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Review Article

Beyond Susceptible and Resistant, Part II: Treatment of Infections Due to Gram-Negative Organisms Producing Extended-Spectrum β-Lactamases

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The production of β-lactamase is the most common mechanism of resistance to β-lactam antibiotics among gram-negative bacteria. Extended-spectrum β-lactamases (ESBLs) are capable of hydrolyzing most penicillins, extended-spectrum cephalosporins, and aztreonam, but their activity is suppressed in the presence of a β-lactamase inhibitor. Serious infections with ESBL-producing isolates are associated with high rates of mortality, making early detection and adequate medical management essential to ensure optimal patient outcomes. Much controversy has centered on the recommendations for testing and reporting of antibiotic susceptibility of potential ESBL-producing organisms. The latest version of the Clinical Laboratory Standards Institute (CLSI) susceptibility reporting guidelines, published in 2010, no longer advocates for phenotypic testing of ESBL-producing isolates. From newer studies demonstrating a correlation between organism minimum inhibitory concentration (MIC) and clinical outcome, along with pharmacokinetic/pharmacodynamic (PK/PD) modeling demonstrating the importance of the MIC to achieving therapeutic targets, the CLSI has assigned lower susceptibility breakpoints for aztreonam and most cephalosporins. The new guidelines recommend using the lower MIC breakpoints to direct antibiotic selection. This article reviews the microbiology and epidemiology of ESBLs, the recent change in CLSI susceptibility reporting guidelines for ESBLs, and the clinical and PK/PD data supporting the relationship between *in vitro* susceptibility and clinical outcome. Finally, considerations for antimicrobial selection when treating patients with infections caused by ESBL-producing organisms from various sources are discussed.

INDEX TERMS: beta-lactamases; drug resistance, microbial; *Escherichia coli; Klebsiella pneumoniae*

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INTRODUCTION

β-Lactams are the predominant class of antimicrobials used in children.[1] Resistance to β-lactams among bacteria is typically mediated either through changes in the proteins that are targets of β-lactams (the penicillin-binding proteins) or through production of enzymes that hydrolyze the drugs (β-lactamases). Among Gram-negative bacteria, the most common mechanism of resistance is β-lactamase production.[2] A tremendous variety of β-lactamases are produced by bacteria, ranging from those that hydrolyze only narrow-spectrum agents to those that inactivate all β-lactams in clinical use.[2] Among the most clinically important β-lactamases are the extended-spectrum β-lactamases (ESBLs).[3] Infections due to ESBL-producing organisms are associated with increased mortality among adults[4] and children[5] and are increasing in frequency.[6] Complicating matters, the relationship between the results of *in vitro* susceptibility tests for ESBLs and clinical outcomes of infections is controversial, and guidelines for reporting antimicrobial susceptibility results for ESBL isolates have recently changed. In this article we will review the microbiology and epidemiology of organisms producing ESBLs and the implications for antibacterial therapy for clinicians.

MICROBIOLOGY AND EPIDEMIOLOGY

The initial definition of ESBLs was based on the ability of these enzymes to hydrolyze then

newly developed "extended-spectrum" cephalosporins, what would now be considered second- and third-generation cephalosporins.[7] The first enzymes classified as ESBLs were found to be evolutionary descendants of narrow-spectrum β-lactamases common in organisms such as *Escherichia coli* and *Klebsiella* (TEM and SHV type).[8] Mutations to the active sites of the progenitor enzymes expanded the substrates the enzymes could hydrolyze. These enzymes proliferated through selective antibacterial pressure and via spread on plasmids. Other ESBL types (such as CTX-M) appear to have originated in obscure organisms with subsequent transfer into Gram-negative pathogens.[9]

Setting aside precise molecular definitions, β-lactamases can be characterized by their spectrum of activity (which β-lactams the enzyme efficiently hydrolyzes), the degree to which their activity is inhibited by β-lactamase inhibitors (BLIs) such as clavulanate and tazobactam, and the location and expression of the enzyme.[2] Although spectrum of activity varies across the hundreds of separate enzymes classified as ESBLs, most will hydrolyze penicillins, cephalosporins, and aztreonam.[3] Hydrolysis of cefepime may be less efficient in many ESBLs, and ESBLs as usually defined do not have significant activity against carbapenems (although some variants not discussed here, such as the *Klebsiella pneumoniae* carbapenemase–type enzymes, have activity).[10,11] The activity of β-lactams against ESBLs is usually improved when combined with a BLI; whether this improvement is adequate to render the organism clinically susceptible depends on the β-lactam, the BLI, and the particular ESBL variant.[12] This is in contrast to the AmpC-type enzymes (previously reviewed in this series), which are largely unaffected by BLIs. It should be noted that some less common varieties of ESBL are "inhibitor-resistant" and the addition of a BLI does not enhance activity of a β-lactam to an appreciable extent.[13] Finally, ESBLs are typically located on plasmids, rather than in the chromosome, and are generally expressed even in the absence of their substrate (in contrast again to AmpC-type enzymes). Plasmids carrying genes for ESBLs often carry genes encoding resistance elements to non–β-lactam antibiotics, helping to explain the multidrug-resistant phenotypes frequently seen in ESBL-producing organisms.

In the clinical microbiology laboratory, presumptive phenotypic identification of an ESBL in a clinical isolate of *E coli*, *Klebsiella*, or *Proteus* is performed by comparing the degree of inhibition of microbial growth by a cephalosporin in the presence or absence of the BLI clavulanate.[14] The exact criteria for a positive ESBL test finding varies by the testing methodology (disk diffusion, Etest, or microdilution) used. While the tests themselves have not changed, the recommendations for performing these tests in the clinical laboratory have been substantially revised during the last few years by the Clinical Laboratory Standards Institute (CLSI).[15] The rationale for these changes is discussed below. Because many clinical laboratories are still operating under the prior recommendations, we will describe both approaches.

Under the CLSI guidelines published before 2010, phenotypic tests for ESBL production were recommended for isolates of *E coli*, *Klebsiella*, and *Proteus mirabilis* when standard susceptibility tests (such as disk diffusion or microdilution) indicated decreased susceptibility to cephalosporins or monobactams. The degree of decreased susceptibility could be such that the organism would still fall in the susceptible or intermediate category, but the minimum inhibitory concentration (MIC) would be higher than that of wild-type isolates (for non–ESBL-producing *E coli,* most isolates have MICs < 0.5 mg/L to ceftriaxone).[16] For example, as illustrated in Table 1, an MIC of 1 mg/L to ceftriaxone in an isolate of *E coli* would trigger a phenotypic ESBL test, even though isolates with MICs of less than or equal to 8 mg/L would be considered susceptible. If the phenotypic ESBL test result was negative, susceptibility reports for β-lactams would be reported according to the results of MIC testing. With a positive ESBL test result, all cephalosporins, penicillins, and monobactams would be reported as resistant, regardless of the results of MIC testing. Many laboratories would add a note indicating that ESBL production was identified for that isolate.

Under the revised CLSI guidelines published in 2010, the breakpoints (cutoff MIC value defining susceptibility of a given organism to a particular antibiotic) for a number of cephalosporins and for aztreonam were revised downwards (for reasons discussed in the next section). With this change, phenotypic testing for ESBLs for the purpose of reporting antimicrobial susceptibilities is no

Table 1. Clinical Laboratory Standards Institute Recommendations for Screening and Detection of Extended-Spectrum β-Lactamase Production

CLSI Version	Drug	MIC Breakpoint for Susceptibility, mg/L	ESBL Phenotypic Testing Recommendations
Pre 2010	Ceftriaxone	≤8	For MIC ≥ 1 mg/L, perform ESBL phenotypic test. If test result is negative, report sensitivities according to MIC. If test result is positive, report all cephalosporins, aztreonam, and penicillins (but not β-lactam/β-lactamase inhibitor combinations) as resistant regardless of MIC.
2010 and later	Ceftriaxone	≤1	Do not perform ESBL phenotypic testing for purposes of reporting susceptibility. Report sensitivities according to MIC.

CLSI, Clinical Laboratory Standards Institute; ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration

longer recommended. Thus, a clinician would no longer see a report identifying an organism as an ESBL producer on a microbiology report. This makes reporting consistent with the results of most susceptibility tests for which the presumed mechanism of resistance is not provided.

The prevalence of ESBL production (measured either via phenotypic or genetic tests), while low in most pediatric populations, appears to be increasing. Data from the SENTRY surveillance system found that in 2004, the overall prevalence of an ESBL phenotype was 1.5% in *E coli* and 3.2% in *Klebsiella* among isolates from pediatric patients submitted to North American study hospitals.[17] Blaschke and colleagues[18] reported on the susceptibility of all isolates of *E coli* and *Klebsiella* from a children's medical center in Utah from 2003 to 2007. The proportion of isolates displaying an ESBL phenotype increased almost 3-fold during the study period, from 0.57% to 1.50%. At the Texas Children's Hospital, Chandramohan and Revell[19] found that from 2010 to 2011, 7% of all *Enterobacteriaceae* organisms harbored at least 1 ESBL gene.

Studies have identified children at highest risk for infection due to ESBL-producing organisms as those with chronic medical conditions, prolonged hospitalizations, and recent antibiotic exposure.[5,18,20] However, much as with the community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) epidemic, there is increasing recognition of infections acquired in the community among patients without traditional risk factors.[21] Also analogous to CA-MRSA, community-onset infections due to ESBL producers seem to be caused by different strains than those usually seen in hospitalized patients. In the case of ESBLs, CTX-M–type enzymes appear to be spreading in the community and into the hospital, displacing the TEM and SHV types that previously circulated.[21] Among healthy children in France aged 6 to 24 months, none of whom had been hospitalized the prior 6 months, stool colonization with an ESBL-producing organism was found in 4.6%.[22] The risk was significantly higher among children with recent use of an oral third-generation cephalosporin (11.1%), but among patients with no recent antibiotic exposure it was 4.4%. CTX-M–type enzymes were identified in 88% of the ESBL isolates. Thus, while the risk for infection with ESBL-producing organisms is highest among children who are hospitalized, a substantial reservoir of organisms with this resistance mechanism exists in the community.

RELATIONSHIP BETWEEN *IN VITRO* SUSCEPTIBILITY AND OUTCOMES

These complex and varying recommendations for testing and reporting antibiotic susceptibility in potential ESBL-producing organisms stem from the evolving understanding of the relationship between *in vitro* susceptibility testing of ESBL-producing organisms and clinical outcomes. ESBLs were first recognized in clinical isolates displaying high-level resistance to second- and third-generation cephalosporins, with clearly deleterious clinical consequences when treatment with these antibiotics was used. It took longer to appreciate that ESBLs could also be present in organisms when the organisms tested as susceptible in the clinical laboratory. In a study of *Klebsiella* isolates from European intensive care units (including both adults and children), almost a third of confirmed ESBL producers tested susceptible to ceftriaxone on standard susceptibility tests.[23] Thus, to maximize detection

of ESBL production, CLSI released the guidance advocating phenotypic susceptibility testing for isolates with elevated MICs to cephalosporins.

The concept that ESBLs could be present and affect outcomes even when the organism tested as susceptible was supported when Paterson and colleagues[24] published a series that included 32 patients (age ≥ 16 years) with *Klebsiella* bacteremia where the organism was confirmed by phenotypic tests to produce an ESBL. Despite ESBL production, the MICs of all of these organisms were in the susceptible or intermediate range to the cephalosporins used for treatment. Of the 32 study patients, 19 (59%) experienced clinical failure despite the lack of laboratory-determined resistance to the drug used. Kim et al[20] examined pediatric patients (aged 0-17 years) with bloodstream infections due to *E coli* or *K pneumoniae*. Among patients treated with a cephalosporin that the organism was susceptible to, a favorable clinical response was seen in 9 of 17 patients (52.9%) infected with ESBL-producing organisms compared with 47 of 50 patients (94.0%) infected with non–ESBL-producing organisms ($p<0.001$). The implication of these studies—that an *in vitro–in vivo* disconnect exists among ESBL-producing isolates—quickly became an established paradigm.

A mechanism for the observed lack of correlation between susceptibility tests and clinical treatment outcomes for ESBLs was offered in the form of the "inoculum effect." The standard quantity of organisms used in most *in vitro* susceptibility testing is 10^{5-6} organisms. However, in severe infections the number of organisms can be 10- to 100-fold higher. Burgess and Hall[25] analyzed the killing by piperacillin/tazobactam and cefepime of ESBL- and non–ESBL-producing *K pneumoniae* (all of which were susceptible to the drugs) at high and low inocula. The bactericidal activity of cefepime and piperacillin/tazobactam was greatly reduced at the higher inoculum, in contrast to meropenem, for which bactericidal activity was maintained at the high inoculum.

In the last several years a number of studies have called into question some of the principles underlying the paradigm of an *in vitro–in vivo* disconnect in the treatment of infections due to ESBL-producing organisms. Careful analysis of episodes of clinical failure when treating infections due to "susceptible" ESBL-producing organisms with cephalosporins found a strong relationship between the MIC of the organism and the likelihood of failure. Andes and Craig[26] analyzed the findings from 42 patients (age > 16 years) with bloodstream infections due to ESBL-producing organisms classified as susceptible to cephalosporins and treated with cephalosporin monotherapy. While the failure rate was very high (89%) when the organism's MIC was at the susceptibility breakpoint (8 mg/L), the likelihood of clinical failure decreased substantially with decreasing MIC: 74% at an MIC of 4 mg/L, 33% at 2 mg/L, and 19% at less than or equal to 1 mg/L. In a study by Kim et al[20] of bloodstream infections due to ESBL-producing organisms in children (aged 0-17 years), clinical failure with cephalosporin monotherapy was noted in 100% of patients when the MIC was 8 mg/L, 67% when the MIC was 4 mg/L, and 0% when the MIC was 2 mg/L. Among 8 patients with bloodstream infections due to ESBL-producing *E coli* or *Klebsiella*, Kang et al[27] found that treatment failed for all patients (age ≥ 16 years) with a cephalosporin to which the organism's MIC was 8 mg/L, compared to 25% with an MIC of 4 mg/L and 0% with an MIC of 1 mg/L or less.

Supporting these clinical observations, simulations, *in vitro* studies, and animal models applying pharmacokinetic/pharmacodynamic (PK/PD) principles also support the importance of the organism's MIC. Across diverse drugs and organisms, achievement of a free (i.e., not bound to plasma proteins) drug concentration above the MIC of the organism for 40% to 60% of the dosing interval has been identified as a strong predictor of microbiologic and clinical success and a *target value* for therapy.[28] Pharmacokinetic/PD simulations indicate that the likelihood of achieving these target values at the susceptibility breakpoint for ceftriaxone of 8 mg/L is 1% for a 2-g dose.[26] Animal models of infection with ESBL-producing and non–ESBL-producing organisms demonstrated that attainment of a PK/PD target value in this range led to similar microbiologic outcomes regardless of ESBL production.[26,29] In these studies, the difference between ESBL- and non–ESBL-producing organisms was that larger drug doses were required to achieve the target values, because MICs were typically higher for ESBL producers. However, the underlying relationship between MIC, drug exposure, and outcome was the same, regardless of resistance mechanism.

Taken together, CLSI's interpretation of these data is that, rather than ESBLs representing an *in vitro–in vivo* disconnect, the breakpoints for *E coli* and *Klebsiella* were originally set too high. The likelihood of treatment failure was unacceptably high when treating organisms with MICs near the breakpoint, regardless of the mechanism of resistance. Because ESBLs are the most common cause of elevated MICs in *E coli* and *Klebsiella*, it appeared that it was the resistance mechanism, and not the MIC, that was driving clinical failures. From these data, the CLSI lowered the breakpoints for most parenteral cephalosporins (although not cefepime) and recommended that phenotypic ESBL testing not be performed for the purposes of establishing susceptibility. However, some investigators and clinicians[30] do not agree with CLSI's new recommendations.

Partly because of the widespread implementation of CLSI's prior recommendation to avoid use of non-carbapenem β-lactams when ESBL-producing organisms are identified, observational data supporting the recommendations are limited, and few data are available from clinical trials. In the available studies, patients may have received multiple antibiotics, either together or sequentially, so separating the effects of each antibiotic is challenging. Because a substantial percentage of ESBL-producing isolates may be reported as susceptible to BLI combinations and cefepime under the new breakpoints,[31,32] several investigations have attempted to determine whether the available clinical data support these agents as alternatives to carbapenems for infections due to ESBL-producing organisms.

Vardakas et al[33] performed a meta-analysis of 21 studies that reported the risk of mortality for primarily adult patients with bacteremia due to ESBL-producing organisms and treated with carbapenems, BLI combinations, or "other" agents (primarily cephalosporins and fluoroquinolones). Susceptibility to the agents used was classified according to the individual study from criteria in use at the time of publication. Carbapenems were associated with statistically significantly lower risks of mortality when compared to the "other" agents as empiric or definitive therapy. There was no statistically significant difference in mortality between carbapenems and BLI combinations, or between BLI combinations and "other" agents, when used either empirically or definitively. However, the study was not able to discriminate among patients with community-acquired or nosocomial infection sources, age, degree of severity of illness, site of infection (e.g., urinary vs non-urinary), drug selection within groups (e.g., cefepime vs ceftriaxone among "other" drugs), organism MIC, or *in vitro* activity of the initial antibacterial regimen. Thus, the study cannot speak to potentially important differences between risk groups.

Rodríguez-Baño et al[34] reported on 740 episodes of bacteremia in adult patients, due to ESBL-producing *E coli*, treated with a BLI combination (piperacillin-tazobactam or amoxicillin-clavulanate) or a carbapenem. Separate cohorts for definitive and empiric therapy were analyzed, and multivariable models were created to adjust for potential confounders. The authors found no statistically significant differences in 30-day mortality between BLI combination therapy and carbapenem therapy for empiric or definitive treatment. Most infections (~75%) were considered to have a urinary source. In a separate study, the authors[35] analyzed the relationship between piperacillin-tazobactam MIC, site and mortality among patients in the study cohort, which was composed of adult patients. Among patients receiving empiric therapy, mortality was 0% among those with a urinary source; among those with a non-urinary source, mortality was 0% when the MIC was ≤2 mg/L, 38% for an MIC of 4 or 8 mg/L, and 44% for an MIC of ≥16 mg/L ($p = 0.02$).

Lee et al[36] performed a study comparing mortality among adult patients treated with cefepime or a carbapenem for bacteremia due to ESBL-producing *E coli* or *Klebsiella*. Mortality was higher among patients treated with cefepime either empirically or definitively, compared to carbapenem therapy. Mortality was strongly associated with cefepime MIC; in the empiric therapy group, no patients infected with an organism with a cefepime of MIC ≤ 1 mg/L died, compared to 40% with MIC of 4 or 8 mg/L, and 100% for an MIC ≥16 mg/L ($p=0.04$). In contrast, Chopra et al[37] did not see an association between cefepime MIC and mortality among adult patients with bacteremia due to ESBL-producing *E coli* or *Klebsiella* and treated with empiric cefepime. In this study, receipt of empiric cefepime was associated with a non-significant trend toward a lower survival to discharge, compared with receipt of empiric carbapenem therapy.

Table 2. Considerations for Definitive Therapy of Infections Due To Known or Presumed ESBL-Producing *Escherichia coli* and *Klebsiella*

Patient Characteristics	Infection Site	First-Line Therapy*	Alternative Therapy*
Hospitalized			
Septic shock or immunocompromised	Any	Carbapenem	Fluoroquinolone (for severe β-lactam allergy)
Clinically stable, non-immunocompromised	Pneumonia, intra-abdominal infection without adequate source control, pyelonephritis, intravascular infection	Carbapenem	Piperacillin/tazobactam,† fluoroquinolone
	Intra-abdominal infection with good source control, catheter-related infection with removal of catheter, skin/soft tissue infection with drainage, lower urinary tract infection	Piperacillin-tazobactam,† carbapenems, fluoroquinolone	Cefepime,‡ aminoglycoside (lower urinary tract infection)
Outpatient	Lower urinary tract	Fluoroquinolone, TMP/SMX, nitrofurantoin, fosfomycin	Amoxicillin/clavulanate§

ESBL, extended-spectrum β-lactamase; TMP/SMX, trimethoprim/sulfamethoxazole
** Assumes drugs are documented as susceptible on testing*
† Consider avoiding for MIC of 16 mg/L
‡ Consider avoiding for MIC of 8 mg/L
§ Consider using a formulation with a greater amount of clavulanic acid (e.g., 250/62.5 mg/5 mL suspension instead of 600/42.9 mg/5 mL)

INTERPRETATION OF STUDIES AND RECOMMENDATIONS FOR THERAPY

Until more data on clinical outcomes accumulate, clinicians may be hesitant to embrace the new guidelines wholeheartedly and rely completely on the reported susceptibility categories when an ESBL is suspected. In the absence of confirmatory ESBL phenotypic testing, ceftriaxone susceptibility may be a reasonable "surrogate" for ESBL production. Thus, isolates of *E coli* or *Klebsiella* found to be ceftriaxone resistant could be flagged by the microbiology laboratory as potential ESBL producers. Either through selective microbiologic reporting or expert consultation, clinicians could be guided to select definitive therapies according to the patient's clinical status and site of infection (Table 2). Because no agents have been shown to be more effective than carbapenems, and several studies suggest their superiority, for patients experiencing septic shock or the severely immunocompromised, carbapenems are recommended. In these highly vulnerable patients, any marginal benefit of carbapenems over comparators is more likely to impact clinical outcomes. For clinically stable, hospitalized patients selection of therapy may consider the source of infection. For infections typically associated with a high-organism burden, such as pneumonia, a carbapenem may still be preferred. When good source control (abscess drainage, surgical removal of infected tissue, etc) of the infection has been achieved, or for lower urinary tract infections where high drug concentrations are attained, piperacillin/tazobactam or fluoroquinolones are likely to be effective alternatives to carbapenems. Current data for cefepime are less supportive and this might be considered a second-line regimen. Most studies of serious infections due to ESBL-producing bacteria have not investigated the relative effectiveness of combination therapy (e.g., a β-lactam plus aminoglycoside) vs monotherapy. However, studies have generally not shown a benefit for combination regimens in definitive treatment of serious Gram-negative infections.[38] For outpatient therapy of urinary tract infections, the most likely setting for oral therapy, amoxicillin/clavulanate may be an alternative to non–β-lactam agents. When using amoxicillin/clavulanate it may be beneficial to use formula-

tions with a higher clavulanate component to take advantage of the ESBL inhibition of the BLI.

CONCLUSION

The evolving picture to date suggests that the increased risk of therapeutic failure associated with β-lactam therapy for infection due to ESBL-producing organisms is mediated by the increase in MIC. The apparent violation of *in vitro–in vivo* susceptibility relationships seems to be a result of setting susceptibility breakpoints too high. The CLSI's efforts to move breakpoints to the "right" place reduce complexity associated with performing extra laboratory tests to identify ESBL producers, and also help identify non-susceptible isolates with other resistance mechanisms (e.g., plasmid-mediated AmpC). However, it remains unclear whether the new breakpoints represent the "right" cutoffs for susceptibility. Most of the supporting data for the CLSI changes comes from simulations, *in vitro* studies, and animal studies. In particular, there is a dearth of data for pediatric patients. This is concerning given that much of the supporting data involve PK/PD relationships, and the pharmacokinetics of antibacterials may differ substantially between children and adults. Breakpoints for some β-lactams, such as piperacillin-tazobactam and cefepime, were not changed, despite clinical evidence suggesting an MIC-outcome relationship among ESBL producers. In the face of so much uncertainty, recommendations for identification and treatment of these organisms are likely to continue to evolve.

The next installment in this series will discuss the detection and clinical management of infections due to Gram-negative organisms producing carbapenem-hydrolyzing β-lactamases.

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Abbreviations BLI, β-lactamase inhibitor; CLSI, Clinical Laboratory Standards Institute; ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic

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