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Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection

Mariana Castanheira[1], Patricia J. Simner[2] and Patricia A. Bradford [3]*

[1]*JMI Laboratories, North Liberty, IA, USA;* [2]*School of Medicine, Johns Hopkins University, Baltimore, MD, USA;* [3]*Antimicrobial Development Specialists LLC, Nyack, NY, USA*

*Corresponding author. E-mail: pbradford@antimicrobialdev.com

Extended-spectrum β-lactamase (ESBL)-producing Gram-negative pathogens are a major cause of resistance to expanded-spectrum β-lactam antibiotics. Since their discovery in the early 1980s, they have spread worldwide and an are now endemic in Enterobacterales isolated from both hospital-associated and community-acquired infections. As a result, they are a global public health concern. In the past, TEM- and SHV-type ESBLs were the predominant families of ESBLs. Today CTX-M-type enzymes are the most commonly found ESBL type with the CTX-M-15 variant dominating worldwide, followed in prevalence by CTX-M-14, and CTX-M-27 is emerging in certain parts of the world. The genes encoding ESBLs are often found on plasmids and harboured within transposons or insertion sequences, which has enabled their spread. In addition, the population of ESBL-producing *Escherichia coli* is dominated globally by a highly virulent and successful clone belonging to ST131. Today, there are many diagnostic tools available to the clinical microbiology laboratory and include both phenotypic and genotypic tests to detect β-lactamases. Unfortunately, when ESBLs are not identified in a timely manner, appropriate antimicrobial therapy is frequently delayed, resulting in poor clinical outcomes. Several analyses of clinical trials have shown mixed results with regards to whether a carbapenem must be used to treat serious infections caused by ESBLs or whether some of the older β-lactam-β-lactamase combinations such as piperacillin/tazobactam are appropriate. Some of the newer combinations such as ceftazidime/avibactam have demonstrated efficacy in patients. ESBL-producing Gram-negative pathogens will continue to be major contributor to antimicrobial resistance worldwide. It is essential that we remain vigilant about identifying them both in patient isolates and through surveillance studies.

1. Introduction

Although naturally occurring in some species of bacteria, β-lactamases have become mobilized on plasmids and have become widespread in response to the use and overuse of β-lactam antibiotics. In Gram-negative bacteria, broad-spectrum enzymes such as TEM-1 and SHV-1 arose following the introduction of first- and second-generation cephalosporins.[1] Subsequently, expanded-spectrum β-lactam antibiotics were introduced that were refractory to hydrolysis by these enzymes. In particular, the oxyimino-cephalosporins such as ceftazidime and cefotaxime became widely used. This led to evolution of new β-lactamases that hydrolysed these new drugs.[2] The most epidemiologically important group of such enzymes is the extended-spectrum β-lactamases, which have become endemic worldwide. ESBLs are serine β-lactamases, belonging to Ambler molecular and structural classification as class A. They are biochemically characterized by their ability to hydrolyse expanded spectrum β-lactam antibiotics, and inhibition by β-lactamase inhibitors, specifically clavulanate.[3] ESBLs have been found in many genera of Enterobacterales as well as in *Pseudomonas aeruginosa*. They confer resistance to most β-lactam antibiotics, including expanded-spectrum cephalosporins and monobactams, but not to carbapenems and cephamycins.

The original ESBL enzymes were variants of TEM and SHV variants that had amino acid substitutions leading to a change in their substrate profile to include the expanded-spectrum cephalosporins. With the widespread usage of gene sequencing to identify β-lactamase genes in clinical isolates, multiple variants of the common TEM and SHV enzymes have been identified. As of this writing, 243 variants of TEM and 228 variants of SHV have been identified, although not all of these possess the ESBL phenotype (https://www.ncbi.nlm.nih.gov/pathogens/isolates#/refgene/TEM; https://www.ncbi.nlm.nih.gov/pathogens/isolates#/refgene/SHV).

ESBL-producing Gram-negative pathogens are now commonplace in both the hospital and community settings.[4] The impact

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of ESBL-positive Enterobacterales on the choice of empirical and definitive antimicrobial therapy has been substantial, resulting in the increased use of carbapenems in many institutions, which led to the increase of carbapenem resistance in these organisms.[5,6] This review will focus on the phenotypic and genetic characterization of ESBLs, their epidemiology, the state of the art of detection of these enzymes, and therapeutic options.

2. ESBL placement in β-lactamase classification scheme

The first classification scheme for β-lactamases that recognized ESBLs was established in 1989 by Karen Bush,[7] in which group 2b' was defined as β-lactamase enzymes that can hydrolyse oxyimino-β-lactams such as cefotaxime, ceftazidime and aztreonam at rates at least 10% that of benzylpenicillin and that are strongly inhibited by clavulanate (Figure 1). Subsequently, these enzymes were designated as group 2be in the functional classification scheme of developed by Bush, Jacoby and Mederios.[8] In this scheme, ESBLs retained the strict definition of class A β-lactamases that could hydrolyse these expanded-spectrum β-lactam antibiotics and are also susceptible to inhibition by the original β-lactamase inhibitors clavulanate, sulbactam and tazobactam. The original classification scheme also included only plasmid-mediated enzymes, however the updated scheme now recognizes the fluidity of genes expressing ESBLs between plasmids and chromosomes.[3] Traditional ESBLs are inhibited by all of the β-lactamase inhibitors including the older inhibitors clavulanate, sulbactam and tazobactam as well as by the newer inhibitors such as avibactam, relebactam and vaborbactam. Although the notion of including any enzyme that can hydrolyse the oxyimino-β-lactams in the classification as an ESBL has been proposed, the strict definition of an ESBL remains that the inhibition by clavulanate is a requirement for designation in this group.[3,9–11]

3. ESBL families

Although ESBLs have common biochemical properties with regards to the hydrolysis of expanded-spectrum β-lactam antibiotics and inhibition by clavulanate, the genes encoding these enzymes are diverse in nature and can be grouped into several families (Table 1).[1] Some of these families such as the TEM- and SHV-type ESBLs are highly related, with variants differing by only a few amino acid substitutions. Other families such as the CTX-M-type ESBLs are much more genetically diverse. Each of the ESBL families have some unique characteristics.

3.1 TEM

TEM-type ESBLS are variants of the original plasmid mediated β-lactamase, TEM-1, which was described in the early 1960s.[12] This enzyme was so named because it was originally found in an isolate of *Escherichia coli* isolate that came from a blood culture from a Greek patient named Temoneira.[13] The first derivative of TEM, TEM-2, has a single amino acid substitution of Gln39Lys from the original TEM-1 β-lactamase.[14] This change did not alter the substrate profile from TEM-1, however TEM-2 served as the progenitor for many of the TEM-type ESBLs.[1] The first TEM-type variant that showed the ESBL phenotype was TEM-3, which was reported in 1989.[15] As of this writing, 243 different TEM variants have been described, although not all are ESBLs (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#TEM).

The amino acid substitutions that occur within the TEM enzyme occur at a limited number of positions[1] The amino acid residues (Ambler numbering) are most frequently involved in conferring the ESBL phenotype to TEM-type enzymes are Gly238 and Glu240 located on the b3 β-pleated sheet; Arg164 located on the neck of the Ω loop; and Glu104 located directly across from Gly238 Glu240 at the opening of the active-site cavity (Figure 2).[16,17] Of these, the substitutions Gly238Ser and Glu240Lys appear to be have the most impact on producing the ESBL phenotype.[1] Some of the newer TEM variants have subtle changes in the substrate profile. For example, TEM-184 (amino acid substitutions at Q6K, E104K, I127V, R164S and M182T) hydrolysed aztreonam more efficiently than ceftazidime or cefotaxime.[18] Although so many new variants are being discovered by WGS, few of these are being phenotypically characterized to determine if they have properties of an ESBL. However, computer modelling and network analysis has enabled the prediction of whether a particular sequence is likely to belong to functional groups 2 b (original broad spectrum), 2be (ESBL) or 2br (inhibitor resistant).[19]

At the height of prominence for TEM-type ESBLs, the prevalence of some of the variants were regional in nature. For example, TEM-3 was very common in France, but rarely seen in the USA.[20] In contrast, TEM-10 was the most prevalent TEM-type ESBL in the USA.[21] Interestingly, TEM-26 was detected in isolates from across the globe.[20,22–24] As the CTX-M-type β-lactamases became the most prevalent ESBL worldwide, TEM-type enzymes became more infrequent. In a recent survey of European isolates, TEM-type ESBLs were detected in less than 1% of ESBL-producing *E. coli* and *Klebsiella pneumoniae*.[25]

3.2 SHV

The SHV-type β-lactamases (so named for sulfhydryl reagent variable) originated as chromosomally encoded enzymes in *K. pneumoniae*.[26] The first ESBL described in 1985 was SHV-2 and was found in a single strain of *Klebsiella ozaenae* isolated in Germany that differed from SHV-1 by a single amino acid substitution of Gly to Ser at position 238.[27] Similar to what is seen in TEM-type ESBLs, the majority of SHV-type ESBLs also have mutations at Ambler positions 238 (Gly to Ser) and 240 (Lys to Glu) (Figure 2).[1] The substitution of serine at position 238 appears to be critical for the efficient hydrolysis of ceftazidime, whereas the substitution of Lys at residue 240 is critical for the efficient hydrolysis of cefotaxime.[27] The relevance of the various amino acid substitutions with regards to phenotypic changes in substrate profile has recently been investigated using a mathematical model.[28] To date, 228 sequence variants of SHV have been detected, although not all have been functionally characterized to determine if they possess the ESBL phenotype (https://www.ncbi.nlm.nih.gov/pathogens/isolates#/refgene/SHV). Worldwide, SHV-5 and SHV-12 have been the most common ESBL variants found in Enterobacterales.[29,30] SHV-type ESBLs are most often found in clinical isolates of *K. pneumoniae*, however, these enzymes have also been found in other genera of Enterobacterales and *P. aeruginosa* as well.[29,31]

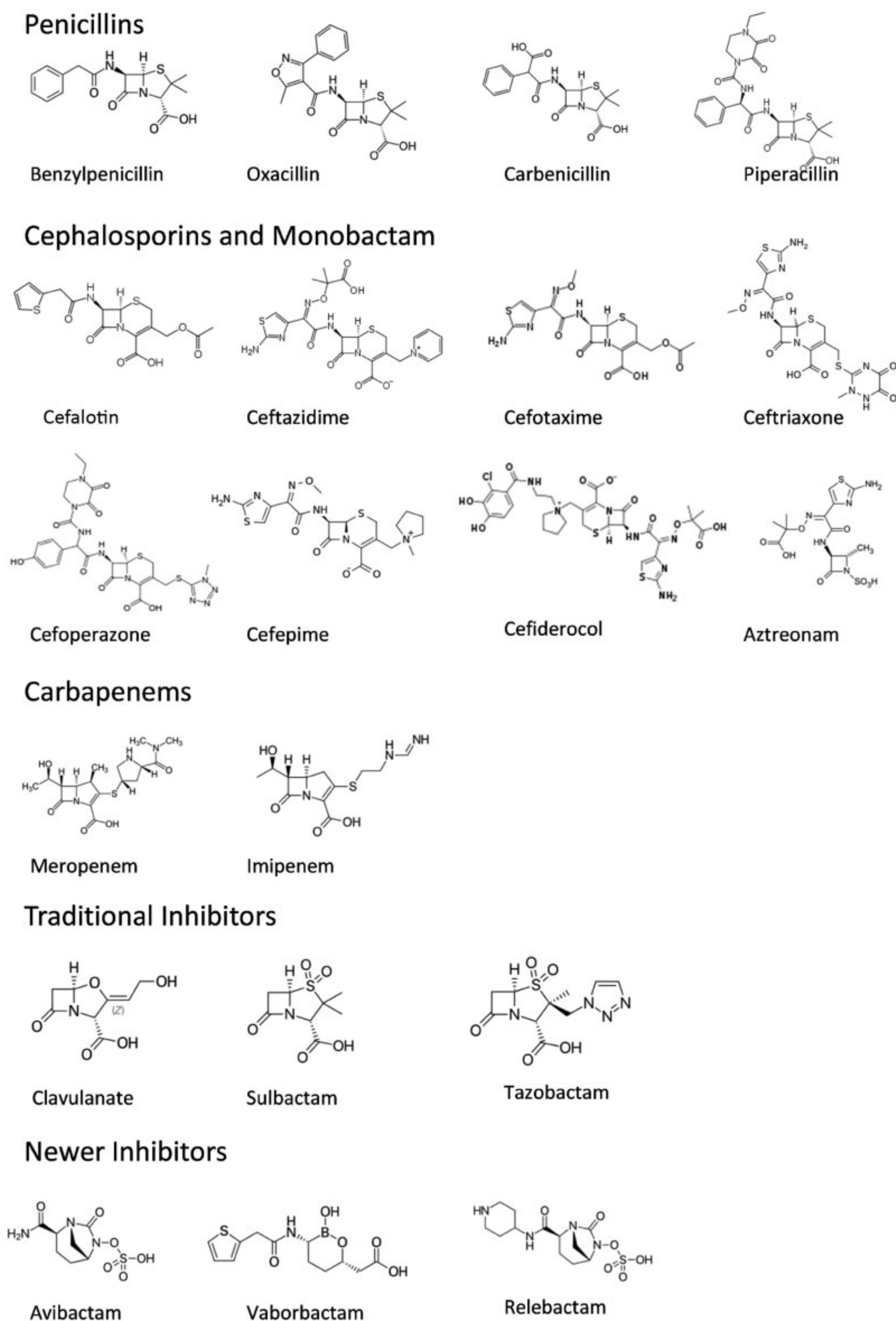

Figure 1. Structures of β-lactam antibiotics and β-lactamase inhibitors.

In recent European surveillance, SHV-type ESBLs were found in 3.1%–17.0% of clinical isolates of *K. pneumoniae*, depending on region.[25] However, in a clinical trial that targeted ceftazidime-resistant pathogens from complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI), SHV-type ESBLs were rarely encountered and were only found in strains that also produced a plasmid-mediated AmpC or carbapenemase.[32] Although TEM- and SHV-type ESBLs are still encountered, it

Table 1. ESBL families

Family	Nomenclature	Characteristics
TEM	Temoneira, the patient infected with the first isolate expressing TEM-1	Point mutation variants of TEM-1 or TEM-2
SHV	Sulfhydryl reagent variable	Point mutation variants of SHV-1
IRT	Inhibitor-resistant TEM	TEM variants that are resistant to inhibition by clavulanate and sulbactam, but do not have ESBL phenotype
CMT	Complex mutant derived from TEM-1	TEM variants that are resistant to inhibition by clavulanate and sulbactam and also have ESBL phenotype
CTX-M	Cefotaxime-hydrolysing β-lactamase isolated in Munich	Derived from the chromosomal β-lactamase from *Kluyvera* spp. Preferentially hydrolyses cefotaxime
GES	Guiana-extended spectrum	More prevalent in *P. aeruginosa* than Enterobacterales Some variants also hydrolyse carbapenems
PER	*Pseudomonas* extended resistant	More prevalent in *P. aeruginosa* and *A. baumannii* than Enterobacterales Inhibition by newer β-lactamase inhibitors is variable
VEB	Vietnam extended-spectrum β-lactamase	Preferentially hydrolyses ceftazidime and aztreonam compared with cefotaxime Inhibition by newer β-lactamase inhibitors is variable
BEL	Belgium extended β-lactamase	Preferentially hydrolyses ceftazidime and aztreonam compared with cefotaxime
TLA	Named after the Tlahuica Indians (Mexico), from whom the first isolate was obtained	Preferentially hydrolyses ceftazidime and aztreonam compared with cefotaxime
SFO	From *Serratia fonticola*	Inducible
OXY	From *Klebsiella oxytoca*	Chromosomally encoded

Adapted from Jacoby.[89]

appears that the impact of their presence among clinical isolates is minimal.

3.3 Inhibitor-resistant β-lactamases

Inhibitor-resistant β-lactamases are derivatives of TEM and SHV enzymes that have amino acid substitutions that confer resistance to inhibition by the β-lactamase inhibitors clavulanate and sulbactam. In the functional classification scheme, they belong to functional group 2br.[3,8] Most of these remain susceptible to inhibition by tazobactam and avibactam.[33,34] The majority of inhibitor-resistant β-lactamases are derivatives of TEM-1 and were formerly called IRT (for inhibitor resistant TEM), but are now given sequential TEM numbering.[35] Common substitutions in the TEM variants have been characterized at amino acid positions Met69, Ser130, Arg244, Arg275 and Asn276 (Figure 2).[36] It appears that the cost of the mutations resulting in resistance to clavulanate and sulbactam is a reduction in the efficiency of hydrolysing some penicillins and cephalosporins such as cefalotin.[37] Although these mutants are rarely detected, a strain of *K. pneumoniae* expressing the inhibitor-resistant TEM-30 was identified in several KPC-producing isolates from an outbreak of carbapenem-resistant Enterobacterales (CRE) in New York City.[33] Several SHV-type β-lactamases have been characterized as inhibitor resistant, including SHV-49, -56 and -107, which were identified in *K. pneumoniae* clinical isolates from patients in Europe.[38–40]

A few complex TEM mutant (CMT) β-lactamases have been described that are mutants of TEM β-lactamases that have both the ESBL phenotype and inhibitor resistance.[36] These CMT variants will not be detected with any of the screening methods used to detect ESBLs because those tests rely on inhibition with clavulanate. One such complex mutant, TEM-152, was found in an isolate of *E. coli* in a patient hospitalized in France.[41] This mutant harboured amino acid substitutions Arg164His and Glu240Lys, previously observed in ESBLs, plus Met69Val and Asn276Asp, previously observed in the inhibitor-resistant enzyme TEM-36, which resulted in efficient hydrolysis of ceftazidime and a 50% reduction in inhibition by clavulanate. Because these complex mutants are not resistant to avibactam, ceftazidime/avibactam or one of the other new β-lactamase inhibitor combinations may be a therapeutic option to treat infections caused by organisms expressing one of these enzymes.[34] It is likely that the prevalence of TEM- or SHV-type inhibitor-resistant β-lactamases is underestimated because there is not a phenotypic test that laboratories can routinely use to identify these strains.[42]

3.4 CTX-M

CTX-M-type β-lactamase enzymes were initially reported in the late 1980s, emerging concomitantly in several locations. The nomenclature CTX-M (cefotaximase from Munich) was initially used in a report from Germany.[43] However, CTX-M-type enzymes identified in other regions received different names, including FEC-1 (Japan), Toho-1 (Japan) and MEN-1 (France in an Italian patient).[44] These initial reports were followed by outbreaks in several countries. The worldwide expansion of isolates carrying these ESBLs later would be referred as the 'CTX-M pandemic'. Since the early 2000s, CTX-M-type enzymes have been recognized as the most common ESBL group, replacing TEM and SHV as the dominant ESBL type. CTX-M variants have been reported among several

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Amino Acid Residue
TEM-1        ---MSIQHFRVALI----PFFAAFCLPVFAH-PETLVKVKDAEDQLGARVGYIELDLNSG
SHV-1        -----MRYIRLCII----SLLATLPLAVHAS-PQPLEQIKLSESQLSGRVGMIEMDLASG
CTX-M-1      MVKKSLRQFTLMATATVTLLLGS--VPLYAQTADVQQKLAELERQSGGRLGVALINTAD-
                 :: : :         ::.:  : :.*   :   ::   * * ..*:*   ::  .

                                 69 70                         104
Amino Acid Residue
TEM-1        KILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLT
SHV-1        RTLTAWRADERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHYRQQDLVDYSPVSEKHLA
CTX-M-1      NSQILYRADERFAMCSTSKVMAVAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAEKHVD
             .    :* :*** * ** **:  .*** : ::  : * :::.  :.***:*.*::***:

                                  130                           164
Amino Acid Residue
TEM-1        DGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIP
SHV-1        DGMTVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
CTX-M-1      GTMSLAELSAAALQYSDNVAMNKLISHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIP
             . *:: **.:**:  *** * * *:: :***  :*** :::**.. **** *  ** *:*

Amino Acid Residue
TEM-1        NDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADK
SHV-1        GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAGPLIRSVLPAGWFIADK
CTX-M-1      GDPRDTTSPRAMAQTLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVGDK
             .* **** * :** ***:*  .: *   .: **: **  : ...  ::: ***.*.:.**

                 238 240  244                          275 276
TEM-1        SGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW-
SHV-1        TGAGERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHWQ
CTX-M-1      TGSGDYGTTNDIAVIWPKDRAPLILVTYFTQPQPKAESRRDVLASAAKIVTNGL-
             :*:*: *: . :* : *..:   *:* *       .  .*.  :*  .  : :
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Figure 2. Amino acid alignments of TEM-1, SHV-1 and CTX-M-1. The amino acid sequences WP_000027057.1 (TEM-1), WP_001620095.1 (SHV-1) and WP_013188473.1 (CTX-M-1) were obtained from NCBI and aligned using Clustal Omega.[224–226] Numbering according to Ambler.[227] Asterisk (*) indicates positions that have a single, fully conserved residue. Colon (:) indicates conservation between groups of strongly similar properties. Period (.) indicates conservation between groups of weakly similar properties. The yellow highlights show the active site Ser70-X-X-Lys active site common to all serine β-lactamases. Red amino acids denote residues where substitutions provide ESBL phenotype (TEM and SHV). Blue amino acids denote where substitutions provide inhibitor resistance phenotype. Green indicates position 240 in CTX-M-1, which has been identified as being associated with increased hydrolysis of cefotaxime.

members of the order Enterobacterales and in *P. aeruginosa* and *Acinetobacter* spp.[45–47] Isolates carrying CTX-M-encoding genes have been detected in nosocomial and community settings as well as in companion animals, the environment, food products and livestock.[48]

Most CTX-M enzymes can be clustered into five groups based on sequence homologies: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25. By far the most common CTX-M-1 group is CTX-M-15, followed by CTX-M-3 and CTX-M-1.[44] In the CTX-M-9 group, CTX-M-9 and CTX-M-14 were the most common enzymes, but more recently CTX-M-27 has often been reported.[49–53] CTX-M-2, CTX-M-8, and CTX-M-25 are the most frequent variants within their own groups. The analysis of the upstream sequences flanking the genes encoding CTX-M-2 groups belonged to *Kluyvera* spp. Further analysis demonstrated that this group derived from KLUA-1, an enzyme from *Kluyvera ascorbate*.[54,55] Similarly, CTX-M-134 (CTX-M-1 group) is derived from KLUC-1 from *Kluyvera cryocrescens* and the CTX-M-9 group have similarity with KLUG-1 from *Kluyvera georgiana*.[56,57] Notably, CTX-M enzymes also exhibit structural similarity and hydrolytic profiles with other class A β-lactamases from environmental organisms, such as *Erwinia persicina* and *Rahnella aquatilis*.[58,59]

The early CTX-M variants efficiently hydrolysed cefotaxime and ceftriaxone, hence the name cefotaximase.[44] Contrary to the TEM- and SHV-type ESBLs reported to that point, the early CTX-M enzymes had limited activity against ceftazidime, however CTX-M variants with enhanced ceftazidime hydrolytic activity were later described. Important examples of CTX-M enzymes displaying ceftazidime hydrolysis are CTX-M-15 and CTX-M-27, which are from the CTX-M-1 and CTX-M-9 groups, respectively.

CTX-M-15 is derived from CTX-M-3 and displays a single amino acid change in position 240 (Asp to Gly) when compared with its ancestor (Figure 2).[60] The Asp240 residue is located in the terminal part of the B3 β-strand and is responsible for the flexibility of this structure and the accommodation of ceftazidime, which is a bulkier molecule than cefotaxime.[61] Despite the modest increase

in hydrolytic activity observed against ceftazidime, this change significantly increased ceftazidime MIC values of constructs carrying CTX-M-15.[60,62] CTX-M-27 has the same residue in position 240 that is present in CTX-M-15. This residue confers elevated ceftazidime MIC values, despite the overall poor activity of CTX-M-27 against other substrates compared with its ancestor CTX-M-14.[63] Data from clinical isolates collected from the SENTRY Antimicrobial Surveillance Program showed that the ceftazidime MIC for CTX-M-producing isolates varies, with MIC values ranging from 0.25 mg/L to >32 mg/L (M. Castanheira, JMI Laboratories, unpublished data).

In 2019, Poirel *et al.*[64] described a new CTX-M variant, CTX-M-33, that had an alteration in position 109 (Asp to Ser) compared with CTX-M-15. This enzyme displayed decreased ceftazidime hydrolysis, but significant meropenem hydrolysis, although this translated into only a modest increase in meropenem MIC in an isogenic pair. However, the clinical isolate of *K. pneumoniae* isolate carrying this new variant also had impaired permeability resulting in a meropenem MIC of 8 mg/L. CTX-M-type β-lactamases are widespread enzymes. Although there are still treatment options for isolates carrying these enzymes alone, the combination of these enzymes in isolates with other resistance mechanisms and the expansion of hydrolytic profiles with single amino acid mutations could limit the activity of meropenem and newer agents.

3.5 ESBL phenotype OXA-type β-lactamases

The OXA-type β-lactamases hydrolyse oxacillin and are grouped as Ambler class D and Bush-Jacoby-Medeiros functional group 2d enzymes.[8] In general, OXA-type enzymes are a broad group that displays variability in substrate profiles and amino acid sequences. However, several OXA-type variants have been noted to hydrolyse cephalosporins, cephems, and/or monobactams. These OXA enzymes with an ESBL phenotype are categorized in Bush functional subgroup 2de.[3] Whether or not these oxacillinases with activity against expanded-spectrum cephalosporins are defined as ESBLs is debatable.[9] Many researchers do not apply the ESBL terminology to oxacillinases because these enzymes are not classified in the 2be group and are refractory to inhibition by clavulanate or other inhibitors in the same manner as the true ESBLs.

According to a recent review, there are 27 oxacillinase enzymes described as extended spectrum. These enzymes' substrates include third- and/or fourth-generation cephalosporins in addition to penicillins and early cephalosporins.[65] Most extended-spectrum oxacillinases derive from OXA-10 (also named PSE-2) and OXA-2. The OXA-10 derivatives include OXA-11, OXA-13, OXA-14, OXA-16, OXA-17, OXA-19 and OXA-28.[66] In addition, OXA-16 has only a partial sequence submitted as its first description (GenBank #AF043100). Among the OXA-2 derivatives, OXA-15, OXA-32, OXA-34, OXA-36 (partial sequence), OXA-53, OXA-141, OXA-161, OXA-210 and OXA-226 have been described.[65] Many OXA-2 and OXA-10 derivatives are detected in isolates of *P. aeruginosa*.

Despite not being considered as extended-spectrum oxacillinases, OXA-1 and OXA-30 have been named for their ability to hydrolyse cefepime.[67–69] OXA-1 and OXA-30 were initially reported to differ by one amino acid; however, it was corrected later that these enzymes were identical.[70] OXA-1 combined with loss of porins has been implicated in false-ESBL phenotypes among *E. coli* isolates and resistance to β-lactamase inhibitor combinations.[71] Contrary to most oxacillinases, which have a dimeric described structure, OXA-1 was reported to be a monomer.[72] An OXA-31 that was detected in an isolate of *P. aeruginosa* had three amino acid substitutions compared with OXA-1, including the amino acid differences from OXA-4 and also displayed activity against cefepime.[73] OXA-48 derivatives, namely OXA-163 and OXA-405, have been described to display activity against extended-spectrum β-lactams with or without many of the OXA-48-like enzyme's characteristic carbapenemase activity.[74,75]

3.6 Other ESBL families

The GES (Guiana extended-spectrum β-lactamase) family is the most prevalent group of the less common ESBLs. The gene encoding GES-1 is not closely related to any other plasmid-mediated β-lactamase but does show 36% homology to a carbenicillin-hydrolysing enzyme from *Proteus mirabilis*.[1] Despite initially being reported among species of Enterobacterales, GES enzymes are more common among isolates of *P. aeruginosa* and *A. baumannii* isolates.[76–80] GES enzymes are notable for their ability to acquire single or double amino acid substitutions and expand their spectrum to carbapenems.

The ESBL GES-1 was first described in 1998 in a *K. pneumoniae* isolate collected in France from a patient who had recently been hospitalized in French Guiana.[81] At the same time, another group described a similar enzyme, named IBC, from an *E. cloacae* isolate from Greece.[82] Subsequent enzymes GES-2 and IBC-2 were both found in isolates of *P. aeruginosa* isolates.[83,84] IBC-1 was later renamed GES-7 and IBC-2, GES-8.

Interestingly, GES-2 had a single amino acid substitution (Gly170Asp) compared with GES-1 and displayed some hydrolytic activity against carbapenems.[83] Later described GES β-lactamases fell into two categories: enzymes that were ESBLs and those that showed some modest carbapenemase activity. The original GES enzymes were ESBLs that hydrolyse penicillins and cephalosporins well, but not aztreonam.[81] These enzymes are inhibited by clavulanate, tazobactam, and the newer β-lactamase inhibitors such as avibactam, relebactam and vaborbactam.[85,86] This means that isolates expressing GES enzymes are often susceptible to ceftazidime/avibactam, but not ceftolozane/tazobactam.[85] GES-1 hydrolyses ceftazidime better than cefotaxime. Amino acid substitutions of Glu104Lys or Gly243Ala/Ser that were detected in GES variants described later have been shown to confer greater resistance to cephalosporins and aztreonam.[87]

The PER-1 β-lactamase (Pseudomonas extended resistant) was initially described from an isolate of *P. aeruginosa* displaying resistance to cephalosporins and inhibition to clavulanate.[88,89] This enzyme hydrolysed most penicillins well and cephalosporins including cefalotin, cefoperazone, cefuroxime, ceftriaxone and ceftazidime. PER-1 did not hydrolyse oxacillin, cephamycins or imipenem. Only a few years later, PER-2 was described in a *P. aeruginosa* isolate from Argentina, which was 86.4% homologous with PER-1.[90] PER enzymes have since been described from *A. baumannii* and *Aeromonas* spp., and in various species of Enterobacterales.

PER-1 and PER-2 are the most common members of the PER family. These enzymes have been reported to be inhibited by avibactam to a lesser extent than other class A β-lactamases, with significant differences in MIC values for avibactam and relebactam when tested in combination with other β-lactams.[85,91] More

detailed studies are warranted due to the difference in activity of these two inhibitors of the same class. Recent analysis demonstrated that *A. baumannii* isolates harbouring PER enzymes can display elevated MIC values against cefiderocol, a siderophore cephalosporin.[92] PER enzymes are most commonly found in isolates from Turkey and Mediterranean countries.[93,94]

VEB-1 (Vietnamese extended-spectrum β-lactamase) was first detected from an *E. coli* isolate recovered from a Vietnamese infant.[95] VEB-1 conferred high MIC values for ceftazidime and aztreonam, but only modest elevation of MIC values for cefotaxime when expressed in an *E. coli* background. A 4-fold increase in cefepime MIC values and no activity against imipenem was observed. This enzyme was well inhibited by clavulanate, but avibactam was initially reported to not reduce the ceftazidime MIC values for *P. aeruginosa* isolates harbouring these enzymes.[96] Further studies demonstrated that when various VEB enzymes were expressed in an *E. coli* isogenic background, the ceftazidime/avibactam MIC values were reduced in a concentration-dependent manner, lowering the ceftazidime MIC values >8-fold when 4 mg/L of inhibitor was used.[97] VEB-1 and other VEB variants have been described among various Gram-negative pathogens of including multiple species of Enterobacterales, *Vibrio* spp., *Achromobacter xylosoxidans* and more clinically relevant species such as *P. aeruginosa* and *A. baumannii*.[98–100]

Less common ESBLs have been described, but their occurrence is limited. These less common ESBLs include SFO-1 from *Serratia fonticola*, TLA-1 from the Mexican indigenous people group Tlahuicas, TLA-2 from Germany that displays only 51% homology to TLA-1, BES-1 from Brazil, and BEL-1 from Belgium.[98] In a review that addresses these rare ESBLs, Naas *et al.*[98] summarized the MIC values for isolates carrying these enzymes against various β-lactams. The cefotaxime MIC value for a $bla_{\text{SFO-1}}$ transconjugant was 8 mg/L and the result for ceftazidime was 4 mg/L. For a $bla_{\text{BEL-1}}$-harbouring recombinant strain, the cefotaxime MIC results was 1 mg/L, but ceftazidime was 4 mg/L. Higher ceftazidime MIC values were noted for clinical isolates carrying $bla_{\text{BES-1}}$ (16 mg/L) or $bla_{\text{TLA-1}}$ (>256 mg/L).

Several other ESBLs have been detected in the chromosome of Enterobacterales and non-fermentative species. Among those, the OXY β-lactamases in *Klebsiella oxytoca* are probably the most common cause of resistance in clinical isolates.[101,102]

4. Molecular characterization of ESBL-producing isolates

4.1 Genetic environment of ESBL genes

Mobile genetic elements (MGEs) such as plasmids, transposons, insertion sequences, integrons and bacteriophages contribute to the dissemination of various ESBL-encoding genes. MGEs can move themselves and/or genes from one location to another within the cell or be transferred from cell to cell horizontally by conjugation, transformation or, in the case of bacteriophages, by transduction.[103] More often than not, MGEs carry multiple resistance genes that confer an MDR phenotype to their hosts.[104] Some of the main elements of MGEs that carry different ESBL types are highlighted in the section below.

Genes encoding TEM-1, TEM-2 and their ESBL derivatives are usually carried by Tn*1*-, Tn*2*-, or Tn*3*-like transposons (Figure 3).[105] These structures were initially named Tn*A* and display 99% nucleotide homology, with most nucleotide differences identified close to their resolvase (*res*) site.[105] A limited number of studies specifically report on the MGE-carrying, bla_{TEM}-encoding ESBL enzymes. In an early study, $bla_{\text{TEM-12}}$ was reported to be part of Tn*841*, which exhibits homology to Tn*3*.[106] The gene encoding TEM-3 was located on an interrupted copy on Tn*1*. Tn*2* was reported to carry $bla_{\text{TEM-10}}$ whereas $bla_{\text{TEM-24}}$ was associated with Tn*1*.[107–109] In all cases, these structures were embedded in plasmids.[110] A study by Marcadé *et al.*[111] that evaluated replicon types of conjugative plasmids carrying ESBL genes revealed that 67% of the plasmids harbouring TEM-type ESBL genes belonged to the IncA/C type. Most of these plasmids carried $bla_{\text{TEM-24}}$, but the plasmids also carried $bla_{\text{TEM-3}}$, $bla_{\text{TEM-10}}$ and $bla_{\text{TEM-21}}$. Others confirmed the occurrence of bla_{TEM}-encoding ESBLs in IncA/C plasmids.[108,112,113] Notably, the $bla_{\text{TEM-52}}$ reported by Marcadé *et al.*[111] was embedded in IncI1 plasmids.

The presence of IS*26* flanking bla_{SHV} was initially described in the early 1990s. In the first report of bla_{SHV}, IS*26* was identified as the mobilizing element for multiple resistance genes and provided a promoter for the expression of bla_{SHV} (Figure 3).[114] Intact copies of IS*26* have been reported in the plasmids or the chromosome of various bacterial species flanking bla_{SHV}, portions of its 5′ proximal termini or defective IS*26* elements.[115–118] Genes encoding SHV-type ESBLs can be found either in plasmids or the chromosome. Seven plasmid replicon types that predominantly carry bla_{SHV}-encoding ESBL enzymes—IncA/C, IncF, IncHI2, IncI1, IncL/M, IncN and IncX3—have been identified.[115,119] Different bla_{SHV} variants have been detected in each of these plasmid types, with the exception of IncX3, which has only been detected carrying $bla_{\text{SHV-12}}$.[115] Additionally, Billard-Pomares *et al.*[120] reported a $bla_{\text{SHV-2}}$-carrying *E. coli* where this gene was embedded in a P1 bacteriophage structure.

IS*Ecp1* has been identified upstream of several $bla_{\text{CTX-M}}$ types (Figure 3).[121] Lartigue *et al.*[122] observed an IS*Ecp1* upstream of the genes belonging to the CTX-M groups 1, 2, and 9. In another study, Eckert *et al.*[123] analysed the genetic environment of 28 isolates carrying 7 unique $bla_{\text{CTX-M}}$ types and observed IS*Ecp1* in 23 of them. Analysis of the sequences surrounding IS*Ecp1* and $bla_{\text{CTX-M}}$ types revealed signature sequences indicating that transposition events were responsible for the mobilization of $bla_{\text{CTX-M.}}$[121] Beyond promoting the dissemination of these genes, IS*Ecp*1 provided a strong promoter for the expression of $bla_{\text{CTX-M}}$.[121] IS*Ecp1* also has been detected flanking other β-lactamase genes, including KLU enzymes in the *Kluyvera* spp.[124]

In addition to IS*Ecp1*, $bla_{\text{CTX-M}}$ have been detected in the 3′ end of complex class 1 integrons between two *qacED1/sul1* elements.[123] The ESBL gene was not part of a gene cassette like the genes upstream of *qacED1/sul1*, but rather in all cases the ESBL gene was flanked upstream by *orf513*. This structure has been named IS*CR1* and was postulated to mobilize genes by rolling circle. Notably, *orf513* might function as a transposase that displays similarities to IS*91*-like transposases.[125] Structures harbouring $bla_{\text{CTX-M}}$, including combinations involving IS*26*, can be observed in several combinations, most likely due to the development of multiple recombination exchanges over time.[126,127]

Elements harbouring $bla_{\text{CTX-M}}$ are usually carried by conjugative plasmids. In a study evaluating CTX-M-15-producing isolates from seven countries located on four continents, Coque *et al.*[128]

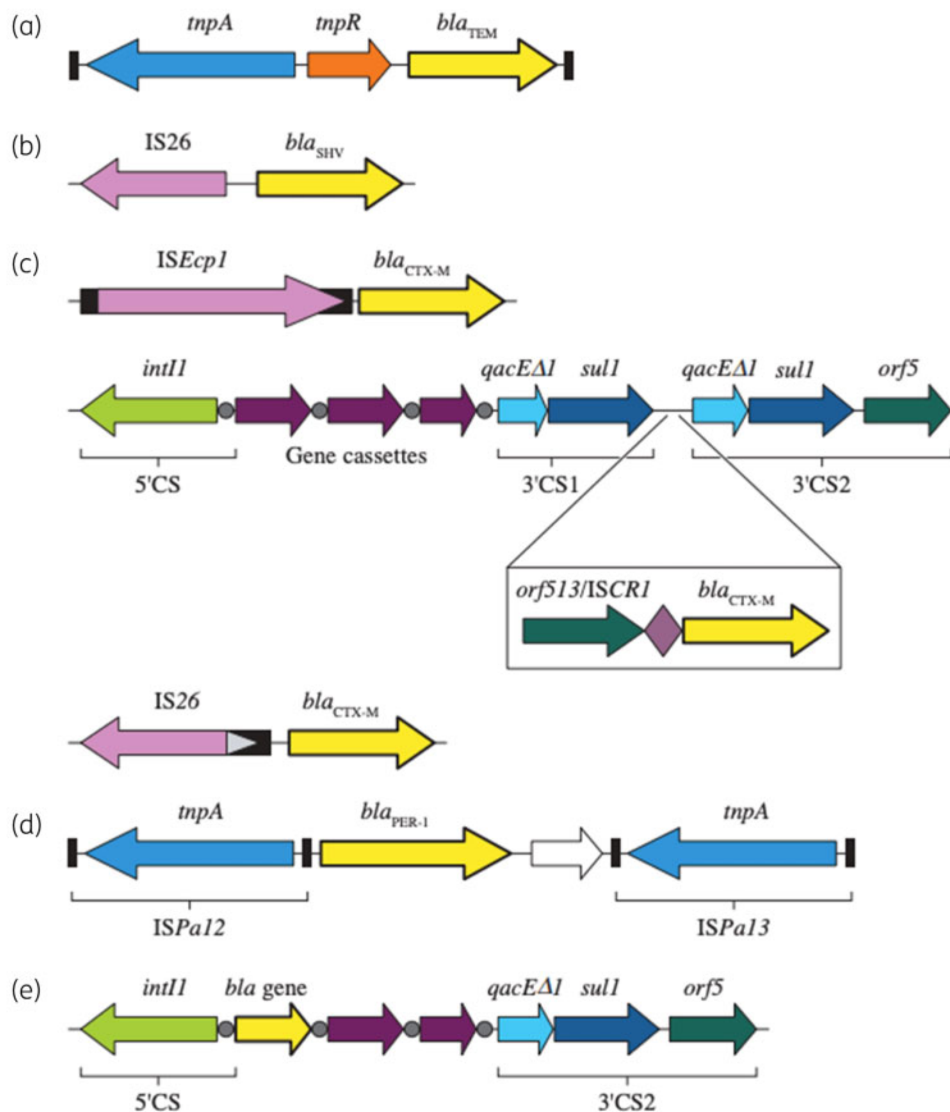

Figure 3. Genetic structures harbouring genes encoding ESBLs. Genetic structures most commonly reported to harbour (a) bla_{TEM}, (b) bla_{SHV}, (c) $bla_{CTX\text{-}M}$, (d) bla_{PER} or (e) class 1 integrons that can carry uncommon ESBL genes. Schematic representations were adapted from Rossolini *et al.*,[60,62] Poirel *et al.*[110] and Diestra *et al.*[62,110,127]

observed that $bla_{CTX\text{-}M\text{-}15}$ was embedded in the narrow host range plasmid IncF with replicon types FII alone or in association with FIA or FIB. Subsequent analysis demonstrated that this was true for various other isolates that harboured $bla_{CTX\text{-}M\text{-}15}$. The dissemination of $bla_{CTX\text{-}M}$ group 9 genes seems to be associated with an IncHI2-type plasmid, but there have also been reports of IncFII-types.[115] Other $bla_{CTX\text{-}M}$ carried various incompatibility-type plasmids, including narrow and broad range conjugative plasmids that may also carry additional resistance genes.

4.2 Common strain types for ESBL-producing isolates

MLST has been used extensively to track and monitor the spread of resistance determinants in bacterial pathogens. Several widely disseminated sequence types have been found in epidemics and outbreaks due to resistant clones that are highly associated with specific resistance mechanisms. Until the mid-2000s, it appeared that CTX-M enzymes spread in an seemingly random pattern, with no major clones responsible for their dissemination.[129] However, in the last two decades, the dissemination of CTX-M-producing enzymes has been mainly associated with spread of *E. coli* belonging to a new clonal group ST131.[130]

E. coli ST131 derives from the phylogenetic group B2 and serotype O25b: H4 and exhibits multiple virulence factors such as adhesins, siderophores, toxins and a group 2 capsule. ST131 isolates differ from most other MDR *E. coli* by being quite pathogenic.[131] *E. coli* belonging to ST131 causes a wide variety of infections, but is most commonly found in urinary tract infections including cystitis, pyelonephritis and urosepsis.[132]

E. coli ST131 isolates have been reported to carry a variety of β-lactamases and several CTX-M types, most commonly CTX-M-15.[133] Five groups (A through E) have been described according

to the virulence factors identified among *E. coli* ST131 isolates. These clones vary according to geographic region. Interestingly, virotypes A, B and over half of virotype C carry $bla_{CTX\text{-}M\text{-}15}$, whereas isolates from virotype D carry other β-lactamase genes, including $bla_{CTX\text{-}M}$ group 9 genes and $bla_{SHV\text{-}12}$.[131,134] In addition to $bla_{CTX\text{-}M\text{-}15}$, other characteristics of virotypes A, B and C include resistance to fluoroquinolones and the Type 1 fimbria gene *fimH30*.[130] This group also carries a ISL3-like transposase within its *fimH* gene. Typing *fimH* highlighted that the subgroups *H*30, *H*30-R, and *H*30-Rx are associated with MDR clones of *E. coli* ST131.[130] These groups seem to have evolved in a stepwise manner, first by acquiring fluoroquinolone resistance for *H*30-R and then incorporating $bla_{CTX\text{-}M\text{-}15}$ for the *H*30-Rx group.[130]

The occurrence of ST131 *E. coli* isolates carrying $bla_{CTX\text{-}M\text{-}15}$ have been well documented globally. In an early survey, Coque *et al.*[128] reported that ST131 *E. coli* isolates producing CTX-M-15 and belonging to ST131 were detected in all seven countries for which isolates were analysed. Among *E. coli* clinical isolates collected as part of the SENTRY and MYSTIC programmes in 2007, it was found that 54/127 (47.1%) isolates belonged to ST131.[135] These isolates were estimated to correspond to 17% of the overall isolates. Almost 70% of the ST131 isolates were resistant to fluoroquinolones or broad-spectrum cephalosporins that was mediated mainly by CTX-M-15.[135] Peirano *et al.*[133] reported that 46% of the ESBL-producing *E. coli* isolates collected in 11 Canadian hospitals belonged to ST131. Most of these isolates harboured $bla_{CTX\text{-}M\text{-}15}$, but other $bla_{CTX\text{-}M}$ types were also observed. More recently, Mendes *et al.*[136] reported that 53.6% of the bloodstream and 58.2% of the urinary tract infection isolates collected in US hospitals as part of the SENTRY programme belonged to ST131 or to clonal complex (CC) 131. These isolates were collected during 2016 in 36 US states and were screened using WGS after displaying elevated MIC values of ceftazidime, ceftriaxone, aztreonam or the carbapenems. A recent study from Colombia showed that *E. coli* isolates from patients with urinary tract infections that expressed CTX-M-15 all belonged to ST131 and the epidemic subclone O25b: H4-B2-*H*30-Rx.[137] The emergence of other *E. coli* and *K. pneumoniae* STs disseminating $bla_{CTX\text{-}M}$ genes has been recently documented, with $bla_{CTX\text{-}M\text{-}15}$ being the most prevalent.[138,139] Among these, ST1193 *E. coli* appears to have rapidly emerged worldwide.[140–143]

Among the less common ESBLs, GES- and VEB-encoding genes are usually gene cassettes within class I integron structures.[79,81,95] These structures can be mobilized as single genes and often are carried alongside other resistance genes that confer resistance to aminoglycosides, quinolones and/or trimethoprim/sulfamethoxazole. The genes encoding PER are a part of a composite transposons such as Tn*1213*, Tn*4176* and IS*CR*. TLA-1 and PME-1 are carried by IS*CR* structures.[104,115,144,145] Lastly, IS*26* has been detected flanking both ends of $bla_{BES\text{-}1}$ and $bla_{SFO\text{-}1}$.[115]

5. Epidemiology

In the early 2000s, reports suggested that CTX-M-producing isolates were becoming widespread in Europe, Latin America and the Asia-Pacific region.[129,146–148] Previously, TEM- and SHV-type enzymes had been the most predominant ESBLs worldwide.[149] Later, this shift in the ESBL population toward higher numbers of CTX-M-producing isolates was observed in the USA with two studies: first CTX-M-producing isolates were found in a single hospital and then these isolates were found in 80% of the hospitals participating in the MYSTIC surveillance programme, with CTX-M-15 and CTX-M-14 being the most prevalent types identified.[150,151] Since the year 2000, the incidence of ESBL infections has risen in the USA with an increase of 53% between 2012 and 2017, largely due to an increase in community-onset cases.[152]

Evaluating the epidemiology of ESBLs from a literature review is challenging. As studies use varying isolate selection criteria and a range of methodologies to detect genes, remarkable disparities in outcomes are generated. Unpublished data (M. Castanheira, JMI Laboratories) from the SENTRY Antimicrobial Surveillance Program demonstrated that among 22 548 non-carbapenem-resistant *E. coli* and *K. pneumoniae* clinical isolates consecutively collected in US hospitals, 3363 isolates exhibited an MIC value $\geq$2 mg/L for two of the following agents: ceftazidime, ceftriaxone or aztreonam. These isolates were screened for β-lactamases using previously described methods.[102,136] An ESBL gene was detected in 2059 (13.3%) *E. coli* and 836 (11.8%) *K. pneumoniae*. Of these, 92.5% carried CTX-M-encoding genes belonging to the CTX-M group 1 (70.0%) or CTX group 9 (22.8%). SHV genes encoding ESBL enzymes were noted among 8.6% of sequenced isolates, mostly in *K. pneumoniae* (6.5%). TEM ESBLs were only detected among 20 isolates, including 16 *E. coli* isolates. The prevalence of ESBLs can vary with geographical location, even within one country. A recent study of Gram-negative blood culture isolates taken across the USA showed an overall prevalence of 11% for $bla_{CTX\text{-}M}$, however the percentages ranged from 5% (Michigan) to 26% (Washington, DC).[153]

Among 15 449 non-CRE *E. coli* and *K. pneumoniae* clinical isolates collected as a part of the SENTRY programme in Europe, Asia-Pacific and Latin America, an ESBL gene was detected among 8.2%, 15.4% and 30.3% of the isolates, respectively (M. Castanheira, JMI Laboratories, unpublished data). These rates varied among individual countries (Figure 4). Similar to the scenario in the USA, most isolates carrying an ESBL gene from the Europe, the Asia-Pacific region and Latin America harboured a CTX-M gene (95.1%, 85.2%, and 98.1%, respectively). Genes belonging to CTX-M group 1 and CTX-M group 9 were the most common.

Canton and Coque[129] reported endemicity of CTX-M-producing isolates in various geographic areas. The authors highlighted a drastic increase among *E. coli* isolates producing CTX-M in the early 2000s. In their analysis, CTX-M-3 and CTX-M-15 were the most common genes detected among the CTX-M group 1 and CTX-M-9. Additionally, CTX-M-14 was most frequent gene observed among group 9. Livermore *et al.*[154] reported similar observations when evaluating several European countries. A literature search by Bevan *et al.*[149] revealed a significant increase in ESBLs in all of the WHO regions analysed. This increase in the prevalence of ESBLs was mainly caused by the dissemination of CTX-M genes. $bla_{CTX\text{-}M\text{-}15}$ was the most common gene in all regions except Latin America, where $bla_{CTX\text{-}M\text{-}2}$ was the most common gene observed. Among other groups, $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}27}$ have also spread globally.

A study evaluating the epidemiology of ESBL-producing *E. coli* in Spain demonstrated a decrease of TEM-producing isolates from $>$19% in 2000 to 1.2% in 2006.[155] These investigators also highlighted the dominance of CTX-M-producing *E. coli*, and noted that CTX-M-14 was the most common type of ESBL. Subsequent

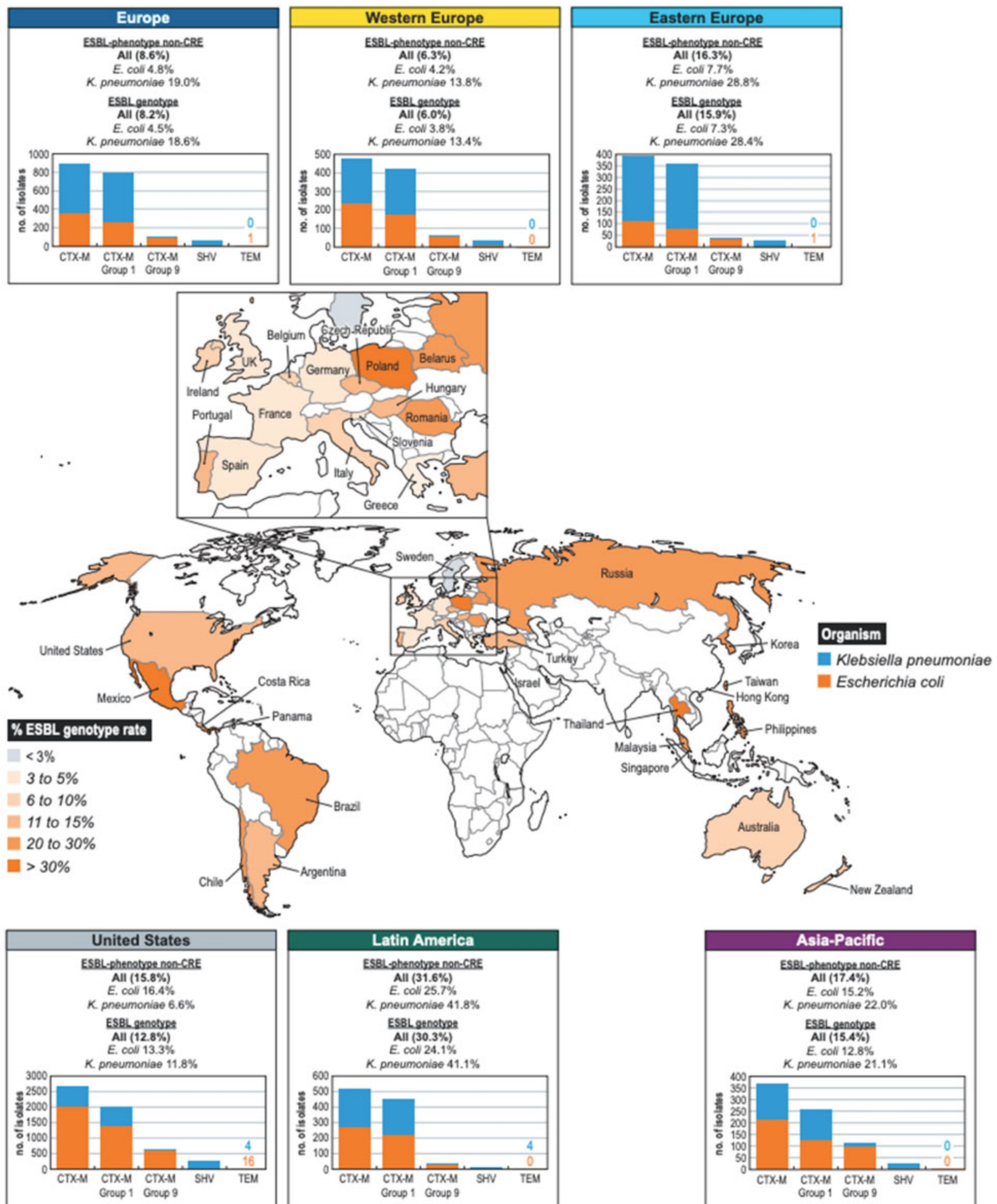

Figure 4. Distribution of CTX-M-, TEM- and SHV-producing isolates in the USA, Asia-Pacific, Europe and Latin America.

studies by the same group highlighted an increase of CTX-M-15-producing isolates that appeared to replace the CTX-M-14-producing population.[156,157] Rodriguez-Villalobos *et al.*[158] highlighted an increase in ESBL production and differences in ESBL types when comparing clinical isolates from 2008 to 2006. These authors evaluated Enterobacterales isolates collected in 118 Belgium clinical laboratories and noted that ESBL rates and CTX-M production increased in *E. coli*, *K. pneumoniae* and *E. cloacae*

isolates. Similar trends were not observed among *K. aerogenes*. Similarly, Peirano *et al.*[159] reported CTX-M enzymes replacing SHV-type ESBLs among *K. pneumoniae* when surveying Canadian isolates, which has also been observed in other regions.[159,160]

ESBLs are less common in *P. aeruginosa* than in isolates of Enterobacterales. Croughs *et al.*[161] evaluated 1528 *P. aeruginosa* isolates from referral hospitals in the Netherlands: 113 isolates displaying ceftazidime MIC values >8 mg/L were screened for ESBL genes and only 6 isolates (0.4% overall; 5.3% among ceftazidime-resistant isolates) possessed ESBLs. These Dutch ESBL-carrying *P. aeruginosa* isolates harboured $bla_{\text{TEM-12}}$ (2 isolates), $bla_{\text{VEB-2}}$ (2), $bla_{\text{BEL-1}}$ (1) and oxacillinase genes (2). Laudy *et al.*[162] reported that among 900 *P. aeruginosa* isolates recovered during 2010–14 from four hospitals in Poland, 99 carried (11.0%) ESBL genes. Among the ESBLs, 69 isolates had VEB-9 and 14 isolates had GES (6 with GES-1, 1 with GES-5, 5 with GES-13 and 2 with GES-15). In Brazil, CTX-M-2 was detected among 19.6% of carbapenem-resistant *P. aeruginosa* screened for ESBLs.[163] In this study, 2/56 isolates carried GES-encoding genes. In Greece, PER-1-producing *P. aeruginosa* isolates belonging to the international high-risk clonal complex 11 were identified among the isolates that displayed a ceftazidime MIC >8 mg/L and a positive ESBL phenotypic test.[94] Additionally, outbreaks of $bla_{\text{SHV-2a}}$-producing *P. aeruginosa* were described in France and Tunisia.[164,165] Data regarding *P. aeruginosa* producing of ESBLs in US isolates is scarce. The analysis of 155 *P. aeruginosa* isolates reported as part of a previous study evaluating resistance mechanisms against various anti-pseudomonal β-lactams revealed that only 3 (1.9%) isolates harboured ESBLs.[166]

ESBL-producing *A. baumannii* isolates have been described in specific locations and/or as part of outbreaks. ESBL genes that have been reported in *A. baumannii* include GES, VEB, PER, TEM and CTX-M-15, among others.[167–170] A study by Endimiani *et al.*[168] screened 407 *A. baumannii* isolates collected in an Italian hospital during a 7 year period for resistance to ceftazidime. Of the 119 that had MIC values >8 mg/L, 31 isolates were found to possess $bla_{\text{TEM-92}}$. In a study from Celenza *et al.*,[46] 150 *A. baumannii* isolates from a Bolivian hospital were screened for ESBLs and found that 106 carried $bla_{\text{CTX-M-2}}$, 32 carried $bla_{\text{CTX-43}}$ and 12 carried $bla_{\text{PER-2}}$. Many other studies highlight single occurrences or groups of isolates harbouring PER, VEB and GES in *A. baumannii* isolates suggesting these are the most common ESBLs in this species.[170–172]

The dissemination of ESBL-producing bacterial pathogens is likely due to many factors such as geographical location, population density, hygiene and usage of antibiotics. For example, the prevalence of ESBLs in *E. coli* is low in Europe but is very high in Southeast Asia, Africa, and Central America.[173] There is even country to country variation with regions. For example, the prevalence of Enterobacterales expressing ESBLs is higher in Mediterranean countries but is very low in the Netherlands and Scandinavia.[174] Today, our global society is quite mobile, whether it be as vacationers, medical tourists or refugees. All of these factors have contributed to outbreaks and the overall global dissemination of ESBL-mediated resistance.

The epidemiology of ESBL-mediated resistance primarily follows the type of infections where the pathogen encountered often require heavy usage of expanded-spectrum β-lactam antibiotics. For ESBL-producing Enterobacterales, the main source of these pathogens is the genitourinary tract of patients, with infections most often caused by strains with which the patient is already colonized.[175] The transmission of ESBL-producing Enterobacterales can occur between patients with or without the involvement of a healthcare worker as an intermediate vector. The rate of transmission is also likely to vary based on differences in various species due to differences in virulence factors. The transmission rate from patients colonized with ESBL-producing *K. pneumoniae* was shown to be 2-fold higher than from those colonized with ESBL-producing *E. coli*.[176]

Initially, ESBL-producing clinical isolates were found only in the hospital setting, however, they quickly spread into nursing homes and then into the community.[4,21] Although most outbreaks of ESBL-producing Enterobacterales occur in the ICU or in immunocompromised patients, other patient populations can also be affected. In Japan, there was an outbreak of ESBL-producing *E. coli* in neonates that was traced to shared breast milk from donor mothers.[177] A 2017 survey of US hospital infections caused by ESBL-producing *E. coli* and *K. pneumoniae* showed that the rates of these infections were increasing.[178]

Both TEM- and SHV-type ESBLs were detected throughout the USA and Europe in the late 1980s and 1990s with specific variants noted to have variations in regional prevalence.[154,179,180] For example, TEM-10 was identified in several unrelated outbreaks of ESBL-producing Enterobacterales in the USA, but was rarely seen in Europe.[21] The prevalence of both TEM- and SHV-type ESBLs has now diminished at the same time as the worldwide dominance of isolates producing CTX-M-type β-lactamases has occurred.[181] ESBLs have also been reported from many environmental, food and veterinary samples.[182]

6. Detection of ESBL-producing Gram-negative organisms in clinical microbiology laboratories

Detection of ESBL-producing Enterobacterales has traditionally relied on phenotypic methods for detection in clinical microbiology laboratories. These methods exploit the fact that ESBLs are inhibited by traditional β-lactamase inhibitors such as clavulanate. Both CLSI and EUCAST have endorsed screening and confirmatory tests for detection of ESBL producers and guidance on use of these tests varies based on the cephalosporin breakpoints applied by the laboratory.[183,184] Although the methods described by these standards setting organizations are similar, differences exist in the recommended organisms to test, screening and confirmatory test methods and interpretations (Table 2). These ESBL methods require overnight incubation and have known limitations that affect both sensitivity (e.g. false negatives due to the co-production of an AmpC β-lactamase) and specificity (e.g. false positivity due to hyperproduction of narrower-spectrum β-lactamases combined with altered permeability). Commercially available automated antimicrobial susceptibility testing systems have adopted comparable built-in ESBL screening and confirmation tests on their panels. However, these systems are known to report false positive ESBL results and some lack US FDA clearance for *P. mirabilis* due to poor performance.[185–187]

Over a decade ago, both CLSI and EUCAST lowered the cephalosporin breakpoints to increase the sensitivity of identifying

Table 2. ESBL screen and confirmatory tests as recommended by CLSI and EUCAST

Criteria	CLSI	EUCAST
Organisms	*E. coli, K. oxytoca, K. pneumoniae* and *P. mirabilis*	Group 1: *E. coli, Klebsiella* spp. [not including *Klebsiella* (formerly *Enterobacter*) *aerogenes*], *P. mirabilis*, *Raoultella* spp., *Salmonella* spp. and *Shigella* spp. Group 2 (Enterobacterales with inducible chromosomal AmpC): *Enterobacter* spp., *Citrobacter freundii, Morganella morganii, Providencia stuartii*, *Serratia* spp., *Hafnia alvei*
Screening test methods	Disc diffusion and BMD methods	Broth dilution, agar dilution or disc diffusion
Screening agents and cutoffs	Aztreonam, cefotaxime, ceftazidime and ceftriaxone MIC of ≥2 mg/L Cefpodoxime MIC of ≥2 mg/L for *P. mirabilis* or MIC ≥8 mg/L for *E. coli, K. pneumoniae* and *K. oxytoca*	Cefpodoxime, cefotaxime, ceftazidime and ceftriaxone MIC of ≥2 mg/L
Positive screening results	Either (i) cefpodoxime alone Or (ii) aztreonam (excluding *P. mirabilis*), cefotaxime, ceftazidime or ceftriaxone screen positive	Either (i) cefpodoxime alone Or (ii) cefotaxime or ceftriaxone AND ceftazidime screen positive
Confirmatory test methods	Disc diffusion and BMD methods	CDT, DDST, ESBL gradient test and BMD test
Test	Ceftazidime and cefotaxime ± clavulanate	Group 1: Ceftazidime and cefotaxime ± clavulanate; add cefepime ± clavulanate if cefoxitin has been tested and has an MIC of ≥16 mg/L Group 2: Cefepime ± clavulanate
Positive interpretation	Disc diffusion: ≥5 mm increase in zone diameter for either agent tested in combination with clavulanate versus the zone diameter of the agent tested alone	CDT: Same interpretation as the CLSI disc diffusion test
		DDST: Zones of inhibition around cephalosporin discs are augmented or there is a keyhole in the direction of the disc containing clavulanate
	BMD: ≥3 2-fold concentration decreases in an MIC for either agent tested in combination with clavulanate versus the MIC of the agent tested alone	BMD: ≥8-fold reduction is observed in the MIC of the cephalosporin combined with clavulanate compared with the MIC of the cephalosporin alone Gradient diffusion: The same as above for BMD or if a phantom zone or deformed ellipse is present
Reporting cephalosporin results for ESBL-producing isolates		
use of obsolete cephalosporin breakpoints	Report all penicillins, cephalosporins and aztreonam as resistant	
use of current cephalosporin breakpoints	Report the MICs and interpretations as tested	

BMD, broth microdilution; CDT, combination disc test; DDST, double-disc synergy test.
Adapted from CLSI[183] and EUCAST.[184]

ESBL-producing organisms, to decrease the burden confirmatory testing placed on microbiology laboratories and because of updated pharmacokinetics (PK)/pharmacodynamics (PD) data, MIC distributions and limited clinical outcome data suggesting improved patient outcomes with lower breakpoints.[183,188] With the lowering of the breakpoints, they revised the recommendations to perform ESBL confirmatory tests for epidemiological or infection control purposes only.[183,189] Based on this guidance, many laboratories updated their cephalosporin breakpoints and stopped performing routine ESBL confirmatory testing. As such, the MICs and interpretations are reported as tested for the penicillins, cephalosporins and aztreonam without identifying the mechanism leading to third-generation cephalosporin resistance.

The MERINO trial was the first randomized clinical trial comparing the outcomes of patients receiving piperacillin/tazobactam and meropenem for the treatment of presumed ESBL-producing bloodstream infections.[190] The original analyses found inferior outcomes for patients treated with piperacillin/tazobactam (although they were later modified due to inaccurate susceptibility testing). This has led to a renewed interest in understanding the role of ESBL tests in clinical practice. In the absence of ESBL confirmation testing, some clinicians and more recently the IDSA antimicrobial

resistance treatment guidance recommended using a ceftriaxone MIC ≥2 mg/L (not susceptible) as a proxy for predicting ESBL production to guide treatment-based decisions.[191,192] In one study, the use of a ceftriaxone MIC ≥2 mg/L to predict ESBL production resulted in overestimation of ESBL production due to a less than ideal specificity which led to increased prescribing of carbapenems.[193] Thus, further guidance may be on the horizon for use of ESBL tests to not only guide infection control practices but to identify ESBL producers to help guide therapeutic decision-making. It remains controversial whether ESBL testing should occur or not, although international thought leaders agree that if an accurate, timely and comprehensive ESBL test was available it could be helpful in clinical decision-making.[194–196]

In contrast to traditional phenotypic ESBL tests, rapid phenotypic methods have more recently been developed with same day results for the detection of ESBL producers including colorimetric and immunological lateral flow assays. The rapid colorimetric methods provide results within 15 min to 2 h and include methods that specifically detect ESBL producers [Rapid ESBL NDP or the Rosco Diagnostica Rapid ESBL Screen (Taastrup, Denmark)] or more broadly detect ESBL, AmpC and carbapenemases without distinction due to cleavage of an expanded-spectrum chromogenic cephalosporin (β Lacta Test; Bio-Rad, Marnes-La-Coquette, France).[197] These tests have been evaluated from cultured isolates and directly from various specimen types (e.g. blood, urine, respiratory specimens) with good sensitivity (>90%) and variable specificity depending on the test.[197–201] The rapid calorimetric tests detect a phenotype broadly associated with ESBL production without discriminating between the various enzymes. Recently, a lateral flow immunoassay (NG-Test CTX-M MULTI assay, NG Biotech, Guipry, France) was developed to detect and differentiate the five groups of CTX-M enzymes (i.e. groups 1, 2, 8, 9, 25) from colonies and from positive blood cultures within 15 min with excellent sensitivity and specificity (≥98%).[202,203]

In addition to phenotypic methods, molecular methods that target specific ESBL genes have been developed and implemented in clinical microbiology laboratories. The most widely adopted include the commercially available syndromic sepsis panels performed from positive blood culture broths that include CTX-M as the sole ESBL target associated with the detection of the Enterobacterales, *P. aeruginosa* and/or *A. baumannii*. The detection of the globally dominant ESBL gene helps with more rapid selection of appropriate therapy. These rapid panels (1 to 4 h) have good sensitivity and specificity for detection of $bla_{\text{CTX-M}}$ and include the GenMark Dx ePlex® Blood Culture Identification Gram-Negative (BCID-GN) Panel (Carlsbad, CA, USA), BioFire BCID2 Panel (Salt Lake City, UT, USA) and the Verigene Gram-Negative Blood Culture Nucleic Acid Test BC-GN panel (Austin, TX, USA).[204,205] More recently, multiplex pneumonia panels that include $bla_{\text{CTX-M}}$ as a marker have been introduced, including the Unyvero LRT panel (Curetis, Holzgerlingen, Germany) and BioFire FilmArray pneumonia panel (Salt Lake City, UT, USA), for detection directly from respiratory specimens. The Unyvero LRT demonstrated 95.7% sensitivity for the detection of $bla_{\text{CTX-M}}$ from bronchoalveolar lavage (BAL) specimens, whereas evaluation of the BioFire pneumonia panel showed 85.7% (6/7 specimens) and 80% (8/10 specimens) sensitivity from BAL and sputum specimens, respectively.[206,207] The lack of inclusion of TEM- and SHV-type ESBL variants on these panels likely reflect the challenge that only a few single nucleotide polymorphisms differentiate narrower-spectrum variants from ESBL variants. However, future molecular diagnostic panels would benefit from the inclusion of SHV- and/or TEM-type ESBL targets as geographic and species-specific differences occur in the distribution of ESBL genes as highlighted in the epidemiology section of this review.[208,209] Last, several research use only molecular assays such as DNA microarray assays, PCR and/or sequencing and WGS have been described for the characterization of ESBL genes and/or variants.[210] However, they are not broadly implemented in clinical laboratories due to the complexity of the methods. The limitations of molecular methods include the expense, requirement of instrumentation and/or requirement of highly trained staff with molecular and/or bioinformatics expertise.

7. Current therapies

The presence of ESBL-producing Enterobacterales in serious infections has had a significant impact on the choice of empirical antimicrobial therapy and is associated with a delay in the initiation of appropriate therapy.[211] The failure to initiate appropriate antibiotic therapy from the start is associated with prolonged hospital stays, increasing hospital costs and higher patient mortality. This has led to the increased use of carbapenems in many institutions, which has subsequently resulted in increased resistance to carbapenems.[5,6] There are few randomized controlled clinical trials that study the treatment of infections due to ESBL-producing bacteria. However, there have been a number of observational studies such as retrospective cohorts, case series and anecdotal reports that examine different treatment regimens for infections caused by ESBL-producing organisms. Several of these studies have been focused on the β-lactam/β-lactamase inhibitor (BL/BLI) combinations amoxicillin/clavulanate and piperacillin/tazobactam.[212–214] Each of these retrospective examinations of clinical data concluded that retrospective data that included patients with urosepsis and other bloodstream infections (BSI), BL/BLI combinations were non-inferior to the carbapenems and could be used as carbapenem-sparing therapy. In another study, the INCREMENT project developed a scoring tool to predict whether or not a patient was at high or low risk for mortality following a BSI caused by an ESBL-producing organism.[215] This study collected data on over 1000 patients from 7 tertiary hospitals in 11 different countries. With regards to therapy, they found that when the isolates demonstrated *in vitro* susceptibility to BL/BLI combinations (mainly piperacillin/tazobactam), aminoglycosides (mainly amikacin) or fluoroquinolones (mainly ciprofloxacin), these agents appeared to be as effective as the carbapenems for both the empirical and targeted therapy.

In 2018 the results of the MERINO trial—an international, non-inferiority, open-label randomized controlled study that compared piperacillin/tazobactam (4.5 g every 6 h) with meropenem (1 g every 8 h) for the treatment of BSIs due to cephalosporin-resistant Enterobacterales—were published.[190] The trial included 379 patients in 26 hospitals in 9 countries. The results showed that BSI patients infected with ceftriaxone-resistant *E. coli* or *K. pneumoniae,* treatment with piperacillin/tazobactam was not shown to be non-inferior when compared with meropenem with the endpoint of 30 day mortality. The authors concluded that piperacillin/tazobactam should not be used in this patient population.[190] This study received a great deal of attention with many

interpreting the results to mean that no infections caused by any ESBL-positive pathogen, regardless of body site, should be treated with piperacillin/tazobactam. As a consequence, there has been a large increase in the usage of carbapenems to treat ESBLs. Unfortunately, there were many limitations of the study design and subsequent analysis. For example, empirical treatments were allowed prior to randomization, acceptable therapies for both empirical and step-down were not pre-specified, crossover of patients from one group to the other was allowed, and there was an unusually low mortality of patients in the meropenem arm of the study.[211] In addition, it was an open-labelled trial; piperacillin/tazobactam was administered with a 30 min infusion, even though the current recommendations are for extended infusion for serious infections, the trial was prematurely stopped, and the imbalance of groups for some variables might not have been corrected in the analysis, and finally, the endpoint of all-cause mortality was skewed as most of them were unrelated to the infection but occurred mostly in patients with advanced cancer.[216]

From a microbiology standpoint, the trial included piperacillin/tazobactam resistant organisms, but none for meropenem and although the authors claim that there was no correlation of MIC with mortality, no MIC by outcome data were provided. Furthermore, susceptibility tests were conducted using the Vitek automated susceptibility testing system, disc diffusion or gradient strip devices at the site lab, then confirmed by in a central lab using Etest strips. Unfortunately, none of these is the standard reference method. This is especially important to note, as Etest strips had previously been noted to be unreliable for testing piperacillin/tazobactam.[217]

To address some of the concerns with regards to the microbiological data from the MERINO trial, the authors then performed a post hoc analysis of MIC values and resistance genes detected comparative to the 30 day mortality of patients treated with both piperacillin/tazobactam and meropenem.[218] MICs of both test drugs for all organisms isolated from BSI in the MERINO trial were retested using the reference method of broth microdilution and all of the β-lactamase genes present were determined. In retesting, they found that a significant number of isolates that had previously been reported as susceptible to piperacillin/tazobactam were non-susceptible (i.e. intermediate or resistant). This, in turn, had a significant impact on the 30 day all-cause mortality endpoint for the trial, as when patients infected with these non-susceptible isolates were removed from the analysis, the absolute difference from meropenem was reduced. The authors found that a piperacillin/tazobactam MIC of >16 mg/L was the strongest predictor of mortality. Many of the piperacillin/tazobactam-resistant isolates expressed AmpC or OXA-1 in addition to the ESBL, which are not expected to be susceptible to piperacillin/tazobactam. Therefore, the previous conclusion that all ceftriaxone-resistant organisms should be treated with carbapenems is not valid. The authors revised their conclusion to the recommendation of allowing susceptibility testing (reference method) to guide therapy with piperacillin/tazobactam for ESBL-producing strains.[218] Despite these findings and the revised mortality assessment, the authors of the MERINO trial continue to be proponents of the notion that all infections caused by ceftriaxone-resistant organisms (and by association ESBL-positive) should be treated with a carbapenem.[219] Other experts have concluded that there has been an overinterpretation of the MERINO trial results, which has caused an overuse of carbapenems and potentially contributed to the dramatic increase of carbapenem-resistant organisms.[216] They agree that patients with severe or difficult-to-treat infections caused by ceftriaxone-resistant Enterobacterales should be treated with carbapenems. However, they believe that many infections can be safely treated with other options if therapy is guided by strong microbiological data. Clinicians should carefully weigh both arguments as they navigate the management of these increasingly common infections.[220]

Some of the new BL/BLI combinations may be suitable therapies for ESBL-producing organisms. In randomized clinical trials, ceftolozane/tazobactam showed comparable efficacy to levofloxacin (cUTI) or meropenem (cIAI) against ESBL-producing Enterobacterales.[221] A recent meta-analysis of five randomized controlled trials with ESBL- and AmpC-specific outcome data showed that of the 246 patients infected with an ESBL-producing pathogen in the ceftazidime/avibactam treatment arm 91% had a favourable clinical response at test of cure compared with 89% in the carbapenem arm.[222] The authors warned that this dataset largely consisted of patients with cUTI or cIAI, therefore caution should be taken before extrapolating to more serious infections. Although ESBL-specific clinical experience in the literature is scarce, the carbapenem-based BL/BLI combinations of meropenem/vaborbactam and imipenem/relebactam show *in vitro* activity against ESBL-producing strains.[223]

With regards to choosing therapy for less severe infections such as community-onset urinary tract infections due to ESBL-producing Enterobacterales, agents such as ciprofloxacin, amoxicillin/clavulanate, nitrofurantoin and fosfomycin show good *in vitro* activity against ESBL-producing bacteria and may be good options.[211] However, resistance is also increasing among these isolates, therefore susceptibility testing is essential for guiding therapy.

8. Conclusions

Antimicrobial resistance is a significant problem worldwide that has been an unwelcome result of modern medical care. ESBL-producing Enterobacterales remain the most commonly encountered mechanism providing resistance to expanded-spectrum cephalosporins in these pathogens, both in healthcare and community settings. ESBLs have spread in virulent clones such as *E. coli* ST131. With today's modern technologies, the detection and molecular characterization of ESBLs has become commonplace. However, with so many ESBL variants that are often produced in combination with other β-lactamases, decoding this information is not always easy. Unfortunately, appropriate therapy is frequently delayed in these patients, who then may suffer clinical outcomes. Although there have been recent debates about whether a carbapenem must be used to treat serious infections caused by ESBLs or whether some of the BL/BLI combinations are appropriate, the fact remains that we have at our disposal a good armamentarium to treat these infections. As ESBLs are now endemic in clinical isolates of Enterobacterales, it will continue to be essential that we remain vigilant about identifying them both in patient isolates and in surveillance.

Transparency declarations

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Amplyx Pharma, Artugen Therapeutics USA Inc., Astellas, Basilea, Beth Israel Deaconess Medical Center, BIDMC, bioMérieux Inc., BioVersys Ag, Bugworks, Cidara, Cipla, Contrafect, Cormedix, Crestone Inc., Curza, CXC7, Entasis, Fedora Pharmaceutical, Fimbrion Therapeutics, Fox Chase, GlaxoSmithKline, Guardian Therapeutics, Hardy Diagnostics, IHMA, Janssen Research & Development, Johnson & Johnson, Kaleido Biosciences, KBP Biosciences, Luminex, Matrivax, Mayo Clinic, Medpace, Meiji Seika Pharma Co. Ltd, Melinta, Menarini, Merck, Meridian Bioscience Inc., Micromyx, MicuRx, N8 Medical, Nabriva, NIH, National University of Singapore, North Bristol NHS Trust, Novome Biotechnologies, Paratek, Pfizer, Prokaryotics Inc., QPEX Biopharma, Rhode Island Hospital, RIHML, Roche, Roivant, Salvat, Scynexis, SeLux Diagnostics, Shionogi, Specific Diagnostics, Spero, SuperTrans Medical LT, T2 Biosystems, The University of Queensland, Thermo Fisher Scientific, Tufts Medical Center, Universite de Sherbrooke, University of Iowa, University of Iowa Hospitals and Clinics, University of Wisconsin, UNT System College of Pharmacy, URMC, UT Southwestern, VenatoRx, Viosera Therapeutics, and Wayne State University. There are no speakers' bureaus or stock options to declare.

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