (KT), leading to unfavorable clinical and allograft outcomes. Gram-negative uropathogenic bacteria are frequently encountered especially extended-spectrum cephalosporin-resistant (ESC-R) Enterobacterales (EB), causing UTI early after KT.

Methods: A retrospective single transplant study was conducted between January 2016 and December 2019. We performed 1:1 nearest-neighbor propensity score matching without replacement using recipient age, recipient sex, induction, transplant year, human leukocyte antigen, cold ischemia time, and panel-reactive antibody before analyses. Cumulative incidence of ESC-R EB early (within 14 days after KT) UTI was estimated by the Kaplan–Meier method. Risk factors for ESC-R EB early UTI were analyzed by a Cox proportional hazards model. Variables measured after transplantation were considered time-dependent covariates.

Results: We included 620 KT recipients (37% women; mean age ± SD, 43 ± 11 years). Overall, 64% and 76% received deceased-donor allograft and induction therapy. Sixty-five (10%) and 555 (90%) received carbapenems and cefuroxime peri-transplant prophylaxis, respectively. Early UTI occurred in 183 (30%) patients, 52% caused by ESC-R EB. Propensity score matching produced 65 well-balanced pairs. During a 14-day follow-up, the cumulative incidence of ESC-R EB early UTI was 5 and 28% in

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the carbapenems and cefuroxime groups, respectively (log-rank test = 0.003). Peri-transplant carbapenems prophylaxis was a protective factor against ESC-R EB after KT (hazard ratio, 0.19; 95% confidence interval, 0.05–0.64; $p = 0.008$). Clinical and allograft outcomes did not differ significantly between the groups.

Conclusions: In the setting where ESC-R EB UTI is common among KT recipients, carbapenems peri-transplant prophylaxis could protect against the occurrence of early ESC-R EB UTI after KT. Further prospective studies should focus on this specific infection prevention strategy.

Keywords: antibiotic prophylaxis, kidney transplantation, propensity score-matched analysis, extended-spectrum beta-lactamase, pyelonephritis

INTRODUCTION

Chronic kidney disease is a significant national public health problem in patients reaching end-stage renal failure. One of the most effective treatments is kidney transplantation (KT) (1). Despite noticeable progress in surgical procedures and immunosuppression after KT, urinary tract infection (UTI) remains an important problem in KT recipients (2–5). UTI is the most frequent infectious complication after KT, occurring in up to 86% of cases (6). Therefore, prevention of UTI must be considered for successful transplantation (7, 8).

The etiological pathogens in UTIs vary depending on environments, hosts' immune status, anatomical structure, virulence factors, or drug susceptibilities. In KT recipients, an emerging multi-drug resistant pathogen, especially Enterobacterales (EB), has been emerging and challenging in clinical practice (9). Uropathogenic *Escherichia coli* (UPEC) is a common bacterial pathogen causing UTIs in KT recipients. Several virulence factors have offered an opportunity to infect vulnerable hosts, mainly, Chaperone-Usher fibers (10, 11). Furthermore, fimbriae have been reported to affect biofilm growth, especially during the early course after KT since KT recipients are indwelled with a urinary catheter and ureteral stent placement (12, 13).

Extended-spectrum cephalosporin-resistant (ESC-R) EB such as *E. coli* and *Klebsiella pneumoniae* were pathogenic for UTI, causing high incidences of infection in KT patients in our setting and internationally (8, 14). Even when antibiotics are used peri-operatively, this pathogen can still survive because it has many mechanisms for antibiotic resistance. However, selecting an appropriate antibiotic is believed to decrease the chance of UTI from this particular bacterium (15). Cefuroxime has been utilized as routine perioperative prophylaxis for KT recipients at our center, although some clinicians sometimes switch to carbapenems due to a concern of an emerging ESC-R EB early UTI after the transplant lately at our institution. In the meantime, using broader spectrum antibiotics could place patients at risk of acquitting multi-drug resistant pathogens is concerning. Therefore, in the present study, we aimed to determine the impact of carbapenems given perioperatively on the incidence of ESC-R EB early UTI among KT recipients whether carbapenems proposed as an appropriate antibiotic that provide an adequate coverage could decrease the rate of ESC-R EB UTI in these vulnerable population.

MATERIALS AND METHODS

Population Study

We included all patients aged ≥18 years who were scheduled to undergo KT at Ramathibodi Hospital between January 2016 and December 2019. The patients were administered intravenous cefuroxime 1.5 g every 8 h or intravenous carbapenems with peri-transplant (first 24 h) prophylaxis started at 30 min before the incision. Patients who received antibiotics other than cefuroxime and carbapenems were excluded from the study. The primary objective was to investigate the effectiveness of carbapenems as prophylactic antibiotics in KT surgery compared with a routine antibiotic (cefuroxime) in preventing ESC-R EB early UTI in KT recipients. The secondary objective was to assess the clinical characteristics, other risk factors, and ESC-R EB early UTI outcomes in KT recipients.

UTI Definitions

UTI was defined in accordance with the guidelines from the American Society of Transplantation Infectious Diseases Community of Practice 2019 (**Supplementary Table S1**) (16). Early UTI was defined as UTI that occurred within 14 days after KT. All KT recipients were preemptively screened for UTI after surgery. Patients with asymptomatic bacteriuria were considered to have a UTI in this study because there is no definite recommendation for treatment of these patients and most responsible teams would provide an antibiotic for this condition during the perioperative period. Urine analysis and culture were performed on days 3, 5, 7, 10, and 14 after KT. Only early UTIs caused by *E. coli* and *K. pneumoniae* were evaluated for ESC-R EB. These organisms (including *E. coli* and *K. pneumoniae*) demonstrated resistance to ESC such as ceftriaxone or cefotaxime with a minimum inhibitory concentration (MIC) > 1 mg/L) according to the criteria of the Clinical and Laboratory Standards Institute (17). The Sensititre system (Thermo Fisher Scientific, Oakwood Village, OH) was used as an *in vitro* diagnostic product by the clinical and laboratory standard institute broth microdilution method for clinical susceptibility testing of EB.

The cefuroxime group comprised KT recipients who received cefuroxime for perioperative prophylaxis, while the carbapenem group comprised KT recipients who received meropenem, imipenem, or ertapenem for perioperative prophylaxis.

Data Collection

The following data were collected: demographic data, comprising sex, age, and etiology of end-stage renal disease; transplant factors, comprising year of transplant, type of allograft, type of immunosuppressants, human leukocyte antigen (HLA) mismatch number, percentage of panel reactive antibody (PRA), donor age and sex, and operation time; UTI data, comprising observed symptoms, type of UTI, pathogens, peri-transplant antibiotic prophylaxis, durations of urinary catheterization and stent after surgery, history of fever in donor, date of diagnosis, and date of initiation and discontinuation of treatment; and outcome data, comprising date of treatment termination, complications such as peri-allograft collection, bacteremia, total length of hospital stay, overall and UTI-related mortality, and allograft function.

Analyses

Data analyses were carried out using Stata version 12.0 software (StataCorp, College Station, TX) in both whole cohort and propensity score-matched cohort. One-to-one nearest-neighbor propensity score matching using recipient age, recipient sex, induction, transplant year, HLA match, PRA antibody, and cold ischemic time (CIT) without replacement was performed before analyses. The demographic analysis was based on reports of ESC-R EB UTI in patients in a descriptive analysis, and the demographic data were reported as percentage or median. Cumulative incidence of ESC-R EB UTI after KT was estimated by the Kaplan–Meier method. Risk factors for ESC-R EB UTI were analyzed by a Cox proportional hazards model. Variables measured after transplantation were considered time-dependent covariates. *P*-values < 0.05 were considered statistically significant. The study protocol was reviewed and approved by the Institutional Review Board of the Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (Approval number: ID MURA 2019/806).

RESULTS

Baseline Characteristics

A total of 691 KT recipients were identified during the study period (**Figure 1**), 620 eligible participants were included. Of those, 37 and 63% were women and men with a mean age ± SD of 43 ± 11 years. Overall, 64 and 76% received deceased-donor allograft and induction therapy. Sixty-five (10%) and 555 (90%) patients received carbapenem and cefuroxime peri-transplant prophylaxis, respectively.

After propensity score matching, there were 65 matched pairs between the two antibiotic prophylaxis groups. The baseline characteristics of the matched groups were well-balanced when evaluating standardized biases. Comparisons of the baseline characteristics between the cefuroxime and carbapenem prophylaxis groups before and after propensity score matching are shown in **Table 1.** The mean recipient age ± SD was 43 ± 11 years, 44% were women, and 56% were men. Most cases of end-stage renal disease occurred for unknown reasons, diabetic nephropathy, or IgA nephropathy. The baseline characteristics after propensity score matching did not differ significantly except for type of KT, because none of the living-related KT recipients received carbapenems for peri-operation prophylaxis.

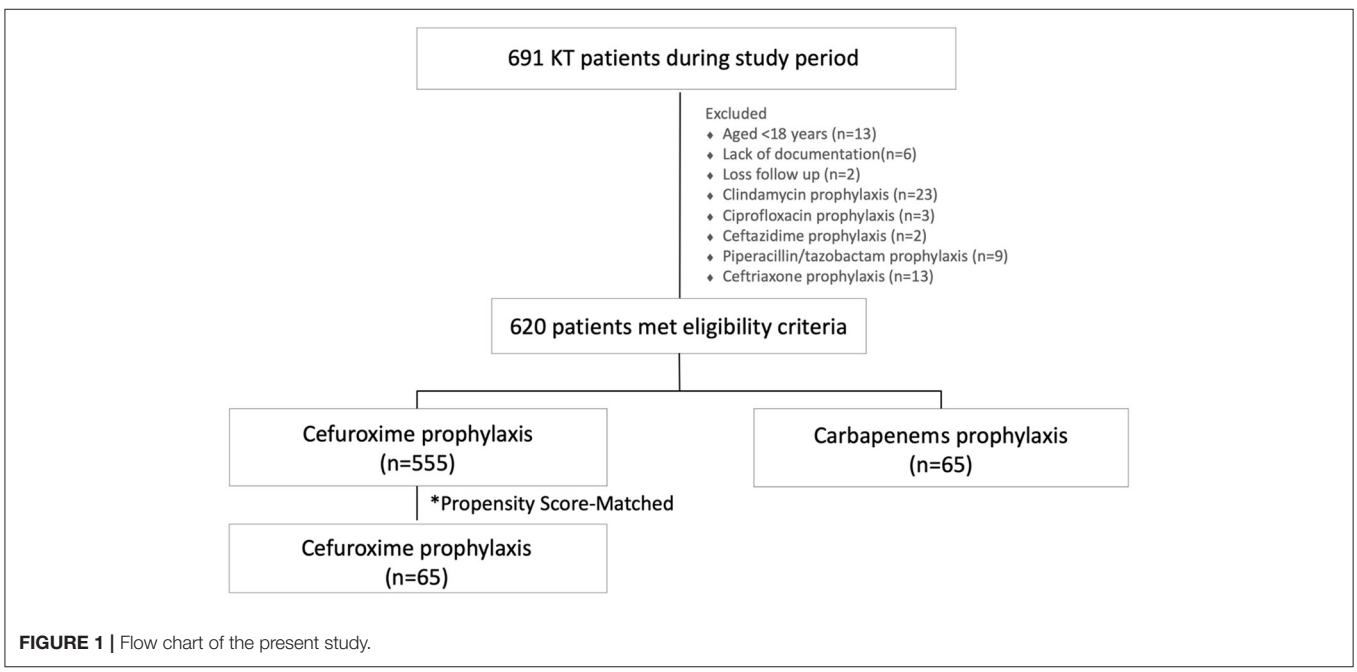

FIGURE 1 | Flow chart of the present study.

TABLE 1 | Baseline characteristics of the kidney transplant recipients.

Characteristic	Whole cohort			Propensity score-matched cohort*		
	Cefuroxime Group (*n* = 555)	Carbapenem group (*n* = 65)	*p*-value	Cefuroxime group (*n* = 65)	Carbapenem group (*n* = 65)	*p*-value
Recipient age (years), mean (SD)	43 (12)	41 (11)	0.345	43 (12)	41 (11)	0.592
Recipient sex			0.091			0.596
Female	197 (36)	30 (46)		27 (42)	30 (46)	
Male	358 (64)	35 (54)		38 (58)	35 (54)	
Induction therapy, *n* (%)	409 (74)	62 (95)	<0.001	62 (95)	62 (95)	>0.999
Year of KT, *n* (%)			<0.001			0.812
2016	145 (26)	10 (15)		14 (22)	10 (15)	
2017	137 (25)	33 (51)		32 (49)	33 (51)	
2018	138 (25)	18 (28)		15 (23)	18 (28)	
2019	135 (24)	4 (6)		4 (6)	4 (6)	
HLA mismatch groups, *n* (%)			<0.001			0.758
0, 1	148 (27)	2 (3)		3 (5)	2 (3)	
2, 3	341 (61)	60 (92)		61 (94)	60 (92)	
4, 5, 6	66 (12)	3 (5)		1 (2)	3 (5)	
CIT (h), median (IQR)	14 (0.5, 18)	17 (15, 21)	<0.001	18 (15, 21)	17 (15, 21)	0.730
PRA (%), *n* (%)			0.555			1.000
≤50	528 (95)	61 (94)		60 (92)	61 (94)	
>50	27 (5)	4 (6.2)		5 (8)	4 (6)	
ESKD cause, *n* (%)			0.413			0.345
Unknown	366 (66)	38 (58)		36 (55)	38 (58)	
DN	49 (9)	4 (6)		9 (14)	4 (6)	
MN	1 (0.2)	0 (0)		0 (0)	0 (0)	
IgA nephropathy	37 (7)	11 (17)		3 (5)	11 (17)	
IgM nephropathy	1 (0.2)	0 (0)		0 (0)	0 (0)	
MPGN	1 (0.2)	0 (0)		0 (0)	0 (0)	
CGN	26 (5)	1 (1.5)		2 (3)	1 (1.5)	
FSGS	11 (2)	1 (1.5)		3 (5)	1 (1.5)	
LN	19 (3)	4 (6)		5 (8)	4 (6)	
HTN	15 (3)	3 (5)		4 (6)	3 (5)	
Others	30(5.4)	3 (5)		3 (5)	3 (5)	
Donor age (years), mean (SD)	39 (13)	38 (15)	0.459	40 (13)	38 (15)	0.472
Donor female sex, *n* (%)	210 (39)	18 (28)	0.127	17 (26)	18 (28)	0.801
Type of KT, *n* (%)			<0.001			<0.001
LRKT	222 (40)	0 (0)		10 (15)	0 (0)	
DDKT	333 (60)	65 (100)		55 (85)	65 (100)	
Operation time (min), mean (SD)	280 (74)	292 (76)	0.224	280 (60)	292 (76)	0.301
Duration of urinary catheter (days), median (IQR)	6 (5–7)	6 (5–7)	0.971	6 (5–7)	6 (5–7)	0.805
Duration of stent (days), median (IQR)	15 (14–16)	15 (14–16)	0.525	15 (14–16)	15 (14–16)	0.698
ESC-R EB UTI, *n* (%)	94 (17)	3 (5)	0.01	18 (28)	3 (5)	<0.001
Non-ESC-R EB, *n* (%)	460 (83)	62 (95)		47 (72)	62 (95)	
Non-ESC-R EB UTI	74 (13)	11 (17)		7 (11)	11 (17)	
No UTI	386 (70)	51 (78)		40 (61)	51 (78)	

**Propensity scores were calculated by logistic regression on two groups (carbapenem/cefuroxime) using a set of characteristics including recipient age, recipient sex, induction, transplant year, HLA, CIT, and PRA and grouped into 10 categories for 1:1 matching (carbapenem-to-cefuroxime).*

SD, standard deviation; KT, kidney transplantation; HLA, human leukocyte antigen; CIT, cold ischemic time; PRA, panel-reactive antibody; ESKD, end-stage kidney disease; DN, diabetic nephropathy; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; CGN, chronic glomerulonephritis; FSGS, focal segmental glomerulosclerosis; LN, lupus nephritis; HTN, hypertension; LRKT, living-related kidney transplantation; DDKT, deceased-donor kidney transplantation; IQR, interquartile range; UTI, urinary tract infection; ESC-R EB, extended-spectrum cephalosporin-resistant Enterobacterales.

ESC-R EB Early UTI

After KT, early UTI occurred during the follow-up period in 182 (29%) patients in a whole cohort. Those included *E. coli* ($n = 122$), *K. pneumoniae* ($n = 17$), *Pseudomonas aeruginosa* ($n = 9$), *Enterobacter* spp. ($n = 1$), *Enterococcus* spp. ($n = 19$), *Staphylococcus* spp. ($n = 11$), *Streptococcus* spp. ($n = 1$), *Proteus mirabilis* ($n = 5$), *Candida* spp. ($n = 12$), and *Cryptococcus* spp. ($n = 1$). There were 167 (92%) and 15 (8%) patients developed monomicrobial and polymicrobial UTI, respectively. Of the latter, one patient had three isolated organisms. ESC-R EB accounted for 52% of the UTI cases. *E. coli* were susceptible to ertapenem (100%), meropenem (100%), amikacin (99%), piperacillin/tazobactam (92%), cefepime (67%), ceftazidime (63%), ceftriaxone (50%), cefotaxime (49%), amoxicillin/clavulanic acid (76%), trimethoprim/sulfamethoxazole (47%), levofloxacin (44%), and ciprofloxacin (43%). *K. pneumoniae* were susceptible to ertapenem (93%), meropenem (93%), amikacin (97%), piperacillin/tazobactam (79%), cefepime (75%), ceftazidime (67%), ceftriaxone (69%), cefotaxime (65%), amoxicillin/clavulanic acid (68%), trimethoprim/sulfamethoxazole (59%), levofloxacin (69%), and ciprofloxacin (65%).

Of those cases, 74 and 26% % had asymptomatic bacteriuria and symptomatic UTI, respectively. Among those with urinary symptoms, 3% had cystitis, and 23% had acute allograft pyelonephritis (**Figure 2A**). ESC-R EB accounted for the majority (54%) of the UTI cases. Of these cases, 76% had asymptomatic bacteriuria and 24% had symptomatic UTI (cystitis 5%, pyelonephritis 19%) (**Figure 2B**).

In the cox proportional hazards model for factors associated with ESC-R EB UTI within 14 days after KT in the whole cohort (**Table 2**), cefuroxime use at peri-transplant period and female sex were associated with early ESC-R EB UTI in univariate analysis (hazard ratio [HR] 0.19; 95% confidence interval [CI], 0.05–0.75; $p = 0.019$ and HR 3.11; 95%CI, 2.03–4.77; $p < 0.001$). In multivariate analysis, carbapenems and female KT recipients remained independently associated with early ESC-R EB UTI (HR 0.14; 95%CI, 0.04–0.59; $p = 0.007$ and HR 3.24; 95%CI, 2.10–5.00 $p < 0.001$).

After propensity score matching, the distributions of pathogens were *E. coli* ($n = 24$), *K. pneumoniae* ($n = 4$), *Pseudomonas aeruginosa* ($n = 2$), *Enterococcus* spp. ($n = 6$), *Staphylococcus* spp. ($n = 2$), *Proteus mirabilis* ($n = 1$), *Candida* spp. ($n = 1$), and *Cryptococcus* spp. ($n = 1$). There were 126 (97%) and 4 (3%) patients who were diagnosed with monomicrobial and polymicrobial UTI, respectively. ESC-R EB accounted for the majority (54%) of the UTI cases. Of these cases, 69% had asymptomatic bacteriuria and 31% had symptomatic UTI (cystitis 7%, pyelonephritis 24%) (**Figure 2B**). There were 3 (5%) KT recipients in the carbapenem group had ESC-R EB UTI, compared with 18 (28%) KT recipients with the cefuroxime group ($p < 0.001$) (**Table 1**). The cumulative incidences of ESC-R EB UTI estimated by the Kaplan–Meier method were 0.33% per day (5% per 14 days) in the carbapenem group and 2.20% per day (28% per 14 days) in the cefuroxime group (log-rank test = 0.003) (**Figure 3**).

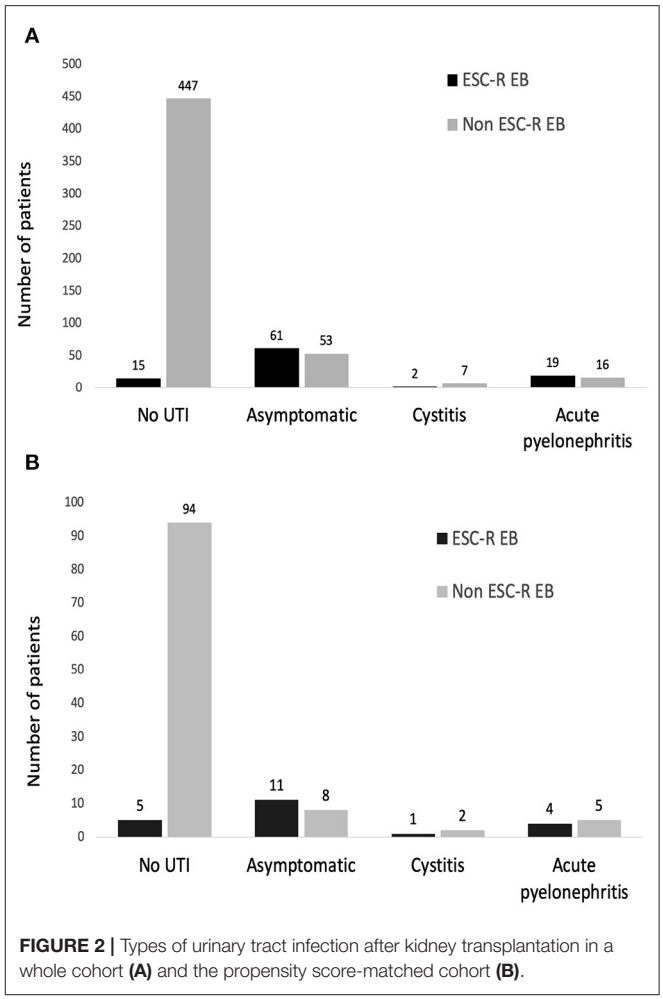

FIGURE 2 | Types of urinary tract infection after kidney transplantation in a whole cohort **(A)** and the propensity score-matched cohort **(B)**.

In the Cox proportional hazards model, antibiotic prophylaxis was identified as a risk factor significantly associated with ESC-R EB UTI within 14 days after KT. In addition, carbapenems were found to be a protective factor against ESC-R EB UTI compared with cefuroxime (HR, 0.19; 95%CI, 0.05–0.64; $p = 0.008$) (**Table 3**).

Outcomes

In the whole cohort ($n = 620$), There were no differences between groups in composite outcomes (such as bacteremia, pyelonephritis, perinephric collection, nephrectomy, slow graft function, delayed graft function, and early allograft dysfunction). However, the duration of antibiotic use and length of hospital stay was significantly longer in the ESC-R EB UTI group.

In a propensity score-matched cohort, there were no differences in composite outcomes between KT recipients who did and did not develop ESC-R EB UTI (**Table 4**). However, KT recipients who developed ESC-R EB UTI had significantly increased median duration of antibiotic use (8 days; interquartile

TABLE 2 | Cox proportional hazards model for factors associated with ESC-R EB UTI within 14 days after kidney transplantation in a whole cohort ($n = 620$).

Factor	ESC-R EB UTI group ($n = 97$)	Non-ESC-R EB UTI group ($n = 523$)	Univariate analysis		Multivariate analysis	
			Hazard ratio (95%CI)	*p*-value	Adjusted hazard ratio (95%CI)	*p*-value
Drug, *n* (%)						
Cefuroxime	94 (17)	461 (83)	Reference		Reference	
Carbapenems	3 (5)	62 (95)	0.19 (0.05–0.75)	0.019	0.14 (0.04–0.59)	0.007
Recipient age (year)	42 (12)	43 (11)	0.99 (0.97–1.01)	0.123		
Recipient sex, *n* (%)						
Male	38 (10)	355(90)	Reference		Reference	
Female	59(26)	168(74)	3.11 (2.03–4.77)	<0.001	3.24 (2.10–5.00)	<0.001
ESKD cause, *n* (%)						
Unknown	37(17)	179(83)	Reference			
Known	60(15)	344(85)	0.96 (0.62–1.48)	0.857		
Donor age (year), mean (SD)	40(14)	39(14)	1.00 (0.98–1.01)	0.919		
Donor sex, *n* (%)						
Male	59(15)	332(85)	Reference			
Female	38(17)	190(83)	1.12 (0.73–1.72)	0.596		
History of donor fever, *n* (%)						
No	55(17)	267(83)	Reference			
Yes	42(14)	255(86)	0.88 (0.58–1.34)	0.558		
CIT (hours), median (IQR)	14.5 (0.5–19)	15 (0.6–8.5)	1.00 (0.98–1.02)	0.943		
Operation time(min), mean (SD)	281 (68)	281 (76)	1.00 (0.92–1.09) per 30-min increment	0.968		
HLA mismatch, *n* (%)						
0, 1	20 (13)	130(87)	Reference			
2, 3	64(16)	337(84)	1.14 (0.68–1.91)	0.620		
4, 5, 6	13(19)	56(81)	1.09 (0.72–2.03)	0.359		
PRA, *n* (%)						
≤50%	89 (15)	500/ (85)	Reference		Reference	
>50%	8 (26)	23 (74)	1.71 (0.79–3.70)	0.173	1.09 (0.50–2.38)	0.836
Induction, *n* (%)						
No	15 (10)	134 (90)	Reference		Reference	
Yes	82 (17)	389 (83)	1.60 (0.92–2.79)	0.096	1.74 (0.99–3.04)	0.053

ESC-R EB, extended-spectrum cephalosporin-resistant Enterobacterales; ESKD, end-stage kidney disease; UTI, urinary tract infection; CI, confidence interval; CIT, cold ischemic time; HLA, human leukocyte antigen; LRKT, living-related kidney transplantation; PRA, panel-reactive antibody; DDKT, deceased-donor kidney transplantation; IQR, interquartile range.

range, 7–14 days; $p < 0{,}001$). Furthermore, the rate of bacteremia was significantly higher in patients with ESC-R EB UTI than in patients without (24 vs. 1%, $p < 0.001$). Overall, 1 and 0% developed allograft loss and died, respectively.

DISCUSSION

We have reported a propensity score-matched cohort study involving adult KT recipients with similar baseline characteristics in a setting where an ESC-R UTI has been emerging among KT recipients. The results revealed that patients who received carbapenem perioperative prophylaxis had significantly decreased incidence of ESC-R EB UTI within 14 days after KT compared with patients who routinely received cefuroxime perioperative prophylaxis. The incidence of ESC-R EB infection increased the antibiotic duration and likely led to bloodstream infection after transplantation.

UTI is a common complication after KT that was reported to occur in 34–42% of cases (18, 19), similar to the incidence of 30% in the present cohort. A recent retrospective study revealed a significantly high incidence of UTI after KT. Specifically, approximately half of the patients developed UTI within the first month postoperatively, with a median onset of 5 days. Although female sex was identified as an independent factor for UTI (within 1 month after KT) among KT recipients in a single transplant center (20), which is comparable to the results of an analysis in a whole cohort, therefore, we decided to match gender as one of the factors to omit the confounding effect and truly investigate the effect of antibiotic agents. Additionally, KT recipients are subjected to innate immunity impairment, mainly toll-like receptors (TLR). TLR2,

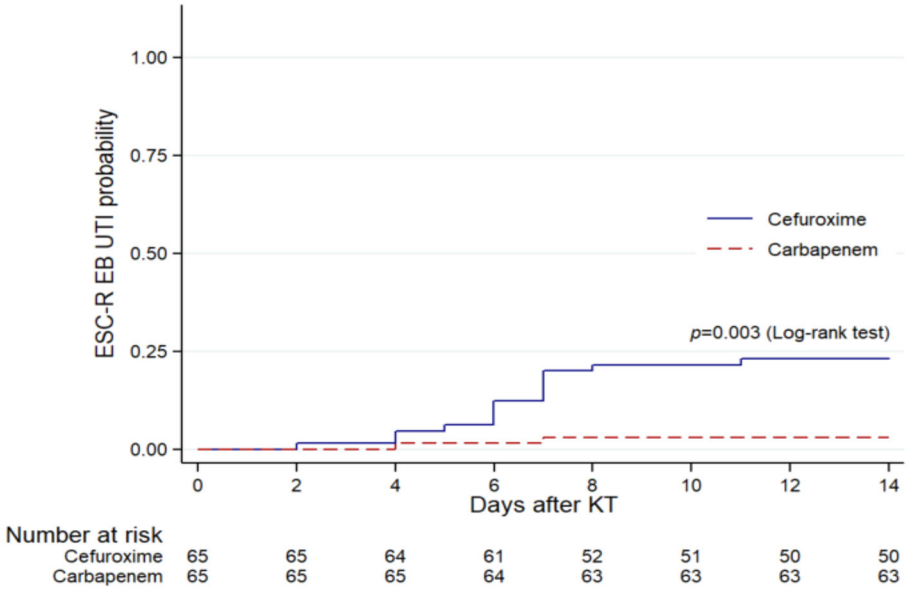

FIGURE 3 | Kaplan–Meier plots of ESC-R EB UTI within 14 days after KT in the cefuroxime and carbapenem groups within the propensity score-matched cohort ($n = 130$).

TABLE 3 | Cox proportional hazards model for factors associated with ESC-R EB UTI within 14 days after kidney transplantation in the propensity score-matched cohort ($n = 130$).

Factors *n* (%)	ESC-R EB UTI group ($n = 21$)	Non-ESC-R EB UTI group ($n = 109$)	Univariate analysis	
			Hazard ratio (95% CI)	*p*-value
Perioperative prophylaxis				
Cefuroxime	18 (28)	47 (72)	Reference	
Carbapenems	3 (5)	62 (95)	0.19 (0.05–0.64)	0.008
History of donor fever				
No	7 (25)	21 (75)	Reference	
Yes	14 (14)	88 (86)	0.85 (0.31–2.36)	0.757
Allograft type				
LRKT	4 (40)	6 (60)	Reference	
DDKT	17 (14)	103 (86)	1.32 (0.25–6.94)	0.742
Operation time (min) (per 30 min)	276 (58)	288 (70)	0.88 (0.69–1.13)	0.318

ESC-R EB, extended-spectrum cephalosporin-resistant Enterobacterales; UTI, urinary tract infection; CI, confidence interval; LRKT, living-related kidney transplantation; DDKT, deceased-donor kidney transplantation.

TLR4, and TLR5 are essential molecules in innate immunity to defend against pathogens in the genitourinary tract, and calcineurin inhibitors, especially tacrolimus, have been reported to decrease TLR5 expression in bladder macrophages while developing UTI (21, 22). Furthermore, most pathogens were Gram-negative bacilli which lately have been broader resistant to available antibiotics (20). Therefore, comprehensive prevention and treatment measures against known risk factors should be undertaken as early as possible to reduce UTI incidence. Due to a lacking of new effective antibiotics for multi-drug resistant Gram-negative pathogens, the properties of TLRs, as mentioned above, could provide an opportunity for ligand-drug alternatives (23).

A large meta-analysis of KT recipients found an overall rate of 10% for development of extended-spectrum beta-lactamase (ESBL)-producing EB UTI; however, this number could be as high as 33% among Asian KT recipients (24). A retrospective study from a single transplant center in China revealed rate of *E. coli* UTI in KT recipients of 12.5%, of that 73% were identified as having ESBL-producing pathogen and 64% carried adhesions-coding gene (25). In comparison, the incidence of ESC-R EB UTI in our cohort was relatively higher at 16%.

TABLE 4 | Outcomes of the kidney transplant recipients in the propensity score-matched cohort.

Factors	ESC-R EB group ($n = 21$)	Non-ESC-R EB group ($n = 109$)	*p*-value
Duration of antibiotic (days), median (IQR)	8 (7–14)	0 (0–0)	<0.001
Composite complications, n (%)	8 (38)	30 (28)	0.329
Bacteremia	5 (24)	1 (1)	<0.001
Peri-allograft collection	1 (5)	6 (5.5)	>0.999
Recurrent UTI	0 (0)	0 (0)	>0.999
Nephrectomy	0 (0)	1 (1)	>0.999
Delayed allograft function	4 (19)	25 (23)	>0.999
Total length of hospital stays (days), median (IQR)	19 (15–21)	15 (11–23)	0.123
Hospital mortality, *n* (%)	0 (0)	0 (0)	>0.999
Allograft function: GFR (mL/min/1.73 m^2), mean (SD)			
On day of discharge	58.1 (23.5)	54.7 (20.0)	0.488
At day 30	60.0 (23.2)	57.7 (21.9)	0.666
At day 365	57.5 (22.6)	57.7 (24.6)	0.966

ESC-R EB, extended-spectrum cephalosporin-resistant Enterobacterales; IQR, interquartile range; GFR, glomerular filtration rate; SD, standard deviation.

A previous retrospective propensity score-matched study revealed no significant impact of carbapenems compared with any other regimens within the first 72 h in preventing ESBL-producing EB bloodstream infection (26). Our recent study showed that carbapenems did not prevent ESC-R EB bloodstream infection (27). However, ESC-R EB was found to be the leading causative pathogen (50%) for bacteremia within the first year after KT, and 85% of the pathogens were considered to be derived from genitourinary sources (27).

ESC-R EB UTI was presented to be a cause of prolonged antibiotic duration both before and after matched analysis; these data emphasized a significant burden of antimicrobial stewardship, which could conserve patients' microbiome and avoid dysbiosis. However, the hospital stay was longer in the whole cohort, which was not substantially different in the propensity score-matched cohort. Therefore, the length of hospitalization is subjected to multiple factors, including infections and other transplant-related issues. ESC-R EB UTI was found to be associated with high mortality rates in several previous studies (28–30), while our results indicated that it did not increase mortality. Additionally, we also did not observe an increasing rate of carbapenem-resistant Enterobacterales, especially Metallo-ß-Lactamases, in our setting, which could threaten an emerging difficult-to-treat bacterial genitourinary tract infection in these vulnerable populations (31). Although no deaths were recorded during ESC-R EB-related UTI in our cohort, one patient developed allograft loss, and the rate of bacteremia was significantly higher in patients with ESC-R EB UTI, which could place patients at risk of unfavorable outcomes and morbidities.

The main strengths of the present study were the data from a large KT center with a high prevalence of ESC-R EB UTI and the presence of a preemptive urine analysis and culture monitoring protocol. This would have allowed us to retrieve the whole spectrum of UTI, including asymptomatic and symptomatic cases. However, our study had some limitations. The first was its retrospective design, which means that some of the collected patient data in the medical records may have been missing. The second was that the data did not enable us to postulate an effective method against ESBL-producing EB because phenotypic tests were not conducted to confirm ESBL enzyme production. The third was that although a relatively large number of participants were enrolled, the number of participants after matching was limited, which could have prevented us from exploring other risk factors. However, we corrected the unbalanced background characteristics between the two groups to accurately evaluate the effectiveness of carbapenems in preventing ESC-R EB UTI. Finally, the follow-up time was relatively short. Therefore, a prospective study design with a larger number of matched patients together with a more extended follow-up period would yield more statistically significant differences.

Furthermore, the American Society of Transplantation Infectious Diseases Community of Practice recommended using single first-generation cephalosporins such as cefazolin for perioperative prophylaxis in KT recipients (32). Instead, our data propose the potential use of antibiotic prophylaxis based on local epidemiology, predominant with ESC-R EB early UTI, which could lead to post-surgical complications.

In conclusion, ESC-R EB UTI is a potentially serious condition that can arise after KT. Consequently, prevention of this infection should be considered. In a well-balanced retrospective analysis, the present study showed that administration of carbapenem peri-transplant prophylaxis can significantly protect against ESC-R EB UTI early after KT. Appropriate antibiotics coverage during the peri-transplant period could potentially omit infection among KT recipients. Further prospective studies should focus on this particular infection prevention strategy. Furthermore, non-pharmacological interventions such as early urinary prosthesis removal should be encouraged to avoid complicated UTIs from the uropathogenic pathogen.

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

The studies involving human participants were reviewed and approved by Faculty of Medicine Ramathibodi

Hospital, Mahidol University (MURA 2019/806). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JB conceptualized and designed the study. SA, NA, PC, and JB collected the data. SA and JB analyzed data and draft manuscript. All authors reviewed and approved a final version of the manuscript.

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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.2022.841293/full#supplementary-material

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Risk Factors for Postoperative Pneumonia: A Case-Control Study

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Background: Postoperative pneumonia is a preventable complication associated with adverse outcomes, that greatly aggravates the medical expenses of patients. The goal of our study is to identify risk factors and outcomes of postoperative pneumonia.

Methods: A matched 1:1 case-control study, including adult patients who underwent surgery between January 2020 and June 2020, was conducted in the Second Affiliated Hospital of Kunming Medical University in China. Cases included all patients developing postoperative pneumonia within 30 days after surgery, defined using consensus criteria. Controls were selected randomly from the matched eligible population.

Results: Out of 17,190 surgical patients, 264 (1.54%) experienced postoperative pneumonia. Increased age, chronic obstructive pulmonary disease, emergency surgery, postoperative reduced albumin, prolonged ventilation, and longer duration of bed rest were identified as significant risk factors independently associated with postoperative pneumonia. Regarding prognostic implications, postoperative pneumonia was associated with longer length of hospital stay, higher ICU occupancy rate, higher unplanned re-operation rate, and higher in-hospital mortality rate. Postoperative pneumonia was most commonly caused by Gram-negative pathogens, and multidrug resistant bacteria accounted for approximately 16.99% of cases.

Conclusions: Postoperative pneumonia is associated with severe clinical outcomes. We identified six independent risk factors that can aid in risk stratification and management of patients at risk of postoperative pneumonia, and the distribution of causative pathogens can also help in the implementation of effective interventions.

Clinical Trial Registration: www.chictr.org.cn, identifier: chiCTR2100045986.

Keywords: postoperative pneumonia, perioperative, risk factors, pathogen distribution, outcomes

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BACKGROUND

Every year, more than 300 million patients worldwide undergo surgery (1). Estimates of procedure-related mortality in surgical patients range from 1 to 4%, of which more than one-fifth are due to perioperative complications, with an incidence ranging from 3 to 16% (1, 2). Studies have shown (3) that almost half of perioperative complications can be effectively prevented, and the current incidence of permanent disability or death caused by these complications still accounts for 0.4% to 0.8%. Even with timely treatment, related complications will still reduce the long-term survival time of surgical patients.

Postoperative pneumonia (POP) is one most common complication of these and it is defined as hospital-acquired pneumonia or ventilator-associated pneumonia in post-surgical patients. Currently, postoperative pneumonia has the highest incidence of hospital-acquired pneumonia in the world, accounting for approximately 50% of all nosocomial pneumonias, with an incidence of 1.5 to 15.8% (4–7). Postoperative pneumonia can adversely affect the outcomes of surgical patients and may even threaten their lives. Mortality related to postoperative pneumonia among surgical patients has been reported to range from 20 to 50%, and the mortality rate varies by the type of surgery (8). Studies have shown that the fatality rate caused by postoperative pneumonia can be up to 9–50%, and even after risk adjustment, the patients' 5-year survival rate after surgery is reduced by 66% (9). Among the remaining survivors, there is also evidence that postoperative pneumonia adversely affects the patients' early postoperative recovery and late quality of life. In addition, postoperative pneumonia can significantly prolong the hospital stay of surgical patients and significantly increase their postoperative ICU occupancy rate, readmission rate, reoperation rate and mortality rate (8, 9), which greatly aggravate the burden of medical expenses of patients and leads to an average increase by approximately 2–10 times of additional medical expenses (5, 9).

Therefore, it is obviously worthwhile to identify the perioperative risk factors for postoperative pneumonia and investigate the distribution of causative bacteria. The result would suggest the measures for risk reduction through action on modifiable factors, or increase vigilance in the presence of non–modifiable conditions. The result of causative bacteria could also aid in selection of antibiotics for post-infection treatment especially considered against the worldwide escalation of infection caused by multidrug resistant microorganisms. Our primary aim was to identify perioperative risk factors and outcomes of postoperative pneumonia. Our secondary aim was to investigate the distribution of causative bacteria and surgical specialty.

METHODS

Study Design and Participants

This study protocol was approved by the Institutional Ethics Committee of the Second Affiliated Hospital of Kunming Medical University (Kunming, China, approval number: PJ-2021-39). Informed consent was waived due to the retrospective design of the study. The study was registered in the Chinese Clinical Trial Registry (Clinical Trials identifier: ChiCTR2100045986). This study is a single-center retrospective 1:1 case-control study. From the hospital's complete electronic medical record "Donghua", a total of 17,190 patients who underwent surgery from January 1, 2020 to June 31, 2020 were included. The case group included all adult patients who followed for hospital acquired pneumonia (HAP) occurrence for 30 days after surgery. Controls were matched by surgical specialty and randomly selected at 1:1 from the remaining surgical patients without pneumonia. Exclusion criteria were age under 18 years, procedures outside an operating room, patients already intubated, procedures related to postoperative complications of previous surgery, outpatient procedures (hospital stay <24 h), patient's medical records missing or inadequate.

Diagnosis of Pneumonia

The US Centers for Disease Control definition of pneumonia was used (7). Two or more serial chest radiographs with at least one of the following (one radiograph is sufficient for patients with no underlying pulmonary or cardiac disease): (i) New or progressive and persistent infiltrates, (ii) consolidation, (iii) cavitation; and at least one of the following: (a) fever ($>38^{\circ}$C) with no other recognized cause, (b) leucopenia (white cell count $< 4 \times 10^9$ liter^{-1}) or leukocytosis (white cell count $> 12 \times 10^9$ liter^{-1}), (c) for adults $>$ 70 years old, altered mental status with no other recognized cause; and at least two of the following: (a) new onset of purulent sputum or a change in character of the sputum, or increased respiratory secretions, or increased suctioning requirements, (b) new onset or worsening cough, or dyspnea, or tachypnea, (c) rales or bronchial breath sounds, (d) worsening gas exchange (hypoxemia, increased oxygen requirement, increased ventilator demand).

Data Collection

Perioperative data were collected retrospectively. Demographic factors, including age, sex, weight, height, body mass index (BMI) and factors assessing general condition [such as the Glasgow Coma Scale (GCS) and American Society of Anesthesiologists (ASA) classification], were recorded. Patients' past medical history, including smoking, drinking, hypertension, diabetes, malignancy, stroke, chronic obstructive pulmonary disease (COPD), coronary heart disease, liver disease and renal dysfunction was assessed. Laboratory measurements were reviewed as last values before operation or first values after operation, such as albumin, hemoglobin (Hb), blood urea nitrogen and creatinine levels. Factors associated with surgery, including surgical specialty, duration of surgery, type of surgery (scheduled or emergency), surgery period (day or night), were also evaluated. Intraoperative variables, including blood loss, red blood cells (RBC) transfusions, human albumin infusion, amount of liquid input and invasive procedure (such as radial artery cannulation, deep vein catheterization and gastric tube intubation) were recorded. Additionally, perioperative factors pertaining to the respiratory system, such as the duration of mechanical ventilation (duration until tracheal extubation) and duration of bed rest (duration until patients' first off-bed activity) were also evaluated. Causative bacteria and Multi-drug-resistance (MDR, defined as non-susceptibility to at least one agent in three or more antimicrobial categories) were recorded. To assess the prognostic implications of postoperative pneumonia, length of hospital stay, admission to the intensive care unit (ICU) and in-hospital mortality rates were reviewed.

Abbreviations: POP, postoperative pneumonia; HAP, hospital acquired pneumonia; BMI, body mass index; GCS, Glasgow Coma Scale; ASA, American Society of Anesthesiologists; COPD, chronic obstructive pulmonary disease; Hb, hemoglobin; RBC, red blood cells; MDR, Multi-drug-resistance; ICU, Intensive Care Unit; OR, odds ratio; CI, confidence interval.

TABLE 1 | Distribution of postoperative pneumonia in each surgical specialty.

Surgical specialty	The operation frequency	Frequency of POP	Rate of POP
Neurosurgery	600	102	17.00%
Hepatobiliary	2,305	52	2.26%
General and digestive	1,300	35	2.69%
Thoracic	300	26	8.67%
Urology	2,624	16	0.61%
Obstetric	1,450	8	0.55%
Traumatology	542	6	1.11%
Gynecology	593	4	0.67%
Cardiac and vascular	218	5	2.29%
Burns	357	4	1.12%
Ear–nose–throat (ENT)	456	2	0.44%
Neurology	367	2	0.54%
Orthopedic	851	1	0.12%
Other	2,227	0	0%

TABLE 2 | Distribution and ratio of pathogens.

Pathogens	Isolates ($N = 153$)	Ratio (%)
Gram-negative bacteria	98	64.05
Klebsiella pneumoniae	34	22.22
Escherichia coli	18	11.76
Acinetobacter baumannii	13	8.50
Pseudomonas aeruginosa	8	5.23
Stenotrophomonas maltophilia	5	3.27
Other enterobacteria	20	13.07
Gram-positive bacteria	42	27.45
Staphylococcus aureus	14	9.15
Staphylococcus hemolyticus	4	2.61
Staphylococcus epidermidis	5	3.27
Streptococcus	7	4.58
Enterococcus	12	7.84
Fungi	13	8.50
Candida albicans	8	5.23
Other candida	5	3.27

Statistical Analysis

In this study, the mean and standard deviation ($x \pm s$) were used to represent the measurement data conforming to a normal distribution and a homogenous variance, and the independent sample *t*-test was used for comparisons between the case group and the control group. The median (interquartile range) was used to represent the measurement data with a non–normal distribution, and the two groups were compared by the rank-sum test. All enumeration data were represented by frequency and percentage, and the two groups were compared by X^2 test. All statistically significant factors on univariate analysis were selected for inclusion in the multivariate regression analysis conducted by a binary logistic regression analysis model. Bivariate odds ratios (OR) and 95% confidence intervals (CI) were also estimated. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software (version 24, SPSS Inc., United States).

RESULTS

Patient Characteristics

A total of 17,190 surgical patients were selected for the study, of which 264 patients were diagnosed with postoperative pneumonia. Overall, the incidence of postoperative pneumonia was 1.54% (264/17,190). **Table 1** summarizes the operative frequency and the incidence of postoperative pneumonia in each surgical specialty. Among patients undergoing neurosurgery, the frequency (102/600) and incidence (17.00%) of postoperative pneumonia were much higher than the others, followed by the thoracic (8.67%), general and digestive (2.69%), cardiac and vascular (2.29%), and hepatobiliary (2.26%). The postoperative pneumonia rates of these five surgical specialty all exceeded 2%.

Causative Pathogens

The distribution of pathogenic bacteria is shown in **Table 2**. A total of 153 different strains of pathogens were isolated from the sputum specimens of patients with postoperative pneumonia by coculture. Among which 98 isolates (64.05%) were Gram-negative bacteria, 42 isolates (27.45%) were Gram-positive bacteria, and 13 isolates (8.50%) were fungi. The main pathogens were *Klebsiella pneumoniae* (22.22%, 34/153), followed by *Escherichia coli* (11.76%, 18/153), *Staphylococcus aureus* (9.15%, 14/153) and *Acinetobacter baumannii* (8.50%, 13/153). In addition, the results showed that a total of 26 isolates (16.99%) were multidrug resistant bacteria, of which 13 isolates (50%) were carbapenem-resistant Enterobacteriaceae, 8 isolates (30.77%) were carbapenem-resistant *Acinetobacter baumannii*, 3 isolates (11.54%) were methicillin-resistant *Staphylococcus aureus*, and 2 isolates (7.69%) were carbapenem-resistant *Pseudomonas aeruginosa*. We found that 73.08% (19/26) of the multidrug-resistant bacteria were isolated from neurosurgery patients.

Univariate Analysis

Significant risk factors associated with postoperative pneumonia on univariate analysis are presented in **Table 3**. We found 25 perioperative risk factors that were significantly associated with postoperative pneumonia ($P < 0.05$), as follows: age, sex, BMI, smoking, drinking, hypertension, diabetes, malignancy, COPD, coma (GCS < 8), Surgical difficulty classification criteria, duration of surgery, emergency surgery, night operation, intraoperative blood loss, ASA physical status, duration of ventilation, deep vein catheterization, gastric tube intubation, amount of intraoperative liquid input, intraoperative RBC transfusion, postoperative hemoglobin level, postoperative albumin level and duration of postoperative bed rest.

TABLE 3 | Risk factors for POP—univariate analysis.

Risk factors	Cases (*N* = 264)	Controls (*N* = 264)	*P* value
Age (yr)	54.70 ± 14.85	48.73 ± 14.86	<0.001
Age grading			
≥60 yr	102 (38.64%)	59 (22.35%)	<0.001
<60 yr	162 (61.36%)	205 (77.65%)	
Sex			
Male	155 (58.71%)	132 (50.0%)	0.044
Female	109 (41.29%)	132 (50.0%)	
Weight (kg)	61.12 ± 10.88	60.35 ± 10.25	0.408
BMI			
≥24 kg/m2	103 (46.8%)	92 (35.4%)	0.011
<24 kg/m2	117 (53.2%)	168 (64.6%)	
ASA physical status			
≥ 3	143 (54.2%)	106 (40.2%)	0.001
< 3	121 (45.8%)	158 (59.8%)	
Coma (GCS < 8)	34 (12.9%)	5 (1.9%)	<0.001
Smoking	112 (42.4%)	83 (31.4%)	0.009
Drinking	91 (34.5%)	66 (25.0%)	0.017
Hypertension	81 (30.7%)	52 (19.7%)	0.004
Diabetes	28 (10.6%)	14 (5.3%)	0.024
Malignancy	86 (32.6%)	52 (19.7%)	0.001
COPD	33 (12.5%)	6 (2.3%)	<0.001
Coronary heart disease	7 (2.7%)	6 (2.3%)	0.779
Stroke	15 (5.7%)	8 (3.0%)	0.136
Liver disease	7 (2.7%)	10 (3.8%)	0.460
Renal dysfunction	6 (2.3%)	2 (0.8%)	0.154
Preoperative prophylactic antimicrobial use	254 (96.2%)	257 (97.3%)	0.460
Preoperative hemoglobin			
<100 g/L	19 (7.2%)	15 (5.7%)	0.478
≥100 g/L	245 (92.8%)	249 (94.3%)	
Postoperative hemoglobin			
<100 g/L	87 (33.0%)	44 (16.7%)	<0.001
≥100 g/L	177 (67.0%)	220 (83.3%)	
Preoperative albumin			
<35 g/L	47 (17.8%)	40 (15.2%)	0.412
≥35 g/L	217 (82.2%)	224 (84.8%)	
Postoperative albumin			
<35 g/L	198 (75.0%)	117 (44.3%)	<0.001
≥35 g/L	66 (25.0%)	147 (55.7%)	
Surgical difficulty classification criteria			
≥ 4	182 (68.9%)	151 (57.2%)	0.005
<3	82 (31.1%)	113 (42.8%)	
Duration of surgery (h)	4.67 ± 3.20	3.40 ± 2.18	<0.001
≥ 3 h	178 (67.4%)	132 (50.0%)	<0.001
< 3 h	86 (32.6%)	132 (50.0%)	
Emergency surgery	58 (22.1%)	19 (7.2%)	<0.001
Night operation	66 (25.0%)	12 (4.5%)	<0.001
Intraoperative blood loss (ml)	586.1 ± 1,428.0	243.2 ± 256.6	<0.001
≥ 400 ml	107 (40.5%)	67 (25.4%)	<0.001
<400 ml	157 (59.5%)	197 (74.6)	
Intraoperative RBC transfusion	48 (18.2%)	19 (7.2%)	<0.001
Intraoperative albumin infusion	16 (6.1%)	21 (8.0%)	0.394
Amount of intraoperative liquid			
≥ 4,000 ml	104 (39.4%)	56 (21.2%)	<0.001
<4,000 ml	160 (60.6%)	208 (78.8%)	
Radial artery cannulation	239 (90.5%)	233 (88.3%)	0.396
Deep vein catheterization	196 (74.2%)	148 (56.1%)	<0.001
Gastric tube intubation	84 (31.8%)	52 (19.7%)	0.001
Duration of ventilation			
≥ 24 h	81 (30.7%)	16 (6.1%)	<0.001
<24 h	183 (69.3%)	248 (93.9%)	
Duration of postoperative bed rest			
≥ 3 days	177 (67.0%)	86 (32.6%)	<0.001
<3 days	87 (33.0%)	178 (67.4%)	

BMI, Body mass index; ASA, American Society of Anaesthesiologists physical status classification; GCS, Glasgow Coma Scale; COPD, chronic obstructive pulmonary disease; RBC, red blood cell.

Multivariate Regression Analysis

To further identify the independent risk factors for postoperative pneumonia, multivariate logistic regression analysis was performed using the 25 factors significantly associated with postoperative pneumonia in the univariate analysis. The results of the multivariate analysis are presented in **Table 4**. We found that there were six independent risk factors for postoperative pulmonary disease, as follows: increased age ($P = 0.047$, OR = 1.622, 95% CI: 1.006–2.614), COPD ($P = 0.001$, OR = 5.521, 95% CI: 2.093–14.565), emergency surgery ($P = 0.004$, OR = 3.407, 95% CI: 1.487–7.804), postoperative reduced albumin ($P < 0.001$, OR = 2.226, 95% CI: 1.447–3.423), prolonged mechanical ventilation ($P = 0.047$, OR = 1.949, 95% CI: 1.008–3.766), and longer duration of bed rest ($P < 0.001$, OR = 2.671, 95% CI: 1.694–4.212).

Outcomes

The outcomes of the patients with postoperative pneumonia were retrospectively analyzed and are presented in **Table 5**. The results show that the hospital stay of patients in the case group (24.32 ± 14.64) was significantly longer than that in the control group (16.16 ± 8.36). In the case group, the proportion of patients with hospital stays over 14 days or 30 days were significantly higher than those in the control group ($P < 0.05$). In addition, the postoperative ICU occupancy rate, re-operation rate and

TABLE 4 | Results of the multivariate analysis of factors associated with POP.

Risk factors	B	S.E.	Wald	*P* value	OR	OR 95%CI
Age (≥ 60 yr)	0.483	0.244	3.940	0.047	1.622	1.006, 2.614
COPD	1.709	0.495	11.919	0.001	5.521	2.093, 14.565
Emergency surgery	1.226	0.423	8.401	0.004	3.407	1.487, 7.804
Postoperative albumin (< 35 g/L)	0.800	0.220	13.281	<0.001	2.226	1.447, 3.423
Duration of ventilation (≥ 24 h)	0.667	0.336	3.938	0.047	1.949	1.008, 3.766
Duration of bed rest (≥ 3 days)	0.983	0.232	17.892	<0.001	2.671	1.694, 4.212

TABLE 5 | The outcomes of postoperative pneumonia.

Outcomes	Cases (*N* = 264)	Controls (*N* = 264)	*P* value
The hospital stay (days)	24.32 ± 14.64	16.16 ± 8.36	<0.001
≥ 14 days	202 (76.5%)	154 (58.3%)	<0.001
≥ 30 days	69 (26.1%)	14 (5.3%)	<0.001
ICU occupancy rate	123 (46.6%)	49 (18.6%)	<0.001
Re-operation rate	92 (34.8%)	7 (2.7%)	<0.001
Mortality rate	8 (3.0%)	0 (0%)	0.004

postoperative mortality rate of patients in the case group were significantly higher than those in the control group ($P < 0.05$).

DISCUSSION

Among the 17,190 surgical patients included in this study, 264 cases of postoperative pneumonia occurred, with an incidence of 1.54%, which is similar to previously reported rates (5–7, 10). The incidence of postoperative pneumonia in neurosurgery (17.00%) was significantly higher than that in other surgical specialties. Obviously, neurosurgery patients are susceptible to pneumonia after surgery. Such patients usually suffer from complicated diseases and long coma, impairing their respiratory and immune function. In addition, the neurosurgery (102 cases), hepatobiliary (52 cases), general and digestive (35 cases), and thoracic (26 cases) were the four surgical specialties with the highest frequency of postoperative pneumonia, accounting for approximately 81.44% of the total. This suggests that it is particularly important to strengthen the management of patients at risk of postoperative pneumonia in these four surgical specialties.

The main pathogens were *Klebsiella pneumoniae* (22.22%), followed by *Escherichia coli* (11.76%), *Staphylococcus aureus* (9.15%) and *Acinetobacter baumann* (8.50%), accounting for half of the total pathogens. These four pathogens are conditional pathogenic bacteria that usually exist in the hospital environment and oropharynx of patients. Obviously, surgical trauma destroys the integrity of the body's skin and tissues, damaging the patient's first line of immune defense and providing opportunities for these bacterial infections. Meanwhile, *Staphylococcus aureus* can be transmitted by hand contact, indicating the need for strict hand hygiene prior to invasive procedures such as tracheal intubation. The drug resistance of pathogens has also become a serious problem, which makes clinical treatment more difficult. In this study, the incidence of multidrug-resistant bacteria was approximately 16.99%, of which 73.08% occurred in neurosurgery patients.

This study has demonstrated that age over 60 years old is an independent risk factor for postoperative pneumonia, which is consistent with previous research (11–13). Kunisaki C et al. (11) reported a significant variation in the postoperative pneumonia rate between patients aged over 75 years and those aged 45–65 years (13.3% and 6.3%). Furthermore, Yamada H et al. (12) reported that the postoperative pneumonia incidence in patients aged over 85 years was significantly higher than that in patients aged 75 to 85 years (16.7% and 3.3%). These two studies showed that the risk of postoperative pneumonia increased significantly with patient age, which was also confirmed by Miki Y et al. (13).

Our study found that the incidence of postoperative pneumonia in patients with COPD was 4.5 times greater than that in unaffected patients ($P = 0.001$, OR = 5.521, 95% CI: 2.093–14.565). Pulmonary chronic inflammation in patients with COPD is a characteristic pathological change that will continue to destroy the alveolar wall septum and result in pulmonary interstitial fibrosis (9, 14). Some previous studies have confirmed that preoperative treatment for COPD can reduce the incidence of postoperative pneumonia (15, 16). Numata T et al. (15) have demonstrated that long-acting anticholinergic drugs and long-acting β2 receptor agonists can effectively reduce the rate of postoperative pulmonary complications in patients with COPD. Du Z et al. (16) have further demonstrated that perioperative aerosol inhalation of ipratropium bromide can reduce the incidence of postoperative pneumonia in COPD patients undergoing thoracic surgery.

We also found that the rate of postoperative pneumonia in emergency surgery patients was 2.4 times greater than that in non–emergency surgery patients ($P = 0.004$, OR = 3.407, 95% CI: 1.487–7.804), which is similar to the results reported by Kim Th et al. (17). Due to the urgency of the surgery, the preoperative preparations appear to be particularly poor, and the prevention of infection is usually not strict enough. Furthermore, McCoy CC et al. (18) reported that compared with elective surgery, emergency surgery was associated with an increased risk of serious postoperative complications and increased the risk of postoperative death by approximately 1.39 times.

The serum albumin level is the most common indicator used to evaluate the nutritional status of patients. A serum

albumin level below 35 g/L is generally considered malnutrition and has been identified as a potential risk factor for poor postoperative outcomes (19, 20). Our findings suggest that postoperative albumin levels under 35 g/L in surgical patients is an independent risk factor for postoperative pneumonia. A decrease in postoperative albumin can directly reflect that the metabolism of the body is in a negative nitrogen balance and engaged in high protein consumption. The results of univariate analysis in our study showed that intraoperative albumin infusion did not increase the risk of postoperative pneumonia, indicating that a timely albumin infusion can be applied to correct hypoalbuminemia if necessary.

Our study demonstrated that the duration of mechanical ventilation over 24 h was an independent risk factor for postoperative pneumonia ($P = 0.047$, OR = 1.949, 95% CI: 1.008–3.766). For patients sent to the ICU after surgery, the duration of ventilation usually exceeds 24 h. Thus, extubation timely after surgery can significantly reduce the incidence of postoperative pneumonia. Vera Urquiza R et al. (21) found that extubation 6 h later was an independent risk factor for postoperative pneumonia ($P = 0.005$, OR: 15.81, 95% CI: 2.2–110.7). Savardekar A et al. (22) confirmed that endotracheal intubation for more than 48 h was an independent risk factor for pneumonia ($P = 0.041$, OR = 6.638, 95% CI: 1.08–40.8).

Duration of bed rest after surgery over 3 days was an independent risk factor for postoperative pneumonia ($P < 0.001$, OR = 2.671, 95% CI: 1.694–4.212).

Therefore, patients ought to start off-bed activity early after surgery if there is no special contraindication. When it is necessary to stay in bed for a long time, the patient should be encouraged to expectorate regularly and clear their airway secretions. Cassidy MR et al. (23) implemented a multidisciplinary team cooperation model, proving that early out of bed after surgery can effectively reduce the occurrence of postoperative pneumonia.

Furthermore, we found that postoperative pneumonia usually caused poor outcomes. The hospital stay of cases (24.32 ± 14.64) was significantly longer than that of the matched controls (16.16 ± 8.36), and postoperative pneumonia significantly increased the ICU occupancy rate, reoperation rate, and perioperative mortality rate. A retrospective analysis of 1,415 consecutive gastric cancer patients reported that postoperative pneumonia was associated with poor long-term outcomes (24). Fujishima S et al. (25) found that postoperative pneumonia in patients with esophageal cancer was associated with skeletal muscle consumption and asymptomatic pneumonia within 6 months after surgery, and the survival time of patients with postoperative pneumonia was significantly lower than that of patients without pneumonia. Obviously, the real value of study is in improving quality of life (26).

A formal statement of shortcomings could keep authors and the public from overstating a study's claims (27). As a single-center study, the external validity of the findings is limited. Due to the retrospective collection of most clinical data, information on the exposures is subject to observation bias. Specifically, although our diagnostic criteria are very specific, our researchers may still make diagnostic errors, even in the same patient. In addition, the rate of postoperative pneumonia varied greatly in each surgical department, and the representation of patients included in the study is prone to bias. Many surgical patients receive prophylactic antibiotics, which tends to skew the study results and obscure the risk factors associated with postoperative pneumonia. Furthermore, this study did not follow up on the long-term outcomes of patients with postoperative pneumonia.

CONCLUSIONS

Postoperative pneumonia is associated with severe clinical outcomes. In this retrospective single-center study, we identified six independent risk factors that can aid in risk stratification and management of patients at risk of postoperative pneumonia. The distribution of causative pathogens can also help in the implementation of effective preventions and interventions, which has great implications for the formulation of infection control policies.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

BX collected, analyzed and interpreted all data, and was a major contributor in writing the manuscript. YS participated in study design and data analysis. FY was involved in data acquisition and analysis. RC was involved in data acquisition and manuscript writing. YY conducted statistics and analysis of data. SJ guided paper writing and provided financial and technical support. All authors read and approved the final submitted manuscript. All authors contributed to the article and approved the submitted version.

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It's about the patients: Practical antibiotic stewardship in outpatient settings in the United States

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Antibiotic-resistant pathogens cause over 35,000 preventable deaths in the United States every year, and multiple strategies could decrease morbidity and mortality. As antibiotic stewardship requirements are being deployed for the outpatient setting, community providers are facing systematic challenges in implementing stewardship programs. Given that the vast majority of antibiotics are prescribed in the outpatient setting, there are endless opportunities to make a smart and informed choice when prescribing and to move the needle on antibiotic stewardship. Antibiotic stewardship in the community, or "smart prescribing" as we suggest, should factor in antibiotic efficacy, safety, local resistance rates, and overall cost, in addition to patient-specific factors and disease presentation, to arrive at an appropriate therapy. Here, we discuss some of the challenges, such as patient/parent pressure to prescribe, lack of data or resources for implementation, and a disconnect between guidelines and real-world practice, among others. We have assembled an easy-to-use best practice guide for providers in the outpatient setting who lack the time or resources to develop a plan or consult lengthy guidelines. We provide specific suggestions for antibiotic prescribing that align real-world clinical practice with best practices for antibiotic stewardship for two of the most common bacterial infections seen in the outpatient setting: community-acquired pneumonia and skin and soft-tissue infection. In addition, we discuss many ways that community providers, payors, and regulatory bodies can make antibiotic stewardship easier to implement and more streamlined in the outpatient setting.

KEYWORDS

antibiotic stewardship, antimicrobial stewardship, therapeutic antibacterial agents, microbial drug resistance, pneumonia, infectious skin diseases, overprescribing, inappropriate prescribing

Introduction

Every year in the United States (US), antibiotic-resistant pathogens are implicated in at least 35,000 deaths and over 2.8 million infections (1). Fundamentals of antibiotic stewardship dictate that clinicians can reduce the impact of antibiotic resistance by carefully prescribing antibiotics only when needed, with the right drug, dosage, and duration (2). While hospital-based stewardship programs have demonstrated remarkable value and healthcare benefit, the expansion of stewardship to the outpatient setting—including primary care clinics, urgent care (UC) settings, and skilled nursing facilities—may be less successful unless consideration is given to the unique nature of outpatient healthcare. This article describes the scope of the problem with outpatient stewardship in the US and systematic challenges limiting implementation, offering some pragmatic solutions to facilitate implementation.

What's the problem?

In the US in 2019, 250 million oral antibiotic prescriptions were written in the outpatient setting—roughly the equivalent of eight antibiotic prescriptions for every 10 people (**Figure 1A**) (3–5). One-third (∼47 million) of these outpatient antibiotic prescriptions are considered unnecessary (6). This is largely attributable to antibiotics prescribed for viral infections (e.g., viral upper respiratory infections, pharyngitis, and middle ear infections), as well as non-bacterial conditions such as allergy/asthma and bronchitis (7, 8).

Antibiotics for common acute infections are often prescribed for 10 or more days of therapy, which is longer than needed (9–11). At 129 Veteran's Affairs medical centers, 40% of antibiotic prescriptions for pneumonia were for 8 days or longer (11). In a single-center study, 42% of uncomplicated skin infections treated in the ambulatory setting were prescribed antibiotic therapy for ≥ 10 days (9). Excessive antibiotic duration is associated with a higher risk of *Clostridioides difficile*–associated diarrhea and drug toxicity (12–14).

Depending on the infection type, some 25–50% of antibiotic prescriptions for bacterial infections do not align with current guidelines (6, 9, 15, 16) or may fail to adequately consider local resistance patterns. The current guidelines from the Infectious Disease Society of America and American Thoracic Society indicate that macrolide monotherapy is a first-line treatment option for the typical patient with community-acquired pneumonia (CAP; those with no comorbidities or risk factors for methicillin-resistant *Staphylococcus aureus* [MRSA] or *Pseudomonas aeruginosa*), but only if local *Streptococcus pneumoniae* resistance rates are < 25% (17). *S. pneumoniae* is resistant to macrolides in around 40–50% of isolates in the US, and most US regions exhibit resistance rates > 25% (**Figure 1B**) (18–21). Despite relatively clear guidance from the CAP guidelines and established patterns of antimicrobial resistance, azithromycin, a macrolide, remains the most commonly prescribed agent in the US, accounting for about 30–40% of outpatient CAP prescriptions written (22).

While some local public health agencies and health systems provide clinicians with local resistance information, these data are becoming more challenging to obtain (23). Furthermore, even if an antibiogram (i.e., antibiotic susceptibility test report) is available, primary care providers may benefit from expert interpretation of the data, including the data source and how they affect the risk/benefit decision for therapy. Antibiotic resistance profiles can differ substantially between isolates collected in the outpatient setting versus inpatient setting and, therefore, antibiograms produced by hospitals should be interpreted carefully when applied to outpatients.

Why don't we just have antibiotic stewardship in all outpatient settings?

Primary care physicians, advanced practice providers, and dentists account for the majority of outpatient antibiotic prescriptions written (24). Prescribers come from diverse specialties, geographic locations, and practice types (e.g., private vs. health system affiliates) (25). Implementation of effective antibiotic stewardship must be customized to each specific care setting and requires some expertise to establish. Moreover, for any substantial change in outpatient antibiotic use to be successfully implemented, outpatient clinicians need the resources and time to address inappropriate antibiotic prescribing.

Though antibiotic stewardship was originally introduced in inpatient care, regulatory bodies, and public health agencies are now implementing antimicrobial stewardship requirements in outpatient settings (26). The Centers for Disease Control and Prevention (CDC) adapted their inpatient stewardship recommendations to the outpatient setting, noting that clinicians should **demonstrate a commitment** to optimizing antibiotic prescribing and patient safety, take at least one **action for policy or practice** to improve antibiotic prescribing,

Abbreviations: CAP, community-acquired pneumonia; CDC, Centers for Disease Control and Prevention; EMR, electronic medical record; MRSA, methicillin-resistant *Staphylococcus aureus*; SSTI, skin and soft-tissue infection; UC, urgent care; US, United States.

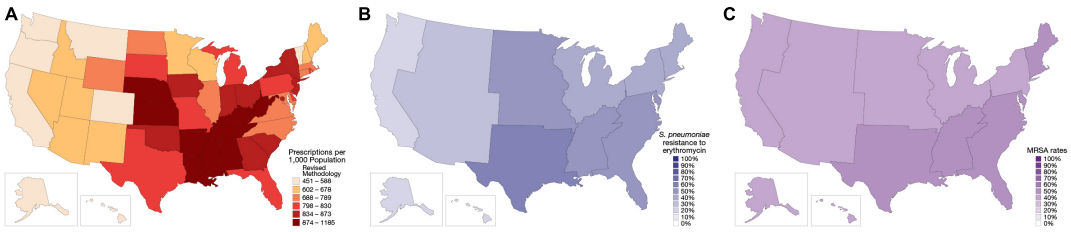

FIGURE 1
Regional distribution of antibiotic prescribing patterns and antibiotic resistance within the United States (US). **(A)** Outpatient antibiotic prescription rates from the Centers for Disease Control and Prevention, 2018 (3). **(B)** Erythromycin-resistant *Streptococcus pneumoniae* phenotype rates, 2019 (98). **(C)** Methicillin-resistant *Staphylococcus aureus* (MRSA) rates, as a percentage of all *S. aureus* isolates, 1997–2017 (51). Resistance rates were derived from isolates collected at US hospitals in the SENTRY surveillance program.

track prescribing practices and **provide regular feedback** to clinicians, and provide **educational resources and expertise** on optimizing antibiotic prescribing (26). The Los Angeles County Department of Public Health has incorporated many of the CDC's Core Elements of antibiotic stewardship into their Targeting Appropriate Prescribing in Outpatient Settings (TAP Out) program, which reduced inappropriate prescribing and provided well-received peer comparison reports on prescribing habits (27).

Recently, the Joint Commission, which is the largest healthcare accrediting body in the US, has been applying new antibiotic prescribing standards to accredited ambulatory healthcare (i.e., outpatient clinics, UC, or worksite clinics; **Supplementary Table 1**) (28, 29). One barrier to implementing antibiotic stewardship in outpatient settings is the lack of accountability for outpatient antibiotic stewardship through traditional regulatory bodies, i.e., the Centers for Medicare and Medicaid Services. Alternatively, payors may be able to play an important role in outpatient stewardship.

Several antibiotic stewardship programs have been developed specifically for implementation in skilled nursing facilities. For instance, the Agency for Health Care Quality created a four-part approach that includes methods to monitor and maintain a stewardship program (30). However, data from this program do not seem to have been published to date. Concurrently, one large health insurance organization has created its own antibiotic stewardship program, but again the effects are not publicly known (31). Full compliance at the participating sites may be difficult due to staffing shortages and lack of systems or protocols. While skilled nursing facilities have successfully implemented infection control measures (32), there is a need for more education and administrative oversight to fully implement the intended nature of antibiotic stewardship (33).

According to a 2018 Pew Trust report, almost 46% of antibiotic prescriptions written in the UC setting were unnecessary (24). These were mainly for respiratory tract infections. However, despite recent efforts by the Academy of Urgent Care Medicine, which developed an antibiotic stewardship education program, very few sites have completed the training to gain accreditation in antibiotic stewardship.

"It's not me"

Prescribers don't think they're part of the antibiotic prescribing problem. Almost all surveyed physicians say that, in general, there is a problem with antibiotic resistance and inappropriate prescribing in the US (34, 35). However, only about 50% of these surveyed physicians see the problem as occurring in their specific practice. This disconnect continues to fuel the problem, and we all need to accept responsibility and survey our prescribing habits.

"I don't have the data, and I don't have the support to implement"

The average healthcare provider seeing patients in the community is not supported by health system-based education, interventions, and staff to guide appropriate prescribing practices. Therefore, the provider is left to navigate this complex field independently, sourcing guidelines and continuing education materials, and implementing stewardship practices. The prime example of this is the UC provider who usually works in isolation without regular peer-to-peer interaction, which is a crucial component of a successful antibiotic stewardship program.

Guideline disconnect

National health agencies (the CDC) and professional organizations (Infectious Diseases Society of America) have published a variety of resources for clinicians on antibiotic prescribing, for particular infections and for more appropriate use of antibiotics in general (17, 36–42). However, the complexity of the documents, the length of

time between document updates, and the inclusion of some content that doesn't reflect real-world practice leads many community providers to turn instead to alternative resources, including decision support information sites such as UpToDate and Epocrates, or rely on their medical training (8, 43). Some of the guidelines lack specific recommendations on duration of therapy, therapy choice, or how to interpret local resistance patterns.

Pressure to prescribe

Patients (and parents of young patients) often expect and may even pressure a provider for an antibiotic prescription when it is not indicated. About 84% of providers surveyed said they feel at least moderate pressure from patients for an antibiotic prescription (34). Patients' and parents' expectations for an antibiotic prescription can increase antibiotic prescribing (44). However, some of the perceived pressure from the perspective of the provider may not be the intention of the patient/parent, who instead is looking for reassurance and a better explanation of the management plan (2). For the independent practitioner in the outpatient setting, leaving the patient's expectations unfulfilled risks having a "dissatisfied customer." Some providers practice defensive prescribing of antibiotics, out of concern for missing bacterial infections and the possible medicolegal ramifications (45).

Smart prescribing for outpatients

Here, we want to address smart prescribing for two of the most common bacterial infections seen in the outpatient setting, CAP and skin and soft-tissue infection (SSTI). For CAP and SSTI, several organizations have released updated clinical practice guidelines within the last 7 years (17, 38, 42). Despite the advances in therapeutic options, many prescribers in the outpatient setting are unaware of these updates or have not received continuing education about updates from previous guidelines.

Community-acquired pneumonia

S. pneumoniae is the most commonly isolated bacterial pathogen in patients with pneumonia without underlying chronic lung disease; other causative pathogens include *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *S. aureus*, and *Legionella pneumophila* (21, 46). A bacterial pathogen is isolated in about 25–50% of CAP cases, with many patients having no pathogen detected, and viral pathogens occurring in some cases (46, 47).

In the community and UC/emergency department settings, the most commonly prescribed antibiotics for CAP are azithromycin and fluoroquinolones, accounting for 50–66% of all prescriptions for CAP treatment (7, 11, 22). While many providers prescribe an antibiotic empirically for CAP, local data on pathogens and susceptibility (if available) could better inform the treatment approach. From a robust collection of isolates from North America, the susceptibility rates of *S. pneumoniae* to levofloxacin were high (97–99%) and remained stable from 2010 to 2014. There was a decrease in susceptibility rates over this period for other common antibiotics, such as amoxicillin, erythromycin, tetracycline (which can be used as a surrogate for doxycycline susceptibility), and trimethoprim–sulfamethoxazole (also known as co-trimoxazole; **Figure 2**), which may have the potential to render these agents less appropriate for empiric treatment of CAP (20). More recent studies show that resistance rates of *S. pneumoniae* to macrolides (e.g., azithromycin) are approximately 40–50% in the US (18, 19, 21). Inappropriate use of antibiotics can lead to selection of resistant mutants either within a class or, less commonly, with other agents, known as co-resistance. Thus, such collateral damage has to be considered. Based on national rates of antimicrobial resistance to *S. pneumoniae*, azithromycin monotherapy for CAP is not recommended. Lack of specificity in our national guidelines leaves most providers guessing at best available therapy rather than following expert guidance.

Skin infection

S. aureus is the most commonly isolated pathogen from SSTIs, with Group A streptococci and *P. aeruginosa* also found to a lesser extent (48, 49). About half of all *S. aureus* isolates from SSTI cases in the US are MRSA strains (**Figure 1C**) (49, 50). Gram-negative pathogens, when they occur in SSTI, are more likely to be associated with surgical-site infections of the abdominal wall, or infections in the anal and perineal region (49).

Global susceptibility of *S. aureus* isolates between 1997 and 2016 showed susceptibility of methicillin-susceptible *S. aureus* (MSSA) isolates to many older agents was > 95%, except for penicillin and erythromycin (**Figure 3**) (51). The susceptibility of MRSA to these older antibiotics was generally lower than methicillin-susceptible *S. aureus*. However, the susceptibility rates did increase over the last two decades, possibly as a result of the spread of MRSA clones that are more susceptible to these agents. Many of the more recently approved antibiotics demonstrated susceptibility rates of > 99% against MRSA, except for levofloxacin (23% susceptible, from 72,000 isolates), delafloxacin (74% susceptible, from > 10,000 isolates), and ceftaroline (92% susceptible, from > 40,000 isolates).

Uncomplicated (superficial), purulent SSTIs can often be treated by incision and drainage alone, while non-purulent

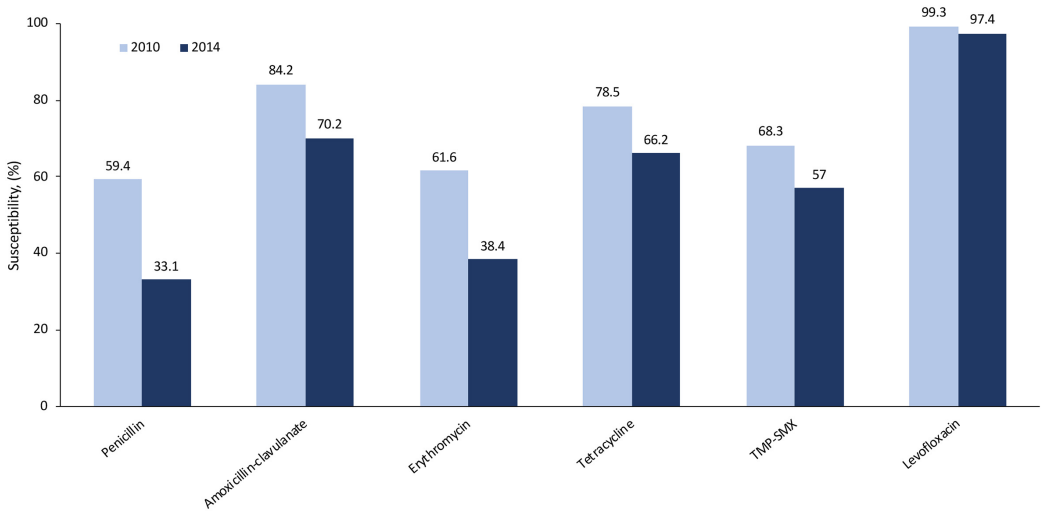

FIGURE 2
Susceptibility rates of *Streptococcus pneumoniae* to common antibiotics in North America (2010, 2014) using CLSI breakpoints (20). Amoxicillin–clavulanate rates were determined using non-meningitis breakpoints. CLSI, Clinical and Laboratory Standards Institute; TMP/SMX, trimethoprim–sulfamethoxazole.

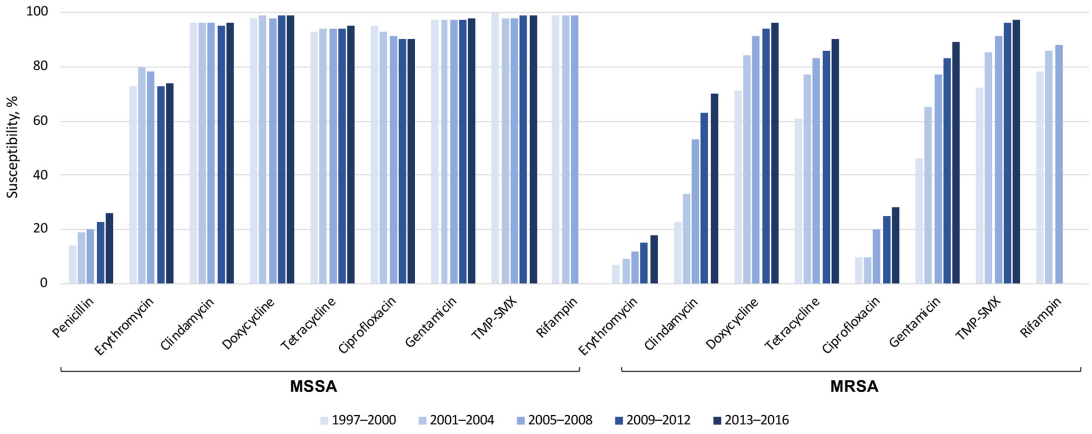

FIGURE 3
Susceptibility of > 191,000 *S. aureus* isolates to older antibiotics, from a global surveillance program (51). MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; TMP-SMX, trimethoprim–sulfamethoxazole.

SSTIs require antibiotics (38, 39, 52, 53). Antibiotic therapy when added to incision and drainage for abscesses can lead to a moderate improvement in efficacy (in one randomized study: 82–83% clinical success, depending on which antibiotic regimen was selected, vs. 68.9% in the incision and drainage only group), though this improvement may be limited to patients who have a positive culture for *S. aureus* (53). Antibiotic therapy for SSTI often consists of cephalexin and/or trimethoprim–sulfamethoxazole, though different agents may be used by provider choice and for certain types of infections (9, 54, 55). If MRSA is known or suspected to be present in the lesion, guideline-recommended treatments include vancomycin, linezolid, clindamycin, daptomycin, ceftaroline, doxycycline, minocycline, and trimethoprim–sulfamethoxazole; of note, cephalexin is not a recommended agent for treating known or suspected MRSA infections (38, 40, 42). SSTIs are often treated in the UC/emergency department setting, where providers may justifiably err on the side of treating with antibiotics because of the episodic nature of patient care and the lack of follow-up. This episodic nature of care occurs for many patients in the US with various conditions, including other infectious diseases, and may not readily be addressable without a systemic change in the availability and interconnectedness of electronic medical records (EMRs) or in the architecture of healthcare delivery and reimbursement.

TABLE 1 Smart prescribing recommendations for community-acquired pneumonia.

General recommendations	
Duration of treatment	• Initial duration of antibiotic treatment should be 5–7 days (10) • Short course associated with fewer adverse reactions (12) • Evidence in CAP (84–88)
Choice of treatment	• Choose antibiotic based on local resistance patterns, known/suspected pathogen; national resistance rates are suitable alternative • If local macrolide resistance rates are unknown, choose other first-line monotherapy (89) • If local rates are known to be < 25%, can consider a macrolide • Informed by prior microbiological culture if available; revised when microbiological culture is available • Common treatments to consider: beta-lactams + macrolides, tetracyclines, fluoroquinolones
Reasons to deviate	**What to change**
Recent antibiotic use	Do not repeat recent drug; increased likelihood that the pathogen is resistant to the specific antibiotic
Drug Resistance in Pneumonia (DRiP) score ≥ 4*	Likely need for extended-spectrum antibiotics (90)
Structural lung disease (e.g., fibrosis, lung cancer)	Risk factor for *Pseudomonas aeruginosa*
Lung cancer, post-obstructive pneumonia	Consider longer therapy duration
Exposure to birds, farm animals, water reservoirs	Risk factors for atypical pathogens
Immunocompromised	Consider longer therapy duration

*DRiP score calculation: antibiotic use within 60 days (2 points); residence in long-term care facility (2 points); tube feeding (2 points); infection with drug-resistant pathogen within 1 year (2 points); hospitalization within 60 days (1 point); chronic pulmonary disease (1 point); poor functional status (1 point); gastric acid suppression (1 point); wound care (1 point); methicillin-resistant *Staphylococcus aureus* colonization within 1 year (1 point) (90). CAP, community-acquired pneumonia.

Smart prescribing in the outpatient setting

Given the number of infections that occur annually for CAP and SSTI in the US, there are millions of chances to make a smart and informed choice when prescribing antibiotics. Antibiotic stewardship in the community, or "smart prescribing" as we suggest, should factor in antibiotic efficacy, safety, local resistance rates, and overall cost, in addition to patient-specific factors and disease presentation, to arrive at an appropriate therapy.

Almost half of surveyed providers said they would need "a lot of help" to implement antibiotic stewardship practices (34). We recognize the magnitude of the challenge and have assembled this easy-to-use best practice guide for providers in the outpatient setting who lack the time or resources to develop a plan or consult lengthy guidelines.

Measure existing prescribing habits

From EMR prescribing data, providers can identify one or two issues within their practice to address (e.g., inappropriate prescribing for a particular diagnosis code; peer benchmarking for antibiotic duration and dosing), and determine what action to take (56–58). Providers can then monitor the issue(s) periodically (e.g., monthly) to see if the data are improving (59). To obtain an approximate idea of how many patients fail initial treatment, providers can examine antibiotic refills, antibiotic switches, emergency department visits, and hospitalizations within 30 days of the initial prescription, though these data may be limited by the interconnectedness of EMRs or the patient obtaining all of their care within one health system. In the absence of an EMR, providers could review a patient's recent medical history to determine previous treatments, and treatment failures on an individual basis. Other data and aggregate analyses require electronic systems and knowledge to interpret the results.

Choose an appropriate drug, dose, and duration

Recommendations are provided for the most common pathogens and patient populations in CAP (**Table 1**) and SSTI (**Table 2**). These recommendations are for the "standard" patient with one of these bacterial infections; a good rule of thumb is that for ∼80% of cases, your treatment should fall along these lines.

Community prescribing tends to follow standard dosing of antibiotics, but providers should be aware of the potential need for dose adjustments, for example related to body size or comorbid conditions (e.g., renal or hepatic impairment). For some patients, providers will need to take a different approach to antibiotic treatment based on certain patient or infection factors (**Tables 1**, **2**). In all cases, providers should use their

TABLE 2 Smart prescribing recommendations for skin infection.

General recommendations	
Duration of treatment	• Initial duration of antibiotic treatment should be 5–7 days (10) • Short course associated with fewer adverse reactions (12) • Evidence in SSTI (91–94)
Choice of treatment	• Incision and drainage is encouraged when clinically indicated, followed by culture • May be sufficient to resolve superficial infection (38, 39, 52, 53) • Choose antibiotic based on local resistance patterns, known/suspected pathogen; national resistance rates are suitable alternative • Common treatments to consider: cephalosporins (not for MRSA), sulfonamides, glycopeptides, oxazolidinones, tetracyclines
Reasons to deviate	**What to change**
Recent antibiotic use	Do not repeat recent drug; increased likelihood that the pathogen is resistant to the specific antibiotic
Lymphedema	Coverage for Group A streptococci; longer therapy duration (95)
Picking at skin	Educate patient about handwashing and avoiding lesion(s)
Injection drug use	*Staphylococcus*, streptococci (including oral origin), and anaerobes more likely (40, 96, 97)
Lesion below the waist	Coverage for Gram-negative rods more likely needed (42)
Lesion on hand or face	Surgical referral urgently, treat more aggressively than other anatomical locations
Immunocompromised	Consider longer therapy duration

MRSA, methicillin-resistant *Staphylococcus aureus*; SSTI, skin and soft-tissue infection.

best judgment, tailor their treatment choice to each patient (their medical history, presentation, comorbid conditions, risk factors, and lifestyle), and use a shorter course whenever possible. Additionally, providers should watch out for certain safety issues that would suggest choosing an alternate antibiotic (**Supplementary Table 2**).

Delayed prescribing ("watchful waiting") may assist in avoiding inappropriate prescribing related to patient pressure to prescribe, and thus reduce antibiotic resistance, by advising patients to return if symptoms do not improve within a few days or worsen (2, 60). It can also be a useful tool to allay a patient's concerns that they present with at the initial visit. For UC settings, providers can offer the patient an antibiotic prescription with specific directions to fill it only if their symptoms haven't improved in a few days, or write a future date on the prescription to be filled under the same circumstances. In cases where clinicians are uncertain of infections, a delayed prescription may be an appropriate safety net.

Case management

Ideally, a nurse or case manager should follow up with the patient at Day 2–3 after beginning antibiotic treatment to see if there are signs of an early response to treatment or any worsening symptoms. However, additional staffing may be needed to achieve this, which might be difficult to implement in certain practices. Alternatively, groups of providers can hold regular debriefing sessions to discuss cases and note any patterns of disease presentation or treatment failure. For patients with a skin infection, the provider can draw a circle around the initial extent of the infection and instruct the patient to call or send a photo of the lesion size at Day 1–2. This protocol also aids a second provider who sees the patient to assess the treatment response.

Making smart prescribing easier

Simplify guidance documents

Providers need guidance from experts that is easy to find and use, and reflects the real-world scenarios that they are faced with every day (43).

Know your local resistance patterns

Ask your local health department or community hospital for information (61). If those resources can't routinely provide this information, reach out for help to a local infectious disease specialist, who can be found at the hospital or through a local chapter of the Infectious Diseases Society of America, or to the laboratory where you send your routine culture data. Laboratories that are accredited by the College of American Pathologists are required to publish an annual antibiogram.

Rapid assessments

Rapid diagnostic tests are available for various viral and bacterial pathogens for respiratory, gastrointestinal, sexually transmitted, and central nervous system infections, most of which provide results within 15–45 min (62). Using highly

sensitive molecular diagnostic tests can significantly reduce unnecessary testing and treatment, including inappropriate antibiotic prescribing, though the results vary by pathogen and disease state. However, rapid diagnostic tests are often reimbursed in a flat fee payment per patient for outpatient providers. As such, the significantly higher costs of molecular and polymerase chain reaction testing must be absorbed by the provider. Unfortunately, this is not an economically feasible option. Diagnostic stewardship is likely a route to ensuring tests are undertaken in the appropriate patient and that the rapid accurate results assist with case management. Payers should be made aware of this situation and that the use of "expensive" tests upfront can reduce costs in the longer term.

Additional challenges to practical implementation of rapid assessments are sensitivity of the test and time and staff required to train and perform quality control of the test. Providers should also be aware that bacterial colonization (rather than infection) can return a positive result based on highly sensitive molecular diagnostic tests, which would not routinely warrant antibiotic treatment.

Patient/parent education

Suppose providers feel that the patient or parent is expecting an antibiotic prescription. In that case, the provider can explain why an antibiotic isn't needed and give other actionable treatment advice so the patient/parent feels that they walked away from the visit with useful information (**Supplementary Table 3**) (2, 63). Even simple interventions, such as clinicians posting an informational letter in examination rooms with a signed commitment to use antibiotics appropriately, can reduce inappropriate prescribing by 20% (**Supplementary Figure 1** shows an example) (64). The CDC has many handouts, posters, and web images, in English and Spanish, from the "Be Antibiotics Aware" campaign that can be shared with patients and caregivers (65). One effective example is the "Viruses or Bacteria: What's got you sick?" poster, which shows a checklist of common conditions and whether an antibiotic is indicated or not (**Supplementary Figure 2**). When appropriate, hand your patient one of the CDC's "prescription" sheets for symptom relief of common cold/viral illness (66). Providers can also obtain training that's been specifically designed around improving their communication skills regarding antibiotic prescribing (67).

If a patient needs an antibiotic, encourage them to adhere to dosing instructions, and explain why this is important. In some situations, it may be worth explaining why you are prescribing a specific antibiotic (e.g., a narrower-spectrum vs. a broader-spectrum one) for the patient's infection. In all cases, let the patient know about the likely disease course with treatment, potential side effects, and when to follow up.

Market the practice as accredited for antibiotic stewardship

The College of Urgent Care Medicine offers an Antibiotic Stewardship Commendation to practices that provide evidence of their compliance with the CDC's Core Elements (68). Practices that receive this accreditation can advertise their achievement in their clinic and online.

Automated systems

EMR systems can be useful tools toward better antibiotic prescribing practices. In addition to making data collection easier (what was prescribed for a particular diagnosis), the EMR system can include prompts for particular interventions, automatically populated fields that comply with current guidelines, and step-through decision making (69). Unfortunately, the financial and logistical hurdles to implement these features in an EMR may be too high for smaller practices to overcome.

Provider behavioral change

In addition to the concern for missing an infection and the possible consequences (e.g., patient morbidity/mortality and litigation), diagnostic uncertainty drives a substantial amount of unnecessary antibiotic prescribing (70). There is an inherent contradiction between avoiding the downstream consequences of failed therapy and limiting inappropriate prescribing of antibiotics. While some internal factors that motivate providers' prescribing habits would be difficult to change without a larger overhaul of the US healthcare system and law reform, some efforts can affect behavioral change in inappropriate antibiotic prescribing. Programs aimed at re-educating healthcare providers on appropriate antibiotic prescribing, providing individualized feedback, and peer comparisons can significantly reduce inappropriate prescribing (57, 71). For example, a recent study in a rural community setting included physician education through presentations on antibiotic stewardship and appropriate, guideline-concordant prescribing; feedback emails on guideline-discordant prescribing for a particular indication; and recommendations on how physicians could improve their prescribing. Additionally, patient education materials were distributed to clinics, from the CDC's "Be Antibiotics Aware" campaign (71). This resulted in an absolute decrease of ∼15% in inappropriate prescribing during the 6-month influenza season. A randomized controlled trial of three different types of antibiotic prescribing interventions in primary care ($N = 248$ clinicians) found that the most significant reductions in inappropriate prescribing occurred after the providers (1) had

to include written justification in the patient's EMR for why the prescription was necessary, becoming a permanent part of the record; and (2) received regularly updated rankings of their prescribing rate compared with that of the top-performing peers (58, 72).

Risk stratification in community-acquired pneumonia

Common laboratory tests, such as complete blood counts and basic metabolic profiles, can be used to generate a risk score for adults that is highly predictive of 30-day all-cause death (73). Disease-specific risk scores, such as the Pneumonia Severity Index or the Confusion, Urea nitrogen, Respiratory rate, and Blood pressure (CURB) score (or alternatively a CRB65 score), can identify adult patients considered low risk who may be suitable candidates for outpatient therapy, and patients at high risk of death who require inpatient treatment and follow-up (74).

Controversies

Costs are part of the bigger picture of antibiotic treatment. In the outpatient setting, typically the only cost limit to the antibiotic is whether the patient's health insurance will cover the prescription and if the patient can afford the co-pay. In the bigger picture of healthcare and societal costs of infections, while a patient may initially have an inexpensive treatment with an oral generic antibiotic for their particular infection, if the patient experiences treatment failure (potentially due to inappropriate drug, dose, or duration), then the overall cost of treating that infection escalates significantly (75). Cost savings unquestionably come into play when deciding between intravenous and oral drugs, thereby decreasing or eliminating inpatient or outpatient parenteral antibiotic therapy costs (76, 77).

While there have been several new antibiotics developed in the last decade, their use is often limited by institutional policies that they should be "saved" for special/last-resort use (78, 79). In practice, this can have the unintended consequence that non-ideal antibiotics are prescribed instead, potentially adding fuel to the fire of antibiotic resistance. Though there is a push by regulatory bodies to develop new antibiotics to combat antibiotic resistance threats, antibiotic stewardship practices may actually be having a negative effect on the research and development pipeline (1, 78, 80). So, we are left to wonder, what is an appropriate place in infection management for newer agents that have less acquired resistance or were designed to overcome common resistance mechanisms (79, 81)?

Conclusion

Regardless of the treatment setting where it is implemented, antibiotic stewardship is an evolving field (82, 83). Community prescribers can help move the needle on antibiotic stewardship by keeping in mind the "4 Ds": prescribe an antibiotic for a bacterial infectious Disease, with the appropriate Drug, Dose, and Duration. To truly make headway with smart prescribing in the outpatient setting, more help from public health agencies, regulatory bodies, and payors is needed to provide education, practical support for implementation, and financial incentives for smart prescribing, as well as guidance from a multidisciplinary group on a pragmatic approach to appropriate antibiotic use in the community.

Author contributions

All authors contributed to data interpretation and reviewing/editing the manuscript and approved the final version to be published and were accountable for the work.

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Conflict of interest

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.901980/full#supplementary-material

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Whole genome sequencing of the multidrug-resistant *Chryseobacterium indologenes* isolated from a patient in Brazil

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Chryseobacterium indologenes is a non-glucose-fermenting Gram-negative bacillus. This emerging multidrug resistant opportunistic nosocomial pathogen can cause severe infections in neonates and immunocompromised patients. This study aimed to present the first detailed draft genome sequence of a multidrug-resistant *C. indologenes* strain isolated from the cerebrospinal fluid of an infant hospitalized at the Neonatal Intensive Care Unit of Brazilian Tertiary Hospital. We first analyzed the susceptibility of *C. indologenes* strain to different antibiotics using the VITEK 2 system. The strain demonstrated an outstanding resistance to all the antibiotic classes tested, including β-lactams, aminoglycosides, glycylcycline, and polymyxin. Next, *C. indologenes* was whole-genome-sequenced, annotated using Prokka and Rapid Annotation using Subsystems Technology (RAST), and screened for orthologous groups (EggNOG), gene ontology (GO), resistance genes, virulence genes, and mobile genetic elements using different software tools. The draft genome contained one circular chromosome of 4,836,765 bp with 37.32% GC content. The genomic features of the chromosome present numerous genes related to cellular processes that are essential to bacteria. The MDR *C. indologenes* revealed the presence of genes that corresponded to the resistance phenotypes, including genes to β-lactamases (bla_{IND-13}, bla_{CIA-3}, $bla_{TEM-116}$, $bla_{OXA-209}$, bla_{VEB-15}), quinolone (*mcb*G), tigecycline (*tet*(X6)), and genes encoding efflux pumps which confer resistance to aminoglycosides (*Ran*A/*Ran*B), and colistin (*Hly*D/*TolC*). Amino acid substitutions related to quinolone resistance were observed in

GyrA (S83Y) and GyrB (L425I and K473R). A mutation that may play a role in the development of colistin resistance was detected in lpxA (G68D). *Chryseobacterium indologenes* isolate harbored 19 virulence factors, most of which were involved in infection pathways. We identified 13 Genomic Islands (GIs) and some elements associated with one integrative and conjugative element (ICEs). Other elements linked to mobile genetic elements (MGEs), such as insertion sequence (ISEIsp1), transposon (Tn5393), and integron (In31), were also present in the *C. indologenes* genome. Although plasmids were not detected, a ColRNAI replicon type and the most resistance genes detected in singletons were identified in unaligned scaffolds. We provided a wide range of information toward the understanding of the genomic diversity of *C. indologenes*, which can contribute to controlling the evolution and dissemination of this pathogen in healthcare settings.

KEYWORDS

***Chryseobacterium indologenes*, whole-genome sequencing, virulence and resistance genes, mobile genetic elements, neonatal intensive care unit, metabolic features**

Introduction

Chryseobacterium indologenes is a non-motile, catalase-positive, oxidase-positive, indole-positive, non-glucose-fermenting Gram-negative bacilli (1) widely distributed in nature, but it is not normally present in the human microflora (2, 3). In humans, *C. indologenes* was isolated for the first time from a tracheal aspirate of a patient with ventilator-associated pneumonia (4). Although considered a relatively uncommon human pathogen, the number of hospital-acquired infections caused by *C. indologenes* has been increasing (5–8). Since *C. indologenes* can survive in inanimate objects, it may be isolated from hospital environments and cultured from specimens of sinks, indwelling vascular catheters, vials, feeding tubes, and other equipment that are in contact with fluids and water (9). Therefore, *C. indologenes* is considered a potential reservoir for various types of serious infections (8, 10), including pneumonia, meningitis, wound infection, intraabdominal infection, primary bacteremia, intravascular catheter–related bacteremia, and cellulitis (7, 11–14). Generally, major risk factors for *C. indologene* infection are patients with predisposing diseases, immunocompromised status, long-term broad-spectrum antibiotics treatment, and long-term hospitalization with indwelling devices (1, 15, 16).

Although the clinical significance and pathogenicity of *C. indologenes* are not well established (17, 18), *C. indologenes* is known to be naturally resistant to a wide variety of antibiotics including not only aminoglycosides, tetracyclines, chloramphenicol, macrolides, aminopenicillins, clindamycin, teicoplanin but also first-generation cephalosporins, aztreonam, ticarcillin-clavulanate, and carbapenems (10, 19–21). The most potent drugs reported against *C. indologenes* are quinolones (gatifloxacin and levofloxacin), minocycline, and trimethoprim-sulfamethoxazole (18, 22, 23); however, many studies show an alarming trend in resistance to those antibiotics (3, 9, 24).

Whole-genome sequencing (WGS) has become a well-established technique for high-resolution characterization of the genetic repertoire of bacterial pathogens, including antibiotic resistance, molecular epidemiology, and virulence (25). It is a promising technique for surveillance and monitoring infection control and outbreak cases of many microbial pathogens of interest in public health (26). Hence, we conducted the complete genome sequencing of MDR *C. indologenes* to understand the genomic diversity and genes responsible for antibiotic resistance and virulence. To the best of our knowledge, there is no published data on *C. indologenes* whole-genome sequencing from the cerebrospinal fluid sample of a hospitalized infant in Tocantins, Brazil.

Materials and methods

Patient and bacterial isolate

Chryseobacterium indologenes was isolated from the cerebrospinal fluid (CSF) of an infant hospitalized at the Neonatal Intensive Care Unit (NICU) of Hospital Geral de Palmas, Palmas, Tocantins, Brazil. This isolate was initially identified as *C. indologenes* by a clinical microbiology laboratory using conventional methods. It was sent to the Central Laboratory of Public Health of Tocantins (LACEN/TO) for

species confirmation and drug susceptibility testing. LACEN is a healthcare facility in the Brazilian Ministry of Health that receives samples for the surveillance of antimicrobial resistance.

Bacteria information and antimicrobial susceptibility

Once the sample was received at LACEN, bacterial identification and drug susceptibility assays were performed by the Vitek 2 system (bioMérieux, Marcy-l'Etoile, France) and interpreted according to Clinical and Laboratory Standards Institute guidelines (27). *Chryseobacterium indologenes* isolate was tested for susceptibility against 16 antibiotics: ampicillin (AMP), ampicillin/sulbactam (SAM), piperacillin/tazobactam (TZP), cefuroximeaxetil (CXM-AX), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), ertapenem (ETP), imipenem (IPM), meropenem (MEM), amikacin (AMK), gentamicin (GEN), ciprofloxacin (CIP), tigecycline (TGC), and colistin (CST).

Minimum inhibitory concentration (MICs) of colistin and tigecycline was determined by the broth microdilution (BMD) method according to the EUCAST (28) recommendations. The results obtained with the VITEK 2 system were compared to those obtained by the BMD method.

Phenotypic detection for the production of carbapenemases was carried out by modified Hodge test, synergy test, and the ethylenediaminetetraacetic acid (EDTA) test under the CLSI guidelines (27) as described elsewhere (29–32). Multidrug-resistant (MDR) *C. indologenes* isolate was defined by non-susceptibility to at least one agent in three or more antibiotic categories (33).

DNA isolation and genome sequencing

Genomic DNA extraction was done in an overnight culture using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, United States), according to the manufacturer's instructions. The DNA extract concentration and purity were determined by measuring absorbance at wavelengths of 260 and 280 nm (NanoVue Plus; GE Healthcare Life Sciences, Marlborough, MA, United States). The integrity of genomic DNA was tested by way of electrophoresis. Bacterial DNA concentration was also measured fluorometrically (Qubit® 3.0, kit Qubit® dsDNA Broad Range Assay Kit, Life Technologies, Carlsbad, CA, United States). The sample from the isolate was prepared for sequencing using 1 ng of input genomic DNA. Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA, United States) was used for library preparation. The libraries were amplified using a limited cycle PCR program. The PCR step adds Index 1(i7) adapters, Index 2 (i5) adapters, and sequences required for sequencing cluster generation. The purification of the amplified library was performed using 0.6x Agencourt AMPure XP beads (Beckman Coulter). For checking the library quality and size of fragmented DNA, the samples were evaluated on 1.5% electrophoresis agarose gel. The libraries were quantified with a fluorometric method Qubit® 3.0 using kit Qubit® dsDNA Broad Range Assay Kit, Life Technologies, Carlsbad, CA, United States) and normalized to 4 nM using a Standard Dilution Method. The libraries were pooled, denatured with 0.2 N NaOH, and diluted to the final concentration of 1.8 pM. A PhiX control was added to a final concentration of 1.5 pM. The run-length was a paired-end run of 75 cycles for each read (2 × 75), plus up to eight cycles each for two index reads.

Genome assembly and annotation

Raw reads were assessed for quality using FastQC v.0.11.9, a quality control tool for high throughput sequence data,[1] and filtered for quality, length, and adapter regions using TrimGalore! v.0.6.5, a wrapper tool specific for FastQC.[2] The *de novo* genome assembly was made with SPAdes v.3.15.3 ("careful" and "cov-cutoff auto" options selected) (34) and SSPACE software (35). Plasmid detection and assembly attempts were made using PlasmidFinder 2.1[3] (36) and PlasmidSPAdes (37), respectively. Scaffolds of less than 200 bp were discarded. We assessed the general statistics of the assembled genome using QUAST v5.0.2 (38). The graphical map of the circular genome was generated using CGView Server (39).

Genome annotations were conducted with Prokka v.1.14.5 annotation pipeline (40) and Rapid Annotation using Subsytems Technology (RAST) server v.2.0[4] (41). Orthologous groups were analyzed using eggNOG mapper v2[5] (42). Blast2GO (43) and Kyoto Encyclopedia of Genes and Genomes (KEGG)[6] (44) were used for the determination of Gene Ontology (GO) annotation and gene role in metabolism, respectively.

Phylogenetic inferences using 16S rRNA gene, average nucleotide identity, and DNA–DNA hybridization

We identified the *C. indologenes* 16S rRNA gene sequence from our genome annotation. We considered reference sequences of the 16S rRNA gene from other 14

1 https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
2 https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/
3 https://cge.cbs.dtu.dk/services/PlasmidFinder/
4 https://rast.nmpdr.org/
5 http://eggnog-mapper.embl.de/
6 https://www.genome.jp/kegg/

Chryseobacterium species available at the GenBank database, including another *C. indologenes* strain. *Elizabethkingia miricola* was used as the outgroup. The accession numbers of these sequences are shown in the phylogeny. Nucleotide sequences were aligned using MAFFT v.7 (45). With the software MEGA-X (46), we estimated the best-fitting nucleotide model of substitution. This information was used to construct the phylogenetic tree by the Maximum Likelihood (ML) method. The robustness of branches was assessed by bootstrap analysis of 1,000 replicates (47).

The average nucleotide identity (ANI) values among our *C. indologenes* genome and other twelve *Chryseobacterium* genomes (**Supplementary Table 1**) were calculated using OrthoANI[7] (48). From these same genomes, we calculated the *in silico* DNA–DNA hybridization (DDH)-analogous values using the GGDC v.3.0 tool[8] (49). Heat maps of ANI and DDH were generated using CIMminer.[9]

Comparative pan-genome analysis of *Chryseobacterium indologenes* strains

We constructed a whole-genome sequence-based phylogenetic tree considering our *C. indologenes* genome and other 15 *Chryseobacterium indologenes* genomes (**Supplementary Table 2**) using the online pipeline REALPHY v.1.13 (50) with default settings. The four closest strains of our *C. indologenes* were then analyzed with OrthoVenn2[10] (51), a webserver used for genome-wide annotation and comparison of orthologous gene clusters. Bacterial Pangenome Analysis Pipeline (BPGA) (52) was used for the identification of the core, accessory, and unique genes, and their functional distribution in KEGG categories.

Identification of antimicrobial resistance and virulence-associated genes

For the identification of antibiotic resistance genes, we used the AMRFinderPlus tool from NCBI (53), ResFinder 4.1 tool[11] from DTU (54), and the Comprehensive Antibiotic Resistance Database (CARD; (55);[12] RGI tool, with a 70% identity cutoff. We also performed BLASTp analysis against the ARG-ANNOT V6 database (56) with a 1E-5 *e*-value, > 50% identity, and > 90% query coverage cut-off. The results obtained from the KEGG functional annotation were also considered.

Putative virulence genes were predicted through BLASTp analysis against the Virulence Factor Database (VFDB) (57) using a 1E-5 *e*-value, > 50% identity, and > 90% coverage cut-off.

Identifications of mutations associated with quinolone and colistin resistance

Genes responsible for colistin and quinolone resistance were identified through the rapid prokaryotic genome annotation (PROKKA). The amino acid sequences obtained from lpxA, lpxC, lpxD, and pmrC were manually analyzed for known mutations conferring resistance to colistin. *Acinetobacter* multispecies (WP_196075311.1) and *Acinetobacter baumanii* (SUU42982.1) lpxA sequences were manually aligned with our *C. indologenes* sequence (58). Other genes associated with polymyxin resistance, such as *pmr*B, *pmr*A, *pho*P, and *pho*Q were not found in the annotation executed by PROKKA.

The presence of a known mutation responsible for quinolone resistance on the *gyr*A, *gyr*B, and *par*C genes was investigated using BLASTp software of NCBI.[13] The deduced amino acid sequences of the *C. indologenes* gyrA were aligned with *Chryseobacterium* multispecies (WP_027372477.1) and *Chryseobacterium indologenes* (QPQ51520.1). Amino acid sequence alignment for the gyrB. of *C. indologenes* was made with *Chryseobacterium aurantiacum* (WP_106916365.1), *Chryseobacterium joostei* (WP_076354057.1), *Chryseobacterium arachidis* (WP_072953039.1), and *Chryseobacterium taiwanense* (WP_039365989.1). Substitutions of amino acids in parC sequence described in the literature were not found in our *C. indologenes*.

Genomic islands, insertion sequences, and other mobile genetic elements detection

IslandViewer 4 webserver[14] (59) was used for the identification of the genomic island using FDAARGOS_379 as a reference strain. Insertion sequences, transposons, and integrons were predicted using ISFinder[15] (60), TnCentral[16] (61), and Integron Finder[17] (62) webservers,

7 https://www.ezbiocloud.net/tools/orthoani
8 https://ggdc.dsmz.de/ggdc.php#
9 https://discover.nci.nih.gov/cimminer/
10 https://orthovenn2.bioinfotoolkits.net/
11 https://cge.cbs.dtu.dk/services/ResFinder/
12 https://card.mcmaster.ca/
13 http://blast.ncbi.nlm.nih.gov
14 https://www.pathogenomics.sfu.ca/islandviewer/upload/
15 https://www-is.biotoul.fr/
16 https://tncentral.ncc.unesp.br/
17 https://galaxy.pasteur.fr/

respectively. CRISPR sequences were searched with CRISPRCas Finder[18] (63) on default parameters. The PHASTER webserver[19] (64) was used for the detection of phage-associated sequences in the genome. Integrative and conjugative elements (ICEs) and Integrative mobile elements (IMEs) were predicted with the ICEfinder tool[20] (65) from the ICEberg 2.0 database using the default settings.

Sequences accession number

The raw reads were submitted to Sequence Reads Archives,[21] and the Bioproject accession id is PRJNA830910. Moreover, all the sequence data that we analyzed are related to this Bioproject id.

Results

Characteristics of the patient and antibiotic resistance profile of strain

Our *C. indologenes* strain presented resistance to all tested antibiotics, including β-lactams (Ampicillin, Ampicillin-Sulbactam, Piperacillin-Tazobactam, Cefuroximeaxetil, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Meropenem); aminoglycosides (Amikacin, Gentamicin); quinolones (Ciprofloxacin); glycylcycline (Tigecycline); and polymyxin (Colistin) (**Table 1**). The isolate was defined as multidrug-resistant (MDR). However, the following antibiotics were not included in the MDR classification: amikacin and gentamicin (aminoglycosides); ampicillin and ampicillin-sulbactam (aminopenicillins); imipenem, meropenem, and ertapenen (carbapenems). This was because *C. indologenes* is intrinsically resistant to these antibiotics (18, 19, 66, 67).

General features of the *Chryseobacterium indologenes* genome

The total length of the draft genome assembled was 4,836,765 bp, comprising one circular chromosome, with 37.32% G + C content. We detected a COIRNAI plasmid replicon but could not assemble any plasmid. The statistics of assembly and annotation are shown in **Figure 1A**. The assembly comprised 58 scaffolds and 4,409 genes that covered 88.20% of the genome. Of these genes, 4,341 were predicted to be coding sequences (CDSs). Of the 68 RNA genes predicted, three were rRNAs, 64 transfer RNAs (tRNAs), and one transfer-messenger RNA (tmRNA) (**Figures 1A,B**). The RAST analysis showed that the genome of *C. indologenes* comprises 366 subsystems that could be classified into 27 categories. The five most significant categories in this genome were "amino acids and derivatives" that accounted for 384 genes, followed by "carbohydrates" (232 genes), "cofactors, vitamins, prosthetic groups, pigments" (224 genes), "protein metabolism" (222 genes), and "virulence, disease, and defense" (120). In the category of "virulence, disease, and defense," 94 genes were found to be related to "resistance to antibiotics and toxic compounds," including beta-lactamase (13 genes), multidrug resistance efflux pumps (13 genes), multidrug resistance, tripartite systems found in gram-negative bacteria (12 genes), resistance to fluoroquinolones (5 genes), and aminoglycoside adenylyltransferases (1 gene) (**Figure 1C**).

Orthologous genes and gene ontology of *Chryseobacterium indologenes*

The distribution of protein-coding genes into the Cluster of Orthologous Groups (COG) functional category using EggNog resulted in a total of 3,759 genes. The majority of known protein-coding genes were related to "amino acid metabolism and transport" (n = 310; 8.25%), followed by those associated with "cell wall/membrane/envelope biogenesis" (n = 304; 8.09%), and "transcription" (n = 295; 7.85%). The number of genes associated with defense mechanisms was 70 (1.86%), and with "unknown functions" it was 973 (25.88%) (**Figure 2A**).

The Gene Ontology (GO) distribution of our *C. indologenes* strain showed a total of 2,900 genes, which accounted for 65.77% of the entire encoded genes. Of these, 7,460 GO terms were associated with "molecular function," 4,620 GO terms with "biological process," and 2,129 GO terms with "cellular location." The GO distribution showed that within the molecular function, the main subcategories were "catalytic activity (1.717 GO terms, 23.02%), "binding activity" (1.080 GO terms, 14.48%), and "organic cyclic compound binding" (812 GO terms, 10.88%). Among the biological process, most of the genes were characterized to the subcategories like "metabolic process" (1.334 GO terms, 28.87%), "organic substance metabolic process" (1.141 GO terms, 24.70%), and "biosynthetic process" (623 GO terms, 13.48%); Within the cellular location, the most highly assigned GO terms were "membrane" (n = 886, 41.62%), "integral component of membrane"

18 https://crisprcas.i2bc.paris-saclay.fr/CrisprCasFinder/Index

19 https://phaster.ca/

20 https://bioinfo-mml.sjtu.edu.cn/ICEfinder/ICEfinder.html

21 https://www.ncbi.nlm.nih.gov/sra

TABLE 1 Phenotyping and antibiotic resistance genes found within the *Chryseobacterium indologenes* genome.

Phenotyping		Genotyping/properties of proteins					
Antibiotic Class	**Antibiotic resistance (Vitek-2)**	**Reference Sequence**	**Putative resistance genes**	**Resistance gene/protein, mechanism function**	**Size (aa)**	**aa identity (%)**	**Resistance gene characterization**
Beta-lactams	Ampicillin, Ampicillin-Sulbactam, Piperacillin-Tazobactam, Cefuroximeaxetil, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Meropenem	HM245381	bla_{IND-13}	class B carbapenemase IND-13	243	100.00	AMRFinderPlus, ARG-ANNOT, CARD, ResFinder
		AB674567	bla_{CIA-3}	class A extended-spectrum beta-lactamase CIA-3	292	100.00	AMRFinderPlus, ARG-ANNOT, CARD
		AY425988	$bla_{TEM-116}$	class A broad-spectrum beta-lactamase	286	100.00	AMRFinderPlus, ARG-ANNOT, CARD, ResFinder, KEGG
		AEM66528	$bla_{OXA-209}$	class D beta-lactamase	279	60.16	AMRFinderPlus, ARG-ANNOT, CARD
		ALB25886	bla_{VEB-15}	class A extended-spectrum beta-lactamase	303	52.36	AMRFinderPlus, ARG-ANNOT, KEGG
Quinolones	Ciprofloxacin	BAP29388	*mcb*G	MCBG protein family	192	79.00	KEGG
Aminoglycosides	Amikacin, Gentamicin, Streptomycin(-)	AAA27459	*aad*S	aminoglycoside 6-adenylyltransferase AadS	287	100.00	AMRFinderPlus, ARG-ANNOT, CARD
		ADZ12699	*RanA**	multidrug efflux ABC transporter ATP-binding subunit RanA	271	78.52	CARD
		ADZ12700	*Ran*B*	multidrug efflux ABC transporter permease subunit RanB	250	79.44	CARD
Polymyxin	Colistin	BAP31997	*Hly*D*	HlyD-like membrane fusion protein	386	94.00	KEGG
Polymyxin	Colistin	BAP31872	*Tol*C*	TolC family protein	438	95.00	KEGG
Glycylcyclines	Tigecycline	M37699	*tet*(X6)	tetracycline-inactivating monooxygenase Tet(X)	372	61.87	ARG-ANNOT, CARD, KEGG
Tetracycline	(-)	AIL45943	*tet*A*	tetracycline efflux protein TetA	410	77.00	KEGG
Sulfonamide	(-)	ABZ82584	*sul*2	sulfonamide-resistant dihydropteroate synthase Sul2	271	100.00	AMRFinderPlus, ARG-ANNOT, CARD, ResFinder, KEGG
Phenicol	(-)	BAP31605	*cat3*	chloramphenicol acetyltransferase	208	82.00	KEGG
Phenicol	(-)	ACD03314	*cat*B3	type B chloramphenicol O-acetyltransferase	210	78.91	AMRFinderPlus, ARG-ANNOT, CARD, KEGG
Macrolide	(-)	AAA27431	*erm*F	23S rRNA methyltransferase	266	63.16	AMRFinderPlus, ARG-ANNOT, CARD, KEGG

*Multidrug efflux pumps. (-) Susceptibility testing was not performed.

(n = 755, 35.46%), and "cytoplasm" (n = 260, 12.21%) (**Figure 2B**).

Phylogenetic position and similarity of whole genomes

A phylogenetic tree based on the 16S rRNA sequence was constructed with fourteen 16S rRNA reference sequences related to *Chryseobacterium* and one 16S rRNA reference sequence of *Elizabethkingia miricola* as an outgroup. This aimed to define the evolutionary position of our *C. indologenes* strain. Analyses revealed that our *C. indologenes* was most closely related to *Chryseobacterium indologenes* (AM232813). Nevertheless, it also showed a similarity between different species, including *Chryseobacterium cucumeris* (KX146463), *Chryseobacterium arthrosphaerae* (FN398101), *Chryseobacterium gleum* (AM232812), and *Chryseobacterium flavum* (EF154516) (**Figure 3A**).

The genomic similarity between our sample and the twelve *Chryseobacterium* species that had complete genome sequences in the NCBI (**Figure 3A**) was evaluated using *in silico* ANI (**Supplementary Table 3**) and DDH (**Supplementary Table 4**) analyses (**Figures 3B,C**) to confirm the species relatedness inferred from the phylogenetic tree and ensure an accurate assignment at the species level. The ANI analysis found that our *C. indologenes* and *C. indologenes* (GCF_900460995.1) had a high similarity of 99.06% (**Figure 3B**). The DDH value

A

Content	Value
Total Sequence Length (bp)	4,836,765
Gene Number	4,409
Gene Length	4,266,283
GC Content (%)	37.32
Gene Length/Genome (%)	88.20
Gene Average Length (bp)	968
Intergenic Region Length (bp)	570,482
Number of Scaffolds	58
Scaffold N50 (bp)	690,911
Scaffold L50	3
Largest Contig (bp)	951,861
Number of Coding Sequences	4,341
Number of rRNA	3
Number of tRNA	64
Number of tmRNA	1
Number of Subsystems	366

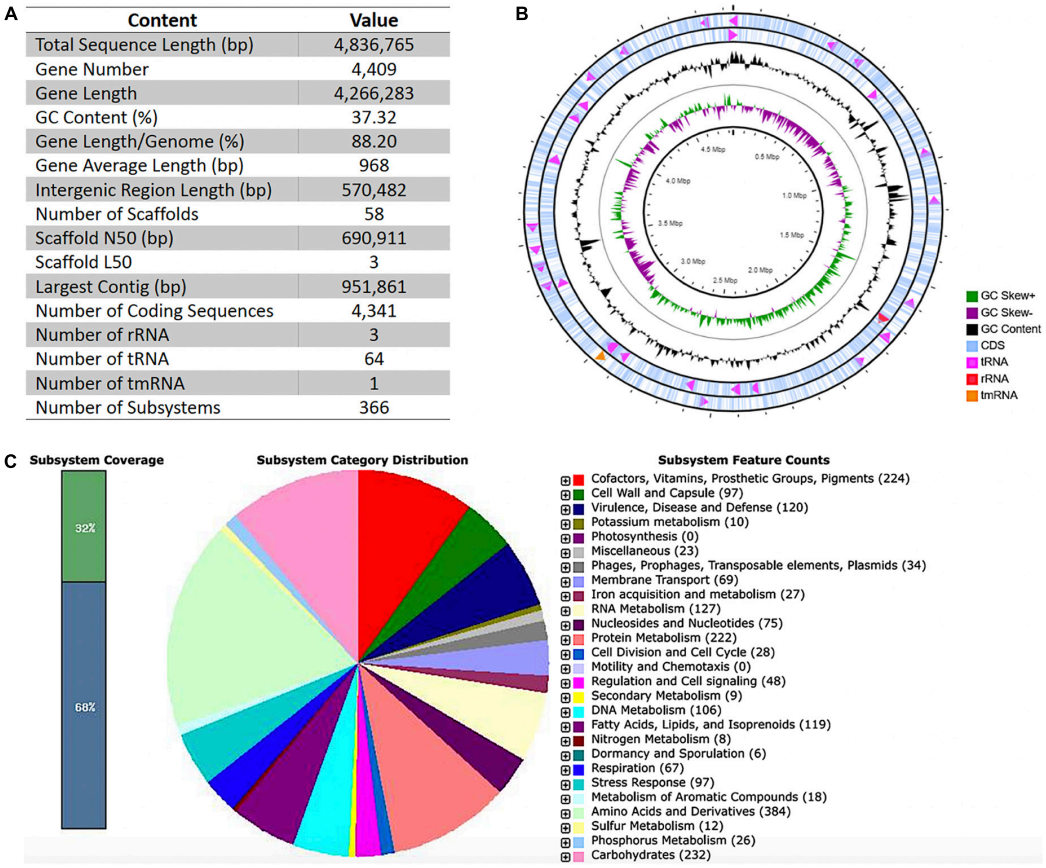


FIGURE 1
Basic data of Whole-Genome Sequencing, circular representations, and subsystem category distributions of *Chryseobacterium indologenes*. **(A)** Assembly and annotation statistics. **(B)** Circles are numbered from 1 (outer) to 4 (inner). The outer two circles represent the coding sequence (CDS), transfer ribonucleic acid (tRNA), ribosomal ribonucleic acid (rRNA), and transfer-messenger RNA (tmRNA). The third circle shows the GC content (black). The fourth circle demonstrates the GC skew curve (positive GC skew, green; negative GC skew, violet). **(C)** The genome of *C. indologenes* annotated by the Rapid Annotation System Technology (RAST) server was classified into subsystems and categories. The green part in the bar chart at the leftmost position corresponds to the percentage of proteins included. The pie chart and count of the subsystem features in the right panel show the percentage distribution and category of the subsystems.

between *C. indologenes* and *C. indologenes* (GCF_900460995.1) was 91.7% (Figure 3C). Therefore, both ANI and DDH analysis indicated that our *C. indologenes* strain belongs to the *C. indologenes* species.

PhyloGenetic tree and genetic relatedness

We performed a phylogenetic analysis based on *C. indologenes* genomes downloaded from the NCBI database. The resulting tree topology was assessed to identify genetic relatedness between our *C. indologenes* isolate and 15 *C. indologenes* strains (Figure 4A). Our analysis showed that our *C. indologenes* strains is more closely related to *Chryseobacterium indologenes* 742 (SAMN22445227), *Chryseobacterium indologenes* FDAARGOS_379 (SAMN07312423), *Chryseobacterium indologenes* FDAARGOS_537 (SAMN10163232), and *Chryseobacterium indologenes* FDAARGOS_648 (SAMN11056363) (Figure 4A). The characteristics of the 16 *C. indologenes* strains are shown in Figure 4B.

Orthologous groups and Kyoto Encyclopedia of Genes and Genomes distribution in the closest *Chryseobacterium indologenes* genomes

Using OrthoVenn web server, comparison and annotation of orthologous gene clusters were performed between our *C. indologenes* and its four most closely related neighbors. Data indicates that there are 4,060 core-conserved genes shared by all five strains and a total of 6 strain-specific gene clusters

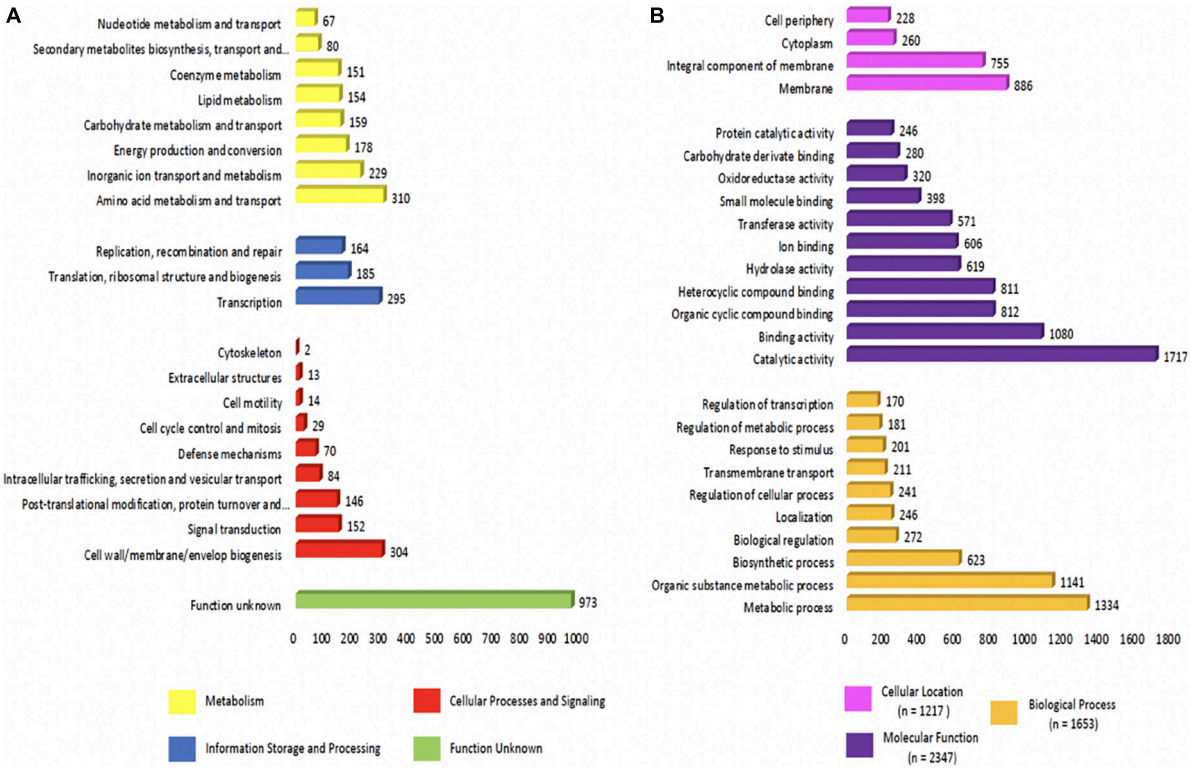


FIGURE 2
Functional annotations of *Chryseobacterium indologenes* strain. (**A**) Clusters of orthologous groups (COGs) based on functional annotation using eggNOG-mapper. (**B**) Distribution of gene ontology (GO).

in our *C. indologenes* strain. These unique genes were related to metal ion transport, response to cadmium ion, and copper ion transport (Figure 5A). The meanings of these unique gene clusters are not clear, and further studies are needed to better understand the functions of these singular genes. Our *Chryseobacterium indologenes* strain contained the highest number of singletons (n = 182), followed by *C. indologenes* 742 (n = 109), *C. indologenes* FDAARGOS_379 (n = 29), *C. indologenes* FDAARGOS_537 (n = 18), and *C. indologenes* FDAARGOS_648 (n = 11) (Figure 5B). We also found *sul*2, $bla_{TEM-116}$, *aad*S, bla_{VEB-15}, $bla_{OXA-209}$, *cat*B e *erm*F resistance genes in singletons of our *C. indologenes* strain.

The KEGG functional distribution showed that genes were associated mainly with "metabolism" and were the most abundant in core (74.62%) compared to unique (33.33%) and accessory (25%) genomes (Figure 5C). In the core genome, genes were mainly related to "amino acid metabolism" (14.36%), "overview" (13.49%), and "carbohydrate metabolism" (12.88%) (Figure 5D). In the accessory genome, "environmental information processing," "genetic information processing," "metabolism," and "organismal systems" accounted for similar portions (25%) (Figure 5C). These genes were associated with "digestive system" (25%), "membrane transport" (25%), "metabolism of cofactors and vitamins" (25%), and "translation" (25%) (Figure 5D). In the unique genome, "metabolism" (33.33%), "human diseases" (33.33%), and "genetic information processing" (16.67%) accounted for most genes (Figure 5C). These genes were mainly associated with "folding, sorting, and degradation" (16.67%), "infectious diseases" (16.67%), and "drug-resistance" (16.67%) (Figure 5D). We found 27 genes related to "drug resistances" in the unique genome. Of these, 11 were related to beta-lactam resistance, 11 were associated with cationic antimicrobial peptide resistance, and 5 were vancomycin resistance genes. Our data suggest that antibiotic resistance plays an important function in all of the *C. indologenes* strains analyzed.

Resistome of *Chryseobacterium indologenes*

The whole-genome sequence analysis of *C. indologenes* corroborated with the phenotypic analyses, which revealed several antibiotic resistance-related genes (Table 1 and Figure 6). These antibiotic resistance genes included 5 β-lactamases (bla_{IND-13}, bla_{CIA-3}, $bla_{TEM-116}$, $bla_{OXA-209}$, bla_{VEB-15}), 1 quinolone gene (*mcb*G), 1 tigecycline gene [tetracycline-inactivating monooxygenase *tet*(X6)], 1 RanA and 1 RanB

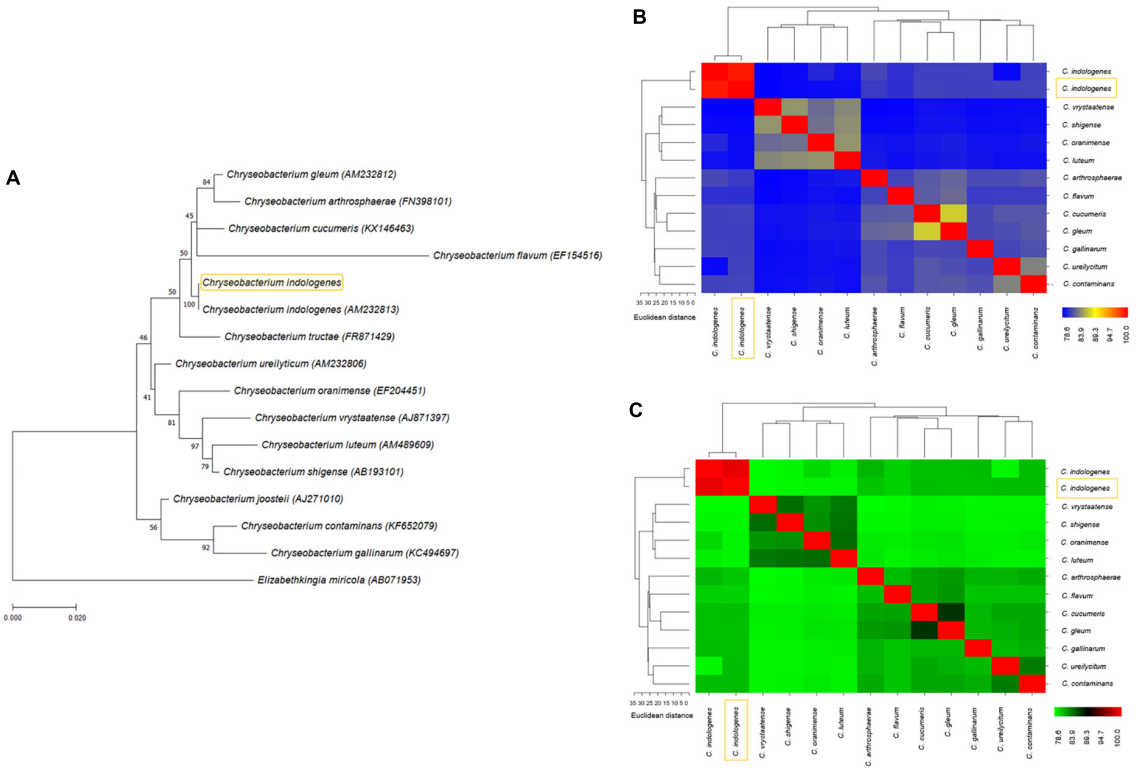
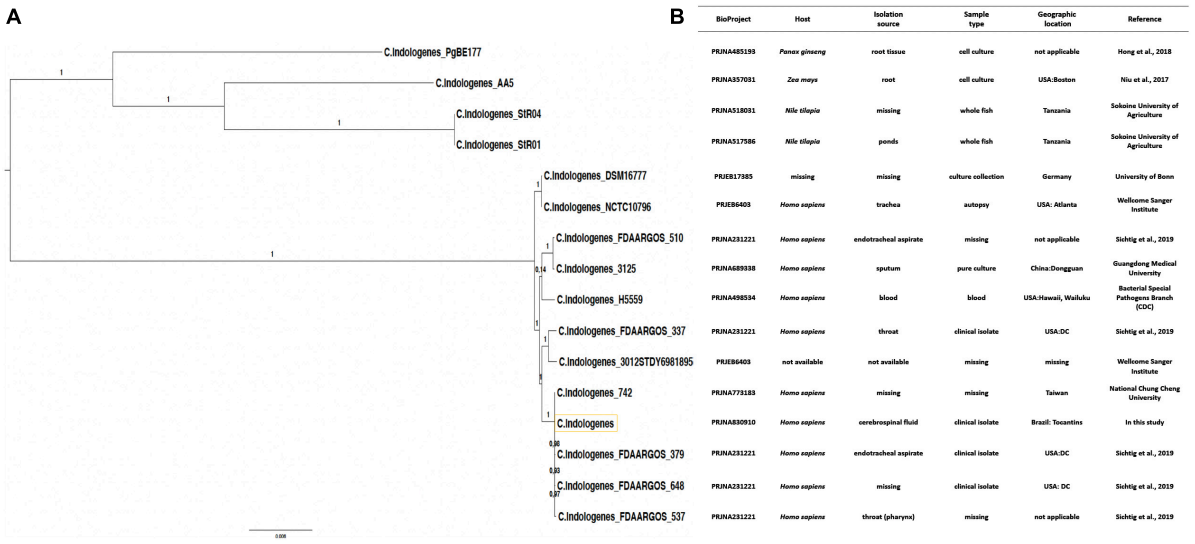
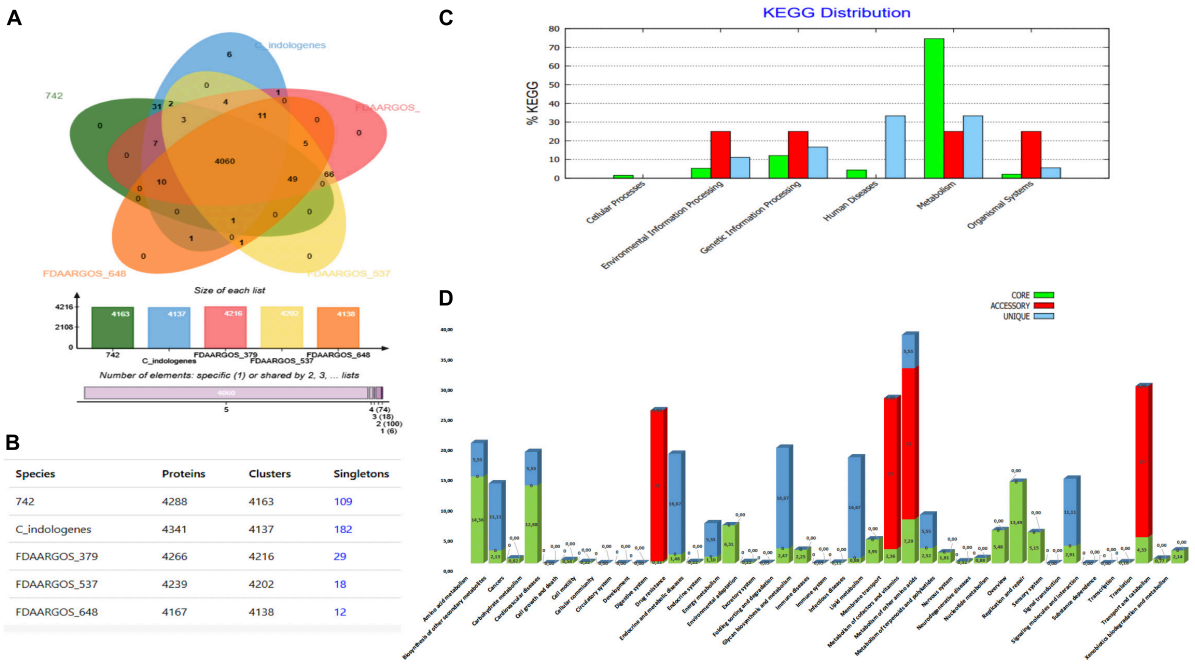


FIGURE 3
(A) Phylogenetic tree based on 16S rRNA showing the relationship among our *C. indologenes* strain and other reference sequences of 16S rRNA obtained from public databases. The number next to the node is the statistical bootstrap value. In brackets are GenBank accession numbers of the 16S rRNA genes. The scale bar indicates 0.020 substitutions per nucleotide position. The heat maps of average nucleotide identity (ANI) (**B**) and *in silico* DNA–DNA hybridization (DDH) (**C**) between our *C. indologenes* genome and twelve *Chryseobacterium* genomes. The yellow box represents our strain.



BioProject	Host	Isolation source	Sample type	Geographic location	Reference
PRJNA485193	*Panax ginseng*	root tissue	cell culture	not applicable	Hong et al., 2018
PRJNA357031	*Zea mays*	root	cell culture	USA:Boston	Niu et al., 2017
PRJNA518031	*Nile tilapia*	missing	whole fish	Tanzania	Sokoine University of Agriculture
PRJNA517586	*Nile tilapia*	ponds	whole fish	Tanzania	Sokoine University of Agriculture
PRJEB17385	missing	missing	culture collection	Germany	University of Bonn
PRJEB6403	*Homo sapiens*	trachea	autopsy	USA: Atlanta	Wellcome Sanger Institute
PRJNA231221	*Homo sapiens*	endotracheal aspirate	missing	not applicable	Sichtig et al., 2019
PRJNA689338	*Homo sapiens*	sputum	pure culture	China:Dongguan	Guangdong Medical University
PRJNA498534	*Homo sapiens*	blood	blood	USA:Hawaii, Wailuku	Bacterial Special Pathogens Branch (CDC)
PRJNA231221	*Homo sapiens*	throat	clinical isolate	USA:DC	Sichtig et al., 2019
PRJEB6403	not available	not available	missing	missing	Wellcome Sanger Institute
PRJNA773183	*Homo sapiens*	missing	missing	Taiwan	National Chung Cheng University
PRJNA830910	*Homo sapiens*	cerebrospinal fluid	clinical isolate	Brazil: Tocantins	In this study
PRJNA231221	*Homo sapiens*	endotracheal aspirate	clinical isolate	USA:DC	Sichtig et al., 2019
PRJNA231221	*Homo sapiens*	missing	clinical isolate	USA: DC	Sichtig et al., 2019
PRJNA231221	*Homo sapiens*	throat (pharynx)	missing	not applicable	Sichtig et al., 2019

FIGURE 4
(A) Phylogenetic tree showing the evolutionary similarity between *C. indologenes* and other 15 selected strains of *Chryseobacterium indologenes*. **(B)** Genome Assembly and Annotation report (https://www.ncbi.nlm.nih.gov/genome/browse/#!/prokaryotes/14653/) of 16 strains of *C. indologenes*. The yellow box represents our strain.



Species	Proteins	Clusters	Singletons
742	4288	4163	109
C_indologenes	4341	4137	182
FDAARGOS_379	4266	4216	29
FDAARGOS_537	4239	4202	18
FDAARGOS_648	4167	4138	12

FIGURE 5
Comparative genomic analysis. **(A)** Venn diagram and bar chart showing the numbers of unique and shared orthologous genes in the five most closely related *C. indologenes*. **(B)** Number of proteins, clusters, and singletons. (**C**) KEGG pathway classification in core, accessory, and unique genomes. **(D)** Distribution of KEGG pathway classification.

genes that encode an efflux pump which confers resistance to aminoglycosides, 1 outer-membrane protein (*Tol*C) and 1 gene of membrane fusion protein (*Hly*D) genes that mediate resistance to colistin antibiotic (Table 1). Additionally, our *in silico* analysis found genes that can confer resistance to tetracycline [*tet*(A)] (68), streptomycin (*aad*S) (69), phenicol (*cat*3, *cat*B3) (70), sulfonamide (*sul*2) (71), and macrolides (*erm*F) (72); however, our strains were not tested phenotypically to these antibiotics (Table 1).

Gene functions annotated using the RAST/PROKKA tool identified *gyr*A, *gyr*B, and *lpx*A gene, in which amino acid substitution can also confer antibiotic resistance for fluoroquinolone and colistin, respectively. Amino acid substitutions related to quinolone resistance were also found at positions 83 of DNA gyrase subunit A (gyrA: Ser83Tyr) and at positions 425 and 473 of DNA gyrase subunit B (gyrB: Leu425Ile and Lys473Arg). Mutations that may play a role in the development of colistin resistance were found at lpxA (G68D) of *C. indologenes* (Figures 6A,B).

Virulence-associated genes detection

Chryseobacterium indologene strain revealed the presence of 19 virulence factorsthat were associated with adhesion (elongation factor *Tu*, *ilp*A), stress response (*kat*A, *kat*G, *clp*P, *and gro*L), environmental adaptation (*ure*G, *ure*B), biofilm formation (*clp*P, *gal*E), metabolism (*clp*E), motility (*rff*G), polysaccharide biosynthesis (tviB), O-antigen nucleotide sugar biosynthesis (*wlb*B), O-antigen-synthesis (*rfb*A), capsular polysaccharide synthesis and antiphagocytosis (*cap*8E, *cap*8G), survival and virulence (*mgt*B), cytokine production and cytotoxicity (*ilp*A), infection and immune evasion (*icl*1), and fatty acid biosynthesis (*acp*P) (Table 2).

Genomic islands and other mobile genetic elements genes in *Chryseobacterium indologenes*

Our analyses indicate that the *C. indologenes* genome contained 13 potential Genomic Islands (GI) (Figure 7A). These had some elements associated with one integrative and conjugative element (ICE), in the 8 and 12 GI regions, such as a copy of a *Trb*C, and a relaxase (*xer*C). The ICE, named ICE*Cind1*, contained further open reading frames (ORFs) encoding putative TrbL, TrbC (1 copy), TrwB, integrase, and it was bordered by a 16-bp direct repeat (attL; 5′-TTGTGGGTCCTGAGG-3′, and attR: 5′-TTGTGGGTCCTGAGGG-3′) at both ends. The attR was included in the 3' end of the tRNA-Val(TAC). In GI, we also found a *sul2* gene encoding for resistance to sulfonamide,

flanked by IS91-like element (ISVsa3 family transposase); an IS91 family transposase ISTha3; 2 copies of *xer*C; and other "accessory" sequences (Figures 7B,C).

Other elements linked to mobile genetic elements (MGEs) were present such as an insertion sequence (ISEIsp1), classified in the IS1595 family (ISPna2 group); and a transposon Tn5393. Sequence examination further indicated a region bordered by a 12-bp direct repeat (aatL 5′-ATTTTCTTAAAT-3 and attR 5′-ATTTTCTTAAAT-3) at both ends that contained the *cat*B3, a chloramphenicol acetyltransferase gene inserted in integron In31; one gene associated with fatty acid biosynthesis (*acp*P); a 3-phenylpropionate-dihydrodiol/cinnamic acid-dihydrodiol dehydrogenase (*hca*B) gene, a transcriptional regulator (*exs*A) gene; a quinone reductase (*chr*R) gene; and a tRNA (Figure 7B).

Through IslandViewer 4, we detected unaligned scaffolds that contained a ColRNAI type of replicons and genes associated with β-lactams ($bla_{TEM-116}$, bla_{VEB-15}, $bla_{OXA-209}$), aminoglycosides (*aad*S), phenicol (*cat*B3), and macrolide (*erm*F) antibiotic resistance. CRISPR and phage elements were not detected in the *C. indologenes* genome.

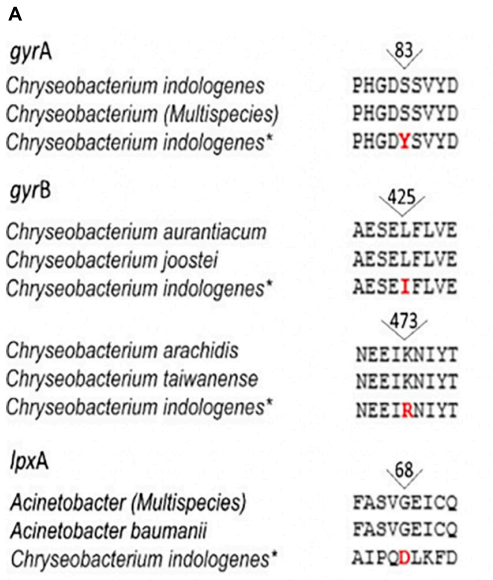
Discussion

Multidrug-resistant Gram-negative bacteria are usually associated with nosocomial infection and pose an important health problem for neonates admitted to neonatal intensive care units worldwide (9, 73). *Chryseobacterium indologenes* is an emerging nosocomial pathogen that has acquired clinical significance due to ubiquitous and intrinsical resistance to several antibiotics, life-threatening infection potential, and ability to persist in hospital settings (9, 74). The number of studies about *C. indologenes* is very limited, mainly in terms of their genomes. To build on current information, we performed a systematic genotypic characterization of a *C. indologenes* strain isolated from the cerebrospinal fluid of an infant.

Although there has been an increasing trend of *C. indologenes* bacteremia related to infants over the last few decades (5, 75, 76), most cases of infections caused by *C. indologenes* are still reported in adults (8, 10). The precise reason for fewer reports of *C. indologenes* infections in children remains unclear. Studies have suggested it may relate to the

TABLE 2 Presence of virulence determinants in *Chryseobacterium indologenes* isolate.

Gene identifier	Putative gene	Encoding	Size (aa)	Reference species	aa identity (%)
NP_206868	*ure*G	Urease accessory protein UreG	212	*Helicobacter pylori*	74.24
NP_206872	*ure*B	Urease subunit β	573	*Helicobacter pylori*	66.26
NP_273273	*kat*A	Catalase KatA	495	*Neisseria meningitidis*	53.72
YP_094248	*kat*G	Catalase/peroxidase KatG	758	*Legionella pneumophila*	61.75
NP_644943	*cap*8E	Capsular polysaccharide synthesis enzyme Cap8E	344	*Straphylococcus aureus*	67.17
NP_644945	*cap*8G	Capsular polysaccharide synthesis enzyme Cap8G	379	*Straphylococcus aureus*	53.58
NP_464522	*clp*E	ATP-dependent protease	845	*Listeria monocytogenes*	50.39
NP_465991	*clp*P	ATP-dependent Clp protease proteolytic subunit	228	*Listeria monocytogenes*	52.38
NP_439034	*rff*G	dTDP-glucose 4,6-dehydratase	359	*Haemophilus influenzae*	51.03
NP_438515	*gal*E	UDP-glucose 4-epimerase GalE	339	*Haemophilus influenzae*	50.30
NP_933683	*ilp*A	MetQ/NlpA family lipoprotein adhesin IlpA	268	*Vibrio vulnificus*	55.56
YP_177728	*icl*1	Isocitrate lyase	426	*Mycobacterium tuberculosis*	61.15
NP_458740	*tvi*B	Vi polysaccharide biosynthesis UDP-N-acetylglucosamine C-6 dehydrogenase TviB	431	*Salmonella enterica*	53.72
NP_462662	*mgt*B	Magnesium-translocating P-type ATPase	890	*Salmonella enterica*	52.75
NP_540392	*acp*P	Acyl carrier protein	79	*Brucella melitensis*	58.67
YP_170388.1	*rfb*A	Glucose-1-phosphate thymidylyltransferase RfbA	287	*Francisella tularensis*	61.03
NP_878994	*wlb*B	O-antigen biosynthesis protein WlbB	210	*Bordetella pertussis*	53.33
YP_094724	*gro*L	Chaperonin GroEL	541	*Legionella pneumophila*	65.72
YP_169203.1	*tuf*	Elongation factor Tu	403	*Francisella tularensis*	67.25



B

Gene	Site	Mutation	Bacteria	Reference
gyrA	83	Ser – Tyr	*Chryseobacterium indologenes; Flavobacterium columnare; Flavobacterium psychrophilum*	Zhang et al., 2021 Mata et al., 2018 Söderlund et al., 2018
gyrB	425	Leu – Ile	*Chryseobacterium indologenes*	Zhang et al., 2021
	473	Lys – Arg	*Chryseobacterium indologenes*	Zhang et al., 2021
lpxA	68	Gly – Asp	*Acinetobacter baumanii; Chryseobacterium oranimense*	Sharma et al., 2015

FIGURE 6
(A) Amino acid sequence alignment for the GyrA QRDR (Ser83Tyr) of *Chryseobacterium ureilyticum*, *Chryseobacterium* (Multispecies), *Chryseobacterium indologenes**; GyrB QRDR (Leu425Ile) of *Chryseobacterium aurantiacum*, *Chryseobacterium joostei*, *Chryseobacterium indologenes** and GyrB QRDR (Lys473Arg) of *Chryseobacterium arachidis*, *Chryseobacterium taiwanense*, *Chryseobacterium indologenes**; and mutations in lpxA (Gly68Asp) of *Chryseobacterium* (Multispecies), Acinetobacter baumanii, *Chryseobacterium indologenes**. **(B)** Known mutations in genes conferring resistance to quinolones (GyrA and GyrB QDDR) and colistin (lpxA). *Strain isolated in this study.

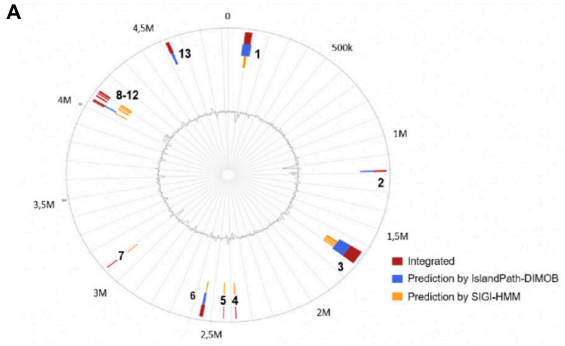
lower frequency of comorbidities in the pediatric population (77). Considering the pediatric population can present significant morbidity and immune dysfunction, we postulate that the relevance of *C. indologenes* isolated from clinical samples of infants can be challenging since novel molecular and phenotypic tests are providing rapid and accurate identification of many bacterial species, including glucose non-fermenting gram-negative bacilli (78, 79) such as *C. indologenes*.

Chryseobacterium indologenes often exhibit resistance to a wide variety of broad-spectrum antibiotics, including aminoglycosides, tetracyclines, chloramphenicol, macrolides, clindamycin, and teicoplanin; extended-spectrum penicillins; and first-, second- and third-generation cephalosporin, aztreonam, ticarcillin-clavulanate and the carbapenems (19, 23, 80). According to our phenotypic results, our *C. indologenes* strain was resistant to most of these antibiotics. It revealed a multidrug resistance profile, presenting *in vitro* resistance to a wide range of antibiotic classes including β-lactams, aminoglycosides, quinolone, glycylcycline, and polymyxin. Although some studies have reported that *C. indologenes* remains susceptible to trimethoprim/sulfamethoxazole, quinolones, minocycline, cefepime, ceftazidime, piperacillin-tazobactam, and rifampin (10, 18, 81, 82), our strain was resistant not only to piperacillin–tazobactam, ciprofloxacin, cefepime, and ceftazidime but also to colistin and tigecycline. Our findings are in accordance with other studies (3, 9, 24) that show an increasing trend in resistance of *C. indologenes* against most commonly used antimicrobial agents.

In recent years, whole-genome sequencing (WGS) has become an efficient method not only for understanding the evolution of a wide range of infectious pathogens, such as emerging bacteria, but also for outbreak surveillance and implementation of rapid infection control protocols (83). Thus, we decided to investigate the *C. indologenes* genome using WGS. Our draft genome revealed one circular chromosome that had a similar length (4,836 kb) to most of the sequenced genomes from *C. indologenes* deposited in NCBI. The genomic features of chromosomes annotated using RAST, eggNOG, and GO showed similar characteristics, presenting cellular processes that are essential to the bacteria (84). The genes related to the disease found in RAST and the defense mechanisms present in eggNOG analysis indicate our *C. indologenes* strain is associated with the multidrug resistance profile. Liang et al. (84) obtained similar results when they analyzed the genomic features and antimicrobial susceptibility patterns of the *Chryseobacterium arthrosphaerae* strain ED882-96 isolated from a patient in Taiwan.

To evaluate the taxonomic position and to confirm the species identification of our strain, we conducted a 16S rRNA analysis and found that our strain is affiliated with the species *C. indologenes* (GenBank: AM232813). Although 16S rRNA gene sequences are highly conserved among strains

A



B

GI number	Puntative GI Family/ Classes	Island start	Island end	Length	Gene	Product	Strand
1	Metabolism associated	81929	118351	36422	*aroE*	Shikimate dehydrogenase (NADP(+))	-1
					rbfA	Ribosome-binding factor A	-1
					epi	Ethylmalonyl-CoA/methylmalonyl-CoA epimerase	1
					xerD	Tyrosine recombinase XerD	1
						30 Hypothetical proteins	
2	-	1182210	1190324	8114		7 Hypothetical proteins	
3	SGI1-K like	1642738	1716241	73503	yycB	putative transporter YycB	-1
					nimR	HTH-type transcriptional regulator NimR	1
					xerC	Tyrosine recombinase XerC	1
					topB	DNA topoisomerase 3	1
					sul2	Dihydropteroate synthase	1
					cadC	Cadmium resistance transcriptional regulatory protein CadC	1
					merA	Mercuric reductase	1
					cadC	Cadmium resistance transcriptional regulatory protein CadC	1
					merA	Mercuric reductase	1
					garB	Glutathione amide reductase	1
					gcvH	Glycine cleavage system H protein	1
					acr3	Arsenical-resistance protein Acr3	1
					topB	DNA topoisomerase 3	-1
						Single-stranded DNA-binding protein	1
						IS91 family transposase ISTha3	1
						61 Hypothetical proteins	
4	-	2375679	2380589	4910		5 Hypothetical proteins	
5	-	2437607	2441852	4245		2 Hypothetical proteins	
6	PRI like	2540026	2558634	18608	*xerC*	Tyrosine recombinase XerC	1
					smc	Chromosome partition protein Smc	-1
					pcrA	ATP-dependent DNA helicase PcrA	-1
						8 Hypothetical proteins	
7	-	3084153	3088999	4846		4 Hypothetical proteins	
8	-	4039126	4044766	5640		7 Hypothetical proteins	
9	-	4045417	4053504	8087	*dnaJ*	Chaperone protein DnaJ	-1
					trbC	Conjugal transfer protein	-1
						Relaxase	-1
						AAA10	-1
						8 Hypothetical proteins	
10	-	4070729	4078836	8107		Single- stranded DNA-binding protein	-1
						11 Hypothetical proteins	
11	Metabolism associated	4086703	4093512	6809		SusD-like protein P25	-1
						TonB-dependent receptor P3	-1
						5 Hypothetical proteins	
12	Metal homeostasis associated	4096722	4107334	10612	*rhaR*	HTH-type transcriptional activator RhaR	1
					pacS	putative Copper- transporting ATPase PacS	1
					copB	Copper-exporting P-type ATPase B	1
						9 Hypothetical proteins	
13	PRI like	4518862	4537715	18853	*ccp*	Cytochrome c551 peroxidase	1
					btuB	Vitamin B12 transporter BtuB	1
						IS1182 family transposase ISMsp1	1
						12 Hypothetical proteins	

C

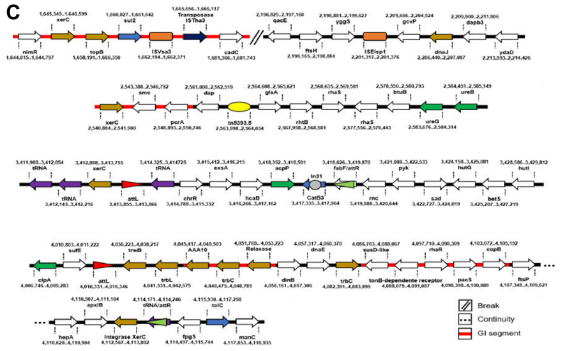


FIGURE 7

Schematic representation of the Genomic Islands (GIs) and Mobile Genetic Elements (MGEs) in *C. indologenes* strain. **(A)** Circular genomic representation of GIs. **(B)** Genetic composition of the GIs. **(C)** MGEs found in *C. indologenes*. Arrow: Light blue (resistance genes), dark blue (transposase genes), golden [elements associated with Integrative and Conjugative Element (ICE)], green (virulence genes), purple (tRNAs). Red/green triangles represent the attL and attR sequences, respectively. Orange squares represent Insertion Sequences. The yellow ellipse represents transposons. The gray circle represents integron.

of the same bacterial species and are frequently used to identify and classify microorganisms, taxonomic classifications based only on the analysis of the 16S rRNA gene can lead to misclassifications in some cases (85). This happens because this analysis lacks sufficient discriminatory power to differentiate species in many genera, such as *Aeromonas*, *Bacillus, Pseudomonas*, *Streptococcus,* etc. (86, 87). Therefore, we used average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) to validate species identity, which confirmed that the most closely related species of our strain was *C. indologenes* (GCF_900460995.1).

The phylogenetic and corresponding taxonomic analysis is fundamental not only to establish the genetic novelty and the genotype–phenotype relationships of the isolates but also to identify the closest relatives of microorganisms within assembled genomes (88, 89). When we assessed the genetic relatedness between *C. indologenes* isolate and fifteen *C. indologenes* strains (90–92), we found that the five closest relatives of *C. indologenes* strains were isolated from clinical human samples. We then analyzed the distribution of shared gene families (sequence clusters) among the proteomes of our sample and four *C. indologenes* strains. Interestingly, our *C. indologenes* strain harbored the highest number of singleton genes compared to its closest four relatives. Some of the singleton genes were related to antibiotic resistance. The genomic variability and diversity among the isolates are highlighted by the analysis of singletons and non-core genes that may be acquired from distal lineages through horizontal gene transfer and represent the genetic source for the emergence of novel functions (89).

The biological functions of the KEGG pathway genes were analyzed to further characterize the genomic differences among five strains. KEGG pathway annotation revealed that most of the genes of the core genome are related to metabolism. Similar results were obtained in previous studies on *Chryseobacterium* genomes (93). Moreover, drug resistance genes that may contribute to the wide resistance of strain *C. indologenes* to antimicrobials were found in a unique genome. All these distinctive features of our *C. indologenes* isolate provide evidence of its genomic plasticity that may contribute to antibiotic resistance and environmental adaptation.

When investigating the genetic basis of multidrug resistance (MDR) profile in *C. indologenes*, we observed a high level of concordance between the phenotypic and the genotypic results. Our analysis showed that the resistance gene profile to β-lactams in *C. indologenes* isolate may be due to the carriage

of bla_{CIA-3}, bla_{IND-13}, $bla_{TEM-116}$, $bla_{VEB,}$ and $bla_{OXA-209}$. The bla_{CIA} and bla_{IND} genes have previously been identified in *C. indologenes* (94), and they usually provide intrinsic resistance to carbapenems and cephalosporins due to their production of class A β-lactamase (CIA) and class B carbapenem-hydrolyzing β-lactamase (IND) (23, 95, 96). The $_{bla}TEM-116$ gene was described for the first time in Korea in a clinical isolate of *E. coli* (97). In Brazil, it was previously found in *Klebsiella pneumoniae* (98), *Vibrio parahaemo*lyticus (99), *Aeromonas hydrophila,* and *Aeromonas jandaei* (100). Studies have related that TEM-116 β-lactamase can confer resistance to ceftazidime, cefotaxime, and aztreonam (97, 101). The bla_{VEB-15} gene was first identified from genomic DNA of *E. coli* isolate that had reduced susceptibility to ceftazidime–avibactam (102). However, the bla_{VEB} group has been identified in a variety of species of *Enterobacteriaceae* and non-fermenting species such as *P. aeruginosa* and *Acinetobacter baumannii* (102). The VEB enzymes confer a high level of resistance to an expanded-spectrum cephalosporin (103, 104). The $bla_{OXA-209}$ is a β-lactamase gene that was first described in *Riemerella anatipestifer* strain isolated from ducks and geese (105). It was also described more recently in pan-drug-resistant *Myroides odoratimimus* PR63039 strain isolated from a patient presenting with post-injury urinary tract infection (106). Although there were a few reports on the substrate profile of beta-lactamase class D OXA-209, we suggest this enzyme can contribute to the resistance of *C. indologenes* to β-lactam antibiotics.

Our analysis revealed the presence of genes that can mediate resistance to tigecycline (*tet*(X)), quinolones (*mcb*G and mutation in the *gyr*A and *gyr*B genes), polymyxin (*Hly*D, *Tol*C, and mutation in the lpxA gene), and aminoglycosides (*Ran*A and *Ran*B). Tet(X) is an enzyme capable of modifying the antibiotic tetracycline and its derivates, including tigecycline and glycylcycline (68, 107). Since the identification of the *tet*(X) gene from the obligately anaerobic *Bacteroides* spp. (108, 109), it has been reported among strains of *Enterobacteriaceae*, *Comamonadaceae, Flavobacteriaceae,* and *Moraxellaceae* (23, 110, 111). Resistance to quinolones can be conferred by mutations in quinolone-resistance-determining region (QRDRs) (gyrA, gyrB, parC, and parE subunits), efflux pumps (QepA and OqxAB), DNA topoisomerase protection protein Qnr, and quinolone acetyltransferase Aac (6′)-Ib-cr (23, 112, 113). Our strain displayed amino acid alternations at position 83 in GyrA (Ser83Tyr) (23, 114) and positions 425 and 473 in gyrB (Leu425 Ile and Lys473Arg) (23). These findings are in line with studies showing these mutations in *C. indologenes* (23). Furthermore, the *mcb*G gene, found in our *C. indologenes* strain, encodes a pentapeptide-repeat protein with 19.6% amino acid identity with QnrA (115). The mcbG protein protects DNA gyrase from the action of microcin B17 (MccB17) and some quinolones antibiotics (115, 116).

We also examined whether mutations in *pmr*C, *lpx*A, *lpx*C, and *lpx*D genes were associated with polymyxin resistance found in our strain. Our analysis revealed only a substitution in lpxA (Gly68Asp). Amino acid substitutions, frameshifts, or truncation of lpxD lpxA and lpxC have been demonstrated to lead to a complete loss of LPS (117). These changes may play a role in colistin resistance, as shown in clinical *A. baumannii* and *Chryseobacterium oranimense* isolates (117, 118).

Efflux systems have been described in several bacteria isolated from clinical specimens and can be related to multidrug resistance phenotypes (119). We identify genes associated with multidrug effux pumps, such as *Hly*D, *Tol*C, *Ran*A, and *Ran*B. HlyD belongs to the membrane fusion protein family (120) and forms a continuous channel by docking to the TolC. HlyD has been shown to contribute to polymyxin resistance in *A. baumannii* (121, 122). TolC, an outer-membrane channel protein, is often the final portal in the pathways of protein toxin transport or export of unwanted molecules, such as antibiotics (123–125). *Ran*A*Ran*B genes encode an efflux pump of the ABC efflux pump system. RanA alongside RanB mediates resistance to aminoglycosides in *Riemerella anatipestifer* (126), a member of the *Flavobacteriaceae* family.

Although some studies suggest that proteases and biofilm production may play an important role in the virulence of invasive infections caused by *C. indologenes* (8, 11), much remains unknown about virulence factors essential for pathogenicity and their mechanism during pathogenesis. Analyzing the virulence profiles of our strain, we found genes that encode conserved virulence factors, which have been previously identified in *C. indologenes* (127) and other pathogens (128). The virulence factors found were associated with oxidative stress such as catalase (*kat*A, *kat*G) (129, 130); adhesion to host cells and extracellular matrix components (elongation factor *Tu*) (131); colonization of a host organism and in maintenance of bacterial cells in tissues (*ure*B) (132); bacterial growth, stress tolerance, and biofilm formation (*clp*P) (133); modulating the expression of virulence determinants and metabolism-related factor (*clp*E) (134); cell envelope structure, swarmer cell elongation, and subsequent swarm motility (*rff*G) (135, 136); serum resistance and biofilm formation (*gal*E) (137–139); O-antigen nucleotide sugar biosynthesis (*wlb*B) (140); O-antigen-synthesis (*rfb*A) (141); polysaccharide biosynthesis (tviB) (142); capsular polysaccharide synthesis and antiphagocytosis (*cap*8E, *cap*8G) (143, 144); survival and virulence (*mgt*B) (145, 146), stress response (*gro*L) (147); induction of cytokine production, adhesion, and cytotoxicity (*ilp*A) (148, 149); and infection and immune evasion capacity (*icl*, *acp*) (150). Our data indicate that *C. indologenes* may be a highly virulent strain, presenting putative virulence factors related to structural functions, physiological activity, defense, or invasion that favor the course of pathogenesis.

Genomic Islands (GIs) are cluster genes in prokaryotic genomes of probable horizontal origin, which harbor components of mobile genetic elements (MGEs) that may be associated with mobilizing DNA (151, 152). GI regions

often carry genes related to pathogenicity, symbiosis, metabolic, fitness, or resistance islands (153) that confer a selective advantage to the host bacterium (152). We searched the *C. indologenes* genome for the MGEs able to transfer genes between DNA molecules (insertion sequences, gene cassettes, integrons, and transposons) and for those able to transfer genes between cells (conjugative and mobilizable plasmids, and integrative and conjugative element (ICEs) (154). Our analysis showed that the most prevalent ORFs are hypothetical proteins, which are frequently found in GIs and ICEs (152). We also found a GI region with ICE features (ICE*Cind*1) that contained copies of a relaxase-encoding gene; genes related to a type IV secretion system such as three conjugative transfer protein-encoding genes (1 copy *trb*L and 2 copies of *trb*C) (155, 156); a gene related to essential integral membrane protein (*Trw*B), important for the conjugation process (157); and a gene encoding a site-specific integrase (*xer*C), which ensures the site-specific chromosomal integration of the ICE as well as effective excision of the element, where it may be aided by an excisionase or recombination directionality factor (158–160). In addition, we found a transposon Tn5393 that usually carries the *str*A and *str*B genes, responsible for the resistance to streptomycin (161, 162). However, these resistance genes were not present in this region. The *sul2* gene, encoding for resistance to sulfonamide, was flanked by ISVsa3 family transposase (IS91-like element). This has been previously described in a plasmid (pEPMS-18199) from *Edwardsiella piscicida* (163). The *cat* variant genes usually participate in the composition of gene cassette or integron, and confer the ability of antibiotic resistance (164). *Chryseobacterium indologenes* contained a *cat*B3, a chloramphenicol acetyltransferase gene, inserted in integron In31. Interestingly, Laraki et al. (165) showed that *cat*B6, a chloramphenicol acetyltransferase—encoding allele of the catB family, may be inserted in this integron (In31). The authors also observed it decreased the *in vitro* antibiotic susceptibilities of *Pseudomonas aeruginosa* strains.

Although WGS allows the analysis of large datasets using *in silico* plasmid typing methods, short reads from popular high-throughput sequencers can be difficult to assemble. Therefore, complete plasmid sequences may not be accurately reconstructed (166). When antimicrobial resistance genes are localized on incomplete contigs, it is uncertain whether they are localized on plasmid or chromosome (167). In our *C. indologenes* strain, most resistance genes ($bla_{TEM-116}$, bla_{VEB-15}, $bla_{OXA-209}$, *aad*S, *cat*B3, *erm*F) detected in singletons and the replicon (ColRNAI_DQ298019) were located on unaligned scaffolds. These findings partially corroborate the results of Evans et al. (168), who found a ColRNAI plasmid harboring genes associated with β-lactams (bla_{OXA-9}, bla_{TEM-1A}), chloramphenicol (*cat*A1) antibiotic resistance. Furthermore, studies have shown that *cat*B3 is one member of the gene cassette *aac*A7-*cat*B3-*aad*B-*oxa*2-*orf*D. It can be mobilized by the integron-encoded DNA integrase and plays a role in chloramphenicol resistance of plasmid pBWH301 (169, 170). However, this complete gene cassette was not found in our analysis.

The scarcity of data on the properties of clinical isolates of *C. indologenes* makes it challenging to characterize the transmission and evolution of this pathogen. Therefore, we believe that our detailed data of the WGS will contribute to the understanding of the genomic diversity, pathogenic potential, and multidrug resistance profile presented by *C. indologenes*. In addition, our data may guide future public health policy and MDR *C. indologenes* infection control.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the link: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA830910. Bioproject accession id is PRJNA830910.

Ethics statement

The studies involving human participants were reviewed and approved by the Committee of Ethics in Human Research of the Federal University of São Carlos (no. 1.595.268). In this work, *C. indologenes* and the anonymous archival data related patient age, gender, and sample type were obtained from the Central Laboratory of Public Health of Tocantins (LACEN/TO, data's owner). Patient consent was not required since the data presented in this study do not relate to any specific person or persons. Written informed consent from the participants or their legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. Permission to conduct the present study was obtained from the Health Department of the State of Tocantins (Secretaria da Saúde do Estado do Tocantins – SESAU) and LACEN/TO.

Author contributions

MSFD, RLF, EBC, GGS, LCC, PML, and ASC performed the experiments. CCMF, LTC, and AFC aided with the bioinformatics analysis. MCSP, MSFD, EBC, and AP-S wrote the manuscript and analyzed the data. MCSP conceived and supervised the study. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.931379/full#supplementary-material

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