

Extended-spectrum β -Lactamases: Implications for the Clinical Laboratory and Therapy

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Production of extended-spectrum β -lactamase (ESBL) is one of the most important resistance mechanisms that hamper the antimicrobial treatment of infections caused by *Enterobacteriaceae*. ESBLs are classified into several groups according to their amino-acid sequence homology. While TEM and SHV enzymes were the most common ESBLs in the 1990s, CTX-M enzymes have spread rapidly among *Enterobacteriaceae* in the past decade. In addition, some epidemiological studies showed that organisms producing CTX-M enzymes had become increasingly prevalent in the community setting in certain areas in the world. Several novel enzymes with hydrolyzing activity against oxyimino-cephalosporins, albeit with additional enzymatic characteristics different from those of original TEM and SHV ESBLs (e.g., inhibitor-resistance), have been discovered and pose a problem on the definition of ESBLs. Although several methods to detect the production of ESBL are available in clinical laboratories, existence of other factors contributing resistance against β -lactams, e.g., inducible production of Amp-C β -lactamase by some species of *Enterobacteriaceae*, or inhibitor-resistance in some ESBLs may hinder the detection of ESBLs with these methods. Carbapenems are stable against hydrolyzing activity of ESBLs and are regarded as the drug of choice for the treatment of infections caused by ESBL-producing *Enterobacteriaceae*. Although several other antimicrobial agents, such as fluoroquinolones and cephamycins, may have some role in the treatment of mild infections due to those organisms, clinical data that warrant the use of antimicrobial agents other than carbapenems in the treatment of serious infections due to those organisms are scarce for now. (*Korean J Lab Med* 2008;28:401-12)

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INTRODUCTION

β -Lactamase production is the cardinal mechanism of resistance to β -lactams in gram-negative organisms. Some of them produce chromosomal β -lactamases, whether constitutively or inducibly. For instance, *Klebsiella pneumoniae* produces class A β -lactamase constitutively, whereas *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia* spp., and *Pseudomonas aeruginosa* pro-

duce inducible class C β -lactamase [1]. On the other hand, plasmid-mediated β -lactamases have become prevalent among gram-negative bacteria during the past 50 yr. The first plasmid-mediated β -lactamase in gram-negative bacteria, TEM-1, was described in the early 1960s [2]. Carried by transposons on plasmids, the TEM-1 genes have spread to several bacterial species and are now distributed around the world. SHV-1 is another frequently encountered plasmid-mediated β -lactamase among gram-negative bacteria.

The first plasmid-mediated β -lactamase capable of hydrolyzing extended-spectrum cephalosporins, now known as SHV-2, was reported in 1983 [3] and a number of other groups of β -lactamases with expanded hydrolytic activity were reported thereafter. The term 'extended-spectrum β -lactamases (ESBLs)' was applied to denote these enzymes

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with activity against extended-spectrum cephalosporins. Sequencing of the encoding genes revealed that most ESBLs described in the 1980s were progenies of the above-mentioned TEM and SHV enzymes. Amino acid sequences of TEM and SHV enzymes are listed on the website dedicated to the nomenclature of β -lactamases hosted by George Jacoby and Karen Bush [4] together with other enzymes (e.g., OXA, CTX-M, CMY, IMP, VIM, and KPC).

In 1989, a clinical isolate of *E. coli* that produced a non-TEM, non-SHV ESBL was reported. The enzyme was designated CTX-M-1, indicating its preferential hydrolytic activity against cefotaxime [5]. Although spread of CTX-M-producing isolates appeared to be limited to specific areas in the world during the 1990s, this situation has changed during the past decade. Recent epidemiological studies of ESBL-producing bacteria demonstrated a dramatic increase in the prevalence of CTX-M enzymes globally [6–8].

DEFINITION OF ESBL

β -Lactamases are commonly classified according to two general schemes: the Ambler molecular classification and the Bush-Jacoby-Medeiros functional classification [9, 10]. The Ambler scheme classifies β -lactamases into four classes according to the protein homology of enzymes. β -Lactamases of class A, C, and D are serine β -lactamase and class B enzymes are metallo- β -lactamases. The Bush-Jacoby-Medeiros functional scheme is based on functional properties of enzymes, i.e., the substrate and inhibitor profiles.

The term ‘extended-spectrum β -lactamses’ was originally applied to the TEM and SHV derivatives that can hydrolyze oxyimino-cephalosporins, and these enzymes were classified as group 2be with the Bush-Jacoby-Medeiros functional scheme. The 2be designation consists of ‘2b’ denoting that the enzyme is derived from a 2b enzyme (e.g., SHV-1, TEM-1, and TEM-2) and ‘e’ representing the ‘extended spectrum of activity’. The definition of extended spectrum of activity is to have a hydrolytic activity against oxyimino-cephalosporins or aztreonam at more than 10% of that against benzylpenicillin. 2be enzymes cannot hydrolyze cephamycins or carbapenems efficiently and

are inhibited by β -lactamase inhibitors such as clavulanate. TEM-ESBLs and SHV-ESBLs belong to class A in the Ambler scheme.

Discovery of several novel enzymes have blurred the original definition of ESBLs [11]. First, several β -lactamses with activity similar to those of TEM and SHV ESBLs, albeit with different origin, have been reported (e.g., CTX-M). Second, some TEM mutants, e.g., TEM-7, and TEM-12, have only slightly increased hydrolytic activity against oxyimino-cephalosporins and do not meet the strict definition of ESBL mentioned above. Third, several enzymes not classified into class A have a hydrolytic profile similar to that of 2be enzymes. For example, some OXA derivatives have broader hydrolytic profiles than their parent enzymes and can confer resistance to oxyimino-cephalosporins.

Typical class C enzymes can confer resistance to oxyimino-cephalosporins if they are hyperproduced as a consequence of mutational derepression [12] or are expressed constitutively on plasmid [13]. Class C enzymes are resistant to the inhibition by β -lactamase inhibitors and are not deemed a member of ESBLs. Recently, AmpC mutants with increased hydrolytic activity against cefepime and ceftipime (extended-spectrum cephalosporinases) have been reported. Although it has been determined that such mutants were mainly located on bacterial chromosome [14–16], two plasmid-mediated extended-spectrum cephalosporinases, CMY-19 [17] and CMY-10 [18], have been reported. Some specialists may insist on regarding such enzymes as ESBLs because of their wide spectrum of activity.

Class A β -lactamases that can hydrolyze carbapenems have been reported (e.g., KPC, NMC/IMI, and SME) [19]. Most of these enzymes also hydrolyze oxyimino-cephalosporins. From the clinical point of view, it is not practical to categorize these enzymes as ESBLs because carbapenems are regarded as the drug of choice for ESBL-producing organisms. GES enzymes pose a more difficult problem on this matter. GES-1 possesses hydrolytic activity similar to the classic class A ESBLs, is inhibited by β -lactamase inhibitors [20], and is generally classified into ESBL. However, some of the GES variants, such as GES-2 and 4, also have hydrolytic activity against carbapenems [21, 22].

ESBL TYPES

1. SHV

SHV-1 is a β -lactamase with activity against penicillins and narrow-spectrum cephalosporins such as cephalothin and cephaloridine [23]. Although *bla*_{SHV-1} and related genes are integrated into the bacterial chromosome in most isolates of *K. pneumoniae* [1, 24], SHV-1 is also common as a plasmid-mediated β -lactamase among gram-negative bacteria.

The first plasmid-mediated resistance mechanism for oxyimino-cephalosporins was demonstrated in clinical isolates of *K. pneumoniae*, *Klebsiella ozaenae*, and *S. marcescens* in 1983 [3]. The new enzyme was designated SHV-2 because of a significant homology between the gene encoding new enzyme and *bla*_{SHV-1} [25]. Sequencing of the structural genes showed that the difference between two enzymes was only one amino-acid substitution of Gly238Ser. A number of SHV variants with ESBL activity have been described thereafter [26]. Most of them have a Gly238Ser substitution in common. In addition, a number of variants related to SHV-5 also have a Glu240Lys substitution. Ser-238 is crucial for cefotaxime hydrolysis whereas additional Glu240Lys substitution increases the hydrolytic activity against ceftazidime [27].

It was suggested that *bla*_{SHV} originated from the chromosome of *K. pneumoniae* and an IS26 element played a role in the mobilization of *bla*_{SHV} to plasmid [28]. Indeed, some reports illustrated the presence of *bla*_{SHV-5} between two IS26 elements together with the sequence identical to part of the *K. pneumoniae* chromosome [29, 30].

2. TEM

TEM-1, first reported in 1965 from an *Escherichia coli* isolate, has substrate and inhibition profiles similar to those of SHV-1 [2]. Ampicillin resistance in clinical isolates of *E. coli* is due to the production of TEM-1 in most instances. TEM-2 has a single amino acid substitution Gln39Lys from TEM-1 and has an almost identical hydrolytic profile with TEM-1. It only differs from TEM-1 by having a different

isoelectric point [31].

In 1987, *K. pneumoniae* isolates exhibiting resistance to multiple antibiotics including oxyimino-cephalosporins were detected and the β -lactamase produced by these isolates was designated as CTX-1 referring to its hydrolytic activity against cefotaxime [32]. Sequencing of the gene encoding the enzyme revealed that the enzyme was related to TEM-2 and differed by two amino acids from its parent enzyme: Lys for Glu at position 102 and Ser for Gly at position of 236 [33]. Since the first TEM variant was reported, more than 150 TEM-type β -lactamases have been described. Most of these enzymes have ESBL activity, whereas other TEM variants reveal the characteristics of inhibitor-resistant β -lactamases [34]. Mutations in several key amino acid residues (e.g., Glu104Lys, Arg164Ser, Gly238Ser, and Glu-240Lys) are important for ESBL activity, similar to those observed in SHV ESBLs. Although inhibitor-resistant TEM enzymes generally do not have a substantial activity against oxyimino-cephalosporins, a few enzymes have hydrolytic activity against oxyimino-cephalosporins together with inhibitor resistance [34]. These enzymes are referred to as complex mutants of TEM (CMT). A CMT enzyme possesses both of the amino acid substitutions observed in TEM-ESBLs and those observed in inhibitor-resistant TEMs. For example, TEM-125, a CMT enzyme reported recently, combines the amino acid substitutions of TEM-12 (ESBL) and those of inhibitor-resistant TEM-39 [35]. CMT-type β -lactamases poses a challenge in detection of ESBLs in clinical laboratories because phenotypic methods used in detection of ESBLs depend on the inhibition of ESBLs by β -lactamase inhibitors such as clavulanate, which is absent in CMT-type β -lactamases.

3. CTX-M

In 1989, a clinical *E. coli* isolate that produced a non-TEM, non-SHV ESBL was recovered and the enzyme was designated CTX-M-1, denoting its hydrolytic activity against cefotaxime [36]. The amino acid sequence of β -lactamase from clinical *E. coli* isolate MEN exhibiting resistance phenotype similar to the *E. coli* isolate producing CTX-M-1

enzyme was determined and the enzyme was designated MEN-1 in 1992 [37]. In the same year, a new plasmid-mediated cefotaximase, designated CTX-M-2, with an isoelectric point different from that of CTX-M-1, was described from multidrug-resistant *Salmonella enterica* serovar Typhimurium [38]. In 1995, Ishii et al. [39] reported a novel enzyme, Toho-1 (renamed as CTX-M-44 later), which was highly homologous to MEN-1. In the following year, nucleotide sequencing studies concluded that the deduced amino acid sequence of CTX-M-1 was identical to the reported sequence of MEN-1, and the amino acid sequence of CTX-M-2 was 84% identical to that of CTX-M-1 [40]. In addition, Toho-1 was found to be more closely related to CTX-M-2 than to CTX-M-1. To date, more than 80 CTX-M enzymes have been described [4]. They are divided into five subgroups, namely CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25, according to the similarity of their amino acid sequences.

The origin of the CTX-M enzymes is different from that of TEM and SHV ESBLs. While SHV-ESBLs and TEM-ESBLs were generated by amino acid substitutions of their parent enzymes, CTX-M ESBLs were acquired by the horizontal gene transfer from other bacteria using genetic apparatuses such as conjugative plasmid or transposon. The gene sequences encoding CTX-M enzymes show a high similarity to those of β -lactamases of *Kluyvera* species. In addition, the gene sequences adjacent to the CTX-M genes of *Enterobacteriaceae* are also similar to those surrounding the β -lactamase genes on the chromosomes of *Kluyvera* species [41–44]. Thus, it is considered that the CTX-M-1 and CTX-M-2 subgroups originate from the chromosomal β -lactamase of *Kluyvera ascorbata*, while the CTX-M-8 and CTX-M-9 subgroups are derived from the chromosomal β -lactamase of *Kluyvera georgiana*.

Two genetic elements have been demonstrated to be involved in the mobilization of *bla*_{CTX-M}: insertion sequence *ISEcp1* and the *ISCR1* element (formerly recognized as CR1 element or orf513) [6–8]. *ISEcp1* has been found upstream of several CTX-M genes belonging to the CTX-M-1, CTX-M-2, CTX-M-9, and CTX-M-25 subgroups. *ISEcp1* belongs to the *ISI380* family and is capable of mobilizing the neigh-

boring genes by one-ended transposition mechanism. A recent *in vitro* study showed that the mobilization of *bla*_{CTX-M-2} from *K. ascorbata* to *E. coli* was achievable in the presence of *ISEcp1* [45]. Genes encoding the CTX-M-2 subgroup and the CTX-M-9 subgroup have also been observed within *ISCR1* associated with class 1 integron. It has been shown that both *ISEcp1* and *ISCR1* provide promoter sequences for high-level expression of CTX-M enzymes [46, 47]. Additionally, a recent study demonstrated the presence of a phage-related sequence immediately upstream of *bla*_{CTX-M-10} in several CTX-M-10-producing isolates [48], suggesting that bacteriophage might be involved in an acquisition of *bla*_{CTX-M-10}.

Organisms harboring CTX-M enzymes are resistant to cefotaxime, whereas they often appear to be susceptible to ceftazidime *in vitro*. Additionally, most CTX-M enzymes hydrolyze cefepime effectively and MIC values of cefepime for bacteria producing CTX-M enzymes tend to be higher than those for bacteria producing other types of ESBLs [49]. Analysis of the crystal structure of CTX-M enzymes has revealed that the active sites of CTX-M enzymes resemble those of narrow-spectrum TEM and SHV enzymes (e.g., TEM-1, SHV-1) and are not large enough to recognize ceftazidime, which is larger than cefotaxime [50, 51]. However, substitution of several amino acids improves the activity of CTX-M enzyme against ceftazidime. Substitutions of Asp240 and Pro167 are known to lead to such alteration in a hydrolytic profile. Asp240Gly substitution appears to increase the flexibility of B3 β -strand allowing an increase in the activity against ceftazidime [50]. Mutation at Pro167 in the omega-loop modifies the interaction between β -lactams and the binding sites as well [52].

β -Lactamase inhibitors such as sulbactam, clavulanate, and tazobactam are generally known as inactivators of class A ESBLs. Interestingly, CTX-M-14 is capable of hydrolyzing sulbactam, while clavulanate and tazobactam retain their ability to inactivate this enzyme [53]. Toho-1 also possesses a similar hydrolytic activity against sulbactam [51].

Although organisms producing TEM-type and SHV-type ESBLs are identified mainly from hospitalized patients, a growing number of infections caused by CTX-M produc-

ing organisms in the community setting have recently been reported [54]. The causative organisms in this situation have been mainly *E. coli* recovered from patients suffering from urinary tract infections. Because ESBL-producing organisms are also often resistant to fluoroquinolones and sulfonamides, this phenomenon has a potential implication in how clinicians empirically manage community-acquired urinary tract infections.

4. GES

GES-1 was initially described in a *K. pneumoniae* isolate from a neonatal patient just transferred to France from French Guiana [20]. GES-1 has hydrolytic activity against penicillins and extended-spectrum cephalosporins, but not against cephamycins or carbapenems, and is inhibited by β -lactamase inhibitors. These enzymatic properties resemble those of other class A ESBLs; thus, GES-1 was recognized as a member of ESBLs.

However, a Gly170 substitution inside the omega-loop appears to alter the substrate profile of the enzyme. GES-2, which has Gly170Asn substitution compared with GES-1, has an increased activity against imipenem and decreased activity against oxyimino-cephalosporins [21]. In addition, GES-2 is only weakly inhibited by β -lactamase inhibitors. GES-4, which has a Gly170Ser substitution compared with GES-3, is also capable of hydrolyzing carbapenems and weakly inhibited by β -lactamase inhibitors. Furthermore, GES-4 extends its hydrolytic activity towards cephamycins [22].

5. OXA

OXA β -lactamases are classified into class D in the Ambler scheme and were placed in group 2d in the Bush-Jacoby-Medeiros functional scheme. OXA enzymes have more than 50% hydrolytic activity against cloxacillin or oxacillin compared with that against benzylpenicillin and variable inhibition profile by β -lactamase inhibitors [55]. Although most OXA β -lactamases have only negligible activity against oxyimino-cephalosporins, OXA-10 and its derivatives (OXA-

11, OXA-14, OXA-16, and OXA-17), OXA-13 and its derivatives (OXA-19 and OXA-32), and some other OXA enzymes (e.g., OXA-18 and OXA-45) have varying degrees of activity against oxyimino-cephalosporins [56, 57]. These enzymes are regarded as OXA-type ESBLs and have been discovered mainly in *Pseudomonas aeruginosa* isolates.

6. Other ESBLs

A number of other groups of β -lactamases capable of hydrolyzing extended-spectrum cephalosporins have been reported. VEB, PER, BEL, BES, TLA, SFO, and IBC are examples of such enzymes and details of these enzymes are reviewed elsewhere [56].

DETECTION OF ESBLs IN CLINICAL LABORATORIES

Detection of organisms harboring ESBLs provides clinicians with helpful information. Treatment of infections caused by ESBL-producing organisms with extended-spectrum cephalosporins or aztreonam may result in treatment failure even when the causative organisms appear to be susceptible to these antimicrobial agents by routine susceptibility testing [57, 58]. In addition, patients colonized or infected with ESBL-producing organism should be placed under contact precautions to avoid hospital transmission [59]. These benefits warrant the detection of ESBL-producing organisms in clinical laboratories. On the other hand, revision of cephalosporin breakpoints has been achieved by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and is under way by the Clinical and Laboratory Standards Institute (CLSI) for better prediction of clinical outcome by MIC values [60]. It is still controversial whether this revision might allow clinical laboratories to dispense with ESBL detection [57, 60].

Since the 1980s, several phenotypic tests for detection of ESBL-producing organisms have been developed. All methods utilize the characteristics of ESBLs: conferring a reduced susceptibility to extended-spectrum cephalosporins and inhibition by clavulanate. Detection of ESBL produc-

tion by organisms with inducible chromosomal AmpC β -lactamase is difficult using these methods because AmpC β -lactamase resists inhibition by clavulanate. In addition, clavulanate may act as an inducer of chromosomal AmpC β -lactamases of these organisms [61].

CLSI RECOMMENDED METHOD

The CLSI recommends screening *E. coli*, *K. pneumoniae*, and *Klebsiella oxytoca* (and *Proteus mirabilis*, if clinically relevant such as bacteremic isolates) for potential production of ESBL. The CLSI method for ESBL detection consists of the Initial Screen Test and the Phenotypic Confirmatory Test [62].

Susceptibilities to more than one of cefpodoxime, ceftazidime, ceftriaxone, cefotaxime, and aztreonam are evaluated using disk diffusion or broth dilution method in the Initial Screen Test. A decrease in susceptibilities to one or more antibiotics tested may indicate production of ESBLs and warrant performance of the subsequent Phenotypic Confirmatory Test. It should be noted that MIC values lower than the breakpoints for susceptibility are applied for the Initial Screen Test. For example, MIC ≥ 2 $\mu\text{g}/\text{mL}$ for ceftazidime is regarded as positive in Initial Screen Test, whereas MIC ≤ 8 $\mu\text{g}/\text{mL}$ is the susceptible range for *Enterobacteriaceae*.

In the Phenotypic Confirmatory Test, susceptibilities to

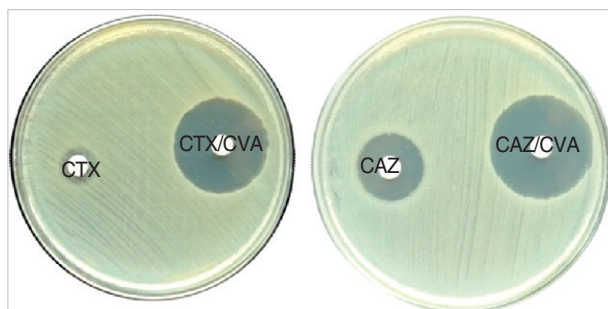


Fig. 1. Positive CLSI recommended method for ESBL producing strains. The inhibition zone around the CTX/CVA disk is apparently larger than that around the CTX disk, indicating ESBL production. Comparable result is obtained with CAZ disk and CAZ/CVA disk. Note that the inhibition zone diameter around the CAZ disk is within the susceptible range (≥ 18 mm). Adherence to the protocol and the use of both CTX and CAZ disks are crucial for the highly sensitive detection of ESBL production. Abbreviations: CTX, cefotaxime; CAZ, ceftazidime; CVA, clavulanic acid.

cefotaxime and ceftazidime alone, and those with clavulanate are compared using disk diffusion or broth dilution method (Fig. 1). If the susceptibility of either antibiotic tested increases significantly (a ≥ 5 mm increase in a zone diameter or a ≥ 3 two-fold decrease in an MIC) in the presence of clavulanate, the result is interpreted as confirmatory of ESBL production. It is important to perform confirmatory tests using both ceftazidime and cefotaxime to improve the sensitivity of the test. A report suggests that the use of ceftazidime alone leads to oversight of production of CTX-M type ESBLs [63].

DOUBLE DISK SYNERGY TEST

Double disk synergy test (DDST) was the first proposed testing method for phenotypic detection of ESBL-producing organisms [64]. DDST is performed on an agar plate with a disk containing cefotaxime (30 μg) and a disk containing amoxicillin/clavulanate (20 $\mu\text{g}/10$ μg , respectively), placed 30 mm apart (center to center). Extension of the inhibition zone around the cefotaxime disk towards the amoxicillin/clavulanate disk indicates production of ESBL. Disks containing other oxyimino- β -lactams (ceftriaxone, ceftazidime, or aztreonam) can be substituted for cefotaxime disk and performance of this test using multiple oxyimino-cephalosporins improves the sensitivity of DDST in the same way as observed in the CLSI method.

If the result of DDST is negative despite the high suspicion of ESBL production, adjustment of disk spacing is advised. Application of the disks closer to each other significantly improves the sensitivity of DDST [65, 66]. In addition, the use of cefepime instead of third-generation cephalosporins improves the sensitivity of DDST when it is applied for AmpC-producing *Enterobacteriaceae* (e.g., *Enterobacter* spp, *K. pneumoniae* producing plasmid-mediated AmpC β -lactamase)[66]. This phenomenon is plausible because cefepime is stable against hydrolysis by most AmpC β -lactamases.

ETEST FOR ESBLs

The Etest ESBL TZ/TZL (AB biodisk, Solna, Sweden) is a

plastic drug-impregnated strip with one side containing a concentration gradient of ceftazidime (0.5–32 $\mu\text{g}/\text{mL}$) and the other side containing a concentration gradient of ceftazidime (0.064–4 $\mu\text{g}/\text{mL}$) plus a constant concentration of clavulanate (4 $\mu\text{g}/\text{mL}$). Similar strips impregnated with cefotaxime/clavulanate (CT/CTL) or cefepime/clavulanate (PM/PML) are now also available. The manufacturer recommends the use of both TZ/TZL and CT/CTL strips for confirmation of ESBL production. PM/PML strip may be useful in the confirmation of ESBL production of organisms that produce an inducible chromosomal AmpC β -lactamase [67]. The result of Etest ESBL is interpreted as positive when a ≥ 3 two-fold decrease in the MIC value of the tested drug is observed in the presence of clavulanate.

The presence of “phantom zone” below the CTL, TZL, or PML gradient and ellipse deformation at the tapered end also indicate ESBL production (Fig. 2). When mutant colonies are observed in the inhibition zone, the MIC value should be determined by reading the drug concentration at which mutant colonies are completely inhibited. If these rules are

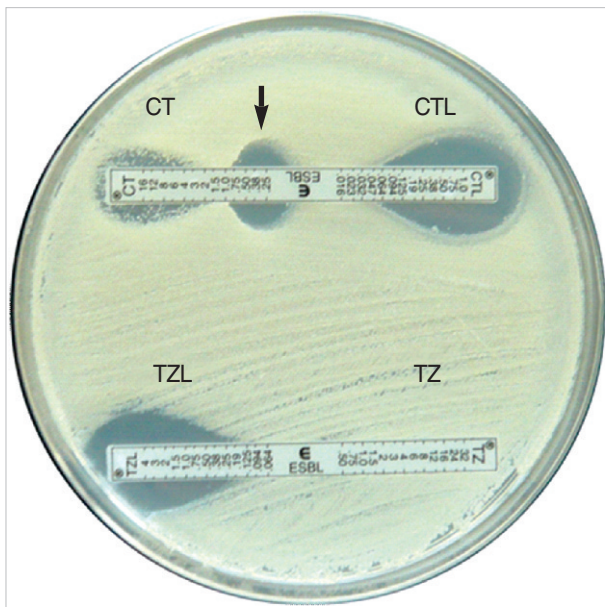


Fig. 2. Positive Etest ESBL test. MICs are CT >16 mg/L, CTL 0.064 mg/L, TZ >32 $\mu\text{g}/\text{mL}$, and TZL 0.25 $\mu\text{g}/\text{mL}$. ESBL production is suggested by a ≥ 3 two-fold reduction of MIC value in the presence of clavulanate. In addition, ‘phantom zone’ is observed below the CT gradient (arrow).

Abbreviations: CT, cefotaxime; CTL, cefotaxime/clavulanate; TZ, ceftazidime; TZL, ceftazidime/clavulanate.

not followed, a high rate of discrepancy between the MICs obtained by experts and those by laboratory technicians may occur [68].

AUTOMATED METHOD

VITEK 2 (bioMérieux, Marcy l’Etoile, France) and Phoenix (Becton Dickinson, Sparks, MD, USA) are automated systems that include ESBL detection test. In both systems, specific panels designed for ESBL confirmation are available and expert systems with which values interpreted are obtained. If production of ESBL is inferred by the expert system, susceptibilities to all penicillins, cephalosporins, and aztreonam are displayed as resistant irrespective of MIC values obtained for these drugs.

Several studies have evaluated the performance of these automated systems in detecting ESBL-producing organisms [68–73]. These studies employed a variety of study designs and showed variable results. For example, several studies used only *E. coli* and *Klebsiella* spp. as test organisms, whereas others included species with chromosomal AmpC β -lactamases production.

DETECTION METHOD WITH BORONIC ACID

Boronic acid is known to inhibit the activity of AmpC β -lactamases, and several studies have reported its utility in the detection of organisms producing both AmpC β -lactamase and ESBL. Addition of 3-aminophenyl boronic acid on antibiotic-containing disks (cefotaxime/ceftazidime with or without clavulanate) has been reported to potentiate the sensitivity and specificity of the combination disk method when this method is applied to organisms with chromosomal [74] or plasmid-mediated [75] AmpC production.

TREATMENT OF INFECTIONS CAUSED BY ESBL-PRODUCING ORGANISMS

Antibiotic choices for infections caused by ESBL-producing organisms are limited [57, 76]. Treatment of these infections with cephalosporins (except for cephamycins) has

been associated with poor clinical outcomes, even if the causative organisms appeared to be susceptible to the antibiotics *in vitro* [58]. Furthermore, ESBL-producing isolates tend to show a high rate of resistance to various other classes of antibiotics such as fluoroquinolones [77–79] and aminoglycosides [77].

Carbapenems (e.g., imipenem and meropenem) are regarded as the drug of choice in treating serious infections caused by ESBL-producing organisms. Carbapenems are stable against hydrolytic activity of ESBLs and treatment with carbapenems showed a significantly better clinical outcome than that with other antibiotics in a prospective observational study involving 85 episodes of ESBL-producing *K. pneumoniae* bacteremia in 12 hospitals in 7 countries [77].

As noted above, treatment of infections due to ESBL-producing organisms with cephalosporins is not generally recommended irrespective of the result of susceptibility testings *in vitro*. Although organisms producing TEM or SHV-type ESBL generally appear susceptible to cefepime, the inoculum effect (i.e., an increase of MIC value with higher inoculum) has been observed [80, 81]. This phenomenon may relate to the inconsistency between the susceptibility *in vitro* and the clinical outcome.

Because most CTX-M enzymes have only a weak activity against ceftazidime, whether this antibiotic is effective against CTX-M producing organisms or not is a matter of debate. Bin et al. [82] showed the efficacy of ceftazidime comparable to that of imipenem/cilastatin in the treatment of bloodstream infection caused by CTX-M producing *E. coli* isolates in a prospective, observational study involving 22 patients. However, further evidence derived from larger studies is necessary to confirm the efficacy of ceftazidime in this situation. This issue would be important for avoiding excessive use of carbapenems if community-acquired CTX-M-type ESBL infections become more common.

Cephamycins (e.g., cefoxitin and cefotetan) are stable to the hydrolytic activity of ESBLs. However, a decrease in the expression of outer membrane protein may occur during the treatment of ESBL-producing organisms and results in resistance to cephamycins [74]. Inducible or constitutive

production of AmpC β -lactamase also leads to resistance to cephamycins. Thus, cephamycins are not recommended as first-line therapy for serious infections caused by ESBL-producing organisms.

β -Lactam/ β -lactamase inhibitor combinations (e.g., amoxicillin/clavulanate and piperacillin/tazobactam) often retain activity against ESBL-producing organisms, but coexistence of other resistance mechanism may lead to the resistance to these drugs. Moreover, the inoculum effect with piperacillin/tazobactam was observed among ESBL-producing isolates (mainly SHV-type) in one study [80].

Fluoroquinolones may be useful for the therapy for mild infections if the causative organisms are susceptible *in vitro*. However, observational studies investigating whether fluoroquinolones are as effective as carbapenems for treating infections caused by ESBL-producing organisms have shown conflicting results [83, 84]. In the study that showed inferiority of treatment with fluoroquinolones [83], the mean fluoroquinolone MICs for responsible organisms were close to the susceptibility breakpoint and suboptimal drug concentrations of the drug in the infected tissue may have caused treatment failure. Treatment with fluoroquinolones of urinary tract infection without bacteremia is relatively safer than that of bacteremia because of the very high drug concentrations achieved in the urine.

CONCLUDING REMARKS

Since the first description of plasmid-mediated ESBL in 1983, ESBL-producing gram-negative organisms have posed a significant threat to hospitalized patients due to their hydrolyzing activity against extended-spectrum cephalosporins, which are often employed in the treatment of hospital-acquired infections. Several methods have been introduced to reliably detect ESBL-producing organisms so that effective antibiotic treatment may be offered and appropriate infection control measures implemented. However, coexistence of multiple resistance mechanisms (e.g., production of ESBL and AmpC β -lactamase simultaneously) can hinder the detection of ESBL with these methods. Furthermore, newly recognized ESBLs that have enzymatic

profiles different from those of TEM-type or SHV-type ESBLs may challenge the definition of ESBL and the clinical strategy in dealing with them. Although introduction of novel detection methods or employment of different strategies, e.g., alteration of susceptibility breakpoints, may solve these problems to some extent, their utility should be carefully evaluated in future studies.

In the past decade CTX-M-type ESBLs have become prevalent globally and their distribution involves not only healthcare environments but also the community. Community-acquired infections due to ESBL-producing organisms pose a serious challenge to clinicians in choosing appropriate empiric therapy. The incidence of such infections is currently low, but we have to pay attention to the trend.

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