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REVIEW ARTICLE

Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae: Update on Molecular Epidemiology and Treatment Options

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Abstract

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are a major global public health concern. Presently, *Escherichia coli* with CTX-Ms are the most common species associated with global ESBLs; CTX-M-15 is the most frequent CTX-M worldwide and is followed by CTX-M-14, which is often found in South-East Asia. Recent surveillance studies showed that CTX-M-27 is emerging in certain parts of the world especially in Japan and Europe. The population structure of ESBL-producing *E. coli* is dominated globally by an high-risk clone named ST131. *Escherichia coli* ST131 belongs to three clades (A, B, and C) and three different subclades (C1, C1-M27, and C2). Clade C1-M27 is associated with $bla_{\text{CTX-M-27}}$, and C2 with $bla_{\text{CTX-M-15}}$. Recent whole genome sequencing studies have shown that clade C has evolved from clade B in a stepwise fashion, resulting in one of the most influential global antimicrobial resistance clones that has emerged during the 2000's. Other important *E. coli* clones that have been detected among ESBL producers include ST405, ST38, ST648, ST410, and ST1193. The INCREMENT project has shown that ertapenem is as effective as other carbapenems for treating serious infections due to ESBL-producing Enterobacteriaceae. The results of the MERINO open-label randomized controlled study has provided clear evidence that piperacillin-tazobactam should be avoided for targeted therapy of blood-stream infections due to ESBL-producing *E. coli* and *K. pneumoniae,* regardless of the patient population, source of infection, bacterial species, and susceptibility result of piperacillin-tazobactam. Research is still warranted to define the optimal therapy of less severe infections due to ESBL-producing Enterobactericeae.

Key Points

Extended-spectrum β-lactamase-producing bacteria are a global antimicrobial-resistant public health concern.

The population structure is dominated by a successful clone named *Escherichia coli* ST131 clade C.

The antibiotic piperacillin-tazobactam should be avoided for treating blood-stream infections due to extended-spectrum β-lactamase-producing *E. coli* and *Klebsiella pneumoniae.*

1 Introduction

The global spread of antimicrobial-resistant organisms (AROs) was recently identified by the World Health Organization, the European Union, the US Government, and the Centers for Disease Control and Prevention (USA) as one of the most significant threats to human health [1]. The spread of AROs is troublesome for medical practitioners because infections caused by such bacteria are often responsible for increased patient mortality and morbidity owing to the delayed administration of suitable antibiotics [2, 3]. Routine medical practices such as chemotherapy for treating patients

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with cancer and extensive surgeries will become obsolete in the future if AROs continue to increase.

β-Lactam antibiotics such as penicillins, cephalosporins, monobactams, and carbapenems are among the most frequently prescribed antibiotics worldwide. These agents bind to and inhibit bacterial enzymes (referred to as penicillin-binding proteins or PBPs) responsible for cell wall synthesis [4]. β-Lactamases are bacterial enzymes that inactivate β-lactam antibiotics by hydrolysis, which results in ineffective compounds [5]. The first enzyme with the ability to hydrolyse penicillin was described nearly 70 years ago in *Escherichia coli* (known then as *Bacillus coli*) and again a few years later from *Staphylococcus aureus* [6].

In Gram-negative bacteria, β-lactamase production remains the most important contributing factor to β-lactam resistance, and their increasing frequency, as well as their continuous evolution, are directly linked to selection by the use of different β-lactam agents [7]. β-Lactamases differ from each another in their substrate profiles (i.e., the different types of β-lactam antibiotics they inactivate), inhibitor profile (i.e., which compounds inactivate them), and sequence homology (i.e., amino acid composition of these enzymes) [5]. Using these different characteristics, two classification systems have been created to divide β-lactamases into Ambler classes (i.e., classes A, B, C, and D, based on amino acid sequence homology), and the Bush-Jacoby-Medeiros groups 1, 2, and 3, based on substrate and inhibitor profiles [8, 9]. The Ambler classification system is more commonly used in the published literature.

The extended-spectrum β-lactamases (ESBLs) are a group of enzymes that cause resistance to the oxyiminocephalosporins (i.e., cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cefepime) and the monobactams (i.e., aztreonam), but not the cephamycins (i.e., cefoxitin, cefotetan) or the carbapenems (i.e., imipenem, meropenem, doripenem, ertapenem) [10]. These enzymes are inhibited by "classical" β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. The majority of ESBLs belong to Ambler class A and include the SHV or TEM types (that have evolved from parent enzymes such as TEM-1, TEM-2, and SHV-1) and CTX-M types (that originate from the chromosomes of *Kluyvera* spp.). During the 1990 s, *Klebsiella pneumoniae* with TEM and SHV ESBL types predominantly caused global nosocomial outbreaks, but since 2000, *E. coli*-producing CTX-M enzymes have emerged as important causes of community-onset infections, especially with the urinary tract as a source [11]. The prevalence of CTX-Ms increased rapidly during the mid-late 2000s, and are currently the most common global ESBL among Enterobacteriaceae (especially prevalent in *E. coli*).

Extended-spectrum β-lactamase-producing Enterobacteriaceae (especially among *E. coli* and *K. pneumoniae*) are important human AROs, and common causes of urinary tract infections and bloodstream infections in both developed and developing countries [12]. Global surveillance studies have shown alarmingly high rates of more than 50% of ESBL-producing *E. coli* and *Klebsiella* spp. in certain areas of Asia, Africa, and Latin America [13]. These bacteria present huge challenges to healthcare, owing to the restricted empiric options available for treating infections with ESBL-producing Enterobacteriaceae. This scenario has led to the subsequent increased use of carbapenems, leading to the emergence and global spread of carbapenemase-producing bacteria, especially among *K. pneumoniae* [14, 15].

Presently, CTX-M-β-lactamases include more than 220 different enzymes clustered into five subfamilies based on their amino acid identities that include the CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 subfamilies [16]. Enzymes that originated from subfamilies CTX-M-1 and CTX-M-9 are widely distributed and commonly reported. As a general rule, CTX-M enzymes are more active against cefotaxime and ceftriaxone than against ceftazidime, but point mutations around the active site of some enzymes, especially those belonging to the CTX-M-1 subfamily (i.e., CTX-M-15) and CTX-M-9 subfamily (i.e., CTX-M-27) have increased their ability to hydrolyze ceftazidime significantly [17].

Currently, the most widely distributed global CTX-M enzymes are CTX-M-15 (forming part of the CTX-M-1 subfamily that was first reported in *E. coli* from India in 2001 [18]) and CTX-M-14 (forming part of the CTX-M-9 subfamily first described during 2001 in *E. coli, K. pneumoniae*, and *Shigella* spp. obtained from Korea) [19]. The prevalence of $bla_{\text{CTX-M-15}}$ has increased significantly during the mid-late 2000s and early 2010s, and is currently the most frequent ESBL described in most regions worldwide. In South-East Asia, especially among countries such as China, South Korea, and Japan, CTX-M-14-producing *E. coli* is the most common ESBL while certain countries in South America (i.e., Argentina), Enterobacteriaceae with $bla_{\text{CTX-M-2}}$, are typically reported [20].

The global distribution of different CTX-M enzymes was recently reviewed in detail by Hawkey and colleagues [20]. The aim of this article is to describe the molecular epidemiology (i.e., the roles of mobile genetic elements and high-risk clones) of Enterobacteriaceae with CTX-M β-lactamases (using *E. coli* as the example) and to update readers regarding recent published studies on treatment options for serious infections due to these bacteria [21].

2 Molecular Epidemiology of Enterobacteriaecae That Produce Extended-Spectrum β-Lactamases

2.1 Introduction: Overview on High-Risk Clones and Mobile Genetic Elements

Molecular epidemiology is a discipline that uses molecular or genetic markers to trace the development of a disease in a population and to understand transmission, as well as the population structure and evolution of bacterial pathogens [22]. Whole genome sequencing has become the key technology for understanding antimicrobial resistance (AMR) pathogen evolution, population dynamics, and genomic epidemiology, as it provides a far greater degree of resolution than previous genotyping methods [23]. Whole genome sequencing has shown that most AMR infections are attributable to the selection and spread of certain clones (referred to as high-risk clones) within the broad microbial population [23]. The movement of AMR genes is also due to mobile genetic elements (MGEs), which act as vehicles for horizontal dissemination. Hence, the major burden of AMR is associated with the global spread of high-risk clones and/or the movement of AMR genes between low-risk/diverse clones, mediated via MGEs (Fig. 1) [24].

'Clone', within the field of microbiology, implies that bacterial isolates share similar traits (as characterized by biochemical or molecular methodologies), indicating that they belong to the same cluster or lineage and have a common ancestor. Baquero et al. in 2013 defined global multi-drug-resistant (MDR) "high-risk or eminent" clones as having the following characteristics [25]: high-risk clones must show international distribution, possess several types of AMR determinants, be able to successfully colonize hosts for prolonged periods of time, be transmitted efficiently among hosts, show extended pathogenicity and fitness, and be able to cause severe and/or recurrent infections [24]. High-risk clones most likely possess some types of biological factors that lead to increased "fitness", providing these strains with a Darwinian edge over other isolates of the same species. Such advantages will provide them with the abilities to outcompete other bacteria and become the principal part of the bacterial populace in that area. This will provide these clones with increased opportunities to spread as well as time to acquire antimicrobial drug resistance determinants from other bacteria. This brings up an intriguing issue. Are high-risk clones inherently more fit and therefore better able to survive in certain environments? Is this "fitness" owing to certain virulence factors that provided them with opportunities to be exposed to and acquire certain plasmids? Is it perhaps

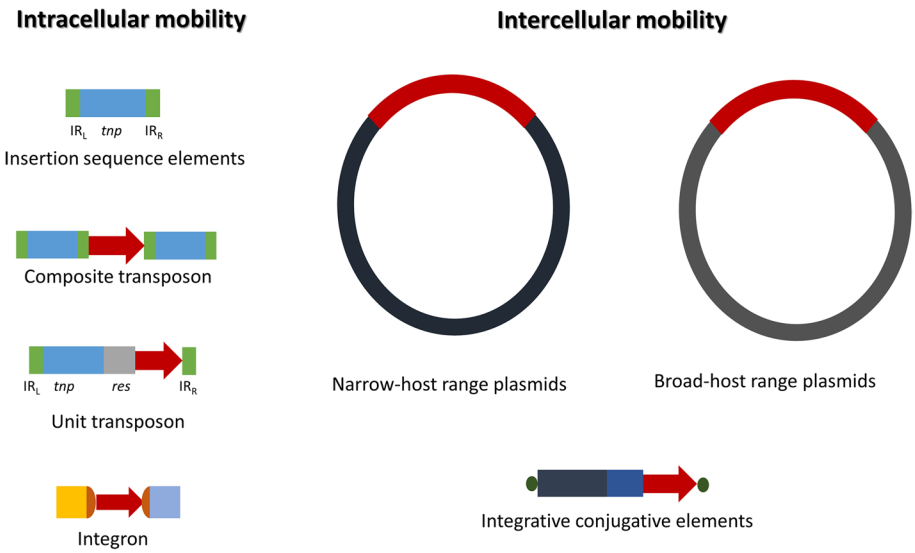

Fig. 1 Mobile genetic element responsible for intracellular and intercellular mobility. Insertion sequence elements, transposons, and integrons are responsible for the intracellular movement of antimicrobial resistance genes. Plasmids and integrative conjugative elements are responsible for the intercellular movement of antimicrobial resistance genes. When a pair of closely related insertion sequence elements insert upstream and downstream of an antimicrobial resistance gene it is then referred to as a composite transposon. Unit transposons are flanked by short inverted repeats rather than longer repeats. Plasmids are extra-chromosomal circular DNA that replicate independently of the host genome and can be divided into narrow and broad-host range types. Insertion sequences are flanked by inverted repeats (IRs) that are short identical sequences in opposite orientation. IR_L inverted repeat left, IR_R inverted repeat right, *res* gene encoding for resolvase, *tnp* gene encoding for transposase

possible that the mutations accommodating plasmids increased the fitness of high-risk clones, enabling them, with the aid of specific virulence factors and AMR determinants to outcompete other clones?

The spread of MDR high-risk clones is especially facilitated by the selective pressures of antimicrobial drugs present in healthcare settings and used during food animal husbandry. Such bacterial clones provide formidable platforms for the dissemination of antimicrobial-resistant genetic components. High-risk AMR clones are able to provide solid platforms for the preservation and multiplication of AMR genes and have been instrumental in the recent global emergence of AROs among several bacterial species. Two prominent, human AMR high-risk clones include *E. coli* ST131 with $bla_{\text{CTX-Ms}}$ (described in detail below) and *K. pneumoniae* ST258 with bla_{KPCs} [24].

Mobile genetic elements are segments of DNA that encode enzymes that mediate the movement of other DNA pieces [26]. Mobile genetic elements can be divided into two major groups: (1) some MGEs have the ability to move DNA segments within the same bacterial genome (i.e., intracellular mobility) and include insertion sequences, transposons, integrons cassettes, and prophages and (2) plasmids and integrative conjugative elements can transfer genes between different bacteria (i.e., intercellular mobility) (Fig. 1).

Mobile genetic elements, through the process of first capture, followed by transposition and recombination, have mobilized AMR genes from bacterial chromosomes (especially from environmental bacteria), onto plasmids, providing them with opportunities to be transferred to other bacteria. It seems that the capture of AMR genes by MGEs are relatively infrequent events and antibiotic selection pressure is vital for the successful accumulation, maintenance, and dissemination of AMR genes in bacterial populations [26].

Insertion sequences or insertion sequence elements are the simplest and smallest type of MGE. They typically contain a single open reading frame that encodes for genes (e.g., *tnp*A encoding for transposase) to facilitate transposition [27]. Insertion sequences are flanked by inverted repeats that are short identical sequences in opposite orientation. The transposase protein recognises the inverted repeat and then moves the insertion sequence to a new location by either a 'cut and paste' or 'copy and paste' mechanism. Insertion sequences have the ability to capture and move AMR genes. When a pair of closely related insertion sequence elements insert upstream and downstream of an AMR gene (either in direct or inverse orientation), it is then referred to as a composite transposon (Fig. 1). A composite transposon can initiate transposition that will include the movement of the region (i.e., including the AMR gene) situated between the insertion sequence elements. A single copy of certain types of insertion sequence elements (such as IS*Ecp1*) are able to capture, mobilise, and express a nearby AMR resistance gene [28].

A unit transposon is usually larger than a composite transposon. The main difference is that unit transposons are flanked by short inverted repeats rather than longer repeats (as seen with composite transposons) (Fig. 1). Unit transposons are responsible for the movement of AMR genes that often belong to the Tn*3* or Tn*21* subfamilies. They contain *tnpA* (transposase), *tnpR* (resolvase) genes, and related resolution (res) sites.

Gene cassettes, consisting of a gene and a recombination site (*att*C), are captured by integrons through the recombination between *att*C and *att*I situated on the integron [26]. This process is initiated by an integrase encoded by the integron. The integron also provides a promoter for expression of the captured cassette genes. Integrons are divided into different classes (I–IV) with class 1 integrons being the most common among AMR clinical isolates.

Plasmids are extra-chromosomal circular DNA that replicate independently of the host genome. The horizontal transfer of MGEs, such as plasmids with AMR genes, play an important role in the dissipation of AMR [29]. Antimicrobial resistance plasmids can broadly be divided into two main groups based on their ability to move between bacteria (Fig. 1). The narrow-host range group most often belongs to the incompatibility (Inc) group F, and the broad-host range group belong to the IncA/C, IncL/M, and IncN. Broad-host range plasmids can easily be transferred between different species while narrow-host range plasmids tend to be restricted to certain species or clones within species [24]. Plasmids often contain various combinations of virulence, fitness, addiction systems, and AMR genes and have contributed significantly to the success of antimicrobial-resistant bacteria [29].

2.2 Roles of Mobile Genetic Elements in the Spread of Extended-Spectrum β-Lactamases

2.2.1 Intracellular Mobility

Insertion elements, transposons, and integrons have played a key role in the capture and dispersal of CTX-M-encoding genes. The insertion element, IS*Ecp1,* is situated upstream of the CTX-M-encoding genes and is responsible for the movement of all types of $bla_{\text{CTX-M}}$ genotypes [30]. IS*Ecp1* acts in an original way, by mobilizing adjacent sequences by using the one-ended transposition mechanism that contributed significantly to the spread of CTX-M-encoding genes [28, 31, 32]. This insertion element encodes for a transposase that was responsible for initial capture and mobilization of KLUA-like genes from the chromosomes of *Kluyvera* spp. onto plasmids [28, 31, 32] (Fig. 2). It is more than likely that

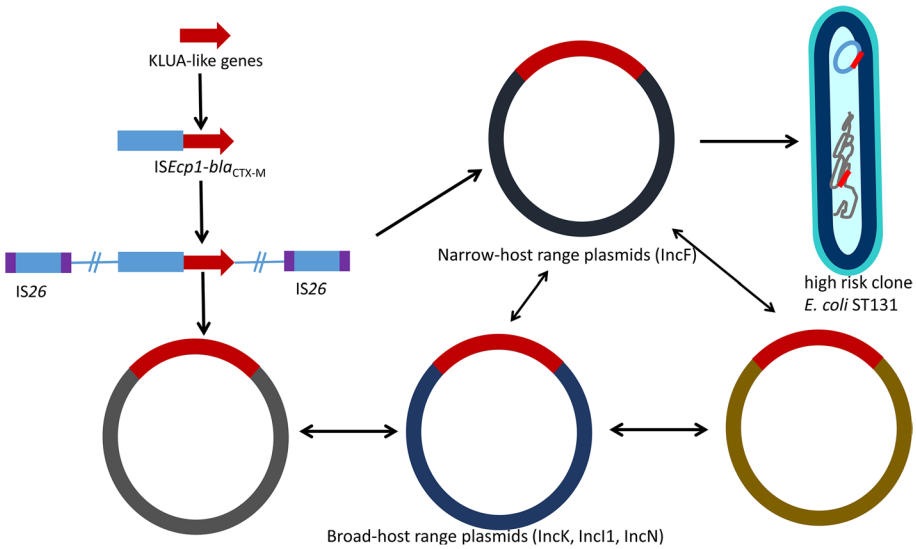

Fig. 2 Mobile genetic elements responsible for the capture and mobilization of CTX-M genes. IS*Ecp1* is situated upstream of the CTX-M genes and is responsible for the initial capture and mobilization of KLUA-like genes from the chromosomes of *Kluyvera* spp. onto narrow [i.e., incompatibility groups with F replicons (IncF)] and broad-host range (i.e., IncK, IncI1, and IncN) plasmids. These plasmids then found their way into the *Escherichia coli* population. IncF plasmids used post-segregation killing and addiction systems to ensure their propagation among high-risk clones such as ST131

IS*26* also played an important role in this process. The plasmids that contained IS*Ecp1*, IS*26*, and $bla_{\text{CTX-M}}$ then found their way into the *E. coli* population (Fig. 2). IS*Ecp1* also served as a strong promoter for the expression of $bla_{\text{CTX-Ms}}$, and with the aid of IS*26*, class 1 integrons and IS*CR1* have mobilized CTX-M-encoding genes onto different types of narrow and broad-host range plasmids [33].

The insertion element IS*26* has also been pivotal in the dissemination of CTX-M-encoding genes: IS*Ecp1*-$bla_{\text{CTX-M-9-like}}$-IS*903* and IS*Ecp1*-$bla_{\text{CTX-M-1-like}}$-*orf477* are often flanked by copies of IS*26* and have played important roles in the distribution of CTX-M-9 (especially CTX-M-14 and CTX-M-27) and CTX-M-1 (especially CTX-M-3 and CTX-M-15) subfamilies, respectively [30]. IS*26* most likely captured $bla_{\text{CTX-M-15}}$ during the 2000s, which has allowed the stable maintenance of $bla_{\text{CTX-M}}$ among *E. coli* populations thereafter [20]. It seems that IS*26*, which is present in high copy numbers across *E. coli* populations, was involved in mediating transposition of $bla_{\text{CTX-M-15}}$ onto a common plasmid backbone (see below for more details on plasmids associated with CTX-M-15). The IS*Ecp1*-$bla_{\text{CTX-M}}$ complex is often inserted in IS*CR1* complexes that are adjacent to class 1 integrons, and these MGEs have also played important roles in the distribution of some enzymes that belong to the CTX-M-9 subfamily (especially CTX-M-9), the CTX-M-2 subfamily, and the CTX-M-25 subfamily [30].

2.2.2 Intercellular Mobility

Molecular epidemiological studies have shown that $bla_{\text{CTX-M}}$ are often harbored on epidemic resistance plasmids that belong to narrow host range incompatibility groups with F replicons (named IncF) while other broad-host range plasmids such as IncI, IncN, IncHI2, IncL/M, and IncK played less important roles in the distribution of CTX-M-encoding genes [24, 30]: IncF plasmids have recently been termed "epidemic resistance plasmids" because of their propensity to acquire resistance genes and then rapidly disseminate among members that belong to the *Enterobacteriaceae* due to a high transfer frequency [24]. The IncF group (with FIA and FII replicons) is mostly associated with $bla_{\text{CTX-M-15}}$, while $bla_{\text{CTX-M-14}}$ are often harbored on broad-host range IncK and IncI1 plasmids (Fig. 2). In addition, the $bla_{\text{CTX-M-1}}$ gene is situated on IncN and IncI1 plasmids, the $bla_{\text{CTX-M-3}}$ gene is situated on IncL/M and IncI1 plasmids, and the $bla_{\text{CTX-M-9}}$ gene is situated on IncHI2 plasmids [30].

IncF plasmids use post-segregation killing and addiction systems to ensure their propagation among high-risk clones such as ST131 [34] (Fig. 2). The $bla_{\text{CTX-M-15}}$ gene has mainly been found on certain IncF plasmids (especially with FIA-FII replicons) in ST131, whereas Inc plasmids with different replicons have been identified in non-ST131 ExPEC [35, 36]. It has been postulated recently that the presence of IncF plasmids harboring $bla_{\text{CTX-M-15}}$ is central to the global success of ST131 and that they have significantly contributed to the evolutionary dominance of subclade C2 (see below for more details on the role of plasmids in the success of ST131 subclades) [24]. There is a clear relationship of *E. coli* ST131 subclade C2 with IncFII_AY458016 containing $bla_{\text{CTX-M-15}}$ and the *pemI*/*pemK* addiction system [37].

2.3 Roles of High-Risk Clones in the Spread of Extended-Spectrum β-Lactamases: *Escherichia coli* ST131 with CTX-Ms

Escherichia coli ST131 is the quintessential example of an high-risk clone and remains one of the most successful global MDR Gram-negative clones among human isolates [24]. This MDR clone was first described in the late 2000s [38]; it typed with serotype O25b:H4, belonged to

phylogenetic group B2, and harbored IncF plasmids containing $bla_{CTXM-15}$. Reports regarding the prevalence of ST131 quickly escalated and currently it is the most common global extra-intestinal pathogenic *E. coli* (ExPEC) clone (e.g., up to 30% of all ExPEC, 60–90% of fluoroquinolone resistant [FQ-R] ExPEC, and 40–80% of ESBL ExPEC belong to ST131) [39]. A molecular epidemiology study from Canada, spanning over an 11-year period (2000–10), showed that ST131 was relatively rare among ESBL-producing ExPEC during the early 2000s, but then exploded in numbers towards the latter part of the 2000s, and by 2010, eight out of every ten ESBL-producing *E. coli* causing blood stream infections in the Calgary Region belonged to ST131 [40].

Population genetics indicated that ST131 consists of different clades [39]: clade A is associated mostly with *fimH*41 and type with O16:H5, clade B is mostly associated with *fimH*22 or *fimH35* and type with O25b:H4, while clade C is mostly associated with *fimH*30 and also type with O25b:H4. The change in *fimH* alleles most likely played a role in the colonization abilities of the different clades [41]. Global longitudinal studies showed that clade B was presiding among ST131 before the 1990s, but since the 2000s clade C has become the most dominant lineage (currently up to 80% of global ST131 belongs to clade C) [38]. Next-generation sequencing identified two subclades within clade C named C1/H30R (associated with FQ-R) and C2/H30-Rx (associated with CTX-M-15) [41, 42]. Both subclades showed extensive global distribution. A molecular epidemiology study from Calgary investigated the distribution of different ST131 clades responsible for blood stream infections over time (2000–10), and showed that subclade C1/H30R were common in the early-mid 2000s while C2/H30Rx only became prominent among FQ-R *E. coli* towards the end of the 2000s [43].

The evolution of ST131 has recently been reviewed in detail and is summarized in the following paragraphs [39]. The acquisition of certain key genomic islands with specific virulence factors combined with the development of FQ-R in clade C played a critical role in the successful global dissemination of subclades C1 and C2. Clade C differs from clade B by approximately 70 substitution single-nucleotide polymorphisms [41]. Clade B is most often FQ susceptible and rarely harbors plasmids with bla_{CTX-Ms}, while clade C is nearly always FQ-R (due to *gyrA* [e.g., *gyrA1AB*] and *parC* [e.g., *parC1aAB*] mutations) and the C2 subclade is often associated with $bla_{CTX-M-15}$ [38].

Recent phylogenetic studies showed that clade C evolved from clade B that likely transpired during the late 1980s in North America (either the USA or Canada) [37, 44]. Next-generation sequencing data suggested that a stepwise evolution process occurred during the 1960s in which clade B sequentially acquired several prophages, genomic islands, the *fimH30* allele, and mutations within *gyrA* and *parC* and evolved into clade C [44]. The C clade-defining *fimH30* allele was acquired by recombination, most likely during the early-mid-1980s, possibly in conjunction with the acquisition of the nearby GI-*leuX*. Clade C then divided into subclades C1 and C2 after the acquisition of the high-level FQ-R mutations (via selection) in *parC* (*parC1aAB*) and *gyrA* (*gyrA1AB*) that defined clade C (Fig. 3). This process transpired during the mid-late 1980s and coincided with the introduction of the FQs (especially ciprofloxacin) in clinical medicine. It seems that the acquisition of genomic islands containing virulence-associated genes (e.g., *sat* and *iutA*), AMR genes (i.e., *parC1a*), and *fimH30* in a stepwise process during the 1960s, 1970s, and early 1980s has primed ST131 for success prior to the acquisition of high-level FQ-R mutations in the late 1980s (Fig. 3).

A study from Minnesota, USA characterized plasmids in a collection of 104 diverse (i.e., clinical, environmental,

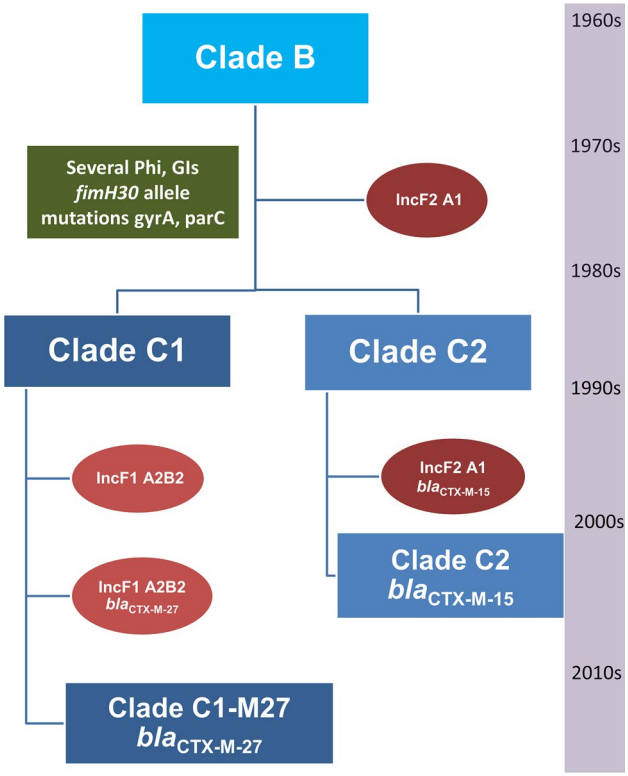

Fig. 3 Sequential evolution of *Escherichia coli* ST131 clade B into subclades C1, C1-M27, and C2. Clade B sequentially acquired several prophages (Phi), genomic islands (GIs), the *fimH30* allele, and mutations within *gyrA* and *parC* to evolve into clade C. The F2:A1 plasmid type (without bla_{CTX-M}) was introduced into clade B. The F2:A1 plasmid was replaced by F1:A2:B2 plasmid (without bla_{CTX-M}) within subclade C1. The F2:A1 plasmid in subclade C2 then acquired antimicrobial resistance cassettes containing $bla_{CTX-M-15}$, *catB4*, bla_{OXA-1}, *aac(6')Ib-cr*, and *tetAR* via IS*26*-mediated events. Some of the F1:A2:B2 plasmids later acquired $bla_{CTX-M-14}$ that in Japan, evolved to $bla_{CTX-M-27}$ in combination with an acquired prophage (i.e., M27PP1) to form the C1-M27 subclade

and veterinary) ST131 isolates [45]. They showed that the IncF variants differ among the C1 and C2 subclades: e.g., F1:A2:B20 replicons (without $bla_{CTX\text{-}Ms}$) are associated with subclade C1, while F2:A1:B replicons (with $bla_{CTX\text{-}M\text{-}15}$) are associated with subclade C2 (the F2:A1:B replicons correlate with IncFII_AY458016 as mentioned before [37]). Different IncF plasmids were introduced over time (during the 1970s and 1980s), thus leading to the current circulating plasmids within the C subclades. The initial step was the introduction of an F2:A1 plasmid type (without $bla_{CTX\text{-}M}$) into clade B. The C clade then evolved from clade B and separated into subclades C1 and C2 (as described above). The F2:A1 plasmid was replaced by F1:A2:B2 plasmid (without $bla_{CTX\text{-}M}$) within subclade C1. The F1:A2:B2 plasmid later acquired $bla_{CTX\text{-}M\text{-}14}$ or $bla_{CTX\text{-}M\text{-}27}$ and some C1 isolates acquired a prophage (i.e., M27PP1) to form the C1-M27 subclade [46] (see below for details on the C1-M27 subclade and Fig. 3). The F2:A1 plasmid in subclade C2 acquired AMR cassettes containing $bla_{CTX\text{-}M\text{-}15}$, *catB4*, $bla_{OXA\text{-}1}$, *aac(6')Ib-cr*, and *tetAR* via IS*26*-mediated events (Fig. 3). It seems that the C1 and C2 subclades have co-adapted with these different plasmids types to harbor them at lower fitness cost to the bacterial cell, and that the plasmids themselves are evolving toward fixation within these clades, playing important roles in the success of their hosts. Toxin–antitoxin systems ensured the plasmids' persistence in the clonal backgrounds in which they are located, preventing promiscuity among different clades/lineages.

Escherichia coli with $bla_{CTX\text{-}M\text{-}15}$ is rare in Japan despite the predominance of ST131 among ESBL-producing isolates [47]. Before 2005, ST131 clade C1 containing $bla_{CTX\text{-}M\text{-}14}$,predominated among Japanese ST131 and subsequently has been replaced by clade C1 with $bla_{CTX\text{-}M\text{-}27}$, which was responsible for a significant increase in ESBL-producing *E. coli* since 2010 in that country [47]. A study from Japan, performed next-generation sequencing on 43 Japanese and ten global ST131 isolates with $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}14}$, and $bla_{CTX\text{-}M\text{-}15}$ to investigate the molecular epidemiology and underlying emergence of *E. coli* with $bla_{CTX\text{-}M\text{-}27}$ in Japan [46]. The investigators identified a discrete ST131:O75:H30 lineage that formed a distinct cluster within the C1 subclade and contained a unique prophage-like region (i.e., M27PP1). This new ST131 C1 subclade was named "C1-M27". Interestingly, subclade C1-M27 was responsible for the recent increase in ESBL-producing ExPEC from Japan and was also present among ST131 obtained from Thailand, Australia, Canada, and the USA, indicating that this subclade is not limited to Japan [46]. The ST131 C1-M27 subclade is currently emerging in Germany [48] and France [49], and 27% of 144 clinical ST131 obtained from different European sites were positive for C1-M27 [50].

2.4 Other *Escherichia coli* Sequence Types Associated with Extended-Spectrum β-Lactamases

A molecular epidemiology study from Canada, spanning an 11-year period (2000–10) characterized ESBL-producing *E. coli* responsible for incident bloodstream-associated infections in a region with a centralized laboratory system [40]. The investigators identified seven sequence types/clones among 91% of isolates that included ST10 clonal complex, ST38, ST131, ST315, ST393, ST405, and ST648. The importance of ST38, ST405, and ST648 among ESBL-producing *E. coli* was also shown in studies from Germany [51] (that investigated a global collection of ST648), Israel [52], USA [53], Japan [54], China [55], Thailand [56], and India [57]. The most common CTX-Ms among these global clones were CTX-M-15 followed by CTX-M-14 that were present on various narrow and broad-host range plasmid replicon types, including IncF, IncI, IncN, IncHI2, IncM, and IncK. Currently, two clones are emerging among ESBL-producing *E. coli*: ST410 with CTX-M-15 [55, 56] and ST1193 with CTX-M-14 and CTX-M-15 [58].

2.5 Clinical Significance of High-Risk Clones and Mobile Genetic Elements

Infection-control practitioners and clinicians need the clinical laboratory to rapidly identify and characterize different types of resistant bacteria efficiently to minimize the spread of such bacteria and help to select more appropriate antibiotics. The recent evolutionary developments within ESBL-producing bacteria had a significant impact on routine laboratory diagnosis and infection control practices. During the 1980s and 1990s, ESBL-producing bacteria, especially due to *K. pneumoniae*, were mainly limited to the hospital setting responsible for nosocomial outbreaks [10]. The molecular epidemiology of ESBL-producing bacteria is becoming more complex with increasingly blurred boundaries between hospitals and the community.

Escherichia coli that produce CTX-M β-lactamases seem to be true community ESBL producers with different behaviors from *Klebsiella* spp. with ESBLs [11]. These bacteria have become widely prevalent in the community setting in most areas of the world and they are most likely being imported into the hospital setting [59]. The emergence of *E. coli* with ESBLs in the community setting is mostly owing to the appearance of ST131 with $bla_{CTX\text{-}M\text{-}15}$ most often in long term care centers. Community-based medical practitioners and laboratories are now encountering infections due to ESBL-producing bacteria on a routine basis.

It is interesting to note that ST131 is rarely responsible for nosocomial outbreaks [60]. The ecology and dynamics of transmission modes of *E. coli* ST131 in the community

setting are presently unknown and are essential for the effective implementation of infection control measures to avoid/limit the transmission of these organisms. Data from a recent mathematical model suggest that avoiding transmission of ST131 would be more effective than reducing antibiotic use in decreasing the rate of infection by this clone [61]. However, the importance of environmental reservoirs and the mechanisms of transmission of *E. coli* ST131 have not been investigated in detail, despite being critical to implement infection control measures. Despite the fact that ST131 have been found in animals and food products, the frequency is much lower than for other *E. coli* lineages, suggesting that person-to-person direct or indirect transmission is key in the epidemiology of this clone. The medical community urgently needs studies that will investigate the roles of environmental reservoirs (i.e., within households and long-term care centers); the contribution of companion animals; and whether direct contact-mediated person-to-person transmission is epidemiologically relevant in the community transmission of ST131.

3 Treatment of Infections due to Extended-Spectrum β-Lactamase (ESBL)-Producing Enterobacteriaceae

3.1 Introduction

The presence of ESBLs complicates antibiotic selection especially in patients with serious infections such as bloodstream-associated infections (BSIs) owing to the MDR nature of such bacteria [21]. Studies have consistently show that infections due to ESBL-producing Enterobacteriaceae are associated with a delay in the initiation of appropriate antibiotic therapy, which consequently prolongs hospital stays associated with increasing hospital costs. More importantly, failure to initiate appropriate antibiotic therapy from the start appears to be responsible for higher patient mortality.

Randomized controlled trials pertaining to the treatment of infections due to ESBL-producing bacteria are rare. The majority of published clinical studies are observational (e.g., retrospective cohort in design) or are case series and anecdotal reports. Therefore, many studies suffer from important limitations, including potential selection and information biases as well as a lack of adequate control for confounding factors [62].

Antibiotic options for the treatment of infections due to ESBL-producing Enterobacteriaceae were reviewed in detail in 2008 [10], 2010 [21], and more recently in 2018 [62]. The carbapenems, including imipenem, meropenem, doripenem, and ertapenem, are the first-choice agents for the treatment of serious infections due to ESBL-producing Enterobacteriaceae [62]. Carbapenems are highly stable to hydrolysis by ESBLs. They are distributed into various body tissues in high concentrations and there is a lack of the inoculum effect (i.e., when the minimal inhibitory concentration of the antibiotic increases [i.e., the antibiotic loses activity] with the increasing size of the inoculum [or number] of bacteria tested] [21]. Potential drawbacks of their use include the relative high cost of these antibiotics, and the selection of carbapenem-resistant bacteria.

There has been a significant movement among specialists in the infectious diseases and medical microbiology fields to find effective carbapenem-sparing therapy for treating serious infections due to ESBL-producing Enterobacteriaceae. β-Lactam-β-lactamase inhibitor combinations (i.e., pipercillin-tazobactam and amoxicillin-clavulanic acid) have activities against ESBL-producing bacteria and the roles of these agents in the treatment of such infections were reviewed in detail during 2015 by Harris et al. [63], in 2017 by Muhammed et al. [64], and again by Rodriquez-Bano in 2018 [62]. These publications concluded that when evaluating retrospective data, β-lactam-β-lactamase inhibitor combinations were non-inferior to the carbapenems and can be used as carbapenem-sparing therapy, especially if the source of the infections are from the urinary tract (i.e., urosepsis). Since the publications of the above-mentioned reviews, the INCREMENT project and the MERINO study have been published and will be described in detail below.

3.2 INCREMENT Project Pertaining to the Treatment of ESBL-Producing Enterobacteriaecae

The INCREMENT project was the brain child of Jesus Rodriguez-Bano and colleagues from Seville, Spain. This project consisted of a multicenter, international retrospective cohort study that included consecutive episodes of BSIs due to ESBLs (or carbapenemase) producing Enterobacteriaceae diagnosed at participating centers from January 2004 through December 2013 [65]. The centers included over a thousand patients from 37 hospitals situated in 11 different countries (i.e., Spain, Germany, Italy, Greece, Israel, Turkey, South Africa, Canada, USA, Argentina, and Taiwan).

A user-friendly, easy-to-collect predictive scoring model (i.e., INCREMENT-ESBL score) was developed and validated for the early identification of patients with high and low risks of mortality [65]. The project also demonstrated significant global differences in empiric and targeted therapy for BSIs caused by ESBL-producing Enterobacteriaceae [66]. The investigators concluded that the 30-day mortality rates were significantly higher in patients with BSIs due to ESBL-producing *K. pneumoniae* than in patients with BSIs due to ESBL-producing *E. coli* [67].

As far as the effect of different antibiotics in the outcomes of BSIs due to ESBL-producing Enterobacteriaceae, the INCREMENT project demonstrated the following results: β-lactam-β-lactamase inhibitor combinations [68] (the majority of patients were treated with piperacillin-tazobactam, but some patients who received intravenous amoxicillin-clavulanic acid were also included), aminoglycosides (especially amikacin), and fluoroquinolones (i.e., ciprofloxacin) [69], when active in vitro, appeared to be as effective as the carbapenems for the empiric and targeted therapy. These results were independent of the source of infections and specific species responsible for the BSIs.

When comparing the outcome differences between the various carbapenems, ertapenem appeared as to be as effective as the other carbapenems (i.e., meropenem, imipenem, doripenem) for the empirical and targeted therapy of BSIs due to ESBL-producing Enterobacteriaceae [70]. Patients with severe sepsis or septic shock showed a non-significant trend favoring the other carbapenems and the INCREMENT investigators do caution the use of ertapenem in such patients.

3.3 MERINO Study

Probably the most significant advancement in the treatment of serious infections due to ESBL-producing Enterobacteriaceae (especially *E. coli* and *K. pneumoniae*) was published in 2018 [71]. The MERINO study was an international, non-inferiority, open-label randomized controlled study that compared piperacillin-tazobactam (4.5 g every 6 hours) with meropenem (1 g every 8 hours) for the treatment of BSIs due to cephalosporin-resistant Enterobacteriaceae (the majority of these patients were infected with ESBL-producing isolates). Patients ($n=391$) were enrolled from 26 hospitals in nine countries (i.e., Australia, New Zealand, Singapore, Italy, Turkey, Lebanon, South Africa, Saudi Arabia, and Canada) during February 2014 to July 2017. Patients were randomized within 72 h of blood culture collection and received either piperacillin-tazobactam or meropenem for at least 4 days. A conservative 5% non-inferiority margin was used to compare the outcomes of piperacillin-tazobactam and meropenem. The study was terminated early on grounds of harm when it was clear that the non-inferiority margin could not be demonstrated for piperacillin-tazobactam [71].

A total of 378 patients were evaluated and in the primary analysis the all-cause 30-day mortality was 12.3% in the piperacillin-tazobactam group and 3.7% in the meropenem group for an absolute risk difference of 8.6% and the number needed to harm was 12 [71]. Of particular interest was that only 4% of the cephalosporin-resistant isolates tested resistant to piperacillin-tazobactam and 30-day mortality was unrelated to piperacillin-tazobactam resistance. Extended-spectrum β-lactamase production was confirmed in 85% of isolates with the majority being positive for *E. coli* ST131 with $bla_{\text{CTX-M-15}}$. These findings do not support the use of piperacillin-tazobactam for treating BSIs due to cephalosporin-resistant Enterobacteriaceae, and practically ended the debate about the potential role of β-lactam-β-lactamase inhibitor combinations in serious infections.

Limitations of the MERINO trial include that the empirical and step-down antibiotic therapies were not specified and allowed before randomization, cross-over of patients from one group to the other was allowed, the low mortality of patients in the meropenem arm of the study, and the susceptibility methodology was not the gold standard (i.e., the MERINO trial used E-tests to determine the MICs for piperacillin-tazobactam and meropenem and micro-dilution methodology would have been more appropriate). The study did not provide information on whether piperacillin-tazobactam would be more effective if administered as an extended infusion and if this agent can be used for non-BSI infections due to ESBL-producing bacteria.

3.4 Role of Newer Agents in Treating Infections due to ESBL-producing Enterobacteriaceae

Ceftazidime–avibactam and ceftolozane–tazobactam are combinations of cephalosporins (i.e., ceftazidime and ceftolozane) with inhibitors (i.e., avibactam and tazobactam) that show good activity against Enterobacteriaceae with ESBLs. Plazomicin is a next-generation aminoglycoside synthetically derived from sisomicin that shows resistance to inactivation by aminoglycoside-modifying enzymes. Clinical studies using these agent to treat various infections due to ESBL-producing bacteria show them to have similar outcomes than standard treatment regimens [62].

4 Summary

Extended-spectrum β-lactamase-producing Enterobacteriaceae have emerged during the 1990s and 2000s as a major global public health concern and currently are important players among AMR per se. Extended-spectrum β-lactamase-producing bacteria were mainly responsible for hospital-acquired infections during the 1990s (due to ESBL-*K. pneumoniae*). Today, ESBL-producing bacteria (mostly due to ESBL-*E. coli*), are common causes of community-onset infections especially, in Asia, South America, and Africa.

Presently, *E. coli* with CTX-Ms are the most common global ESBL; CTX-M-15 is the most frequent CTX-M worldwide, and is followed by CTX-M-14, which is often found in South-East Asia. Recent surveillance studies showed that CTX-M-27 is emerging in certain parts of the world, especially Japan and Europe. The emergence

of CTX-M-27 is linked to the appearance of a new *E. coli* ST131 subclade named C1-M27 (see below for more detail on ST131 subclades).

The population structure of ESBL-producing *E. coli* is dominated globally by an high-risk clone named ST131. *Escherichia coli* ST131 belongs to three clades (e.g., A, B, and C) and three different subclades (e.g., C1, C1-M27, and C2). Clade C is associated with fluoroquinolone resistance and the most common global clade among clinical ST131; subclade C1 is associated to a lesser extent with $bla_{\text{CTX-M-14}}$, C1-M27 is associated with $bla_{\text{CTX-M-27,}}$ while C2 is associated with $bla_{\text{CTX-M-15}}$. Recent WGS studies have shown that clade C had evolved from clade B in a stepwise manner resulting in one of the most influential global AMR clones among Gram-negative bacteria. Other important *E. coli* clones that have been detected among ESBL producers included ST405, ST38, ST648, ST410, and ST1193.

It still remains unclear which features of *E. coli* ST131 clade C resulted in one of the most unparalleled AMR success stories of the 2000s. The selection pressures created by the widespread usage of the fluoroquinolones and oxyimino-cephalosporins have played an important role in the emergence of *E. coli* ST131. However, other *E. coli* clones (e.g., ST405) contain the same AMR determinants and similar virulence gene profiles as ST131, but do not share the success of clade C. The question remains if *E. coli* ST131 clade C is inherently more fit than other ExPEC clones, or even other ST131 clades, and therefore was able to survive and outcompete other susceptible or AMR clones/clades in certain environments, even in the absence of antimicrobial selection pressures.

It has always been unclear if different carbapenems have similar clinical outcomes when treating BSIs caused by ESBL-producing Enterobacteriaceae. The INCREMENT project has shown that ertapenem is as effective as other carbapenems for treating serious infections due to ESBL-producing Enterobacteriaceae. The use of once-daily ertapenem will enable clinicians to confidently treat BSIs due to ESBL-producing bacteria on an outpatient basis through an outpatient parenteral antibiotic therapy program; patients seen at the emergency room with BSI due to suspected ESBL-producing bacteria can be referred to an outpatient parenteral antibiotic therapy clinic that can continue with a once-daily intravenous carbapenem treatment for a few days. However, there are also oral options such as the fluoroquinolones (amoxicillin-clavulanic acid), if they test susceptible.

The results of the MERINO study provided clear evidence that piperacillin-tazobactam should not be used as targeted therapy of serious infections due to ESBL-producing *E. coli* and *K. pneumoniae,* regardless of the patient population, source of infection, bacterial species, and susceptibility result of piperacillin-tazobactam. This has important implications for physicians and microbiologists [72]. Clinical laboratories should refrain from reporting the susceptibilities of piperacillin-tazobactam on cephalosporin-resistant Enterobacteriaceae, especially if such a bacterium is positive for ESBLs. It might be prudent to add a comment to the laboratory report stating that the use of piperacillin-tazobactam should be avoided in BSIs, even if the ESBL-producing bacterium tested susceptible to the drug.

Research is still warranted to define the optimal therapy of less severe infections due to ESBL-producing Enterobactericeae. Agents such as amoxicillin-clavulanic acid, nitrofurantoin, and fosfomycin show good in-vitro activity against ESBL-producing bacteria and are options for the treatment of uncomplicated urinary tract infections due to these MDR bacteria, but randomized controlled studies are lacking.

In summary, ESBL-producing Enterobacteriaceae are important causes of global hospital and community-onset infections. A single high-risk clone namely *E. coli* ST131 clade C is mainly responsible for the global distribution of ESBL-producing bacteria. The use of piperacillin-tazobactam should be avoided for treating serious infections due to ESBL-producing *E. coli* and *K. pneumoniae.*

Compliance with Ethical Standards

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