Overview of the Epidemiological Profile and Laboratory Detection of Extended-Spectrum β-Lactamases

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Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated bacterial enzymes that confer resistance to a broad range of β-lactams. They are descended by genetic mutation from native β-lactamases found in gram-negative bacteria, especially infectious strains of *Escherichia coli* and *Klebsiella* species. Genetic sequence modifications have broadened the substrate specificity of the enzymes to include third-generation cephalosporins, such as ceftazidime. Because ESBL-producing strains are resistant to a wide variety of commonly used antimicrobials, their proliferation poses a serious global health concern that has complicated treatment strategies for a growing number of hospitalized patients. Another resistance mechanism, also common to Enterobacteriaceae, results from the overproduction of chromosomal or plasmid-derived AmpC β-lactamases. These organisms share an antimicrobial resistance pattern similar to that of ESBL-producing organisms, with the prominent exception that, unlike most ESBLs, AmpC enzymes are not inhibited by clavulanate and similar β-lactamase inhibitors. Recent technological improvements in testing and in the development of uniform standards for both ESBL detection and confirmatory testing promise to make accurate identification of ESBL-producing organisms more accessible to clinical laboratories.

Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated bacterial enzymes that are able to hydrolyze a wide variety of penicillins and cephalosporins. Most ESBLs have evolved by genetic mutation from native β-lactamases, particularly TEM-1, TEM-2, and SHV-1. These parent enzymes are commonly found in gram-negative bacteria, particularly Enterobacteriaceae [1]; they are highly active against penicillins and modestly active against early-generation cephalosporins [2]. The genetic mutations that give rise to ESBLs broaden the parental resistance pattern to a phenotype that includes resistance to third-generation cephalosporins (e.g., cefotaxime and ceftazidime) and monobactams (e.g., aztreonam) [3]. In general, ESBL-producing isolates remain susceptible to cephamycins (e.g., cefoxitin) and carbapenems [3]. Nevertheless, their resistance to a wide variety of common antimicrobials has made the proliferation of ESBL-producing strains a serious global health concern that has complicated treatment strategies for a growing number of patients. In this context, routine screening for ESBL-producing organisms is of great importance. Unfortunately, the overall adherence to routine screening among diagnostic microbiology laboratories is relatively low. Efforts are now under way to improve this situation.

ESBLS: CLASSIFICATION AND PROPERTIES

Although ESBLs have been reported most frequently in *Escherichia coli* and *Klebsiella* species [2], they have been found in other bacterial species as well, including *Salmonella enterica, Pseudomonas aeruginosa,* and *Serratia marcescens* [4–6]. The first definitively characterized ESBL, TEM-3 (cefotaxime-hydrolyzing enzyme type 1), was discovered in *Klebsiella pneumoniae* isolates recovered from intensive care unit patients in France [7].

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Since that initial report, TEM-type enzymes have become the most abundant class of ESBLs, with >100 genetic variants now reported [1].

A limited number of mutations are sufficient to convert a parental β-lactamase into an ESBL; TEM-3 is illustrative of the process. It is a plasmid-mediated β-lactamase with a complex resistance phenotype toward antibiotics. The amino acid sequence of TEM-3 differs from that of its parent, TEM-2, by substitutions at just 2 positions [8]. In general, the mutations that give rise to ESBLs tend to be clustered at discrete foci within the nucleotide sequence. There are at least 4 "hot spots" in the coding sequence of TEM-1, where specific amino acid substitutions in TEM-1, or in a descendant of TEM-1, contribute to the ESBL phenotype [1].

Members of the SHV family of β-lactamases trace their descent to SHV-1, a plasmid-encoded enzyme that confers to *K. pneumoniae* high levels of resistance against ampicillin [1]. With >50 unique genetic variants, there are significantly fewer SHV-type β-lactamases than there are enzymes of the TEM type [1]. The earliest reported ESBL belonging to the SHV family (SHV-2) differs from SHV-1 by a single amino acid, a glycine-to-serine substitution at position 213 [9]. Comparing the sequences of the SHV family of ESBLs reveals that the amino acid changes that give rise to the extended-spectrum phenotype are confined to relatively few regions of the enzyme [1]. Two substitutions in particular are important for determining the specificity of SHV-type β-lactamases. Both occur within the catalytic site of the enzyme: one substitution (serine 238 for glycine) is important for degrading cefotaxime, whereas the other substitution (lysine 240 for glutamate in combination with serine 238) strongly increases activity against ceftazidime [10].

On the basis of characterizations of numerous β-lactamases, a classification scheme devised by Bush, Jacoby, and Medeiros [2] assigns most ESBLs to group 2be (table 1)—that is, β-lactamases that are inhibited by clavulanic acid, which can hydrolyze penicillins, narrow- and extended-spectrum cephalosporins, and monobactams [2]. Although susceptibility to β-lactamase inhibitors is a defining property of ESBLs, there are several examples of enzymes derived from TEM and SHV that have a resistance spectrum similar to that of ESBLs but are resistant to inhibitors [1].

In addition to the TEM- and SHV-types, 2 other classes of ESBLs have been identified (table 1). The cefotaxime-hydrolyzing (CTX-M)–type β-lactamases are carried on plasmids and have been found in *Klebsiella* species [11], *Salmonella typhimurium,* and *E. coli* [1, 13]. These enzymes are not closely related to TEM and SHV β-lactamases [1]. Instead, they show a very high degree of sequence homology with the chromosome-encoded AmpC-type β-lactamase of *Kluyvera georgiana,* suggesting that the CTX-M–type β-lactamases might represent genetic variants descended from the β-lactamase of *Kluyvera* species [14]. The CTX-M enzymes show a preference for hydrolyzing cefotaxime, and members of the class are susceptible to inhibition by clavulanate, sulbactam, and tazobactam [1, 15, 16].

The oxacillin-hydrolyzing (OXA)–type β-lactamases are unique among the ESBLs because they are most often found in *P. aeruginosa,* rather than in members of the Enterobacteriaceae [1]. In the Bush-Jacoby-Medeiros classification scheme, the OXA enzymes are assigned to group 2d, apart from most other ESBLs [2]. Their preferred substrates are penicillins and cloxacillin [17, 18], rather than third-generation cephalosporins. The OXA class of ESBLs exhibits appreciable diversity in the properties of its enzymes. For example, although most OXA-type enzymes are resistant to β-lactamase inhibitors [1, 17, 18], OXA-18 has been reported to be totally inhibited by clavulanic acid [19]. Although most OXA-type β-lactamases have significant activity against ceftazidime, OXA-17 has little effect on the MIC of ceftazidime, but has substantial activity against cefotaxime [18].

Selection of ESBLs. Generally speaking, the emergence of ESBLs has been tied to the overuse and misuse of third-generation cephalosporins and other antimicrobials. However, the emergence of one ESBL variant over another at a given medical center can be the result of a complex set of factors, with an-

Table 1. Properties of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC.

Type	Bush-Jacoby-Medeiros group	Preferred substrates	Resistance or susceptibility to β-lactamase inhibitors	Major sources
TEM, SHV	2be	Penicillins, narrow- and extended-spectrum cephalosporin, and monobactams	Susceptible	*Escherichia coli* and *Klebsiella pneumoniae*
Cefotaxime hydrolyzing	…	Penicillins and cefotaxime	Susceptible	*Salmonella enterica, E. coli,* and *K. pneumoniae*
Oxacillin hydrolyzing	2d	Penicillins and cloxacillin	Resistant (except oxacillin hydrolyzing–18)	*Pseudomonas aeruginosa*
Plasmid-mediated AmpC	1	Penicillins and cephalosporins	Resistant	*K. pneumoniae, Klebsiella oxytoca, Salmonella* species, and *Proteus mirabilis*

NOTE. Data are from [1, 2, 11, 12].

tibiotic use a contributing, although not necessarily a determining, factor. The diverse nature of ESBLs, with different enzymes having substrate preferences among the oxyimino-β-lactams, means that selection pressure should favor those ESBLs that are highly active against β-lactams and that are currently used at a center [20]. Reports of ceftazidime-resistant isolates found in hospitals that use high levels of this third-generation cephalosporin illustrate this phenomenon.

At a chronic-care facility in Massachusetts, resistance to ceftazidime resulted from 2 ESBLs, one of which was TEM-26, which has particularly high activity against ceftazidime [21]. A nosocomial outbreak of ceftazidime-resistant *K. pneumoniae* at a large New York hospital was reported to have occurred at a time when the use of ceftazidime had increased. The outbreak was attributed to strains carrying TEM-10 or TEM-26 and, at its peak, the incidence of resistant *K. pneumoniae* isolates was >17% [22]. Curtailing the use of third-generation and older cephalosporins resulted in a dramatic reduction in the hospital-wide prevalence of ceftazidime-resistant *K. pneumoniae* [23]. At the same time, characterization of isolates revealed that the TEM-26 enzyme, which had been associated with resistance to ceftazidime during the outbreak, was now lacking in most *K. pneumoniae* strains. Hence, removal of selective pressure by drug class restriction led to the disappearance of ESBL-producing strains, an observation that is consistent with the fact that in vitro passage of ceftazidime-resistant strains on antibiotic-free media also led to the loss of ceftazidime-degrading enzymes [24].

The scenario in which 1 or 2 ESBL variants are selected as having high activity against a single, high-volume β-lactam is not necessarily the most likely outcome at every center. This is because, in most cases, a new β-lactam is introduced while previous ones continue to be used, making the selection milieu more diverse rather than more focused and restrictive. Blazquez et al. [25] have suggested that a mutation that gives rise to an ESBL-producing strain capable of very efficient hydrolysis of a new β-lactam may, at the same time, undermine the ability of that strain to efficiently degrade older substrates that remain in use. Selection pressure would, therefore, more likely favor mutations or combinations of mutations that give rise to ESBLs capable of adapting to highly fluctuating chemical environments, such as those in most health care facilities.

This proposal is consistent with several observations. For example, the broadening of the resistance phenotype seen in association with the development of ESBLs is often accompanied by an increase in susceptibility to penicillin. Penicillinase activity for several TEM- and SHV-producing strains is reported to be ⩽10% than the activity of strains making the parental TEM-1, TEM-2, or SHV-1 enzymes. When exposed to a complex antimicrobial environment, an ESBL with this spectrum of activity might never be selected for its high activity against extended-spectrum cephalosporins, because the producing strain is vulnerable to penicillins. Selection of ESBLs with a broad-based resistance to antimicrobials may also explain why, of the many enzymes generated in the laboratory by site-directed mutagenesis, relatively few have ever been observed to occur naturally.

Epidemiological profile. Because ESBL-producing strains often arise in focal outbreaks, their prevalence can vary greatly from one site to another and even over time for a given site. As a result, regional and local estimates are probably more useful to clinical decision-making than are more-global assessments. A further caveat when reviewing prevalence data is that different criteria are used to determine whether an organism produces ESBL. However, organisms that test positive for ESBL at screening might possess a resistance mechanism other than ESBL production. Further observation of a significant reduction in the MIC of a screening substrate in the presence of clavulanate is needed to confirm that an isolate is an ESBL producer and to exclude the likelihood that resistance is due to the presence of AmpC-type β-lactamase or to the permeability effects that result from changes in outer membrane porins.

Ongoing large-scale surveillance studies, such as the SENTRY Antimicrobial Surveillance Program, have been a source of useful data regarding the regional prevalence of ESBL-producing isolates. As indicated in table 2 [26–29], the estimated prevalence of ESBL-producing strains of *E. coli* and *K. pneumoniae* in the United States is relatively low. In contrast, the prevalence of ESBL-producing *K. pneumoniae* is high in the Asia-Pacific region and in Latin America. Data from Latin America suggest that the presence of ESBL-producing strains of *K. pneumoniae* has increased dramatically in recent years [27].

The proportion of putative ESBL-producing isolates that are confirmed as such can vary according to region. Jones et al. [26] tested almost 2800 *Klebsiella* species isolates recovered from >30 US medical centers from 1997 through 2000. They found that 6%–7% of *Klebsiella* isolates met the criteria for a potential ESBL-producing phenotype. After confirmatory testing, only approximately half of the isolates showed resistance to clavulanate-inhibitable cephalosporin, confirming the ESBL phenotype (table 2) [26–29]. In a survey of ~2000 *E. coli* and *K. pneumoniae* isolates from the Asia-Pacific region, a relatively larger proportion of potential ESBL producers were confirmed [28]. The issue of the predictive value of screening for the ESBL phenotype was addressed in a study by Winokur et al. [29]. Using a subset of *K. pneumoniae* isolates recovered in 1997–1998 and contributed to the SENTRY program, they tested for evidence of ESBL production by use of clavulanic acid enhancement as the criterion. They found noteworthy variation in the proportion of confirmed isolates. Among North American isolates that exhibited an ESBL phenotype on screening, 43% were confirmed as ESBL producers; for isolates from Latin

Table 2. SENTRY Antimicrobial Surveillance Program: regional prevalence of *Escherichia coli* and *Klebsiella* species that produce extended-spectrum β-lactamase (ESBL).

Study, location, year(s)	Organism (no. of isolates tested)	Prevalence by ESBL status, %	
		Presumptive[a]	Confirmed[b]
Jones et al. [26], United States, 1997–2000	*Klebsiella* species (2768)	6–7	~3
Gales et al. [27], Latin America			
1997–1999[c]	*E. coli* (801)	4–6	…
	Klebsiella species (166)	29–32	…
2000[c]	*E. coli* (320)	5	…
	Klebsiella species (61)	44–46	…
Bell et al. [28], Asia/Pacific and South Africa, 1998–1999	*Escherichia coli* (1377)	10	7
	Klebsiella pneumoniae (678)	25	22
Winokur et al. [29], Europe, 1997–1999	*E. coli* (3822)	5	…
	K. pneumoniae (946)	23	…

[a] According to NCCLS 2002 criteria: MIC, ⩾2 μg/mL for aztreonam, ceftriaxone, or ceftazidime.
[b] Confirmation was made by reduction (>4-fold) in substrate MIC with the addition of clavulanic acid.
[c] Urinary tract infection isolates.

America and the Western Pacific, >80% of possible ESBL producers were confirmed [29]. These results indicate that the predictive value of screening for ESBL phenotype is highest in regions where ESBL-producing organisms are most prevalent.

Risk factors. Several factors have been reported to increase the risk of colonization or infection with ESBL-producing bacilli (table 3) [30–37]. Prior antibiotic use is one very important factor that contributes to the selection of such organisms. In one study that compared patients with infection due to ESBL-producing *E. coli* or *K. pneumoniae* with matched control subjects, total prior antibiotic use was the only independent risk factor for infection due to ESBL-producing *E. coli* or *K. pneumoniae*. Patients infected with ESBL-producing bacteria tended to be those for whom there was a longer delay until treatment with an effective antibiotic was initiated [30]. In another study of patients with nosocomial bacteremia due to *E. coli* or *K. pneumoniae*, previous treatment with a third-generation cephalosporin was the only proven independent risk factor for infection with ESBL-bearing bacteria [31]. A similar observation was made during an outbreak of infection with ESBL-producing *K. pneumoniae* that affected 15 hospitals in Brooklyn, New York. A correlation was found between total cephalosporin use and the prevalence of ESBL-producing strains of *K. pneumoniae* at each hospital [32]. In skilled-care facilities, total dependence on health care workers has been reported to be an important risk factor for acquisition of ESBL-producing *K. pneumoniae* [33].

Certain procedures and invasive medical manipulations seem to contribute to colonization and infection with ESBL-producing organisms. A case-control study designed to identify risk factors for acquisition of ESBL-producing *K. pneumoniae* found that tracheostomy and insertion of a Foley catheter, endotracheal tube, nasogastric tube, or central venous catheter were all associated with infection with ESBL-producing *K. pneumoniae*. Tracheostomy increased the risk by ~5-fold, whereas prior ceftazidime use increased the risk by >13-fold; insertion of a nasogastric tube or a central venous catheter remained as risk factors by multivariate analysis with logistic regression [34].

Pena et al. [35] confirmed the importance of indwelling catheters as a risk factor for infection with ESBL-producing bacteria. They compared 49 episodes of hospital-acquired *K. pneumoniae* bacteremia due to ESBL-producing organisms and 43 episodes due to non–ESBL-producing *K. pneumoniae*. Approximately twice as many ESBL-producing *K. pneumoniae* cases as non–ESBL-producing *K. pneumoniae* cases occurred in the intensive care unit (90% vs. 46%). A significant association between intravascular catheter–related bacteremia and isolation

Table 3. Risk factors associated with infection or colonization with extended-spectrum β-lactamase–producing pathogens.

Prolonged hospital stay
Prolonged intensive care unit or neonatal intensive care unit stay
Residency in long-term care facility
Exposure to third-generation cephalosporins
Exposure to trimethoprim-sulfamethoxazole
Exposure to ciprofloxacin
Total antibiotic use
Delayed appropriate therapy
Indwelling catheter
Gastrostomy or tracheostomy
Severity of illness
Decubitus ulcer
Total dependence on health care workers
Endotracheal or nasogastric tube

NOTE. Data are from [30–37].

of ESBL-producing *K. pneumoniae* was observed. This association is most likely a result of the fact that patients who require an indwelling device are sicker than those who do not and, thus, are more likely to acquire a resistant pathogen.

Severely debilitated residents of nursing homes are at high risk of developing infection with ESBL-producing bacteria. One study of nursing home residents [36] who required transfer to a hospital for treatment of an infection found that the presence of a decubitus ulcer and/or a gastrostomy tube was an independent risk factor for the presence of the ESBL-producing organisms. Interestingly, although there was an association between the occurrence of resistant strains and ceftazidime use, only 5 of 24 patients with resistant strains had received ceftazidime in the 4 months before a positive culture result was noted. The use of trimethoprim-sulfamethoxazole and/or ciprofloxacin during the prior 4 months each was independently associated with the presence of ceftazidime-resistant organisms. Therefore, administration of an antibiotic unrelated to the action of ESBLs can nevertheless select for ESBL-producing organisms. Given these exposures, it is not surprising that ESBL-producing organisms are often resistant not only to the oxyimino-β-lactams but also to other agents and drug classes [38]. This common multidrug-resistant phenotype is most often the result of the presence of plasmids and/or chromosomal mutations encoding for the various resistances.

Further evidence that nursing home residents are at increased risk of infection with ESBL-producing pathogens comes from a study by Schiappa et al. [37]. On the basis of a study of 32 unique isolates recovered from the blood of patients at a large Chicago hospital, they determined risk factors for bloodstream infection with ceftazidime-resistant *K. pneumoniae* and *E. coli*. Infection with presumptive ESBL producers occurred more frequently in debilitated nursing home patients with central venous catheters than in younger, healthier patients, again suggesting that sicker patients are more likely to acquire resistant pathogens.

AmpC* β*-lactamases. The AmpC β-lactamases are not ESBLs by the standard definition. However, the subject of AmpC enzymes is germane to a discussion of ESBLs, because the phenotypes of the 2 classes overlap. Constitutive production of AmpC β-lactamase in *Enterobacter* species confers resistance to ampicillin, amoxicillin, amoxicillin-clavulanate, and most cephalosporins. AmpC-producing strains also remain susceptible to fourth-generation cephalosporins, such as cefepime [39, 40]. Distinguishing between ESBL- and AmpC-producing strains has clinical significance, because susceptibility patterns for these 2 classes of β-lactamases differ in important ways (table 1) [1, 2, 11, 12].

Bacterial species (e.g., *Enterobacter cloacae* [41, 42], *S. marcescens* [43], *E. coli* [44], *P. aeruginosa* [45], and *Citrobacter freundii* [46]) possess chromosomal sequences that encode β-lactamases of the AmpC type (Bush-Jacoby-Medeiros group 1) [2]. The product of the *ampC* gene is a β-lactamase that is broadly active against cephalosporins but is not inhibited by clavulanate, which differentiates AmpC enzymes from ESBLs [2]. Chromosome-encoded AmpC is usually an inducible enzyme that is expressed at low basal levels, although, in some species, such as *E. coli,* the enzyme is noninducible [41]. Nevertheless, strains of *E. coli* in which the *ampC* gene is preceded by a strong promoter can constitutively express the β-lactamase at high levels [47]. Another mechanism by which chromosomal *ampC* can become constitutively expressed at high levels is by derepression. Mutation in *ampD,* a gene that encodes an enzymatic repressor of AmpC synthesis, can produce a resistant phenotype in inducible strains, the result of high-level production of AmpC in the absence of any inducer [48]. According to data from the Meropenem Yearly Susceptibility Test Information Collection survey of European medical centers [49], stably derepressed AmpC-producing strains have been reported to occur at significant rates among *Enterobacter* species, *Citrobacter* species, and *S. marcescens.*

From a public health standpoint, the migration of chromosomal *ampC* genes into plasmids poses a serious threat. When encoded in plasmids, antimicrobial resistance due to AmpC expression is rendered highly mobile, with the trait becoming easily disseminated to diverse bacterial species. In 1990, Papanicolaou et al. [50] described *K. pneumoniae* isolates that were resistant to cefoxitin and ceftibuten, as well as to aztreonam, cefotaxime, and ceftazidime. Antimicrobial resistance was due to expression of a β-lactamase that shared properties with Bush-Jacoby-Medeiros class 1 (AmpC-type) enzymes, even though *K. pneumoniae* is a bacterial species that does not have a chromosomal *ampC* gene. Cloning and sequencing of the gene encoding the enzyme revealed it to be 90% identical to the sequence of *ampC* from *E. cloacae,* providing the first unequivocal demonstration of a plasmid-encoded AmpC β-lactamase. Some plasmid-mediated AmpC β-lactamases are inducible, as is the case for an AmpC identified in a single isolate of *Salmonella enteritidis* [51]. Both the functional control element (AmpR) and the *ampC* gene seem to have originated in *Morganella morganii.*

Since the earliest reports were published, numerous examples of AmpC plasmid–encoded β-lactamases have been reported in diverse bacterial species on most continents [40]. One unsettling observation is that the plasmid-mediated AmpC β-lactamases have usually been identified in bacterial species that do not have a chromosome-encoded version of the enzyme, including *K. pneumoniae, Klebsiella oxytoca, Salmonella* species, and *Proteus mirabilis* [40]. Risk factors for infection with strains that produce plasmid-mediated AmpC include prolonged hospital stay, often in an intensive care unit; surgery; immuno-

suppression accompanying organ transplantation; or serious underlying disease, such as leukemia [50, 52–54].

Because of the ambiguity of many screening tests, the prevalence of AmpC β-lactamases in pathogenic bacteria is not known. Coudron et al. [55] used susceptibility to cefoxitin to screen consecutive isolates of *E. coli, K. pneumoniae,* and *P. mirabilis* for AmpC and found that 3.5% of isolates screened positive and 1.2% displayed AmpC bands after confirmation by isoelectric focusing.

PROBLEMS ASSOCIATED WITH ESBL DETECTION

Detecting the presence of ESBL-producing pathogens in specimens obtained from patients has important implications for clinical decision-making, chiefly by influencing the choice of appropriate therapy. In addition to its clinical value, detection can also aid in infection-control measures by helping to guide patient isolation procedures. However, several factors complicate the detection of ESBL-producing pathogens by means of standard clinical laboratory methods. One difficulty is that different ESBLs have different affinities for the third-generation cephalosporins. In fact, the ESBLs are sometimes broadly divided into subgroups on the basis of their relative preferences for cefotaxime or ceftazidime as a favored substrate [56]. Because test results are sensitive to the substrates used for detection, a given isolate may not be properly categorized as an ESBL-producing strain, depending on the specific substrate used.

The inoculum effect is a further complicating factor that must be considered when interpreting susceptibilities of ESBL-producing organisms. The effect is typically defined as a 4-fold increase in the MIC associated with an increase in inoculum size [57]. In some cases, the change in MIC is sufficient to alter the categorization of the isolate from susceptible at a low inoculum to resistant at a higher inoculum. The inoculum effect can occur when a bacterial species produces an enzyme, such as a β-lactamase, that degrades an antibiotic. If bacteria are killed by the antibiotic at the same time that the antibiotic is degraded, the still-active enzyme liberated in large amounts from the dead bacteria can reduce the effective concentration of an antibiotic in the surrounding medium [58, 59].

Although conventional screening and confirmation protocols are usually reliable for identifying ESBLs, false-negative results can occur when a low inoculum is used in testing [60]. This phenomenon was observed when the effects of inoculum were studied in ESBL-producing *E. coli* and *K. pneumoniae.* When bacteria were screened for ESBL production at a low inoculum, several strains, including strains producing CTX-M-10, TEM-3, TEM-10, TEM-12, TEM-6, and SHV-18, demonstrated false-negative results for ⩾1 antimicrobial, including common screening substrates, such as ceftazidime, cefotaxime, and aztreonam.

The results of in vitro measurements of antimicrobial activity at different inocula are not always consistent with more clinically relevant in vivo assessments. When the activity of piperacillin-tazobactam and cefepime against ESBL-producing strains of *K. pneumoniae* was evaluated in vitro, both antimicrobials were bactericidal against ESBL-producing isolates at the standard inoculum but showed reduced activity at a high inoculum [61]. However, when cefepime was evaluated against ESBL-producing *E. coli* in a murine thigh infection model, similar doses of cefepime produced similar bactericidal effects at a standard inoculum and at a 100-fold higher inoculum [62]. These examples suggest that the type of assay system, the nature of the ESBL enzymes, and the species of bacteria must all be taken into account when interpreting antimicrobial activities at different inocula. The clinical importance of the inoculum effect is a matter of debate [57–62]; however, considerable evidence now suggests that it is an artifact of the in vitro susceptibility test methods and has little clinical consequence [38, 63].

AmpC β-lactamases exhibit a pattern of resistance to screening substrates that is similar to that of ESBLs. As is the case for the ESBLs, AmpC confers resistance to oxyimino-cephalosporins (e.g., ceftazidime, cefotaxime, ceftriaxone, ceftizoxime, and cefuroxime) and to the monobactam aztreonam [12]. This means that subsequent tests designed to confirm that isolates that screen positive produce ESBLs must, at the same time, differentiate ESBLs from AmpC β-lactamases. A difference in the relative activity toward cephalosporins is one means of distinguishing ESBLs from AmpC β-lactamases. Moland et al. [64] tested 75 *E. coli* and *K. pneumoniae* isolates of known β-lactamase specificity. They found that cefpodoxime MICs ⩾2 μg/mL were noted only for isolates that produced ESBLs or AmpC β-lactamases. However, an elevated cefoxitin MIC of >16 μg/mL was noted only for strains that produce AmpC. A ceftazidime MIC ⩾4 μg/mL correctly identified 90% of ESBL-producing isolates and all AmpC-producing isolates. A cefotetan MIC ⩾8 μg/mL identified 7 of 8 AmpC β-lactamase producers, but no ESBL producers.

In addition to a high cefoxitin MIC, resistance to clavulanic acid also distinguishes AmpC β-lactamases from ESBLs [40]. With use of an MIC-based detection procedure, an ESBL-producing pathogen can be distinguished from a potential AmpC-producing strain when the MIC of the cephalosporin in the presence of clavulanate is at least 8-fold lower than that noted when the cephalosporin is tested alone [65]. Although there has been progress in developing methods to distinguish AmpC from ESBLs, the spread of antimicrobial resistance due to *ampC* gene expression has nevertheless been facilitated by a lack of detection and reporting standards for isolates producing these enzymes. Although the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) recommendations are in place for the detection of ESBL-producing isolates of *E. coli* and *Klebsiella* species, similar recommendations have not yet been

developed for the detection of plasmid-mediated AmpC β-lactamases [66].

RECENT PROGRESS IN DETECTION OF ESBLS

The importance of reliable detection of ESBL-producing organisms led the CLSI to develop screening and confirmatory test methods and guidelines for reporting results of susceptibility tests for resistant strains [67]. Some health care institutions have responded aggressively, acknowledging the threat that proliferation of resistant organisms poses. A survey of 28 Connecticut hospitals conducted during 1998–2002 found that the number of hospitals that have instituted ESBL detection systems doubled over the course of the study period. At the end of the study, almost 70% of the laboratories were conducting both screening and confirmatory testing [68].

Although some institutions have embraced routine screening for ESBL-producing organisms, overall adherence to the practice is still relatively low. In a 1998 survey of clinical laboratories conducted by the Centers for Disease Control and Prevention, only 32% of responding laboratories reported performing tests to identify ESBL-producing pathogens [63]. Even where procedures are implemented, they may not yield accurate results when performed by laboratory personnel. Babini and Livermore [69] analyzed *Klebsiella* species isolates collected from selected European intensive care units and determined that up to 40% of ESBL-producing organisms had mistakenly been reported as being susceptible to cefotaxime and/or ceftriaxone. A prospective study sponsored by the Centers for Disease Control and Prevention identified the nature of laboratory deficiencies in greater detail. Thirty-eight clinical laboratories were asked to apply their usual assays to determine the resistance phenotype of a collection of bacterial strains that had previously been characterized by the investigators [70]. Almost 25% of laboratories failed to detect resistance to relevant screening antibiotics in any of the ESBL- or AmpC-producing isolates. The proportion of laboratories unable to detect resistance in the ESBL or AmpC isolates ranged from ~24% to 32%, depending on the type of β-lactamase present in the test organism.

The technology available for the detection of ESBL at the time that the CLSI guidelines were originally issued was poorly suited to identifying the ESBL resistance phenotype. Commercial MIC systems used to detect and report ESBL-producing bacterial strains often used test drug concentrations that were clustered around concentration breakpoints, which allowed in vitro isolates to be characterized as susceptible, moderately susceptible, or resistant to extended-spectrum cephalosporins. However, this assay strategy hindered detection of ESBL, because the MICs defined for the test drugs (i.e., ceftazidime, cefotaxime, cefpodoxime, or aztreonam) often were well within the susceptible range, whereas clinical experience indicated resistance to the drugs [49]. For example, such pathogens as *Klebsiella* species and *E. coli,* which were inhibited at a test substrate concentration of 2–8 μg/mL, were often characterized in vitro as being susceptible to extended-spectrum cephalosporins; however, ESBL-bearing bacteria with MICs to some cephalosporins within this range might still cause clinically resistant infection [65].

Manufacturers have recognized the need for diagnostic tests that are easy to use, selective, and specific for the determination of ESBL production, by developing several new, commercially available products. A list of currently used tests for screening for and confirmation of ESBL production in clinical isolates appears in table 4 [71]. One detection system that overcomes some of the deficiencies of earlier products is MicroScan (Dade Behring), a broth-based system that uses dehydrated microdilution panels. These panels contain broad dilution series for multiple extended-spectrum antibiotics, which enhance their discriminatory power to detect ESBL-bearing bacteria. In a study that used bacterial strains producing well-characterized β-lactamases, MicroScan was able to detect ESBL-producing strains of *E. coli* and *Klebsiella* species with MICs $\geqslant$2 μg/mL for CLSI-recommended substrates [64]. A microdilution panel containing cefpodoxime was found to be the most useful for detecting ESBL-bearing bacteria, although this drug was not able to distinguish ESBL-producing bacteria from those producing an AmpC β-lactamase. Routine screening for ESBL-producing organisms has been further facilitated with the incorporation of cefpodoxime (4 μg/mL) and ceftazidime (1 μg/mL) into all of the latest MicroScan panels [72]. Although the panels demonstrated 100% sensitivity when tested against ESBL-producing and non–ESBL-producing strains (with the latter including AmpC producers), the specificity of the panels was low (61%). As a result, confirmation of a phenotype suspected of being associated with ESBL production by MicroScan screening requires testing by a second method [73].

The MicroScan ESBL Plus test panel is designed to screen for ESBLs and to confirm suspected isolates. The panel incorporates an extended dilution series for ESBL test substrates, which include both ceftazidime and cefotaxime, with or without clavulanate, as a means of distinguishing ESBL-producing strains from those that produce AmpC [72]. Thomson et al. [74] demonstrated the ability of these microdilution panels to recognize β-lactamases by testing the system with isolates of *E. coli, Klebsiella* species, *Enterobacter* species, *C. freundii,* and *S. marcescens.* A minimum panel of 5 tests, including tests using screening drugs with and without β-lactamase inhibitors, may provide maximal differentiation of clinically significant classes of β-lactamases, including ESBLs, AmpC, and the K1 β-lactamase of *K. oxytoca.* A suspected ESBL-producing isolate was confirmed when the individual MICs for ceftazidime and cefotaxime in the presence of clavulanate were sufficiently decreased, compared with the test drugs with the inhibitor. Ac-

Table 4. Laboratory tests for the detection of extended-spectrum β-lactamases (ESBLs).

Test	Method and interpretation
Screening	
Double disk approximation or double disk synergy	Disk of third-generation cephalosporin placed 30 mm from amoxicillin-clavulanic acid. Enhanced inhibition indicates ESBL.
Combination disk	Uses 2 disks of third-generation cephalosporin alone and combined with clavulanic acid. An increase of >5 mm in zone inhibition with use of the combination disk indicates the presence of ESBL.
Microdilution	Growth in a broth containing 1 μg/mL third-generation cephalosporin indicates the presence of ESBL.
Confirmatory	
MIC broth dilution	MIC of third-generation cephalosporin alone or combined with clavulanic acid. A decrease in the MIC of the combination of ⩾3 two-fold dilutions indicates the presence of ESBL.
Etest (MIC ESBL strips)	Two-sided strip containing ceftazidime on one side and ceftazidime–clavulanic acid on the other. If the ratio of the MIC of the combination to the MIC of ceftazidime alone was >8, if a phantom zone was present, or if both were present, ESBL was considered to be present.
Automated instruments (e.g., Vitek)	Measures MICs and compares the growth of bacteria in presence of ceftazidime vs. ceftazidime–clavulanic acid.
Molecular (DNA probes, PCR, RFLP)	Targets specific nucleotide sequences to detect different variants of TEM and SHV genes.

NOTE. Adapted from [71], with permission. RFLP, restriction fragment–length polymorphism.

cording to these criteria, the sensitivity and specificity of the microdilution panels for identifying ESBL-producing strains were 92% and 94%, respectively [72].

The Vitek AutoMicrobic System (Vitek) is a broth-based MIC system widely used by clinical microbiology laboratories for antimicrobial susceptibility testing. The testing card of the latest model, the Vitek 2, assays a wider range of drug concentrations than its predecessor. This change, along with updated optics and new algorithms based on a kinetic analysis of data, enhances the ability of the Vitek 2 to detect ESBL-producing bacteria. An advanced expert system (AES), when used in conjunction with the Vitek 2, can distinguish ESBL-producing phenotypes from other types of resistant bacteria, particularly those that overexpress AmpC. In a validation study by Sanders et al. [75], the Vitek 2 AES correctly identified the β-lactam phenotypes of 93.4% of Enterobacteriaceae and *P. aeruginosa* isolates. The study included isolates characterized previously by a variety of biochemical and molecular methods. ESBL production was just one of several antimicrobial resistance mechanisms exhibited by the isolates, which included some strains with phenotypes rarely encountered in the clinical laboratory. Livermore et al. [76] evaluated the performance of the Vitek 2 AES in 10 European laboratories, using test isolates of known resistance genotypes, including strains that produced ESBLs. Interpretations by the Vitek 2 AES were in full agreement with genotype data for 88%–89% of strains; for an additional 5%–6% of isolates, the mechanism was reduced to 2 possibilities. These studies indicate that the Vitek 2 AES is capable of accurately assigning a phenotype to a wide variety of resistant strains, including ESBL-producing strains.

As noted above, the similarity of behavior for AmpC and ESBLs in most MIC screening tests necessitates a confirmatory test for strains suspected of producing ESBLs. The Vitek ESBL test uses cefotaxime and ceftazidime alone and in combination with clavulanic acid to confirm the detection of ESBLs that are sensitive to inhibition by the β-lactamase inhibitor. When the Vitek ESBL system was tested against a set of 157 isolates producing previously characterized β-lactamases, the sensitivity and specificity for detecting ESBLs were 99.5% and 100%, respectively [75]. As a convenience, the Vitek ESBL test is available incorporated into a standard Vitek antimicrobial susceptibility test card, which allows the confirmatory test to be run with the MIC screening.

The Etest ESBL strip (AB Biodisk) is a commercially available agar-based test that has demonstrated excellent sensitivity and specificity for the confirmation of ESBL-producing strains. The basis of the test is the determination of the MIC of ceftazidime, compared with the MIC of ceftazidime in the presence of clavulanic acid. When the Etest ESBL strip was used to confirm suspected ESBL production in strains of *Klebsiella* species and *E. coli,* it was found to be more sensitive (100% vs. 87%, respectively) and convenient than the disk approximation test [77]. In a study by Leverstein–van Hall et al. [78], the Etest ESBL strip was challenged against 17 control strains of *E. coli* and *Klebsiella* species with genotypically identified β-lactamases and was found to have an accuracy rate of 94%.

Because of its relative ease of use and precision, the Etest ESBL strip has been used as a confirmatory test in large-scale surveillance studies of gram-negative bacteria. Winokur et al. [29] measured the prevalence of ESBL-producing strains of Enterobacteriaceae that tested positive by microdilution susceptibility tests in the SENTRY surveillance project. The sensitivity of screening varied with the choice of substrate for different geographic regions and bacterial species. For ex-

ample, ceftazidime detected the greatest number of suspected ESBL-producing *K. pneumoniae* isolates, regardless of the region. Aztreonam was the most sensitive substrate for *E. coli* isolates from the United States and the Western Pacific. For *Proteus* species, ceftriaxone was the most sensitive substrate in Latin America, whereas ceftazidime was the most sensitive substrate in the United States. *K. pneumoniae* isolates that tested positive for ESBL (ceftazidime MIC, ≥ 2 μg/mL) were confirmed by use of Etest ESBL strip using ceftazidime, with or without clavulanate. Results tended to parallel the prevalence of ESBL-producing strains in the different geographic areas, with 43% of isolates from North America, 84% of isolates from Latin America, and 82% of isolates from the Western Pacific confirmed as ESBL producers. The results demonstrate the ability of the ESBL Etest strip to efficiently evaluate large numbers of suspected ESBL phenotypes in a surveillance program. Recent technological improvements in testing, along with the development of uniform standards of ESBL detection and confirmatory testing, promise to make accurate identification of ESBL-producing organisms more accessible to clinical laboratories and easier to perform.

CONCLUSIONS

A few years after it was first isolated, TEM-1, the first plasmid-mediated β-lactamase found in gram-negative organisms, spread worldwide and across many species. Since then, several β-lactam antibiotics have been developed to treat patients, and, likewise, new β-lactamases have emerged to neutralize antimicrobial effects. It follows that resistance to β-lactam antibiotics arose from the selective use and overuse of these new antimicrobial agents; those β-lactamases with an increased spectrum of activity became the class of ESBLs.

Presently, the proliferation of ESBLs and their purported resistance to a wide variety of antimicrobials are serious public health concerns. To this end, proper detection of ESBLs and corresponding treatment strategies are of paramount importance in curtailing this growing epidemic. A working knowledge of ESBL properties, risk factors for the selection of ESBL-producing organisms, the differentiation between ESBLs and AmpC β-lactamases, and uniform standards of ESBL detection and confirmatory testing will help physicians to best treat patients presenting with resistant organisms.

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