



Global Evolution of Pathogenic Bacteria With Extensive Use of Fluoroquinolone Agents

Miklos Fuzi^{1*}, Jesus Rodriguez Baño² and Akos Toth³

¹ Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary, ² Unit of Infectious Diseases, Clinical Microbiology and Preventive Medicine, Department of Medicine, Hospital Universitario Virgen Macarena, University of Seville – Biomedicine Institute of Seville (IBiS), Seville, Spain, ³ Department of Bacteriology, Mycology and Parasitology, National Public Health Center, Budapest, Hungary

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*Correspondence:

Miklos Fuzi
miklosfuzi@gmail.com

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It is well-established that the spread of many multidrug-resistant (MDR) bacteria is predominantly clonal. Interestingly the international clones/sequence types (STs) of most pathogens emerged and disseminated during the last three decades. Strong experimental evidence from multiple laboratories indicate that diverse fitness cost associated with high-level resistance to fluoroquinolones contributed to the selection and promotion of the international clones/STs of hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA), extended-spectrum β -lactamase-(ESBL)-producing *Klebsiella pneumoniae*, ESBL-producing *Escherichia coli* and *Clostridioides difficile*. The overwhelming part of the literature investigating the epidemiology of the pathogens as a function of fluoroquinolone use remain in concordance with these findings. Moreover, recent *in vitro* data clearly show the potential of fluoroquinolone exposure to shape the clonal evolution of *Salmonella* Enteritidis. The success of the international clones/STs in all these species was linked to the strains' unique ability to evolve multiple energetically beneficial gyrase and topoisomerase IV mutations conferring high-level resistance to fluorquinolones and concomittantly permitting the acquisition of an extra resistance gene load without evoking appreciable fitness cost. Furthermore, by analyzing the clonality of multiple species, the review highlights, that in environments under high antibiotic exposure virulence factors play only a subsidiary role in the clonal dynamics of bacteria relative to multidrug-resistance coupled with favorable fitness (greater speed of replication). Though other groups of antibiotics should also be involved in selecting clones of bacterial pathogens the role of fluoroquinolones due to their peculiar fitness effect remains unique. It is suggested that probably no bacteria remain immune to the influence of fluoroquinolones in shaping their evolutionary dynamics. Consequently a more judicious use of fluoroquinolones, attuned to the proportion of international clone/ST isolates among local pathogens, would not only decrease resistance rates against this group of antibiotics but should also ameliorate the overall antibiotic resistance landscape.

Keywords: multidrug-resistant pathogens, clonal dynamics, fluoroquinolone resistance, fitness cost, virulence

INTRODUCTION

We have been witnessing a genuine metamorphosis in the clonal spectra of many multidrug-resistant (MDR) pathogens during the last three decades. While polyclonal situations also occur a few highly successful international clones/sequence types (STs) of various MDR pathogens achieved dominance in multiple geographic regions replacing local strains and posing a serious challenge for the treatment of patients. The advent of the nowadays principal MDR clones commenced with the widespread dissemination of the first international ST of hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) the New York-Japan clone (ST5) in the 1990s (Oliveira et al., 2001) which was followed by the emergence of the ribotype 027 clone of *Clostridioides difficile* (Kuijper et al., 2006) and the international STs of vancomycin-resistant *Enterococcus faecium* (VRE) (Willems et al., 2005), and the major STs/lineages of ESBL-producing *Klebsiella pneumoniae* (Damjanova et al., 2008) and ESBL-producing *Escherichia coli* (Nicolas-Chanoine et al., 2008). Subsequently we had to face the global clones of MDR *Acinetobacter baumannii* (Zarrilli et al., 2013) and most recently the international STs of MDR *Enterobacter cloacae* (Gomez-Simmonds et al., 2018).

The question arises what could have triggered the metamorphosis of the clonal landscape of MDR pathogens during this time period that resulted in the dominance of a few recently disseminated global “superbugs”?

Virulence factors have been rightly suspected by many authors to have contributed to the emergence of the MDR clones/STs in various pathogens. However, with the exception of the ribotype 027 clone of *C. difficile* in no the other species showed MDR major clone/ST strains greater virulence relative to minor clone isolates and this observation argues strongly against a virulence-based dissemination. Martínez and Baquero (2002) emphasized the primacy of antibiotic resistance over virulence in the selection of international clones in their classic 2002 paper: “If antibiotics are almost ubiquitously present in the hosts, as in an intensive care unit . . . the spread of the resistant bacteria is favored” while, “.the most virulent bacteria are perhaps less exposed to antibiotics than non-virulent ones” which may account for the lower virulence score of major clone strains in the healthcare setting.

Varying fitness effects (diverse speed of replication¹) associated with high-level resistance to fluoroquinolones was demonstrated to confer an indirect growth advantage onto the international clone/ST strains of HA-MRSA (Horváth et al., 2012; Knight et al., 2012; Holden et al., 2013), ESBL-producing *K. pneumoniae* (Toth et al., 2014), ESBL-producing *E. coli* (Johnson et al., 2015a,b) and *C. difficile* (Wasels et al., 2015; Vernon et al., 2019) compared with minor clone isolates that should have contributed to the selection of the international clones/STs in these species [reviewed by Fuzi (2016) and Fuzi et al. (2017)]. A “fluoroquinolone connection” could also account for the puzzling time period – the last three decades – for the emergence and dissemination of the international clones and

STs. Fluoroquinolones were introduced in many countries in the late 1990s and consumption increased considerably in the early 2000s². We are aware that the emergence and successful spread of resistant clones is probably a multifactorial phenomenon; however, we hypothesize that fluoroquinolones may have played a critical role.

Though all of the international clones/STs had existed prior to the fluoroquinolone era they constituted smaller groups of various pathogens. Otherwise we would have been aware of their extensive dissemination.

Strains of the two largest international clones of HA-MRSA – ST5 and ST22 – were uncommon in both hospitals and the community before the widespread use of fluoroquinolones and their incidence remained low in the community where it is rarely promoted by exposure to these antibiotics (Oliveira et al., 2002; Vandenesch et al., 2003; Wijaya et al., 2006).

Though no reliable data are available on the incidence of the international STs of MDR *K. pneumoniae* in “pre-fluoroquinolone times” strains from the ST131 clone of *E. coli* are well-known to have been isolated from the 1960s across Europe and the United States (Olesen et al., 2014). The available information suggest that the clone’s incidence must have been much lower then compared with its global presence subsequent to the advent of fluoroquinolones (Nicolas-Chanoine et al., 2008; Olesen et al., 2014; Peirano et al., 2014).

Moreover, it is well-established that *C. difficile* ribotype 027 used to be an uncommon clone in North America prior to the beginning of the “fluoroquinolone era” (24). It “was first identified in 1987” as “a minor strain accounting for less than 5% of isolates on surveys, without apparent association with large outbreaks or increased lethality” (McDonald et al., 2005; Louie, 2005).

Though the international MDR clones/STs showed distinct expansions the different pace of evolution and diverse transmission features of individual pathogens might have accounted for the varying onset and time scale of dissemination.

Growth tests, epidemiological observations and genetic data all support a role for fluoroquinolones in the selection and dissemination of the international clones/STs of HA-MRSA, MDR *K. pneumoniae*, MDR *E. coli* and *C. difficile* [reviewed by Fuzi (2016) and Fuzi et al. (2017)]. This paper will present additional information, published most recently on the fitness, epidemiology and genetics of these pathogens and will analyze the role of virulence factors in the spread of these agents. It will be shown that virulence factors play only a subsidiary role in the transmission of MDR bacteria compared with speed of replication in a “high fluoroquinolone exposition.”

HA-MRSA

It was reported by our group in 2012 and a couple of months later by others that the clonal shifts observed among competing STs of HA-MRSA was in concordance with diverse fitness cost (resulting in diverse speed of replication) associated with high-level resistance to fluoroquinolones (Horváth et al., 2012;

¹The term fitness always refers to speed of replication in the manuscript.

²<https://ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/esac-net>

Knight et al., 2012; Holden et al., 2013). STs suffering considerable fitness cost upon developing high-level resistance to fluoroquinolones will be replaced by STs retaining much fitness even at high MIC values in “high fluoroquinolone-consumption areas”. In line with experimental results long dominant HA-MRSA clones, especially ST30, ST8, and ST239, have been losing ground to or have been completely replaced by CC5, ST228, and ST22 strains in adult hospital wards across various continents (Mato et al., 2004; Pérez-Roth et al., 2004; Velazquez-Meza et al., 2004; Ma et al., 2006; Amorim et al., 2007; Conceição et al., 2007; Aires-de-Sousa et al., 2008; Knight et al., 2012; Espadilha et al., 2013; Lim et al., 2013; Coombs et al., 2014; Abdulgader et al., 2015; Hsu et al., 2015; Lawes et al., 2015; Zarfel et al., 2016; Chamon et al., 2017; Nikolaras et al., 2018).

Moreover, even the fittest STs are not equal and subtle differences between their speed of replication were also reflected in clonal dynamics. The ST228 clone – that was shown to command inferior fitness associated with resistance to fluoroquinolones relative to the ST5 and ST22 clones (Horváth et al., 2012) – was replaced by ST22 strains in German, Italian, Hungarian and Austrian facilities (Albrecht et al., 2011; Baldan et al., 2012; Grundmann et al., 2014; Zarfel et al., 2016).

The observed “fitness influence” may also account for the characteristic community affiliation of community-associated (CA)-MRSA strains. The substantial fluoroquinolone-associated fitness cost demonstrated with various CA-MRSA STs (Horváth et al., 2012) should prevent the widespread dissemination of CA-MRSA in hospital wards where fluoroquinolones are in extensive use. Consequently most CA-MRSA strains will remain in the community where they can survive and disseminate without having to develop high-level resistance to fluoroquinolones.

Interestingly fitness cost associated with high-level resistance to fluoroquinolones is not static. Some strains from the ST8 clone showed remarkable resilience and proved capable of evolving into a novel major HA-MRSA lineage in the United States (Challagundla et al., 2018a).

The ST8 clone (USA300) was previously considered CA-MRSA, however, a new lineage of the pathogen has recently emerged replacing strains from other STs in the healthcare setting (Challagundla et al., 2018a). The reason for the success of the new ST8 lineage is linked to its skillful genetic evolution. All of the major international STs of MRSA were shown to carry two typical quinolone-resistance determining regions (QRDR) mutations affecting the *gyrA* Ser84 and *grlA* Ser80 residues. These double-serine alterations are characteristic features of both highly successful international HA-MRSA groups of CC5 and ST22 (Holden et al., 2013; Challagundla et al., 2018a).

The replacement of the double-serine residues in the DNA gyrase and topoisomerase IV enzymes involved in the binding of fluoroquinolones is crucial for the preservation of considerable fitness at high MIC values to fluoroquinolones. It was demonstrated in various species that the *gyrA* Ser84 and *grlA* Ser80 (or corresponding) residues are not optimal for the function of the enzymes and their replacement by some other amino acids is associated with a modest fitness gain in the isolates, in a clone-dependent fashion [reviewed by Fuzi et al. (2017)].

The question arises if the double-serine residues are not optimal for the function of gyrase and topoisomerase IV why have these residues been evolutionarily preserved?

Japanese scientists demonstrated in *S. aureus* that these serine residues (in codon 84 in gyrase and codon 80 in topoisomerase IV) confer protection against antibacterial substances of herbal and *Streptomyces* origin (Hiramatsu et al., 2012; Morimoto et al., 2015) permitting survival in diverse environments.

Similarly to the ST5 and ST22 clones the success of the novel lineage of the ST8 strains should be related to their ability to evolve the double-serine QRDR mutations. While the early CA-MRSA ST8 (USA300) strains were either susceptible to fluoroquinolones or carried just one of the serine mutations (*grlA* Ser80) the novel strains harbored also a *gyrA* Ser84 change in a similar fashion to the ST5 and ST22 clones (Holden et al., 2013; Alam et al., 2015; Challagundla et al., 2018b). In their recent review of the USA300 MRSA ST8 clone Challagundla et al. (2018a) also emphasized the relevance of the evolution of the second – *gyrA* Ser84Leu – mutation in the success of the lineage in the United States. Moreover, ST8 strains were reported having evolved double-serine QRDR mutations also in other geographical regions (Khokhlova et al., 2015; Glaser et al., 2016; Wan et al., 2016) that might have contributed to the dissemination of ST8 strains in France (Glaser et al., 2016) and Russia (Gostev et al., 2017). Unfortunately information on the prevalence of QRDR mutations in ST8 MRSA strains are missing from many countries. Data available from some European countries show that ST8 CA-MRSA strains – like other CA-MRSA isolates – are either void of QRDR mutations or carry just a single serine QRDR mutation (Horváth et al., 2012; Lepuschitz et al., 2018).

Though information remains scarce it has to be noted that in some geographical regions some strains of emerging ST239 and ST398 MRSA sublineages also proved capable of developing the double-serine QRDR mutations that may account for the sustenance of these groups in the healthcare setting in some areas (Takano et al., 2008; Lozano et al., 2012; Chakrakodi et al., 2014; Khokhlova et al., 2015).

The superior fitness (speed of replication) of the major international clone strains of HA-MRSA relative to minor clone isolates has serious practical implications. Since these strains multiply faster than isolates from other clones they are expected to achieve higher rates upon occupying novel niches and replacing the local pathogens. This is really what we have been witnessing for more than two decades. The replacement of minor clone strains of HA-MRSA by faster replicating international ST strains showing higher growth rates is well-documented to trigger a significant rise in the incidence of the pathogen (O’Neill et al., 2001; Horváth et al., 2012; Holden et al., 2013; Hsu et al., 2015; Zarfel et al., 2016).

Horváth et al. (2012) and Knight et al. (2012) have demonstrated with *in vitro* propagation assays that the growth rate of international HA-MRSA clone isolates is higher than that of minor ST strains. Since in many countries a large proportion of the HA-MRSA strains belong to the fluoroquinolone-selected major STs a decrease in the use of fluoroquinolones should result in a decline of these major clone strains yielding lower

incidences. The overwhelming part of the literature published until 2016 investigating a link between the incidence of HA-MRSA and fluoroquinolone consumption support the existence of an association. The previous literature on the area was reviewed by one of us (Fuji, 2016). Moreover, a recent report analyzing the impact of antibiotic consumption on the prevalence of MRSA in seven European countries found that the use of both cephalosporins and fluoroquinolones were significantly associated with the occurrence of the pathogen (Kinoshita et al., 2017). This study was performed by using data available in ESAC-NET the European Antimicrobial Consumption database where many countries did not disclose separate figures for antibiotic consumption in the community and in the hospital sector³. Nevertheless, it is obvious that a dramatic decline in the rate for MRSA occurred in the United Kingdom. Though we do not have antibiotic consumption data in the hospital sector for the whole country Dingle et al. (2017) published the figures for England where the use of fluoroquinolones in the hospital sector was reduced by close to 50% between 2005 and 2012 (Dingle et al., 2017). During the same period the proportion of MRSA among invasive infections caused by *S. aureus* decreased from 43.6% to 14.0% in the United Kingdom⁴. The reduction must have been partly associated with the more judicious use of fluoroquinolones. Finally Conlon-Bingham et al. (2019) recently observed a link between the use of some antibiotics including fluoroquinolones and the incidence of HA-MRSA in a local hospital-based study. A significant temporal association was demonstrated between HA-MRSA incidence and fluoroquinolone use (coefficient 0.004, $p < 0.01$), with a 3-month lag time (Conlon-Bingham et al., 2019).

We believe that not just fitness cost associated with high-level resistance to fluoroquinolones but fitness in general governs the clonal dynamics of HA-MRSA. Though not all clonal replacements involving MRSA are associated with resistance to fluoroquinolones, fitness costs – in at least some of these clonal shifts – seem also have been involved. During two recent clonal shifts reported in MRSA the fitness of the isolates was investigated and scientists found that strains from the dominant ST/lineage commanded superior replication capacity compared with the replaced isolates (Shang et al., 2016; Li et al., 2018). The latter clonal shift occurred at the expense of mostly fluoroquinolone resistant ST239 isolates which were replaced by faster growing primarily fluoroquinolone susceptible ST59 strains. A shift of that nature – obviously – must have taken place in wards where fluoroquinolones were not in extensive use (Li et al., 2018).

In summary, substantial circumstantial evidence suggest that fitness (replication capacity) plays a major – probably crucial – role in the clonal dynamics of MRSA in the healthcare setting in adult facilities. However, the contribution of pathogenicity to the epidemiology of MRSA is rightly considered relevant and therefore we must evaluate the significance of virulence factors relative to replication in the clonal dynamics of the pathogen.

³<https://ecdc.europa.eu/en/antimicrobial-consumption/surveillance-and-disease-data/database>

⁴<https://ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/ears-net>

The Virulence Component in MRSA

Strains from successful international clones/STs of HA-MRSA are in general less virulent than isolates of CA-MRSA which are conversely more susceptible to antibiotics.

Monecke et al. (2011) investigating the virulence factors of a huge number of MRSA strains from a variety of clones/STs clearly showed that the production of the Panton-Valentine leukocidin (PVL) remains a hallmark of CA-MRSA isolates. The production of PVL is not rare among well-known CA-MRSA clones like ST30, ST59, and ST80. However, it will be produced mostly by the fluoroquinolone susceptible CA-MRSA variants of the biggest international HA-MRSA clones/STs of CC5, ST8, and ST22 associated almost exclusively with the small-size *scmec*-IV/V elements (Rossney et al., 2007; Pinto et al., 2013; Udo et al., 2016; Yang et al., 2019). These observations, the clear distinction in the production of PVL between susceptible CA-MRSA and major clone/ST HA-MRSA strains, strongly suggests that the fitness gain associated with the “non-production of PVL” may assist the usually MDR HA-MRSA strains to disseminate in the healthcare setting.

The “arginine catabolic mobile element” (ACME) enhancing survival of *S. aureus* on the skin of patients was correctly linked to the success of the ST8 (USA300) CA-MRSA clone in the United States (Strauß et al., 2017; Challagundla et al., 2018a). However, interestingly, ACME was missing from strains of the novel lineages of the ST8 HA-MRSA clone some of which have extensively disseminated in the healthcare setting on various continents [reviewed by Strauß et al. (2017)] In addition, ACME remains uncommon in the international clone/ST strains of ST5 and ST22 (Monecke et al., 2011). Moreover, Hsu et al. (2015) recently observed a clonal displacement of the ST239 MRSA clone by ST22 strains in Singapore and a thorough review of the genetic makeup of both clones showed that in contrast to the ST22 isolates many of the ST239 strains harbored the genes of ACME. Thus, greater speed of replication associated with high-level resistance to fluoroquinolones – permitted by the acquisition of the double-serine QRDR mutations – proved more important for clonal superiority than the carriage of ACME.

Nevertheless, the carriage of ACME should certainly confer an advantage to antibiotic susceptible CA-MRSA strains facilitating transmission in a fluoroquinolone-free environment (Aung et al., 2017; Strauß et al., 2017; Murai et al., 2019).

Biofilm is also rightly considered a relevant determinant of clonal dynamics. However, observations published in the related literature show it plays only a secondary role to fitness, i.e., speed of replication.

Though the production of biofilm was suggested to promote the dissemination of the ST22 (EMRSA-15) clone the general biofilm-producing capacity of the ST22 isolates remain inferior compared with those of ST228 and ST8 strains (Baldan et al., 2012) which they readily replace in the healthcare setting (see above).

Biofilm production was observed to advance the dissemination of ST59 MRSA in the community and also in the healthcare setting in pediatric wards which are supposed to be “fluoroquinolone-free” (Yang et al., 2017). These ST59

strains proved strong producers of biofilm, however, more than 80% of the isolates were susceptible to fluoroquinolones and even the resistant strains might have had relatively low MIC values since all of them were isolated from young children (Yang et al., 2017). Nevertheless, despite the production of substantial biofilm these susceptible ST59 strains have not been observed to disseminate in adult wards where fluoroquinolones are supposed to be in use. Moreover, the production of biofilm has also been shown to be influenced by not just the type of the pathogen but also by resistance to macrolides (Sun et al., 2018), previous exposure to antibiotics and some features of the infected patient (Luther et al., 2018).

Baldan et al. (2015) suggested that ST22 HA-MRSA could replace ST228 strains because of a higher rate of hemolysin production. However, they did not consider the “fluoroquinolone” effect. Figure 1 in their paper (Baldan et al., 2015) clearly shows that the incidence of the ST22 strains was in complete agreement with the consumption of fluoroquinolones and it had been previously demonstrated that ST22 strains of HA-MRSA suffer smaller fitness cost upon developing high-level resistance to fluoroquinolones than ST228 isolates (Horváth et al., 2012).

The primacy of fitness (speed of replication) over virulence in MRSA is also reflected by the observation that the acquisition of methicillin resistance by HA-MRSA is associated with an overall downregulation of virulence gene expression linked to a repression of the activity of the accessory gene regulator system (*agr*) [reviewed by McCarthy et al. (2015)]. The product of the *psm-mec* gene carried by most strains of HA-MRSA on the SCCmec II and III cassettes is responsible for the attenuation of virulence (Kaito et al., 2013).

It is tempting to speculate that the favorable fitness effect gained from the double-serine QRDR mutations combined with the *psm-mec*-induced suppression of multiple virulence genes allowed HA-MRSA to acquire/evolve further antibiotic resistance mechanisms without suffering significant fitness cost permitting the pathogen’s survival in “high-antibiotic” areas.

Strains from the fluoroquinolone resistant lineage of the ST8 MRSA clone in France were reported to carry significantly fewer virulence genes than the fluoroquinolone susceptible ST5 and ST80 isolates (Dauwalder et al., 2008). Thus, strains seem to reduce the carriage/expression of virulence genes as a balancing act for the more important preservation of fitness – if they are capable – when having been “weighted down” by antibiotic resistance.

However, they are not always capable of performing a balancing act. The most virulent clone of *S. aureus* is certainly the ST121 clone harboring a large “armament” of pathogenicity factors (Rao et al., 2015). ST121 is a global clone and an important pathogen in the community, nevertheless, its CA-MRSA variant remains rare (51, 74). Furthermore, high-level resistance to fluoroquinolones has not been reported in ST121 *S. aureus* to date and the clone has never been able to extensively disseminate as a HA-MRSA in hospitals. Thus, it seems, supervirulence has not helped the ST121 clone in the healthcare setting and its obvious inability to preserve fitness upon developing

multidrug-resistance might have prevented it from becoming a major pathogen in hospitals.

Multidrug-Resistant *K. pneumoniae*

Subsequent to the clonal shifts with HA-MRSA we witnessed a substantial “clonal rearrangement” of MDR *K. pneumoniae* in Hungary in 2004–2005 (Damjanova et al., 2008). The investigation of clonal fitness associated with resistance to fluoroquinolones yielded similar results to that with HA-MRSA, however, the difference in loss of robustness proved even greater between minor- and international STs of MDR *K. pneumoniae* than between minor-, and major clones of HA-MRSA (Toth et al., 2014).

Moreover, a clear difference was shown in the use of efflux systems between minor-, and international STs of *K. pneumoniae*. While active efflux could be demonstrated in a Phe-Arg-β-naphthylamide inhibition assay in all of the minor ST strains in which resistance to ciprofloxacin was *in vitro* induced, highly fluoroquinolone resistant major ST isolates proved negative in the test (Toth et al., 2014). Since running an active efflux requires an investment of energy (the area was reviewed recently: Fuzy et al., 2017) it must have impacted the fitness of the fluoroquinolone resistant minor ST isolates (Toth et al., 2014).

Furthermore our previous findings with minor- and major ST strains of *K. pneumoniae* strongly suggest that fitness cost associated with resistance to fluoroquinolones contributed to the widespread dissemination of CTX-M type ESBL genes. We found that all of our fluoroquinolone susceptible minor ST isolates which originally carried plasmids with SHV type ESBL genes eliminated these plasmids upon induction of resistance to ciprofloxacin while strains with plasmids harboring CTX-M type ESBL genes were not cleared from either major-, or minor ST fluoroquinolone resistant isolates (Toth et al., 2014). The process permitting the persistence of the *bla*_{CTX-M-15}-carrying plasmids remains unknown.

In addition, the success of the major ST (ST11, ST15, ST147) MDR *K. pneumoniae* strains was shown to be associated with ability to evolve at least two but rather three energetically favorable QRDR mutations. In contrast to major ST strains minor ST isolates either proved unable to evolve any of these genetic changes or developed just a single alteration (Toth et al., 2014) – a measure reminiscent strongly of observations made with HA-MRSA. Consequently the influence of fluoroquinolones might have substantially contributed to the success of the major clones of *K. pneumoniae*.

Furthermore the carriage of multiple QRDR mutations proved also characteristic for ST258 isolates. Bowers et al. (2015) in their comprehensive review of KPC-producing ST258 *K. pneumoniae* observed: “all CG258 isolates have the fluoroquinolone resistance-conferring mutations in *GyrA* (Ser83 to Ile) and *ParC* (Ser80 to Ile),” which should have contributed to the global dissemination of carbapenem resistance.

Moreover, a most recent paper characterizing strains of the novel international MDR *K. pneumoniae* sequence type, ST101, showed that almost all of the strains carry three “classic” QRDR mutations supporting further the “fluoroquinolone concept” (Roe et al., 2019). In addition, the carriage of the

“double-serine” QRDR mutations was reported characteristic also for the widespread clone of ST307 MDR *K. pneumoniae* (Wyres et al., 2019).

The Virulence Component in MDR *K. pneumoniae*

Similarly to HA-MRSA the potential role of virulence factors vs robust fitness (high speed of replication) in the clonal dynamics of MDR *K. pneumoniae* need to be assessed.

Interestingly fecal carriage of MDR major ST *K. pneumoniae* strains were reported from various continents in both outpatients and the general population with higher prevalence in developing countries (Zhang et al., 2015; Baraniak et al., 2016; Aghamohammad et al., 2018; Li et al., 2019; Büdel et al., 2019; Pan et al., 2019). In addition, most recently MDR major ST strains of *K. pneumoniae* were observed to colonize more frequently the respiratory system than the gastrointestinal tract (Shu et al., 2019). The factors governing the colonization capacity of MDR major ST strains of *K. pneumoniae* in various sites of the human body remain to be determined.

Though some major international ST (ST11, ST14, ST147, ST258) strains of MDR *K. pneumoniae* are called sometimes hypervirulent or “high-risk” an abundance of papers show that these isolates carry significantly fewer virulence factors than strains from the really highly virulent STs, primarily ST23 and ST65 (Lascols et al., 2013; Bialek-Davenet et al., 2014; Liu et al., 2014; Qu et al., 2015; Yan et al., 2015; Paczosa and Meccas, 2016; Gomez-Simmonds and Uhlemann, 2017; Gu et al., 2018; Lam et al., 2018b; Turton et al., 2018). Interestingly – despite the large outfit of virulence factors – ST23 and ST65 strains remain minor STs relative to the major clones of MDR *K. pneumoniae* in the healthcare setting (Cantón et al., 2012; Chen et al., 2014; Pitout et al., 2015; Gomez-Simmonds and Uhlemann, 2017; Runcharoen et al., 2017). Moreover it is well-established that strains from the most virulent *K. pneumoniae* sequence type ST23 remain less resistant to antibiotics – primarily to fluoroquinolones – than isolates from the MDR major international STs (Cejas et al., 2014; Qu et al., 2015; Cheong et al., 2016; Yan et al., 2016; Chen Y.T. et al., 2017; Ku et al., 2017; Lu et al., 2017; Sturm et al., 2018; Shen et al., 2019). Moreover, similarly to the most virulent MRSA clone, ST121, no high-level resistance to fluoroquinolones has been reported in any ST23 *K. pneumoniae* strain to date.

Some virulence factors were supposed to be associated with the successful dissemination of the major international STs of MDR *K. pneumoniae*. Holt et al. (2015) suggested that the production of yersiniabactin was associated with the success of the major international ST strains. Nevertheless, the penetrance of yersiniabactin genes is far from complete in major ST isolates (Bialek-Davenet et al., 2014; Dong et al., 2018; Gu et al., 2018; Lam et al., 2018b; Turton et al., 2018; Marques et al., 2019). In contrast the carriage of yersiniabactin genes is a hallmark of ST23 strains and some other minor ST isolates (Lam et al., 2018a) which remain far less common than major ST MDR *K. pneumoniae* strains in the healthcare setting (Damjanova et al., 2008; D’Andrea et al., 2013; Lee et al., 2017; Ko, 2019).

Colibactin was correctly presumed to contribute to the dissemination of ST23 *K. pneumoniae* (Chen Y.T. et al., 2017), however, if colibactin were the most important determinant of transmission of pathogenic *K. pneumoniae* then ST23 and ST65 should make the most common groups since the production of colibactin is much more characteristic for these STs than for other clones including the major MDR international groups (Chen Y.T. et al., 2017; Lam et al., 2018a). In addition, Lam et al. (2018a) observed that the colibactin locus was often disrupted in ST258 isolates and suggested: “selection against costly colibactin production in hospital adapted strains that already benefit from positive selection under antimicrobial exposure.”

Andrade et al. (2014) suggested that biofilm formation contributed to the advancement of ST11 MDR *K. pneumoniae*. However, Zheng et al. (2018) recently showed that ST23 strains of *K. pneumoniae* produce significantly greater quantities of biofilm than ST11 strains. In addition, Diago-Navarro et al. (2014) demonstrated that ST23 strains also generate much more biofilm than isolates from the global MDR group ST258 which is related to the ST11 clone.

Moreover though aerobactin is considered the prime virulence factor in *K. pneumoniae* (Russo et al., 2015) its prevalence can be low in strains of major international STs (Zhan et al., 2017; Liu et al., 2019).

Nevertheless some gram negative bacteria – including certainly also *K. pneumoniae* – are capable of employing an additional “fitness manoeuvre” to advance their performance. Interestingly some major ST *K. pneumoniae* strains, even though showing an MDR phenotype, may harbor more virulence genes than some more susceptible isolates. However, several groups who investigated the integron content in the isolates reported that an extra virulence gene load is significantly associated with the carriage of type 1 integrons conferring resistance to antibiotics (Derakhshan et al., 2016; Xu et al., 2017; Zaki, 2019). Though the promoter types of *K. pneumoniae* integrons have not been investigated to date it is well-established that type 1 integrons usually contain weak promoters in *E. coli* (Vinué et al., 2011; Wei et al., 2013). Weak promoters remain dominant also in class 1 integrons in *Proteus* species and were recently demonstrated to confer a favorable fitness effect on the isolates (Xiao et al., 2019). A similarly favorable fitness effect mediated by class 1 integrons remains most probable in *K. pneumoniae*.

Finally, similarly to MRSA, in community-acquired *Klebsiella* infections the more virulent and less resistant strains of “non-major ST” *K. pneumoniae* will dominate, like ST23 and ST65 (Zhao et al., 2016; Chen Y.T. et al., 2017; Garza-Ramos et al., 2018; Shi et al., 2018) in which high-level resistance to fluoroquinolones has not been reported to date.

In summary, the available data strongly suggest that – similarly to HA-MRSA – the contribution of virulence factors was only supplementary compared with high growth rate to the success of the major STs of MDR *K. pneumoniae* in the adult hospital setting.

Multidrug-Resistant *E. coli*

One year subsequent to our report demonstrating the impact of fluoroquinolones on the clonal dynamics of MDR *K. pneumoniae*

a similar mechanism was proposed for the emergence of the then single global clone/lineage of MDR *E. coli*: ST131 H30R (Johnson et al., 2015a,b). MDR ST131 H30R strains were demonstrated to command a fitness advantage when showing high-level resistance to fluoroquinolones relative to *E. coli* strains from other STs (Johnson et al., 2015a). Moreover in a similar fashion to MDR *K. pneumoniae* a lower efflux pump activity was observed in major ST strains of *E. coli* than in minor clone isolates (Johnson et al., 2015a).

Interestingly, unlike the major international STs of HA-MRSA and MDR *K. pneumoniae* which mostly carry just two or three alterations in the gyrase and topoisomerase IV genes strains from the ST131 H30R lineage carry four or often five QRDR mutations (Fuzi et al., 2017). Individual QRDR mutations in *E. coli* strains will just modestly raise the MIC values for fluoroquinolones, thus, the isolate needs to acquire at least four mutations to achieve a resistance level that allows its survival in a fluoroquinolone environment [area reviewed by Fuzi et al. (2017)]. Moreover the strain has to evolve the multiple QRDR mutations so “cleverly” so as not to compromise its fitness (growth rate) too much (Marcusson et al., 2009). That remains a real challenge that could have been met by only two international MDR lineages of MDR *E. coli* so far.

Apart from ST131 H30R one more group, ST1193, seems to be emerging as a global pathogen (Zhao et al., 2015; Johnson et al., 2018; Valenza et al., 2019). MDR ST1193 strains similarly to MDR ST131 H30R isolates carry multiple (mostly four) QRDR mutations (Wu et al., 2017; Valenza et al., 2019) and show high-level resistance to fluoroquinolones (Johnson et al., 2019).

The proportion of the ST131 H30Rx *E. coli* is usually lower and more variable among clinical *E. coli* isolates than that of the major STs/lineages of HA-MRSA, thus, an epidemiological study investigating the impact of fluoroquinolone use on the incidence of ST131 H30Rx is more difficult to evaluate. Nevertheless, recommendations for a reduced consumption of fluoroquinolones in the United Kingdom starting in 2007³ similarly to HA-MRSA (see above) yielded spectacular results. ST131 strains had been reported to dominate the *E. coli* landscape in the United Kingdom prior to 2007 (Lau et al., 2008). However, following the intervention a significant decline was observed in the proportion of the ST131 clone relative to some other STs usually susceptible to fluoroquinolones (Day et al., 2016).

Though Kallonen et al. (2017) suggested that the proportion of ST131 strains of *E. coli* remained “stable” among *E. coli* clinical isolates in the United Kingdom between 2004 and 2012 (Figure 2 in their paper) their study design, unfortunately, did not allow for a solid estimate. First, just the “first ten isolates per site” were collected from each participating laboratory and authors themselves admit that the study could include strains from “local epidemics” (Kallonen et al., 2017). More importantly about one third of the isolates were collected in a single facility starting in 2006 (Kallonen et al., 2017). Consequently the study remains inherently biased with respect to the effect of lower fluoroquinolone consumption that commenced in 2007 (Kallonen et al., 2017).

Patients are for obvious reasons considerably more exposed to fluoroquinolones – and some other antibiotics – in the hospital setting than in the community. The incidence of infections caused by MDR ST131 *E. coli* reflects this difference. The proportion of MDR ST131 strains among isolates obtained from infections is significantly higher in the hospital setting than in the community (Banerjee et al., 2013; Goswami et al., 2018).

Though the proportion of infections caused by MDR ST131 *E. coli* is lower in the community than in the hospital sector the fecal carriage of the pathogen is not rare in outpatients and the general population and can last for extensive time periods (Gurnee et al., 2015; Nakane et al., 2016; Ny et al., 2017; Huang et al., 2018; Morales Barroso et al., 2018; Teunis et al., 2018; Birgy et al., 2019; Meijs et al., 2019). The question arises how can MDR ST131 *E. coli* strains persist in the intestinal flora in the community without ostensible antibiotic exposure?

ST131 strains of *E. coli* may wield some metabolic advantage over other groups of *E. coli* (McNally et al., 2019) but probably more important is their relative insensitivity to colicins that may promote colonization of the gastrointestinal tract (Sharp et al., 2019). Moreover, a low-level exposure of the general population to antibiotics and – occasionally even to ST131 *E. coli* – via food and the environment [reviews by: Riaz et al. (2018); Roth et al. (2019), and Rogers et al. (2011)] would deserve a thorough investigation. Fluoroquinolones are well-established to command an extended degradation half-life in moist environments (Felis et al., 2020).

The Virulence Component in MDR *E. coli*

Though virulence factors beyond doubt make a relevant contribution to the success of *E. coli* STs several lines of circumstantial evidence suggest that their role – similarly to HA-MRSA and MDR *K. pneumoniae* – remains auxiliary compared with fitness (speed of replication).

Mathers et al. (2015) emphasized that though the carriage of an important virulence gene – the H30 type 1 fimbrial adhesin – has been characteristic for ST131 strains for a long time the clone became globally disseminated only subsequent to the development of high-level resistance to fluoroquinolones.

Though Ciesielczuk et al. (2015) reported that ST131 strains of *E. coli* showed a higher level of virulence in a *Galleria mellonella* model than strains from some other STs (ST69, ST73, ST95), Goswami et al. (2018) demonstrated that the ST131 clone harbors a significantly more limited virulence gene load than ST73 and ST95 isolates which proved mostly susceptible to fluoroquinolones. In addition, a comparative genomic analysis demonstrated that strains from the important sequence types, ST405, ST648, and ST38, which were not tested by Ciesielczuk et al. (2015) carried considerably more virulence factors than ST131 isolates (Shaik et al., 2017). However, despite this lower virulence gene load compared with some STs, it is well-established, that ST131 H30R remains the most prevalent group among MDR *E. coli* in most countries. Moreover, similarly to MRSA and MDR *K. pneumoniae*, the proportion of the major ST131 H30 group remains much lower among fluoroquinolone susceptible *E. coli* strains relative to other STs (Hertz et al., 2016; Yamaji et al., 2018) arguing strongly for a dominant role for

fluoroquinolone resistance-associated fitness relative to virulence in promoting dissemination.

ST405 strains of *E. coli* command an impressive virulence gene load (Shaik et al., 2017) and were considered promising candidates for global dissemination (Matsumura et al., 2013). However, the ST405 clone has internationally remained a smaller group relative to ST131 H30 probably due to its inability to evolve a sufficient number of favorable mutations in the gyrase and topoisomerase IV genes. While ST131 H30R and ST1193 strains carry four-to-five QRDR mutations ST405 isolates, similarly to the “moderately disseminated” ST410 strains, have evolved just three alterations (Mavroidi et al., 2012; Alouache et al., 2014; Roer et al., 2018) that is not supposed to confer high-level resistance to fluoroquinolones (Marcusson et al., 2009) preventing thereby their extensive spread. Nevertheless, three QRDR mutations may allow a lower level persistence of the clone in a fluoroquinolone environment. Furthermore, some strains of the ST405 group, similarly to ST8 MRSA, may evolve (or might have evolved) an additional favorable QRDR mutation that may turn or (may be turning) the clone into a prime global player. Strains from additional *E. coli* STs may also prove capable of evolving four (or more) favorable QRDR mutations and start to extensively disseminate in fluoroquinolone environments.

Interestingly Cha et al. (2016) observed a greater virulence gene load in ST131 H30 isolates compared with non-ST131 strains of *E. coli*. However, 71.1% of the non-ST131 isolates were also resistant to ciprofloxacin that should have been associated with a considerable fitness cost in these strains preventing the carriage of a substantial virulence gene load.

ST131 H30 strains are assigned to different virotypes determined by their virulence gene profiles (Blanco et al., 2013; Dahbi et al., 2014). Interestingly virotypes B and D associated with a higher virulence gene load are less frequent than virotypes C and A (Merino et al., 2017). Virotypes C strains of ST131 H30 Rx – commanding the smallest virulence gene load – are well-established to be the most common worldwide (Blanco et al., 2013; Nicolas-Chanoine et al., 2014; Merino et al., 2017).

Though virotypes C strains of ST131 H30 strains remain most frequent a few groups reported a higher incidence for virotypes A isolates (Olesen et al., 2014; Ludden et al., 2015; Jamborova et al., 2018). Virotypes A strains harbor a somewhat greater virulence gene load compared with virotypes C. This extra gene load is supposed to be associated with fitness cost, thus, the question arises how could these isolates exceed equally resistant or less resistant virotypes C strains?

The “integron effect” – observed also with MDR *K. pneumoniae* (see above) – may account for the circumstance. Class 1 integrons are common in *E. coli* (Copur-Cicek et al., 2014; Oliveira-Pinto et al., 2017) including ST131 isolates (Pérez-Etayo et al., 2018) and it is well-established that they are mostly associated with weak promoters (Vinué et al., 2011; Wei et al., 2013). It was demonstrated that these “weak-promoter integrons” are “low-cost structures” in *E. coli* and their carriage influences the fitness of the pathogen (Lacotte et al., 2017). The “integron effect” is supported by the high-quality report of Jamborova et al. (2018) who performed a comprehensive investigation of a large number of ST131 H30 isolates and found

that virotypes A was the most common variant among the highly resistant strains. However, they also tested the integron content of the isolates and unequivocally showed that, similarly to MDR *K. pneumoniae*, in their collection, the more virulent virotypes A isolates carried significantly more type 1 integrons than the virotypes C strains.

A few reports were published on ST131 strains harboring a greater virulence gene load than isolates from other STs (Johnson et al., 2009; Blanco et al., 2011; Colpan et al., 2013), however, these observations were made with *E. coli* ST131 isolates which were susceptible to several groups of antibiotics that could have permitted the carriage of a greater virulence gene load. In addition, López-Cerero et al. (2014) showed that “the severity of sepsis, bacteraemia and mortality were similar among ST131 and non-ST131” *E. coli* isolates.

Moreover, Johnson et al. (2019) recently reported that the ST1193-H64 major *E. coli* lineage disseminating extensively in the United States carried a more modest virulence gene load compared with other isolates further supporting the view that virulence factors play only an auxiliary role relative to fitness in determining the transmissibility of pathogens in a fluoroquinolone environment.

In summary we believe that in line with observations obtained with HA-MRSA and MDR *K. pneumoniae* fitness cost associated with resistance to fluoroquinolones is involved more prominently in the dissemination of the global STs of MDR *E. coli* than virulence factors. However, the distinction in virulence between hospital-associated and community-acquired strains is not as unequivocal as with MRSA and MDR *K. pneumoniae* because in contrast to these agents CA-*E. coli* strains, as primarily urogenital pathogens, are often exposed to fluoroquinolones and other antibiotics impacting the fitness of the isolates in a clone-dependent fashion. This is the reason why in contrast to MRSA and MDR *K. pneumoniae* the fluoroquinolone-selected clone of MDR *E. coli*, ST131, may feature prominently in community-acquired infections (Blanco et al., 2013; Doi et al., 2013; Nicolas-Chanoine et al., 2014; Chong et al., 2018; Jamborova et al., 2018; Matsukawa et al., 2019).

C. difficile

Two groups have demonstrated that ability to evolve a particular, energetically favorable *gyrA* mutation (Thr82Ile) is responsible for the rapid replication of the major international ribotypes of *C. difficile* when showing high-level resistance to fluoroquinolones (Wasels et al., 2015; Vernon et al., 2019). *C. difficile* does not carry topoisomerase IV genes, thus, a single mutation in the gyrase gene proved sufficient to confer both high-level resistance to fluoroquinolones and a favorable fitness effect.

Wasels et al. (2015) introduced the *gyrA* Thr82Ile mutation – typical for all major *C. difficile* ribotypes (Spigaglia et al., 2010) – into a non-major clone strain of *C. difficile* and found that the isolate suffered just a minimal (2–3%) fitness cost upon developing resistance to fluoroquinolones. In contrast some other QRDR mutations in the same isolate were associated with a considerable loss of robustness. Authors correctly predicted that the favorable fitness effect could have facilitated the dissemination of this particular clone of *C. difficile*.

Vernon et al. (2019) compared the fitness (speed of replication) of seven fluoroquinolone resistant ribotype 027 strains carrying the *gyrA* Thr82Ile mutation with that of their fluoroquinolone susceptible parent isolates void of the genetic alteration and experienced a fitness gain of 8–22% across the isolates. This paper attests that the fitness effect associated with a favorable QRDR mutation can be substantial and is both clone- and strain specific.

The salience of the *gyrA* Thr82Ile mutation is also reflected by a clonal shift that occurred recently in South Korea. Korean scientists demonstrated that clones which are considered minor in Europe and carry an energetically less favorable mutation will become major clones/ribotypes when they are capable of evolving the Thr82Ile *gyrA* mutation and will replace other ribotypes harboring energetically detrimental *gyrA* alterations (Lee et al., 2014). Consequently, the dissemination of a particular clone was governed by the strains' ability to evolve the favorable QRDR mutation.

Moreover, in the United Kingdom a campaign was launched in 2007 to restrict the use of fluoroquinolones to reduce the incidence of *C. difficile* (Wilcox et al., 2012). The campaign proved highly successful: the incidence of *C. difficile* infections dropped dramatically during the next couple of years (Wilcox et al., 2012; Dingle et al., 2017). Interestingly, the proportion of the major clone strains also declined significantly and polyclonality expanded during the same time period demonstrating clearly the association of major clones with the use of fluoroquinolone type antibiotics (Wilcox et al., 2012). A similar decrease in the rates for HA-MRSA and ST131 *E. coli* subsequent to the reduction of fluoroquinolone use (see above) was also observed.

Furthermore Sarma et al. (2015) reported that a significant decrease in the consumption of fluoroquinolones resulted in a partial replacement of strains from some major clones (all of them characteristically carrying the favorable *gyrA* Thr82Ile mutation) by minor clone isolates.

The observation that the incidence of ribotype 027 strains remains significantly lower in pediatric units (where fluoroquinolones are not supposed to be used) compared with adult wards (McFarland et al., 2016) remains an additional argument for the “fluoroquinolone effect” selecting major clones.

Minor clone strains of *C. difficile* are well-known to often remain susceptible to fluoroquinolones (Wiuff et al., 2011; Knight et al., 2015; Seugendo et al., 2018) or evolve various mutations in the *gyrB* gene and display reduced resistance to this group of antibiotics (Lee et al., 2014; Wasels et al., 2015; Shaw et al., 2019; Vernon et al., 2019). Though some of the genetic alterations in *gyrB* will confer some fitness gain onto the isolates (Wasels et al., 2015; Vernon et al., 2019), it is also well-established that these mutations will accord just a low-level resistance to moxifloxacin rendering the isolates “vulnerable” to exposure (Wasels et al., 2015; Vernon et al., 2019).

The recently observed widespread dissemination of the ribotype 017 clone of *C. difficile* in Asia (Imwattana et al., 2019) may also be associated with the “fluoroquinolone influence”. Ribotype 017 and other expanding-clone strains were reported to mostly carry the favorable *gyrA* Thr82Ile

mutation in various Asian countries (Lee et al., 2014; Wang et al., 2018).

It was recently suggested that efficient trehalose metabolism might have contributed to the advance of some clones of *C. difficile* (Collins et al., 2019). However, Eyre et al. (2019) demonstrated that many STs of *C. difficile* share similar metabolic pathways and some of these clones remain extremely rare, thus, the contribution of the “trehalose effect” should be slight compared with that of fluoroquinolones.

The Virulence Component in *C. difficile*

Apart from favorable fitness various virulence factors produced by ribotype 027 strains have certainly contributed to the clone's dissemination (Stabler et al., 2009; Valiente et al., 2014). Moreover, strains of additional major ribotypes (001 and 106) have also been shown to command superior virulence relative to many other clones (Vohra and Poxton, 2011). Nevertheless, superior virulence could not prevent the demise of strains from any of these major ribotypes once the selecting pressure of fluoroquinolone exposure ceased/diminished (Wilcox et al., 2012; Lee et al., 2014; Sarma et al., 2015; Dingle et al., 2017).

Finally the question arises: if the *gyrA* Thr82Ile mutation will substantially enhance the fitness of ribotype 027 strains (Vernon et al., 2019) how can isolates from this clone be replaced by minor clone strains upon cessation of fluoroquinolone exposure? The answer is that 027 strains replicate far slower – perhaps as a consequence of producing excessive quantities of toxin – than minor clone isolates. This was clearly established by Carlson et al. (2013) conducting propagation assays with strains of *C. difficile* from diverse genetic backgrounds. They found that “isolates of ribotype 027 produced higher levels of toxin and exhibited slower growth compared to other ribotypes”. This may account for the swift replacement of ribotype 027 strains by isolates from minor clones upon decreased use of fluoroquinolones.

Moreover Carlson et al.'s (2013) report clearly shows that toxin production is not related to transmissibility in *C. difficile*. Carlson et al. (2013) found an abundance of strains from diverse genetic backgrounds which, similarly to ribotype 027 strains, produced large quantities of toxin, nevertheless, their prevalence remained very low or were single isolates and no data is available on any of these ribotype strains carrying the favorable *gyrA* Thr82Ile mutation.

Since major ribotype strains feature prominently in *C. difficile* infections it is well-established that a reduction in the use of fluoroquinolones results in a decline in the incidence of the pathogen (area was reviewed by one of us) (Fuzi, 2016).

Other Species

All bacterial species causing human infections and exposed to the “fluoroquinolone effect” particularly in adult hospital wards have to adapt to the fluoroquinolone environment by evolving resistance mechanisms. We have seen how MRSA, MDR *K. pneumoniae*, MDR *E. coli* and *C. difficile* responded to the challenge: exclusively strains from a few international/global clones/STs proved capable of developing favorable QRDR mutations and emerged commanding superior fitness and replacing minor clone/ST strains in fluoroquinolone

environments. The available literature strongly suggest that the major international clones/STs of additional MDR pathogens might also have been shaped by the fluoroquinolone effect. The related literature on MDR *E. faecium* (VRE) was recently reviewed (Fuzi, 2016; Fuzi et al., 2017).

Although *Streptococcus pneumoniae* is primarily a community-acquired pathogen the extensive use of fluoroquinolones might have contributed to the selection of some clones of the species which managed evolved the energetically favorable double-serine mutations. *S. pneumoniae* strains from these clones proved prevalent in some European, Asian and Latin-American countries (Canton et al., 2003; de la Campa et al., 2009; Hsieh et al., 2010; Ardanuy et al., 2014; Chen H.H. et al., 2017), but could not disseminate in others (Ceysens et al., 2016; Metcalf et al., 2016; Schmitz et al., 2017). The most widespread “fluoroquinolone resistance-influenced” *S. pneumoniae* sequence type is certainly the ST81 group (de la Campa et al., 2009; Hsieh et al., 2010; Chen H.H. et al., 2017).

Moreover, international STs showing high-level resistance to fluoroquinolones were reported also in *Neisseria gonorrhoeae* with three or more QRDR mutations, including consistently the double-serine alterations (Cámara et al., 2012; Chen et al., 2013; Endimiani et al., 2014; Kubanov et al., 2016). These MDR *N. gonorrhoeae* STs are also suspect of having been influenced by diverse fitness cost associated with resistance to fluoroquinolones.

Furthermore, several papers were recently published on fluoroquinolone resistant *Haemophilus influenzae* strains carrying multiple QRDR mutations and showing clonal relatedness (Kuo et al., 2014; Puig et al., 2015; Fuursted et al., 2016) making these groups promising candidates for fluoroquinolone-selected pathogens.

Though enteric pathogens are less exposed to fluoroquinolones a lineage of fluoroquinolone resistant *Salmonella* Kentucky (ST198) might have been selected by fluoroquinolone pressure in the veterinary sphere. Isolates in this international ST also carry multiple QRDR mutations including the double-serine alterations (Le Hello et al., 2013) that should confer a fitness advantage in a fluoroquinolone environment.

In addition American scientists recently investigated *in vitro* the diverse mechanisms and related varying fitness costs associated with resistance to fluoroquinolones in *Salmonella* Enteritidis. Their findings remain in complete agreement with those obtained with MDR *K. pneumoniae* and MDR *E. coli* (Toth et al., 2014; Johnson et al., 2015a,b). The single isolate acquiring three QRDR mutations – including the double-serine alterations – retained much fitness (speed of replication) and used little efflux. All the other strains evolved either fewer-, or “non-double-serine” QRDR mutations, employed more efflux and suffered significant fitness costs (Vidovic et al., 2019). Consequently a few proportion of *S. enteritidis* isolates are potentially capable of generating major clones if strains of the serotype were extensively exposed to fluoroquinolones.

Campylobacter jejuni has also been exposed to the fluoroquinolone effect, however, this pathogen proved more adept relative to other bacteria to comply with the challenge, certainly due to the hyperplasticity of its genome (area was reviewed by us) (Fuzi, 2016; Fuzi et al., 2017). Consequently no

major clones of *C. jejuni* are known to have been selected by fluoroquinolones.

In addition, the involvement of fluoroquinolone resistance-associated fitness cost in the selection of the global/international clones of MDR *A. baumannii*, *Enterobacter cloacae* and MDR *Pseudomonas aeruginosa* would deserve a thorough investigation.

DISCUSSION

In summary fluoroquinolones have been shown to select for the major international clones/STs of various MDR pathogens and have potentially affected all species which were exposed to these antibiotics for extensive time periods. Since the evolution of the double-serine QRDR mutations were often associated with a fitness gain – though in a clone-dependent fashion – the assumption of high-level resistance to fluoroquinolones permitted the major clone/ST isolates to acquire resistance mechanisms against additional groups of antibiotics without suffering a substantial loss of vitality. This is in sharp contrast to minor clone/ST isolates which proved less capable of evolving energetically favorable QRDR mutations and – consequently – had to apply alternative mechanisms for developing resistance to fluoroquinolones, like enhanced efflux, that requires considerable energy. This was, however, associated with substantial fitness cost resulting in compromised growth that allowed the major clone/ST isolates to replace minor clone/ST strains in fluoroquinolone environments. The faster growth rate of these newly dominant major clone/ST isolates then brought about a steep rise in the incidence of MDR pathogens.

Moreover, the available data clearly show that fitness cost suffered by minor clone/ST strains can hardly be recovered by compensatory mutations. Toth et al. (2014) failed to reverse lost fitness in fluoroquinolone resistant minor ST strains of *K. pneumoniae*. Furthermore, it should not be an accident that the clonal landscape of multidrug-resistant pathogens remains largely stable in the hospital sector. If minor clone strains could easily reverse the fitness cost associated with resistance to fluoroquinolones by evolving compensatory mutations novel international clones of various multidrug-resistant pathogens hailing from previous minor ST strains should regularly emerge. However, this is what we have not been witnessing. What we see is just the infrequent dissemination of previous minor clone strains which with some delay proved competent to evolve the energetically favorable QRDR mutations (see above).

We believe the data presented in the paper demonstrate the crucial role fluoroquinolones played in the selection of the international clones/STs of MDR bacteria. Nevertheless, a variety of other groups of antibiotics are well-established to have also promoted the dissemination of MDR pathogens including strains of the international clones of HA-MRSA (Monnet et al., 2004; Lawes et al., 2015), MDR *K. pneumoniae* (da Silva et al., 2012; Ryu et al., 2018), MDR *E. coli* (López-Lozano et al., 2019) and *C. difficile* (Owens et al., 2008; Vardakas et al., 2016). However, the impact of these antibiotics remain second rate relative to that

of fluoroquinolones as shown above. The comprehensive review of this literature is beyond the scope of this paper.

Interestingly virulence-, and colonization factors seem to have exerted only a smaller impact relative to growth rate on the dissemination of major bacterial clones/STs in adult hospital wards. This observation is not surprising and remains consistent with features of an environment characterized by the extensive use of antibiotics, primarily that of fluoroquinolones. In this “high-antibiotic environment” a favorable fitness (high growth rate) will confer a considerable advantage onto a particular MDR isolate vs. other competing MDR strains from the same species showing superior virulence/colonization efficiency but inferior replication capabilities. Moreover, an MDR isolate with relatively lower virulence but almost “wild-type fitness” will also command considerable advantage against a highly virulent and fit strain that remains susceptible to antibiotics. Though the latter strain should transmit more readily it can be – in contrast to our “super bug” – easily controlled in the hospital setting with antibiotics.

Consequently, it is not an accident that the most widely disseminated types of the major international ST/lineage strains of HA-MRSA, MDR *K. pneumoniae* and MDR *E. coli* show less virulence than isolates from many other STs/lineages of the same species (see above). *C. difficile* remains an exception as ribotype 027 strains are highly toxic, however, the extensive dissemination of this clone was also the consequence of substantial fitness gain conferred by the *gyrA* Thr82Ile mutation (Vernon et al., 2019) and not the production of excessive toxin.

Nevertheless, the situation is different in some “low-antibiotic areas” in the community. In a “low-antibiotic” sphere pathogens can afford to remain less resistant and consequently retain fitness and harbor more virulence factors. Since in these areas antibiotics will usually not restrict the bacteria the virulence factors should confer a considerable advantage onto the pathogen that is reflected in the well-established high incidence of virulent and “non-major ST” strains (CA-MRSA, *K. pneumoniae*) in community-acquired infections (see above).

Excessive virulence is well-established even in a low-antibiotic environment to be actually detrimental for the evolutionary success because killing the host will arrest further propagation of the infectious agent. So the equilibrium must be maintained to avoid excessive expression or acquiring too lethal combinations of pathogenic traits that might impact the virulence of both community-acquired and healthcare-associated pathogens.

The fluoroquinolone effect implies that in a fluoroquinolone-free hospital environment the incidence of the international clone/ST strains should decrease. This is exactly what has been

observed with both HA-MRSA and *C. difficile*, the two MDR pathogens in which major ST strains comprise the highest proportion of the respective species in adult hospital wards. The regress of the major clones of both pathogens was demonstrated to have been associated with a decline in the incidence of both HA-MRSA and *C. difficile* upon a more judicious use of fluoroquinolones (see above).

The impact of a reduction in the consumption of fluoroquinolones on the incidence of major clone pathogens from other species remains less conspicuous since the proportion of major clone/ST bacteria is smaller among these clinical isolates. Nevertheless, some positive effect should also be observed with other species. Further epidemiological studies involving the judicious use of fluoroquinolones and investigating the clonal composition of the local pathogens are needed.

Finally, the judicious use of fluoroquinolones will ameliorate resistance rates only if infection control measures are complied with and poor hygiene will not compromise the expected favorable outcome.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

The manuscript was based on MF’s perception and he did much of the writing. JR and AT contributed significantly to both writing the manuscript and raising its professional standard by making relevant supplements.

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REFERENCES

- Abdulgader, S. M., Shittu, A. O., Nicol, M. P., and Kaba, M. (2015). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Africa: a systematic review. *Front. Microbiol.* 6:348. doi: 10.3389/fmicb.2015.00348
- Aghamohammad, S., Badmasti, F., Solgi, H., Aminzadeh, Z., Khodabandelo, Z., and Shahcheraghi, F. (2018). First report of extended-spectrum betalactamase-producing *Klebsiella pneumoniae* among fecal carriage in Iran: high diversity of clonal relatedness and virulence factor profiles. *Microb. Drug Resist.* [Epub ahead of print].
- Aires-de-Sousa, M., Correia, B., de Lencastre, H., and Multilaboratory Project Collaborators, (2008). Changing patterns in frequency of recovery of five methicillin-resistant *Staphylococcus aureus* clones in Portuguese hospitals: surveillance over a 16-year period. *J. Clin. Microbiol.* 46, 2912–2917. doi: 10.1128/JCM.00692-08
- Alam, M. T., Read, T. D., Petit, R. A. I. I., Boyle-Vavra, S., Miller, L. G., Eells, S. J., et al. (2015). Transmission and microevolution of USA300 MRSA in

- U.S. households: evidence from whole-genome sequencing. *mBio* 6:e00054. doi: 10.1128/mBio.00054-15
- Albrecht, N., Jatzwauk, L., Slickers, P., Ehrlich, R., and Monecke, S. (2011). Clonal replacement of epidemic methicillin-resistant *Staphylococcus aureus* strains in a German university hospital over a period of eleven years. *PLoS One* 6:e28189. doi: 10.1371/journal.pone.0028189
- Alouache, S., Estepa, V., Messai, Y., Ruiz, E., Torres, C., and Bakour, R. (2014). Characterization of ESBLs and associated quinolone resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolates from an urban wastewater treatment plant in Algeria. *Microb. Drug Resist.* 20, 30–38. doi: 10.1089/mdr.2012.0264
- Amorim, M. L., Faria, N. A., Oliveira, D. C., Vasconcelos, C., Cabeda, J. C., Mendes, A. C., et al. (2007). Changes in the clonal nature and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* isolates associated with spread of the EMRSA-15 clone in a tertiary care Portuguese hospital. *J. Clin. Microbiol.* 45, 2881–2888. doi: 10.1128/JCM.00603-07
- Andrade, L. N., Vitali, L., Gaspar, G. G., Bellissimo-Rodrigues, F., Martinez, R., and Darini, A. L. (2014). Expansion and evolution of a virulent, extensively drug-resistant (polymyxin B-resistant), QnrS1-CTX-M-2-, and KPC-2-producing *Klebsiella pneumoniae* ST11 international high-risk clone. *J. Clin. Microbiol.* 52, 2530–2535. doi: 10.1128/JCM.00088-14
- Ardanuy, C., de la Campa, A. G., García, E., Fenoll, A., Calatayud, L., Cercenado, E., et al. (2014). Spread of *Streptococcus pneumoniae* serotype 8-ST63 multidrug-resistant recombinant clone, Spain. *Emerg. Infect. Dis.* 20, 1848–1856. doi: 10.3201/eid2011.131215
- Aung, M. S., Kawaguchiya, M., Urushibara, N., Sumi, A., Ito, M., Kudo, K., et al. (2017). Molecular characterization of methicillin-resistant *Staphylococcus aureus* from outpatients in Northern Japan: increasing tendency of ST5/ST764 MRSA-IIa with arginine catabolic mobile element. *Microb. Drug Resist.* 23, 616–625. doi: 10.1089/mdr.2016.0176
- Baldan, R., Rancoita, P. M., Di Serio, C., Mazzotti, M., Cichero, P., Ossi, C., et al. (2015). Epidemic MRSA clone ST22-IV is more resistant to multiple host- and environment-related stresses compared with ST228-I. *J. Antimicrob. Chemother.* 70, 757–765. doi: 10.1093/jac/dku467
- Baldan, R., Testa, F., Lorè, N. I., Bragonzi, A., Cichero, P., Ossi, C., et al. (2012). Factors contributing to epidemic MRSA clones replacement in a hospital setting. *PLoS One* 7:e43153. doi: 10.1371/journal.pone.0043153
- Banerjee, R., Johnston, B., Lohse, C., Chattopadhyay, S., Tchesnokova, V., Sokurenko, E. V., et al. (2013). The clonal distribution and diversity of extraintestinal *Escherichia coli* isolates vary according to patient characteristics. *Antimicrob. Agents Chemother.* 57, 5912–5917. doi: 10.1128/AAC.01065-13
- Baraniak, A., Izdebski, R., Fielt, J., Gawryszewska, I., Bojarska, K., Herda, M., et al. (2016). NDM-producing *Enterobacteriaceae* in Poland, 2012–14: inter-regional outbreak of *Klebsiella pneumoniae* ST11 and sporadic cases. *J. Antimicrob. Chemother.* 71, 85–91. doi: 10.1093/jac/dkv282
- Bialek-Davenet, S., Criscuolo, A., Ailloud, F., Passet, V., Jones, L., Delannoy-Vieillard, A. S., et al. (2014). Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. *Emerg. Infect. Dis.* 20, 1812–1820. doi: 10.3201/eid2011.140206
- Birgy, A., Levy, C., Nicolas-Chanoine, M. H., Cointe, A., Hobson, C. A., Magnan, M., et al. (2019). Independent host factors and bacterial genetic determinants of the emergence and dominance of *Escherichia coli* sequence type 131 CTX-M-27 in a community pediatric cohort study. *Antimicrob. Agents Chemother.* 63:e382-19. doi: 10.1128/AAC.00382-19
- Blanco, J., Mora, A., Mamani, R., López, C., Blanco, M., Dahbi, G., et al. (2011). National survey of *Escherichia coli* causing extraintestinal infections reveals the spread of drug-resistant clonal groups O25b:H4-B2-ST131, O15:H1-D-ST393 and CGA-D-ST69 with high virulence gene content in Spain. *J. Antimicrob. Chemother.* 66, 2011–2021. doi: 10.1093/jac/dkr235
- Blanco, J., Mora, A., Mamani, R., López, C., Blanco, M., Dahbi, G., et al. (2013). Four main virotypes among extended-spectrum- β -lactamase-producing isolates of *Escherichia coli* O25b:H4-B2-ST131: bacterial, epidemiological, and clinical characteristics. *J. Clin. Microbiol.* 5, 3358–3367. doi: 10.1128/JCM.01555-13
- Bowers, J. R., Kitchel, B., Driebe, E. M., MacCannell, D. R., Roe, C., Lemmer, D., et al. (2015). Genomic analysis of the emergence and rapid global dissemination of the clonal group 258 *Klebsiella pneumoniae* pandemic. *PLoS One* 10:e0133727. doi: 10.1371/journal.pone.0133727
- Büdel, T., Kuenzli, E., Clément, M., Bernasconi, O. J., Fehr, J., Mohammed, A. H., et al. (2019). Polyclonal gut colonization with extended-spectrum cephalosporin- and/or colistin-resistant *Enterobacteriaceae*: a normal status for hotel employees on the island of Zanzibar, Tanzania. *J. Antimicrob. Chemother.* 74, 2880–2890. doi: 10.1093/jac/dkz296
- Cámara, J., Serra, J., Ayats, J., Bastida, T., Carnicer-Pont, D., Andreu, A., et al. (2012). Molecular characterization of two high-level ceftriaxone-resistant *Neisseria gonorrhoeae* isolates detected in Catalonia, Spain. *J. Antimicrob. Chemother.* 67, 1858–1860. doi: 10.1093/jac/dks162
- Cantón, R., Akóva, M., Carmeli, Y., Giske, C. G., Glupczynski, Y., Gniadkowski, M., et al. (2012). Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. *Clin. Microbiol. Infect.* 18, 413–431. doi: 10.1111/j.1469-0691.2012.03821.x
- Canton, R., Morosini, M., Enright, M. C., and Morrissey, I. (2003). Worldwide incidence, molecular epidemiology and mutations implicated in fluoroquinolone-resistant *Streptococcus pneumoniae*: data from the global PROTEKT surveillance programme. *J. Antimicrob. Chemother.* 52, 944–952. doi: 10.1093/jac/dkg465
- Carlson, P. E. Jr., Walk, S. T., Bourgis, A. E., Liu, M. W., Kopluku, F., Lo, E., et al. (2013). The relationship between phenotype, ribotype, and clinical disease in human *Clostridium difficile* isolates. *Anaerobe* 24, 109–116. doi: 10.1016/j.anaerobe.2013.04.003
- Cejas, D., Fernández Canigia, L., Rincón Cruz, G., Elena, A. X., Maldonado, L., Gutkind, G. O., et al. (2014). First isolate of KPC-2-producing *Klebsiella pneumoniae* sequence type 23 from the Americas. *J. Clin. Microbiol.* 52, 3483–3485. doi: 10.1128/JCM.00726-14
- Ceyssens, P. J., Van Bambeke, F., Mattheus, W., Bertrand, S., Fux, F., Van Bossuyt, E., et al. (2016). Molecular analysis of rising fluoroquinolone resistance in Belgian non-invasive *Streptococcus pneumoniae* isolates (1995–2014). *PLoS One* 11:e0154816. doi: 10.1371/journal.pone.0154816
- Cha, M. K., Kang, C. I., Kim, S. H., Cho, S. Y., Ha, Y. E., Wi, Y. M., et al. (2016). Comparison of the microbiological characteristics and virulence factors of ST131 and non-ST131 clones among extended-spectrum β -lactamase-producing *Escherichia coli* causing bacteremia. *Diagn. Microbiol. Infect. Dis.* 84, 102–104. doi: 10.1016/j.diagmicrobio.2015.10.015
- Chakrakodi, B., Prabhakara, S., Nagaraj, S., Etienne, J., and Arakere, G. (2014). High prevalence of ciprofloxacin resistance in community associated *Staphylococcus aureus* in a tertiary care Indian hospital. *Adv. Microbiol.* 4, 133–141. doi: 10.4236/aim.2014.42018
- Challagundla, L., Luo, X., Tickler, I. A., Didelot, X., Coleman, D. C., Shore, A. C., et al. (2018a). Range expansion and the origin of USA300 North American epidemic methicillin-resistant *Staphylococcus aureus*. *mBio* 9, e2016–e2017. doi: 10.1128/mBio.02016-17
- Challagundla, L., Reyes, J., Rafiqullah, I., Sordelli, D. O., Echaniz-Aviles, G., Velazquez-Meza, M. E., et al. (2018b). Phylogenomic classification and the evolution of clonal complex 5 methicillin-resistant *Staphylococcus aureus* in the Western Hemisphere. *Front. Microbiol.* 9:1901. doi: 10.3389/fmicb.2018.01901
- Chamon, R. C., Ribeiro, S. D., da Costa, T. M., Nouér, S. A., and Dos Santos, K. R. (2017). Complete substitution of the Brazilian endemic clone by other methicillin-resistant *Staphylococcus aureus* lineages in two public hospitals in Rio de Janeiro, Brazil. *Braz. J. Infect. Dis.* 21, 185–189. doi: 10.1016/j.bjid.2016.09.015
- Chen, C. C., Yen, M. Y., Wong, W. W., Li, L. H., Huang, Y. L., Chen, K. W., et al. (2013). Tracing subsequent dissemination of a cluster of gonococcal infections caused by an ST1407-related clone harbouring mosaic *penA* alleles in Taiwan. *J. Antimicrob. Chemother.* 68, 1567–1571. doi: 10.1093/jac/dkt059
- Chen, H. H., Li, H. C., Su, L. H., and Chiu, C. H. (2017). Fluoroquinolone-nonsusceptible *Streptococcus pneumoniae* isolates from a medical center in the pneumococcal conjugate vaccine era. *J. Microbiol. Immunol. Infect.* 50, 839–845. doi: 10.1016/j.jmii.2016.05.003
- Chen, L., Mathema, B., Chavda, K. D., DeLeo, F. R., Bonomo, R. A., and Kreiswirth, B. N. (2014). Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends Microbiol.* 22, 686–696. doi: 10.1016/j.tim.2014.09.003
- Chen, Y. T., Lai, Y. C., Tan, M. C., Hsieh, L. Y., Wang, J. T., Shiau, Y. R., et al. (2017). Prevalence and characteristics of pks genotoxin gene cluster-positive

- clinical *Klebsiella pneumoniae* isolates in Taiwan. *Sci. Rep.* 7:43120. doi: 10.1038/srep43120
- Cheong, H. S., Chung, D. R., Lee, C., Kim, S. H., Kang, C. I., Peck, K. R., et al. (2016). Emergence of serotype K1 *Klebsiella pneumoniae* ST23 strains co-producing the plasmid-mediated AmpC beta-lactamase DHA-1 and an extended-spectrum beta-lactamase in Korea. *Antimicrob. Resist. Infect. Control* 5:50.
- Chong, Y., Shimoda, S., and Shimono, N. (2018). Current epidemiology, genetic evolution and clinical impact of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Infect. Genet. Evol.* 61, 185–188. doi: 10.1016/j.meegid.2018.04.005
- Ciesielczuk, H., Betts, J., Phee, L., Doumith, M., Hope, R., Woodford, N., et al. (2015). Comparative virulence of urinary and bloodstream isolates of extra-intestinal pathogenic *Escherichia coli* in a *Galleria mellonella* model. *Virulence* 6, 145–151. doi: 10.4161/21505594.2014.988095
- Collins, J., Danhof, H., and Britton, R. A. (2019). The role of trehalose in the global spread of epidemic *Clostridium difficile*. *Gut Microbes* 10, 204–209. doi: 10.1080/19490976.2018.1491266
- Colpan, A., Johnston, B., Porter, S., Clabots, C., Anway, R., Thao, L., et al. (2013). *Escherichia coli* sequence type 131 (ST131) subclone H30 as an emergent multidrug-resistant pathogen among US veterans. *Clin. Infect. Dis.* 57, 1256–1265. doi: 10.1093/cid/cit503
- Conceição, T., Aires-de-Sousa, M., Fuzi, M., Tóth, A., Pászti, J., Ungvári, E., et al. (2007). Replacement of methicillin-resistant *Staphylococcus aureus* clones in Hungary over time: a 10-year surveillance study. *Clin. Microbiol. Infect.* 13, 971–979. doi: 10.1111/j.1469-0691.2007.01794.x
- Conlon-Bingham, G. M., Aldeyab, M., Scott, M., Kearney, M. P., Farren, D., Gilmore, F., et al. (2019). Effects of antibiotic cycling policy on incidence of healthcare-associated MRSA and *Clostridioides difficile* infection in secondary healthcare settings. *Emerg. Infect. Dis.* 25, 52–62. doi: 10.3201/eid2501.180111
- Coombs, G. W., Nimmo, G. R., Daly, D. A., Le, T. T., Pearson, J. C., Tan, H. L., et al. (2014). Australian group on antimicrobial resistance. Australian *Staphylococcus aureus* sepsis outcome programme annual report, 2013. *Commun. Dis. Intell. Q. Rep.* 38, E309–E319.
- Copur-Cicek, A., Ozgumus, O. B., Saral, A., and Sandalli, C. (2014). Antimicrobial resistance patterns and integron carriage of *Escherichia coli* isolates causing community-acquired infections in Turkey. *Ann. Lab. Med.* 34, 139–144. doi: 10.3343/alm.2014.34.2.139
- da Silva, R. M., Traebert, J., and Galato, D. (2012). *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae*: a review of epidemiological and clinical aspects. *Expert Opin. Biol. Ther.* 12, 663–671. doi: 10.1517/14712598.2012.681369
- Dahbi, G., Mora, A., Mamani, R., López, C., Alonso, M. P., Marzoa, J., et al. (2014). Molecular epidemiology and virulence of *Escherichia coli* O16:H5-ST131: comparison with H30 and H30-Rx subclones of O25b:H4-ST131. *Int. J. Med. Microbiol.* 304, 1247–1257. doi: 10.1016/j.ijmm.2014.10.002
- Damjanova, I., Tóth, A., Pászti, J., Hajbel-Vékony, G., Jakab, M., Berta, J., et al. (2008). Expansion and countrywide dissemination of ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-type beta-lactamase-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005–the new 'MRSA's?'. *J. Antimicrob. Chemother.* 62, 978–985. doi: 10.1093/jac/dkn287
- D'Andrea, M. M., Arena, F., Pallecchi, L., and Rossolini, G. M. (2013). CTX-M-type β -lactamases: a successful story of antibiotic resistance. *Int. J. Med. Microbiol.* 303, 305–317. doi: 10.1016/j.ijmm.2013.02.008
- Dauwalder, O., Lina, G., Durand, G., Bes, M., Meugnier, H., Jarlier, V., et al. (2008). Epidemiology of invasive methicillin-resistant *Staphylococcus aureus* clones collected in France in 2006 and 2007. *J. Clin. Microbiol.* 46, 3454–3458. doi: 10.1128/JCM.01050-08
- Day, M. J., Doumith, M., Abernethy, J., Hope, R., Reynolds, R., Wain, J., et al. (2016). Population structure of *Escherichia coli* causing bacteraemia in the UK and Ireland between 2001 and 2010. *J. Antimicrob. Chemother.* 71, 2139–2142. doi: 10.1093/jac/dkw145
- de la Campa, A. G., Ardanuy, C., Balsalobre, L., Pérez-Trallero, E., Marimón, J. M., Fenoll, A., et al. (2009). Changes in fluoroquinolone-resistant *Streptococcus pneumoniae* after 7-valent conjugate vaccination, Spain. *Emerg. Infect. Dis.* 15, 905–911. doi: 10.3201/eid1506.080684
- Derakhshan, S., Najar Peerayeh, S., and Bakhshi, B. (2016). Association between presence of virulence genes and antibiotic resistance in clinical *Klebsiella pneumoniae* isolates. *Lab. Med.* 47, 306–311. doi: 10.1093/labmed/lmw030
- Diago-Navarro, E., Chen, L., Passet, V., Burack, S., Ulacia-Hernando, A., Kodianplakkal, R. P., et al. (2014). Carbapenem-resistant *Klebsiella pneumoniae* exhibit variability in capsular polysaccharide and capsule associated virulence traits. *J. Infect. Dis.* 210, 803–813. doi: 10.1093/infdis/jiu157
- Dingle, K. E., Didelot, X., Quan, T. P., Eyre, D. W., Stoesser, N., Golubchik, T., et al. (2017). Effects of control interventions on *Clostridium difficile* infection in England: an observational study. *Lancet Infect. Dis.* 17, 411–421. doi: 10.1016/S1473-3099(16)30514-X
- Doi, Y., Park, Y. S., Rivera, J. I., Adams-Haduch, J. M., Hingwe, A., Sordillo, E. M., et al. (2013). Community-associated extended-spectrum β -lactamase-producing *Escherichia coli* infection in the United States. *Clin. Infect. Dis.* 56, 641–648. doi: 10.1093/cid/cis942
- Dong, N., Zhang, R., Liu, L., Li, R., Lin, D., Chan, E. W., et al. (2018). Genome analysis of clinical multilocus sequence type 11 *Klebsiella pneumoniae* from China. *Microb. Genom.* 7:146. doi: 10.1099/mgen.0.000149
- Endimiani, A., Guilarte, Y. N., Tinguely, R., Hirzberger, L., Selvini, S., Lupo, A., et al. (2014). Characterization of *Neisseria gonorrhoeae* isolates detected in Switzerland (1998–2012): emergence of multidrug-resistant clones less susceptible to cephalosporins. *BMC Infect. Dis.* 14:106. doi: 10.1186/1471-2334-14-106
- Espadinha, D., Faria, N. A., Miragaia, M., Lito, L. M., Melo-Cristino, J., de Lencastre, H., et al. (2013). Extensive dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) between the hospital and the community in a country with a high prevalence of nosocomial MRSA. *PLoS One* 8:e59960. doi: 10.1371/journal.pone.0059960
- Eyre, D. W., Didelot, X., Buckley, A. M., Freeman, J., Moura, I. B., Crook, D. W., et al. (2019). *Clostridium difficile* trehalose metabolism variants are common and not associated with adverse patient outcomes when variably present in the same lineage. *EBioMedicine* 43, 347–355. doi: 10.1016/j.ebiom.2019.04.038
- Felis, E., Kalka, J., Sochacki, A., Kowalska, K., Bajkacz, S., Harnisz, M., et al. (2020). Antimicrobial pharmaceuticals in the aquatic environment - occurrence and environmental implications. *Eur. J. Pharmacol.* 866:172813. doi: 10.1016/j.ejphar.2019.172813
- Fuursted, K., Hartmeyer, G. N., Stegger, M., Andersen, P. S., and Justesen, U. S. (2016). Molecular characterisation of the clonal emergence of high-level ciprofloxacin-monoresistant *Haemophilus influenzae* in the region of Southern Denmark. *J. Glob. Antimicrob. Resist.* 5, 67–70. doi: 10.1016/j.jgar.2015.12.004
- Fuzi, M. (2016). Dissimilar fitness associated with resistance to fluoroquinolones influences clonal dynamics of various multiresistant bacteria. *Front. Microbiol.* 7:1017. doi: 10.3389/fmicb.2016.01017
- Fuzi, M., Szabo, D., and Cserecsik, R. (2017). Double-serine fluoroquinolone resistance mutations advance major international clones and lineages of various multi-drug resistant bacteria. *Front. Microbiol.* 8:2261. doi: 10.3389/fmicb.2017.02261
- Garza-Ramos, U., Barrios-Camacho, H., Moreno-Domínguez, S., Toribio-Jiménez, J., Jardón-Pineda, D., Cuevas-Peña, J., et al. (2018). Phenotypic and molecular characterization of *Klebsiella* spp. isolates causing community-acquired infections. *New Microbes New Infect.* 23, 17–27. doi: 10.1016/j.nmni.2018.02.002
- Glaser, P., Martins-Simões, P., Villain, A., Barbier, M., Tristan, A., Bouchier, C., et al. (2016). Demography and intercontinental spread of the USA300 community-acquired methicillin-resistant *Staphylococcus aureus* lineage. *mBio* 7:e2183-15. doi: 10.1128/mBio.02183-15
- Gomez-Simmonds, A., Annavaajhala, M. K., Wang, Z., Macesic, N., Hu, Y., Giddins, M. J., et al. (2018). Genomic and geographic context for the evolution of high-risk carbapenem-resistant *Enterobacter cloacae* complex clones ST171 and ST78. *mBio* 9:e542-18. doi: 10.1128/mBio.00542-18
- Gomez-Simmonds, A., and Uhlemann, A. C. (2017). Clinical Implications of Genomic adaptation and evolution of carbapenem-resistant *Klebsiella pneumoniae*. *J. Infect. Dis.* 215(Suppl. 1), S18–S27. doi: 10.1093/infdis/jiw378
- Gostev, V., Kruglov, A., Kalinogorskaya, O., Dmitrenko, O., Khokhlova, O., Yamamoto, T., et al. (2017). Molecular epidemiology and antibiotic resistance

- of methicillin-resistant *Staphylococcus aureus* circulating in the Russian Federation. *Infect. Genet. Evol.* 53, 189–194. doi: 10.1016/j.meegid.2017.06.006
- Goswami, C., Fox, S., Holden, M., Connor, M., Leanord, A., and Evans, T. J. (2018). Genetic analysis of invasive *Escherichia coli* in Scotland reveals determinants of healthcare-associated versus community-acquired infections. *Microb. Genom.* 4:e000190. doi: 10.1099/mgen.0.000190
- Grundmann, H., Schouls, L. M., Aanensen, D. M., Pluister, G. N., Tami, A., Chlebowicz, M., et al. (2014). The dynamic changes of dominant clones of *Staphylococcus aureus* causing blood stream infections in the European region: results of a second structured survey. *Euro Surveill.* 19:20987. doi: 10.2807/1560-7917.ES2014.19.49.20987
- Gu, D., Dong, N., Zheng, Z., Lin, D., Huang, M., Wang, L., et al. (2018). A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. *Lancet Infect. Dis.* 18, 37–46. doi: 10.1016/S1473-3099(17)30489-9
- Gurnee, E. A., Ndao, I. M., Johnson, J. R., Johnston, B. D., Gonzalez, M. D., Burnham, C. A., et al. (2015). Gut colonization of healthy children and their mothers with pathogenic ciprofloxacin-resistant *Escherichia coli*. *J. Infect. Dis.* 212, 1862–1868. doi: 10.1093/infdis/jiv278
- Hertz, F. B., Nielsen, J. B., Schønning, K., Littauer, P., Knudsen, J. D., Løbner-Olesen, A., et al. (2016). Population structure of drug-susceptible, -resistant and ESBL-producing *Escherichia coli* from community-acquired urinary tract. *BMC Microbiol.* 16:63. doi: 10.1186/s12866-016-0681-z
- Hiramatsu, K., Igarashi, M., Morimoto, Y., Baba, T., Umekita, M., and Akamatsu, Y. (2012). Curing bacteria of antibiotic resistance: reverse antibiotics, a novel class of antibiotics in nature. *Int. J. Antimicrob. Agents* 39, 478–485. doi: 10.1016/j.ijantimicag.2012.02.007
- Holden, M. T., Hsu, L. Y., Kurt, K., Weinert, L. A., Mather, A. E., Harris, S. R., et al. (2013). A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. *Genome Res.* 23, 653–664. doi: 10.1101/gr.147710.112
- Holt, K. E., Wertheim, H., Zadoks, R. N., Baker, S., Whitehouse, C. A., Dance, D., et al. (2015). Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc. Natl. Acad. Sci. U.S.A.* 112, E3574–E3581. doi: 10.1073/pnas.1501049112
- Horváth, A., Dobay, O., Kardos, S., Ghidán, Á., Tóth, Á., Pászti, J., et al. (2012). Varying fitness cost associated with resistance to fluoroquinolones governs clonal dynamic of methicillin-resistant *Staphylococcus aureus*. *Eur. J. Clin. Microbiol. Infect. Dis.* 31, 2029–2036. doi: 10.1007/s10096-011-1536-z
- Hsieh, Y. C., Chang, L. Y., Huang, Y. C., Lin, H. C., Huang, L. M., and Hsueh, P. R. (2010). Circulation of international clones of levofloxacin non-susceptible *Streptococcus pneumoniae* in Taiwan. *Clin. Microbiol. Infect.* 16, 973–978. doi: 10.1111/j.1469-0691.2009.02951.x
- Hsu, L. Y., Harris, S. R., Chlebowicz, M. A., Lindsay, J. A., Koh, T. H., Krishnan, P., et al. (2015). Evolutionary dynamics of methicillin-resistant *Staphylococcus aureus* within a healthcare system. *Genome Biol.* 16:81. doi: 10.1186/s13059-015-0643-z
- Huang, I. F., Lee, W. Y., Wang, J. L., Hung, C. H., Hu, H. H., Hung, W. Y., et al. (2018). Fecal carriage of multidrug-resistant *Escherichia coli* by community children in southern Taiwan. *BMC Gastroenterol.* 18:86. doi: 10.1186/s12876-018-0807-x
- Imwattana, K., Knight, D. R., Kullin, B., Collins, D. A., Putsathit, P., Kiratisin, P., et al. (2019). *Clostridium difficile* ribotype 017 - characterization, evolution and epidemiology of the dominant strain in Asia. *Emerg. Microbes Infect.* 8, 796–807. doi: 10.1080/22221751.2019.1621670
- Jamborova, I., Johnston, B. D., Papousek, I., Kachlikova, K., Micekova, L., Clabots, C., et al. (2018). Extensive genetic commonality among wildlife, wastewater, community, and nosocomial isolates of *Escherichia coli* sequence type 131 (H30R1 and H30Rx Subclones) that carry bla(CTX-M-27) or bla(CTX-M-15). *Antimicrob. Agents Chemother.* 62:e000519-18. doi: 10.1128/AAC.00519-18
- Johnson, J. R., Johnston, B., Kuskowski, M. A., Sokurenko, E. V., and Tchesnokova, V. (2015a). Intensity and mechanisms of fluoroquinolone resistance within the H30 and H30Rx subclones of *Escherichia coli* sequence type 131 compared with other fluoroquinolone-resistant *E. coli*. *Antimicrob. Agents Chemother.* 59, 4471–4480. doi: 10.1128/AAC.00673-15
- Johnson, J. R., Johnston, B. D., Porter, S. B., Clabots, C., Bender, T. L., Thuras, P., et al. (2019). Rapid emergence, subsidence, and molecular detection of *Escherichia coli* sequence type 1193- fimH64 (ST1193-H64), a new disseminated multidrug-resistant commensal and extraintestinal pathogen. *J. Clin. Microbiol.* 57:JCM.1664-18. doi: 10.1128/JCM.01664-18
- Johnson, J. R., Menard, M., Johnston, B., Kuskowski, M. A., Nichol, K., and Zhanel, G. G. (2009). Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. *Antimicrob. Agents Chemother.* 53, 2733–2739. doi: 10.1128/AAC.00297-09
- Johnson, J. R., Porter, S. B., Thuras, P., Johnson, T. J., Price, L. B., Tchesnokova, V., et al. (2015b). Greater ciprofloxacin tolerance as a possible selectable phenotype underlying the pandemic spread of the H30 subclone of *Escherichia coli* sequence type 131. *Antimicrob. Agents Chemother.* 59, 7132–7135. doi: 10.1128/AAC.01687-15
- Johnson, T. J., Elnekave, E., Miller, E. A., Munoz-Aguayo, J., Flores Figueroa, C., Johnston, B., et al. (2018). Phylogenomic analysis of extraintestinal pathogenic *Escherichia coli* sequence type 1193, an emerging multidrug-resistant clonal group. *Antimicrob. Agents Chemother.* 63, e1913–e1918. doi: 10.1128/AAC.01913-18
- Kaito, C., Saito, Y., Ikuo, M., Omae, Y., Mao, H., Nagano, G., et al. (2013). Mobile genetic element SCCmec-encoded psm-mec RNA suppresses translation of agrA and attenuates MRSA virulence. *PLoS Pathog.* 9:e1003269. doi: 10.1371/journal.ppat.1003269
- Kallonen, T., Brodrick, H. J., Harris, S. R., Corander, J., Brown, N. M., Martin, V., et al. (2017). Systematic longitudinal survey of invasive *Escherichia coli* in England demonstrates a stable population structure only transiently disturbed by the emergence of ST131. *Genome Res.* 27, 1437–1449. doi: 10.1101/gr.216606.116
- Khokhlova, O. E., Hung, W. C., Wan, T. W., Iwao, Y., Takano, T., Higuchi, W., et al. (2015). Healthcare-and community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and fatal pneumonia with pediatric deaths in Krasnoyarsk, Siberian Russia: unique MRSA's multiple virulence factors, genome, and stepwise evolution. *PLoS One* 10:e0128017. doi: 10.1371/journal.pone.0128017
- Kinoshita, T., Tokumasu, H., Tanaka, S., Kramer, A., and Kawakami, K. (2017). Policy implementation for methicillin-resistant *Staphylococcus aureus* in seven European countries: a comparative analysis from 1999 to 2015. *J. Mark. Access Health Policy* 5:1351293. doi: 10.1080/20016689.2017.1351293
- Knight, D. R., Giglio, S., Huntington, P. G., Korman, T. M., Kotsanas, D., Moore, C. V., et al. (2015). Surveillance for antimicrobial resistance in Australian isolates of *Clostridium difficile*, 2013–14. *J. Antimicrob. Chemother.* 70, 2992–2999. doi: 10.1093/jac/dkv220
- Knight, G. M., Budd, E. L., Whitney, L., Thornley, A., Al-Ghusein, H., Planche, T., et al. (2012). Shift in dominant hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) clones over time. *J. Antimicrob. Chemother.* 67, 2514–2522. doi: 10.1093/jac/dks245
- Ko, K. S. (2019). Antibiotic-resistant clones in Gram-negative pathogens: presence of global clones in Korea. *J. Microbiol.* 5, 195–202. doi: 10.1007/s12275-019-8491-2
- Ku, Y. H., Chuang, Y. C., Chen, C. C., Lee, M. F., Yang, Y. C., Tang, H. J., et al. (2017). *Klebsiellapneumoniae* isolates from meningitis: epidemiology, virulence and antibiotic resistance. *Sci. Rep.* 7:6634. doi: 10.1038/s41598-017-06878-6
- Kubanov, A., Vorobyev, D., Chestkov, A., Leinsoo, A., Shaskolskiy, B., Dementieva, E., et al. (2016). Molecular epidemiology of drug-resistant *Neisseria gonorrhoeae* in Russia (Current Status, 2015). *BMC Infect. Dis.* 16:389. doi: 10.1186/s12879-016-1688-7
- Kuijper, E. J., Coignard, B., Tüll, P., Escmid Study Group for *Clostridium difficile*, Eu Member States, and European Centre for Disease Prevention and Control, (2006). Emergence of *Clostridium difficile*-associated disease in North America and Europe. *Clin. Microbiol. Infect.* 12(Suppl. 6), 2–18. doi: 10.1111/j.1469-0691.2006.01580.x
- Kuo, S. C., Chen, P. C., Shiau, Y. R., Wang, H. Y., Lai, J. F. I., Huang, W., et al. (2014). Levofloxacin-resistant *Haemophilus influenzae*, Taiwan, 2004–2010. *Emerg. Infect. Dis.* 20, 1386–1390. doi: 10.3201/eid2008.140341
- Lacotte, Y., Ploy, M. C., and Raheirison, S. (2017). Class 1 integrons are low-cost structures in *Escherichia coli*. *ISME J.* 11, 1535–1544. doi: 10.1038/ismej.2017.38
- Lam, M. M. C., Wick, R. R., Wyres, K. L., Gorrie, C. L., Judd, L. M., Jenney, A. W. J., et al. (2018a). Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICEKp in *Klebsiella pneumoniae* populations. *Microb. Genom.* 4:e000196. doi: 10.1099/mgen.0.000196
- Lam, M. M. C., Wyres, K. L., Duchêne, S., Wick, R. R., Judd, L. M., Gan, Y. H., et al. (2018b). Population genomics of hypervirulent *Klebsiella pneumoniae*

- clonal-group 23 reveals early emergence and rapid global dissemination. *Nat. Commun.* 9:2703. doi: 10.1038/s41467-018-05114-7
- Lascols, C., Peirano, G., Hackel, M., Laupland, K. B., and Pitout, J. D. (2013). Surveillance and molecular epidemiology of *Klebsiella pneumoniae* isolates that produce carbapenemases: first report of OXA-48-like enzymes in North America. *Antimicrob. Agents Chemother.* 57, 130–136. doi: 10.1128/AAC.01686-12
- Lau, S. H., Kaufmann, M. E., Livermore, D. M., Woodford, N., Willshaw, G. A., Cheasty, T., et al. (2008). UK epidemic *Escherichia coli* strains A-E, with CTX-M-15 beta-lactamase, all belong to the international O25:H4-ST131 clone. *J. Antimicrob. Chemother.* 62, 1241–1244. doi: 10.1093/jac/dkn380
- Lawes, T., López-Lozano, J.-M., Nebot, C., Macartney, G., Subbarao-Sharma, R., Dare, C. R. J., et al. (2015). Turning the tide or riding the waves? Impacts of antibiotic stewardship and infection control on MRSA strain dynamics in a Scottish region over 16 years: non-linear time series analysis. *Br. Med. J. Open* 5:e006596. doi: 10.1136/bmjopen-2014-006596
- Le Hello, S., Bekhit, A., Granier, S. A., Barua, H., Beutlich, J., Zajac, M., et al. (2013). The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. *Front. Microbiol.* 4:395. doi: 10.3389/fmicb.2013.00395
- Lee, C. R., Lee, J. H., Park, K. S., Jeon, J. H., Kim, Y. B., Cha, C. J., et al. (2017). Antimicrobial resistance of hypervirulent *Klebsiella pneumoniae*: epidemiology, hypervirulence-associated determinants, and resistance mechanisms. *Front. Cell. Infect. Microbiol.* 7:483. doi: 10.3389/fcimb.2017.00483
- Lee, J. H., Lee, Y., Lee, K., Riley, T. V., and Kim, H. (2014). The changes of PCR ribotype and antimicrobial resistance of *Clostridium difficile* in a tertiary care hospital over 10 years. *J. Med. Microbiol.* 63(Pt 6), 819–823. doi: 10.1099/jmm.0.072082-0
- Lepuschitz, S., Huhulescu, S., Hyden, P., Springer, B., Rattei, T., Allerberger, F., et al. (2018). Characterization of a community-acquired-MRSA USA300 isolate from a river sample in Austria and whole genome sequence based comparison to a diverse collection of USA300 isolates. *Sci. Rep.* 8:9467. doi: 10.1038/s41598-018-27781-8
- Li, S., Sun, S., Yang, C., Chen, H., Yin, Y., Li, H., et al. (2018). The changing pattern of population structure of *Staphylococcus aureus* from bacteremia in China from 2013 to 2016: ST239-030-MRSA replaced by ST59-t437. *Front. Microbiol.* 9:332. doi: 10.3389/fmicb.2018.00332
- Li, Y., Shen, H., Zhu, C., and Yu, Y. (2019). Carbapenem-resistant *Klebsiella pneumoniae* infections among ICU admission patients in central China: prevalence and prediction model. *Biomed. Res. Int.* 2019:9767313. doi: 10.1155/2019/9767313
- Lim, K. T., Hanifah, Y. A., Mohd Yusof, M. Y., Ito, T., and Thong, K. L. (2013). Comparison of methicillin-resistant *Staphylococcus aureus* strains isolated in 2003 and 2008 with an emergence of multidrug resistant ST22:SCCmecIV clone in a tertiary hospital, Malaysia. *J. Microbiol. Immunol. Infect.* 46, 224–233. doi: 10.1016/j.jmii.2013.02.001
- Liu, Y., Li, X. Y., Wan, L. G., Jiang, W. Y., Yang, J. H., and Li, F. Q. (2014). Virulence and transferability of resistance determinants in a novel *Klebsiella pneumoniae* sequence type 1137 in China. *Microb. Drug Resist.* 20, 150–155. doi: 10.1089/mdr.2013.0107
- Liu, Z., Gu, Y., Li, X., Liu, Y., Ye, Y., Guan, S., et al. (2019). Identification and characterization of NDM-1-producing hypervirulent (Hypermucoviscous) *Klebsiella pneumoniae* in China. *Ann. Lab. Med.* 39, 167–175. doi: 10.3343/alm.2019.39.2.167
- López-Cerero, L., Navarro, M. D., Bellido, M., Martín-Peña, A., Viñas, L., Cisneros, J., et al. (2014). *Escherichia coli* belonging to the worldwide emerging epidemic clonal group O25b/ST131: risk factors and clinical implications. *J. Antimicrob. Chemother.* 69, 809–814. doi: 10.1093/jac/dkt405
- López-Lozano, J. M., Lawes, T., Nebot, C., Beyaert, A., Bertrand, X., Hocquet, D., et al. (2019). A nonlinear time-series analysis approach to identify thresholds in associations between population antibiotic use and rates of resistance. *Nat. Microbiol.* 4, 1160–1172. doi: 10.1038/s41564-019-0410-0
- Louie, T. J. (2005). How should we respond to the highly toxigenic NAP1/ribotype 027 strain of *Clostridium difficile*? *CMAJ* 173, 1049–1050. doi: 10.1503/cmaj.051212
- Lozano, C., Rezusta, A., Gómez, P., Gómez-Sanz, E., Báez, N., Martín-Saco, G., et al. (2012). High prevalence of spa types associated with the clonal lineage CC398 among tetracycline-resistant methicillin-resistant *Staphylococcus aureus* strains in a Spanish hospital. *J. Antimicrob. Chemother.* 67, 330–334. doi: 10.1093/jac/dkr497
- Lu, B., Zhou, H., Zhang, X., Qu, M., Huang, Y., and Wang, Q. (2017). Molecular characterization of *Klebsiella pneumoniae* isolates from stool specimens of outpatients in sentinel hospitals Beijing, China, 2010–2015. *Gut Pathog.* 9:39. doi: 10.1186/s13099-017-0188-7
- Ludden, C., Cormican, M., Vellinga, A., Johnson, J. R., Austin, B., and Morris, D. (2015). Colonisation with ESBP-producing and carbapenemase-producing *Enterobacteriaceae*, vancomycin-resistant *Enterococci*, and methicillin-resistant *Staphylococcus aureus* in a long-term care facility over one year. *BMC Infect. Dis.* 15:168. doi: 10.1186/s12879-015-0880-5
- Luther, M. K., Parente, D. M., Caffrey, A. R., Daffinee, K. E., Lopes, V. V., Martin, E. T., et al. (2018). LaPlante KL. Clinical and genetic risk factors for biofilm-forming *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 62:e2252-17. doi: 10.1128/AAC.02252-17
- Ma, X. X., Ito, T., Chongtrakool, P., and Hiramoto, K. (2006). Predominance of clones carrying Panton-Valentine leukocidin genes among methicillin-resistant *Staphylococcus aureus* strains isolated in Japanese hospitals from 1979 to 1985. *J. Clin. Microbiol.* 44, 4515–4527. doi: 10.1128/JCM.00985-06
- Marcusson, L. L., Frimodt-Møller, N., and Hughes, D. (2009). Interplay in the selection of fluoroquinolone resistance and bacterial fitness. *PLoS Pathog.* 5:e1000541. doi: 10.1371/journal.ppat.1000541
- Marques, C., Menezes, J., Belas, A., Aboim, C., Cavaco-Silva, P., Trigueiro, G., et al. (2019). *Klebsiella pneumoniae* causing urinary tract infections in companion animals and humans: population structure, antimicrobial resistance and virulence genes. *J. Antimicrob. Chemother.* 74, 594–602. doi: 10.1093/jac/dky499
- Martínez, J. L., and Baquero, F. (2002). Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. *Clin. Microbiol. Rev.* 15, 647–679. doi: 10.1128/cmr.15.4.647-679.2002
- Mathers, A. J., Peirano, G., and Pitout, J. D. (2015). *Escherichia coli* ST131: the quintessential example of an international multiresistant high-risk clone. *Adv. Appl. Microbiol.* 90, 109–154. doi: 10.1016/bs.aams.2014.09.002
- Mato, R., Campanile, F., Stefani, S., Crisóstomo, M. I., Santagati, M., Sanches, S. I., et al. (2004). Clonal types and multidrug resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) recovered in Italy during the 1990s. *Microb. Drug Resist.* 10, 106–113. doi: 10.1089/1076629041310109
- Matsukawa, M., Igarashi, M., Watanabe, H., Qin, L., Ohnishi, M., Terajima, J., et al. (2019). Epidemiology and genotypic characterisation of dissemination patterns of uropathogenic *Escherichia coli* in a community. *Epidemiol. Infect.* 147:e148. doi: 10.1017/S0950268819000426
- Matsumura, Y., Yamamoto, M., Nagao, M., Ito, Y., Takakura, S., Ichijima, S., et al. (2013). Association of fluoroquinolone resistance, virulence genes, and IncF plasmids with extended-spectrum-β-lactamase-producing *Escherichia coli* sequence type 131 (ST131) and ST405 clonal groups. *Antimicrob. Agents Chemother.* 57, 4736–4742. doi: 10.1128/AAC.00641-13
- Mavroidi, A., Miriagou, V., Liakopoulos, A., Tzelepi, E., Stefanos, A., Dalekos, G. N., et al. (2012). Ciprofloxacin-resistant *Escherichia coli* in Central Greece: mechanisms of resistance and molecular identification. *BMC Infect. Dis.* 12:371. doi: 10.1186/1471-2334-12-371
- McCarthy, H., Rudkin, J. K., Black, N. S., Gallagher, L., O'Neill, E., and O'Gara, J. P. (2015). Methicillin resistance and the biofilm phenotype in *Staphylococcus aureus*. *Front. Cell. Infect. Microbiol.* 5:1. doi: 10.3389/fcimb.2015.00001
- McDonald, L. C., Killgore, G. E., Thompson, A., Owens, R. C. Jr., Kazakova, S. V., Sambol, S. P., et al. (2005). An epidemic, toxin gene-variant strain of *Staphylococcus aureus*. *N. Engl. J. Med.* 353, 2433–2441.
- McFarland, L. V., Ozen, M., Dinleyici, E. C., and Goh, S. (2016). Comparison of pediatric and adult antibiotic-associated diarrhea and *Clostridium difficile* infections. *World J. Gastroenterol.* 22, 3078–3104. doi: 10.3748/wjg.v22.i11.3078
- McNally, A., Kallonen, T., Connor, C., Abudahab, K., Aanensen, D. M., Horner, C., et al. (2019). Diversification of colonization factors in a multidrug-resistant *Escherichia coli* lineage evolving under negative frequency-dependent selection. *mBio* 10:e644-19. doi: 10.1128/mBio.00644-19
- Meijs, A. P., Gijssbers, E. F., Hengeveld, P. D., Veenman, C., van Roon, A. M., van Hoek, A. H. A. M., et al. (2019). Do vegetarians less frequently carry ESBP/pAmpC-producing *Escherichia coli*/*Klebsiella pneumoniae* compared

- with non-vegetarians? *J. Antimicrob. Chemother.* 2019;dkz483. doi: 10.1093/jac/dkz483
- Merino, I., Porter, S. B., Johnston, B. D., Clabots, C., Shaw, E., Horcajada, J. P., et al. (2017). Virulence genes and subclone status as markers of experimental virulence in a murine sepsis model among *Escherichia coli* sequence type 131 clinical isolates from Spain. *PLoS One* 12:e0188838. doi: 10.1371/journal.pone.0188838
- Metcalfe, B. J., Chochua, S., Gertz, R. E. Jr., Li, Z., Walker, H., Tran, T., et al. (2016). Active bacterial core surveillance team. Using whole genome sequencing to identify resistance determinants and predict antimicrobial resistance phenotypes for year 2015 invasive pneumococcal disease isolates recovered in the United States. *Clin. Microbiol. Infect.* 22, 1002.e1–1002.e8. doi: 10.1016/j.cmi.2016.08.001
- Monecke, S., Coombs, G., Shore, A. C., Coleman, D. C., Akpaka, P., Borg, M., et al. (2011). A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS One* 6:e17936. doi: 10.1371/journal.pone.0017936
- Monnet, D. L., MacKenzie, F. M., López-Lozano, J. M., Beyaert, A., Camacho, M., Wilson, R., et al. (2004). Antimicrobial drug use and methicillin-resistant *Staphylococcus aureus*, Aberdeen, 1996–2000. *Emerg. Infect. Dis.* 10, 1432–1441.
- Morales Barroso, I., López-Cerero, L., Navarro, M. D., Gutiérrez-Gutiérrez, B., Pascual, A., and Rodríguez-Baño, J. (2018). Intestinal colonization due to *Escherichia coli* ST131: risk factors and prevalence. *Antimicrob. Resist. Infect. Control* 7:135. doi: 10.1186/s13756-018-0427-9
- Morimoto, Y., Baba, T., Sasaki, T., and Hiramatsu, K. (2015). Apigenin as an anti-quinolone-resistance antibiotic. *Int. J. Antimicrob. Agents* 4, 666–673. doi: 10.1016/j.ijantimicag.2015.09.006
- Murai, T., Okazaki, K., Kinoshita, K., Uehara, Y., Zuo, H., Lu, Y., et al. (2019). Comparison of USA300 with non-USA300 methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Int. J. Infect. Dis.* 79, 134–138. doi: 10.1016/j.ijid.2018.11.020
- Nakane, K., Kawamura, K., Goto, K., and Arakawa, Y. (2016). Long-term colonization by bla(CTX-M)-harboring *Escherichia coli* in healthy Japanese people engaged in food handling. *Appl. Environ. Microbiol.* 82, 1818–1827. doi: 10.1128/AEM.02929-15
- Nicolas-Chanoine, M. H., Bertrand, X., and Madec, J. Y. (2014). *Escherichia coli* ST131, an intriguing clonal group. *Clin. Microbiol. Rev.* 27, 543–574. doi: 10.1128/CMR.00125-13
- Nicolas-Chanoine, M. H., Blanco, J., Leflon-Guibout, V., Demarty, R., Alonso, M. P., Caniça, M. M., et al. (2008). Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX- M-15. *J. Antimicrob. Chemother.* 61, 273–281. doi: 10.1093/jac/dkm464
- Nikolaras, G. P., Papaparaskivas, J., Samarkos, M., Tzouveleki, L. S., Psychogiou, M., Pavlopoulou, I., et al. (2018). Changes in the rates and population structure of MRSA from bloodstream infections. A single center experience (2000–2015). *J. Glob. Antimicrob. Resist.* 17, 117–122. doi: 10.1016/j.jgar.2018.11.023
- Ny, S., Löfmark, S., Börjesson, S., Englund, S., Ringman, M., Bergström, J., et al. (2017). Community carriage of ESBL-producing *Escherichia coli* is associated with strains of low pathogenicity: a Swedish nationwide study. *J. Antimicrob. Chemother.* 72, 582–588. doi: 10.1093/jac/dkw419
- Olesen, B., Frimodt-Møller, J., Leihof, R. F., Struve, C., Johnston, B., Hansen, D. S., et al. (2014). Temporal trends in antimicrobial resistance and virulence-associated traits within the *Escherichia coli* sequence type 131 clonal group and its H30 and H30-Rx subclones, 1968 to 2012. *Antimicrob. Agents Chemother.* 58, 6886–6895. doi: 10.1128/AAC.03679-14
- Oliveira, D. C., Tomasz, A., and de Lencastre, H. (2001). The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated mec elements. *Microb. Drug Resist.* 7, 349–361. doi: 10.1089/10766290152773365
- Oliveira, D. C., Tomasz, A., and de Lencastre, H. (2002). Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect. Dis.* 2, 180–189. doi: 10.1016/s1473-3099(02)00227-x
- Oliveira-Pinto, C., Diamantino, C., Oliveira, P. L., Reis, M. P., Costa, P. S., Paiva, M. C., et al. (2017). Occurrence and characterization of class 1 integrons in *Escherichia coli* from healthy individuals and those with urinary infection. *J. Med. Microbiol.* 66, 577–583. doi: 10.1099/jmm.0.000468
- O'Neill, G. L., Murchan, S., Gil-Setas, A., and Aucken, H. M. (2001). Identification and characterization of phage variants of a strain of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-15). *J. Clin. Microbiol.* 39, 1540–1548. doi: 10.1128/jcm.39.4.1540-1548.2001
- Owens, R. C. Jr., Donskey, C. J., Gaynes, R. P., Loo, V. G., and Muto, C. A. (2008). Antimicrobial-associated risk factors for *Clostridium difficile* infection. *Clin. Infect. Dis.* 46(Suppl. 1), S19–S31. doi: 10.1086/521859
- Paczosa, M. K., and Meccas, J. (2016). *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiol. Mol. Biol. Rev.* 80, 629–661. doi: 10.1128/MMBR.00078-15
- Pan, F., Tian, D., Wang, B., Zhao, W., Qin, H., Zhang, T., et al. (2019). Fecal carriage and molecular epidemiology of carbapenem-resistant *Enterobacteriaceae* from outpatient children in Shanghai. *BMC Infect. Dis.* 19:678. doi: 10.1186/s12879-019-4298-3
- Peirano, G., van der Bij, A. K., Freeman, J. L., Poirel, L., Nordmann, P., Costello, M., et al. (2014). Characteristics of *Escherichia coli* sequence type 131 isolates that produce extended-spectrum β -lactamases: global distribution of the H30-Rx sublineage. *Antimicrob. Agents Chemother.* 58, 3762–3767. doi: 10.1128/AAC.02428-14
- Pérez-Etayo, L., Berzosa, M., González, D., and Vitas, A. I. (2018). Prevalence of integrons and insertion sequences in ESBL-producing *E. coli* isolated from different sources in Navarra, Spain. *Int. J. Environ. Res. Public Health* 15:E2308. doi: 10.3390/ijerph15102308
- Pérez-Roth, E., Lorenzo-Díaz, F., Batista, N., Moreno, A., and Méndez-Alvarez, S. (2004). Tracking methicillin-resistant *Staphylococcus aureus* clones during a 5-year period (1998 to 2002) in a Spanish hospital. *J. Clin. Microbiol.* 42, 4649–4656. doi: 10.1128/jcm.42.10.4649-4656.2004
- Pinto, A. N., Seth, R., Zhou, F., Tallon, J., Dempsey, K., Tracy, M., et al. (2013). Emergence and control of an outbreak of infections due to Pantone-Valentine leukocidin positive, ST22 methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Clin. Microbiol. Infect.* 19, 620–627. doi: 10.1111/j.1469-0691.2012.03987.x
- Pitout, J. D., Nordmann, P., and Poirel, L. (2015). Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. *Antimicrob. Agents Chemother.* 59, 5873–5884. doi: 10.1128/AAC.01019-15
- Puig, C., Tirado-Vélez, J. M., Calatayud, L., Tubau, F., Garmendia, J., Ardanuy, C., et al. (2015). Molecular characterization of fluoroquinolone resistance in nontypeable *Haemophilus influenzae* clinical isolates. *Antimicrob. Agents Chemother.* 59, 461–466. doi: 10.1128/AAC.04005-14
- Qu, T. T., Zhou, J. C., Jiang, Y., Shi, K. R., Li, B., Shen, P., et al. (2015). Clinical and microbiological characteristics of *Klebsiella pneumoniae* liver abscess in East China. *BMC Infect. Dis.* 15:161. doi: 10.1186/s12879-015-0899-7
- Rao, Q., Shang, W., Hu, X., and Rao, X. (2015). *Staphylococcus aureus* ST121: a globally disseminated hypervirulent clone. *J. Med. Microbiol.* 64, 1462–1473. doi: 10.1099/jmm.0.000185
- Riaz, L., Mahmood, T., Khalid, A., Rashid, A., Ahmed Siddique, M. B., Kamal, A., et al. (2018). Fluoroquinolones (FQs) in the environment: a review on their abundance, sorption and toxicity in soil. *Chemosphere* 191, 704–720. doi: 10.1016/j.chemosphere.2017.10.092
- Roe, C. C., Vazquez, A. J., Esposito, E. P., Zarrilli, R., and Sahl, J. W. (2019). Diversity, virulence, and antimicrobial resistance in isolates from the newly emerging *Klebsiella pneumoniae* ST101 lineage. *Front. Microbiol.* 1:542. doi: 10.3389/fmicb.2019.00542
- Roer, L., Overballe-Petersen, S., Hansen, F., Schønning, K., Wang, M., Røder, B. L., et al. (2018). *Escherichia coli* sequence type 410 is causing new international high-risk clones. *mSphere* 3:e337-18. doi: 10.1128/mSphere.00337-18
- Rogers, B. A., Sidjabat, H. E., and Paterson, D. L. (2011). *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. *J. Antimicrob. Chemother.* 66, 1–14. doi: 10.1093/jac/dkq415
- Rossney, A. S., Shore, A. C., Morgan, P. M., Fitzgibbon, M. M., O'Connell, B., and Coleman, D. C. (2007). The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Pantone-Valentine leukocidin gene (pvl) reveal that pvl is a poor marker for community-acquired MRSA strains in Ireland. *J. Clin. Microbiol.* 45, 2554–2563. doi: 10.1128/jcm.00245-07
- Roth, N., Käsböhrer, A., Mayrhofer, S., Zitz, U., Hofacre, C., and Domig, K. J. (2019). The application of antibiotics in broiler production and the resulting

- antibiotic resistance in *Escherichia coli*: a global overview. *Poult. Sci.* 98, 1791–1804. doi: 10.3382/ps/pey539
- Runcharoen, C., Moradigaravand, D., Blane, B., Paksanont, S., Thammachote, J., Anun, S., et al. (2017). Whole genome sequencing reveals high-resolution epidemiological links between clinical and environmental *Klebsiella pneumoniae*. *Genome Med.* 9:6. doi: 10.1186/s13073-017-0397-1
- Russo, T. A., Olson, R., MacDonald, U., Beanan, J., and Davidson, B. A. (2015). Aerobactin, but not yersiniabactin, salmochelin, or enterobactin, enables the growth/survival of hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* *ex vivo* and *in vivo*. *Infect. Immun.* 83, 3325–3333. doi: 10.1128/IAI.00430-15
- Ryu, S., Klein, E. Y., and Chun, B. C. (2018). Temporal association between antibiotic use and resistance in *Klebsiella pneumoniae* at a tertiary care hospital. *Antimicrob. Resist. Infect. Control* 7:83. doi: 10.1186/s13756-018-0373-6
- Sarma, J. B., Marshall, B., Cleeve, V., Tate, D., Oswald, T., and Woolfrey, S. (2015). Effects of fluoroquinolone restriction (from 2007 to 2012) on *Clostridium difficile* infections: interrupted time-series analysis. *J. Hosp. Infect.* 91, 74–80. doi: 10.1016/j.jhin.2015.05.013
- Schmitz, J., van der Linden, M., Al-Lahham, A., Levina, N., Pletz, M. W., and Imöhl, M. (2017). Fluoroquinolone resistance in *Streptococcus pneumoniae* isolates in Germany from 2004–2005 to 2014–2015. *Int. J. Med. Microbiol.* 307, 216–222. doi: 10.1016/j.ijmm.2017.04.003
- Seugendo, M., Janssen, I., Lang, V., Hasibuan, I., Bohne, W., Cooper, P., et al. (2018). Prevalence and strain characterization of *Clostridioides (Clostridium) difficile* in representative regions of Germany, Ghana, Tanzania and Indonesia - A comparative multi-center cross-sectional study. *Front. Microbiol.* 9:1843. doi: 10.3389/fmicb.2018.01843
- Shaik, S., Ranjan, A., Tiwari, S. K., Hussain, A., Nandanwar, N., and Kumar, N. (2017). Comparative genomic analysis of globally dominant ST131 clone with other epidemiologically successful extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. *mBio* 8:e1596-17. doi: 10.1128/mBio.01596-17
- Shang, W., Hu, Q., Yuan, W., Cheng, H., Yang, J., Hu, Z., et al. (2016). Comparative fitness and determinants for the characteristic drug resistance of ST239-MRSA-III-t030 and ST239-MRSA-III-t037 strains isolated in China. *Microb. Drug Resist.* 22, 185–192. doi: 10.1089/mdr.2015.0226
- Sharp, C., Boinett, C., Cain, A., Housden, N. G., Kumar, S., Turner, K., et al. (2019). O-antigen-dependent colicin insensitivity of uropathogenic *Escherichia coli*. *J. Bacteriol.* 201:e545-18. doi: 10.1128/JB.00545-18
- Shaw, H. A., Preston, M. D., Vendrik, K. E. W., Cairns, M. D., Browne, H. P., Stabler, R. A., et al. (2019). The recent emergence of a highly related virulent *Clostridium difficile* clade with unique characteristics. *Clin. Microbiol. Infect.* [Epub ahead of print].
- Shen, D., Ma, G., Li, C., Jia, X., Qin, C., Yang, T., et al. (2019). Emergence of a multidrug-resistant hypervirulent *Klebsiella pneumoniae* sequence type 23 strain with a rare bla (CTX-M-24)- harboring virulence plasmid. *Antimicrob. Agents Chemother.* 63:e2273-18. doi: 10.1128/AAC.02273-18
- Shi, Q., Lan, P., Huang, D., Hua, X., Jiang, Y., Zhou, J., et al. (2018). Diversity of virulence level phenotype of hypervirulent *Klebsiella pneumoniae* from different sequence type lineage. *BMC Microbiol.* 18:94. doi: 10.1186/s12866-018-1236-2
- Shu, L. B., Lu, Q., Sun, R. H., Lin, L. Q., Sun, Q. L., Hu, J., et al. (2019). Prevalence and phenotypic characterization of carbapenem-resistant *Klebsiella pneumoniae* strains recovered from sputum and fecal samples of ICU patients in Zhejiang Province, China. *Infect. Drug Resist.* 12, 11–18. doi: 10.2147/IDR.S175823
- Spigaglia, P., Carattoli, A., Barbanti, F., and Mastrantonio, P. (2010). Detection of *gyrA* and *gyrB* mutations in *Clostridium difficile* isolates by real-time PCR. *Mol. Cell. Probes* 24, 61–67. doi: 10.1016/j.mcp.2009.10.002
- Stabler, R. A., He, M., Dawson, L., Martin, M., Valiente, E., Corton, C., et al. (2009). Comparative genome and phenotypic analysis of *Clostridium difficile* 027 strains provides insight into the evolution of a hypervirulent bacterium. *Genome Biol.* 10:R102. doi: 10.1186/gb-2009-10-9-r102
- Strauß, L., Stegger, M., Akpaka, P. E., Alabi, A., Breurec, S., Coombs, G., et al. (2017). Origin, evolution, and global transmission of community-acquired *Staphylococcus aureus* ST8. *Proc. Natl. Acad. Sci. U.S.A.* 114, E10596–E10604. doi: 10.1073/pnas.1702472114
- Sturm, E., Tai, A., Lin, B., Kwong, J., Athan, E., Howden, B. P., et al. (2018). Bilateral osteomyelitis and liver abscess caused by hypervirulent *Klebsiella pneumoniae*-a rare clinical manifestation (case report). *BMC Infect. Dis.* 18:380. doi: 10.1186/s12879-018-3277-4
- Sun, X., Lin, Z. W., Hu, X. X., Yao, W. M., Bai, B., Wang, H. Y., et al. (2018). Biofilm formation in erythromycin-resistant *Staphylococcus aureus* and the relationship with antimicrobial susceptibility and molecular characteristics. *Microb. Pathog.* 124, 47–53. doi: 10.1016/j.micpath.2018.08.021
- Takano, T., Higuchi, W., Zaraket, H., Otsuka, T., Baranovich, T., Enany, S., et al. (2008). Novel characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* strains belonging to multilocus sequence type 59 in Taiwan. *Antimicrob. Agents Chemother.* 52, 837–845. doi: 10.1128/aac.01001-07
- Teunis, P. F. M., Evers, E. G., Hengeveld, P. D., Dierikx, C. M., Wielders, C. C. H., and van Duikeren, E. (2018). Time to acquire and lose carriage of ESBL/pAmpC producing *E. coli* in humans in the Netherlands. *PLoS One* 13:e0193834. doi: 10.1371/journal.pone.0193834
- Toth, A., Kocsis, B., Damjanova, I., Kristóf, K., Jánvári, L., Pászti, J., et al. (2014). Fitness cost associated with resistance to fluoroquinolones is diverse across clones of *Klebsiella pneumoniae* and may select for CTX-M-15 type extended-spectrum β -lactamase. *Eur. J. Clin. Microbiol. Infect. Dis.* 33, 837–843. doi: 10.1007/s10096-013-2022-6
- Turton, J. F., Payne, Z., Coward, A., Hopkins, K. L., Turton, J. A., Doumith, M., et al. (2018). Virulence genes in isolates of *Klebsiella pneumoniae* from the UK during 2016, including among carbapenemase gene-positive hypervirulent K1-ST23 and 'non-hypervirulent' types ST147, ST15 and ST383. *J. Med. Microbiol.* 67, 118–128. doi: 10.1099/jmm.0.000653
- Udo, E. E., Boswih, S. S., and Al-Sweih, N. (2016). High prevalence of toxic shock syndrome toxin-producing epidemic methicillin-resistant *Staphylococcus aureus* 15 (EMRSA-15) strains in Kuwait hospitals. *New Microbes New Infect.* 12, 24–30. doi: 10.1016/j.nmni.2016.03.008
- Valenza, G., Werner, M., Eisenberger, D., Nickel, S., Lehner-Reind, L. V., Höller, C., et al. (2019). First report of the new emerging global clone ST1193 among clinical isolates of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* from Germany. *J. Glob. Antimicrob. Resist.* 17, 305–308. doi: 10.1016/j.jgar.2019.01.014
- Valiente, E., Cairns, M. D., and Wren, B. W. (2014). The *Clostridium difficile* PCR ribotype 027 lineage: a pathogen on the move. *Clin. Microbiol. Infect.* 20, 396–404. doi: 10.1111/1469-0691.12619
- Vandenesch, F., Naimi, T., Enright, M. C., Lina, G., Nimmo, G. R., Heffernan, H., et al. (2003). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg. Infect. Dis.* 9, 978–984.
- Vardakas, K. Z., Triggidis, K. K., Boukouvala, E., and Falagas, M. E. (2016). *Clostridium difficile* infection following systemic antibiotic administration in randomised controlled trials: a systematic review and meta-analysis. *Int. J. Antimicrob. Agents* 48, 1–10. doi: 10.1016/j.ijantimicag.2016.03.008
- Velazquez-Meza, M. E., Aires, de Sousa, M., Echaniz-Aviles, G., Solórzano-Santos, F., Miranda-Novales, G., et al. (2004). Surveillance of methicillin-resistant *Staphylococcus aureus* in a pediatric hospital in Mexico City during a 7-year period (1997 to 2003): clonal evolution and impact of infection control. *J. Clin. Microbiol.* 42, 6877–6880. doi: 10.1128/JCM.42.8.3877-3880.2004
- Vernon, J. J., Wilcox, M. H., and Freeman, J. (2019). Effect of fluoroquinolone resistance mutation Thr-82→Ile on *Clostridioides difficile* fitness. *J. Antimicrob. Chemother.* 74, 877–884. doi: 10.1093/jac/dky535
- Vidovic, S., An, R., and Rendahl, A. (2019). Molecular and physiological characterization of fluoroquinolone-highly resistant *Salmonella enteritidis* strains. *Front. Microbiol.* 10:729. doi: 10.3389/fmicb.2019.00729
- Vinué, L., Jové, T., Torres, C., and Ploy, M. C. (2011). Diversity of class 1 integron gene cassette Pc promoter variants in clinical *Escherichia coli* strains and description of a new P2 promoter variant. *Int. J. Antimicrob. Agents* 38, 526–529. doi: 10.1016/j.ijantimicag.2011.07.007
- Vohra, P., and Poxton, I. R. (2011). Comparison of toxin and spore production in clinically relevant strains of *Clostridium difficile*. *Microbiology* 157, 1343–1353. doi: 10.1099/mic.0.046243-0
- Wan, T. W., Khokhlova, O. E., Iwao, Y., Higuchi, W., Hung, W. C., Reva, I. V., et al. (2016). Complete circular genome sequence of successful ST8/SCCmecIV community-associated methicillin-resistant *Staphylococcus aureus* (OC8) in Russia: one-megabase genomic inversion, IS256's spread, and evolution of Russia ST8-IV. *PLoS One* 11:e0164168. doi: 10.1371/journal.pone.0164168
- Wang, B., Peng, W., Zhang, P., and Su, J. (2018). The characteristics of *Clostridium difficile* ST81, a new PCR ribotype of toxin A- B+ strain with

- high-level fluoroquinolones resistance and higher sporulation ability than ST37/PCR ribotype 017. *FEMS Microbiol. Lett.* 365:fny168. doi: 10.1093/femsle/fny168
- Wasels, F., Kuehne, S. A., Cartman, S. T., Spigaglia, P., Barbanti, F., Minton, N. P., et al. (2015). Fluoroquinolone resistance does not impose a cost on the fitness of *Clostridium difficile* in vitro. *Antimicrob. Agents Chemother.* 59, 1794–1796. doi: 10.1128/AAC.04503-14
- Wei, Q., Jiang, X., Li, M., Li, G., Hu, Q., Lu, H., et al. (2013). Diversity of gene cassette promoter variants of class 1 integrons in uropathogenic *Escherichia coli*. *Curr. Microbiol.* 67, 543–549. doi: 10.1007/s00284-013-0399-1
- Wijaya, L., Hsu, L. Y., and Kurup, A. (2006). Community-associated methicillin-resistant *Staphylococcus aureus*: overview and local situation. *Ann. Acad. Med. Singapore* 35, 479–486.
- Wilcox, M. H., Shetty, N., Fawley, W. N., Shemko, M., Coen, P., Birtles, A., et al. (2012). Changing epidemiology of *Clostridium difficile* infection following the introduction of a national ribotyping-based surveillance scheme in England. *Clin. Infect. Dis.* 55, 1056–1063. doi: 10.1093/cid/cis614
- Willems, R. J., Top, J., van Santen, M., Robinson, D. A., Coque, T. M., Baquero, F., et al. (2005). Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *Emerg. Infect. Dis.* 11, 821–828. doi: 10.3201/1106.041204
- Wiuff, C., Brown, D. J., Mather, H., Banks, A. L., Eastaway, A., and Coia, J. E. (2011). The epidemiology of *Clostridium difficile* in Scotland. *J. Infect.* 62, 271–279. doi: 10.1016/j.jinf.2011.01.015
- Wu, J., Lan, F., Lu, Y., He, Q., and Li, B. (2017). Molecular characteristics of ST1193 Clone among phylogenetic group B2 non-ST131 fluoroquinolone-resistant *Escherichia coli*. *Front. Microbiol.* 8:2294. doi: 10.3389/fmicb.2017.02294
- Wyres, K. L., Hawkey, J., Hetland, M. A. K., Fosterovold, A., Wick, R. R., Judd, L. M., et al. (2019). Emergence and rapid global dissemination of CTX-M-15-associated *Klebsiella pneumoniae* strain ST307. *J. Antimicrob. Chemother.* 74, 577–581. doi: 10.1093/jac/dky492
- Xiao, L., Wang, X., Kong, N., Cao, M., Zhang, L., Wei, Q., et al. (2019). Polymorphisms of gene cassette promoters of the class 1 Integron in clinical *Proteus* isolates. *Front. Microbiol.* 10:790. doi: 10.3389/fmicb.2019.00790
- Xu, X., Li, X., Luo, M., Liu, P., Su, K., Qing, Y., et al. (2017). Molecular characterisations of integrons in clinical isolates of *Klebsiella pneumoniae* in a Chinese tertiary hospital. *Microb. Pathog.* 104, 164–170. doi: 10.1016/j.micpath.2017.01.035
- Yamaji, R., Rubin, J., Thys, E., Friedman, C. R., and Riley, L. W. (2018). Persistent pandemic lineages of uropathogenic *Escherichia coli* in a college community from 1999 to 2017. *J. Clin. Microbiol.* 56:e1834-17. doi: 10.1128/JCM.01834-17
- Yan, J. J., Zheng, P. X., Wang, M. C., Tsai, S. H., Wang, L. R., and Wu, J. J. (2015). Allocation of *Klebsiella pneumoniae* bloodstream isolates into four distinct groups by ompK36 typing in a Taiwanese University Hospital. *J. Clin. Microbiol.* 53, 3256–3263. doi: 10.1128/JCM.01152-15
- Yan, Q., Zhou, M., Zou, M., and Liu, W. E. (2016). Hypervirulent *Klebsiella pneumoniae* induced ventilator-associated pneumonia in mechanically ventilated patients in China. *Eur. J. Clin. Microbiol. Infect. Dis.* 35, 387–396. doi: 10.1007/s10096-015-2551-2
- Yang, X., Dong, F., Qian, S., Wang, L., Liu, Y., Yao, K., et al. (2019). Accessory gene regulator (agr) dysfunction was unusual in *Staphylococcus aureus* isolated from Chinese children. *BMC Microbiol.* 19:95. doi: 10.1186/s12866-019-1465-z
- Yang, X., Qian, S., Yao, K., Wang, L., Liu, Y., Dong, F., et al. (2017). Multiresistant ST59-SCCmec IV-t437 clone with strong biofilm-forming capacity was identified predominantly in MRSA isolated from Chinese children. *BMC Infect. Dis.* 17:733. doi: 10.1186/s12879-017-28337
- Zaki, A. (2019). Molecular study of *Klebsiella pneumoniae* virulence genes from patients with hospital acquired sepsis. *Clin. Lab.* 65:138. doi: 10.7754/Clin.Lab.2018.180709
- Zarfel, G., Luxner, J., Folli, B., Leitner, E., Feiler, G., Kittinger, C., et al. (2016). Increase of genetic diversity and clonal replacement of epidemic methicillin-resistant *Staphylococcus aureus* strains in South-East Austria. *FEMS Microbiol. Lett.* 363:fnw137. doi: 10.1093/femsle/fnw137
- Zarrilli, R., Pournaras, S., Giannouli, M., and Tsakris, A. (2013). Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. *Int. J. Antimicrob. Agents* 41, 11–19. doi: 10.1016/j.ijantimicag.2012.09.008
- Zhan, L., Wang, S., Guo, Y., Jin, Y., Duan, J., Hao, Z., et al. (2017). Outbreak by hypermucoviscous *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in a tertiary hospital in China. *Front. Cell Infect. Microbiol.* 7:182. doi: 10.3389/fcimb.2017.00182
- Zhang, H., Zhou, Y., Guo, S., and Chang, W. (2015). High prevalence and risk factors of fecal carriage of CTX-M type extended-spectrum beta-lactamase-producing *Enterobacteriaceae* from healthy rural residents of Taian, China. *Front. Microbiol.* 6:239. doi: 10.3389/fmicb.2015.00239
- Zhao, J., Chen, J., Zhao, M., Qiu, X., Chen, X., Zhang, W., et al. (2016). Multilocus sSequence types and virulence determinants of hypermucoviscosity-positive *Klebsiella pneumoniae* isolated from community-acquired infection cases in Harbin, North China. *Jpn. J. Infect. Dis.* 69, 357–360. doi: 10.7883/yoken.JJID.2015.321
- Zhao, L., Zhang, J., Zheng, B., Wei, Z., Shen, P., Li, S., et al. (2015). Molecular epidemiology and genetic diversity of fluoroquinolone-resistant *Escherichia coli* isolates from patients with community-onset infections in 30 Chinese county hospitals. *J. Clin. Microbiol.* 53, 766–770. doi: 10.1128/JCM.02594-14
- Zheng, J. X., Lin, Z. W., Chen, C., Chen, Z., Lin, F. J., Wu, Y., et al. (2018). Biofilm formation in *Klebsiella pneumoniae* bacteremia strains was found to be associated with CC23 and the presence of wcaG. *Front. Cell. Infect. Microbiol.* 8:21. doi: 10.3389/fcimb.2018.00021

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

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