Clinical Management of Infections Caused by Enterobacteriaceae that Express Extended-Spectrum β-Lactamase and AmpC Enzymes

Patrick N. A. Harris, BSc, MBBS, MRCP, DTM&H, FRACP, FRCPA

1 Infection and Immunity Theme, University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia

Semin Respir Crit Care Med 2015;36:56–73.

Address for correspondence Patrick N. A. Harris, BSc, MBBS, MRCP, DTM&H, FRACP, FRCPA, University of Queensland Centre for Clinical Research (UQCCR), Building 71/918, Royal Brisbane and Women’s Hospital Campus, Herston, Queensland 4029, Australia (e-mail: p.harris@uq.edu.au).

It is thought that β-lactamase enzymes have evolved in bacteria over many millions of years as a protective mechanism against naturally occurring compounds produced by other microorganisms.\(^1\)–\(^3\) Environmental bacteria found in underground caverns, isolated from the outside world for more than 4 million years, show extensive resistance to commercial antibiotics, including penicillins and cephalosporins mediated by hydrolyzing β-lactamases.\(^4\) As such, bacterial resistance to β-lactam antibiotics may be nothing “new.” Even before penicillin had been used to treat clinical infections, Abraham and Chain in 1940 observed a substance produced by Escherichia coli (then named Bacillus coli) that would reduce the inhibitory effect of penicillin on Staphylococcus aureus.\(^5\) Although not known at that time, this was the first scientific description of β-lactamase activity, in this case the low-level AmpC activity seen in E. coli.\(^6\) However, it is clear that the diversity, distribution, host range, and prevalence of β-lactamases have expanded dramatically since the introduction of widespread commercial use of antibiotics.\(^7\)

**Extended-Spectrum β-Lactamases**

Production of β-lactamase is the primary mechanism by which gram-negative bacteria express resistance to β-lactams—our most useful and effective antibiotics (see –Fig. 1). When first recognized, most β-lactamase enzymes showed narrow spectrum activity. For instance, TEM-1 in E. coli or SHV-1 in Klebsiella pneumoniae are both able to effectively hydrolyze ampicillin, yet most other β-lactam classes remain unaffected to any clinically significant degree (unless these enzymes become expressed at very high levels). In response to the increasing prevalence of these β-lactamases in gram-negative bacteria and their spread to other new host species.
(e.g., Haemophilus influenzae or Neisseria gonorrhoeae), third-generation cephalosporins (such as ceftriaxone or cefotaxime) were developed and showed stability to the effects of these narrow spectrum β-lactamases. As such, these agents became “workhorse” antibiotics in many hospitals, with a spectrum of activity that covered common pathogens implicated in many infectious syndromes. However, within a few years of their introduction into clinical use a bacterial isolate showing transmissible resistance to third-generation cephalosporins, a key feature of “extended-spectrum” β-lactamase (ESBL) activity, was described in a nosocomial K. pneumoniae isolate following a point mutation in its “parent” β-lactamase.7 There are now more than 1,300 unique β-lactamase types described7 (see www.lahey.org/Studies for a comprehensive list), many of which possess activity against “expanded-spectrum” cephalosporins—a term used to include third-generation (e.g., ceftriaxone, cefotaxime, ceftazidime) and fourth-generation (e.g., cefepime) cephalosporins, as well as novel antistaphylococcal agents such as ceftaroline.9 ESBLs also typically render bacteria resistant to monobactams such as aztreonam.

**Classification of β-Lactamases**

Several classification schemes for β-lactamases have been proposed over the years, but two main systems have predominated. The Bush–Jacoby–Medeiros functional classification scheme defines three main groups of β-lactamase enzymes according to their substrate and inhibitor profiles: group 1 cephalosporinas not inhibited well by clavulanate; group 2 enzymes with penicillinase, cephalosporinase, and broad-spectrum β-lactamase activity generally inhibited by β-lactamase inhibitors; and group 3 metallo-β-lactamase that hydrolyze penicillins, cephalosporins, and carbapenems that are poorly inhibited by most β-lactamase inhibitors.10 This scheme also incorporates several subcategories that have evolved over the years with the discovery of new β-lactamase types.7 The Ambler classification scheme relies upon amino acid sequences of β-lactamase types and includes four categories: types A, C, and D with a serine residues at the active site and class B metalloenzymes with a Zn$^{2+}$ cofactor11 (see Table 1).

Although these schemes have been helpful in categorizing β-lactamase types, they have several drawbacks. The nomenclature can seem impenetrable to the nonspecialist and has evolved significant complexity to accommodate the expanding variety of β-lactamase types.12 Some β-lactamases do not fit neatly into the category definitions. As a result, the clinical applicability of these schemes, in terms of determining therapy, defining infection control responses or policy decisions, may be obscure. The narrow definition of an ESBL suggests an Ambler class A type, clavulanate-inhibited, Bush-Jacoby group 2e (“e” standing for “extended spectrum”) enzyme that can hydrolyze an oxyimino-cephalosporin at a rate at least 10% of that for benzylpenicillin. Yet many other enzymes, such as OXA-type cephalosporinase or carbapenemase, plasmid-mediated AmpC, metallo-β-lactamases, or KPC-type carbapenemases all share some activity in common with ESBLs, and lead to key resistance patterns such as resistance to expanded spectrum cephalosporins. Such β-lactamase types are not considered as “true” or “classical” ESBLs, yet have equal or greater consequences for infection control and therapeutic decision making. A simplified nomenclature has been proposed, whereby the term ESBL applies to any broad-spectrum β-lactamase with a suffix to suggest underlying mechanisms (e.g., using ESBL-ARB for a KPC-type carbapenemase, ESBL-MEC for a “miscellaneous” plasmid AmpC type, or ESBL-ARB-D for OXA-type carbapenemase). This nomenclature is yet to find widespread use or acceptance.13,14

**The Problem with AmpC**

In addition to ESBL-type enzymes, Ambler class C (Bush-Jacoby group 1) enzymes may also effectively hydrolyze third-generation cephalosporins. These enzymes have been recognized since the 1960s and were termed AmpC-type β-lactamases—a nomenclature that remains today. Many gram-negative species contain chromosomally located genes encoding and regulating AmpC. Yet, in several species AmpC is only expressed at clinically insignificant levels (e.g., E. coli, Shigella spp.), and do not alter the effect of β-lactams, unless their expression is upregulated by mutations in promoter regions.15 In some species, AmpC production is controlled by transcription factors that respond to changes in cell-wall cycling pathways under the influence of β-lactam exposure, leading to marked increases in AmpC levels—so-called inducible expression.16 Inducible ampC genes are usually chromosomally located and are intrinsic to certain species: particularly Enterobacter cloacae, Enterobacter aerogenes, Serratia marcescens, Citrobacter freundii, Providencia spp., and Morganella morgani. These species have been informally labeled as the “ESCPM” or “SPACE” organisms.17,18 However, there is no clear definition for the term; it can include variable species (such as Proteus vulgaris or P. penneri, which have a weakly inducible cephalosporinase, but of a class A type19) and underestimates the variability in AmpC expression in each species and the clinical consequences of this. It should also be noted that nonfermenters such as Pseudomonas aeruginosa also possess inducible AmpC enzymes with homology to those seen in Enterobacteriaceae.20 There are several other additional species that possess AmpC-type enzymes, with variation in the levels of expression and subsequent clinical significance.6 Nevertheless, the “ESCPM”
penicillin-binding proteins, the complex interaction of peptidoglycan breakdown products, is now understood to be linked to cell-wall recycling involving an inducing agent, but when exposed to a β-lactam inducer, AmpC expression increases to 10- to 200-fold. For instance, the AmpR protein found in E. coli, Klebsiella spp., and Proteus spp. but have been described in most Enterobacteriaceae and Pseudomonas spp. KPC seen in Klebsiella pneumoniae

Class B

Group 3

Contain metal ions (e.g., Zn²⁺). Carbapenemase activity, not inhibited by clavulanate/ tazobactam. Aztreonam not hydrolyzed

Highly transmissible on plasmids carrying multiple other resistance determinants

E. coli, Klebsiella spp. But described in many Enterobacteriaceae

Carbapenemase: IMP, NDM (Often called "metallo-β-lactamases")

Class C

Group 1

Contain serine residues at active site. Also known as “AmpC” enzymes. Broad cephalosporinase activity including hydrolysis of third-generation cephalosporins and cephamycins, but cepime usually stable. Not inhibited well by clavulanate, and only limited tazobactam effect

Chromosomally encoded in several species, and may be inducible by exposure to β-lactams. Expression regulated by complex systems; mutations in key regulatory genes can lead to “derepression” and high-level AmpC production. Increasing plasmid transmission seen

Enterobacter cloacae, E. aerogenes, Serratia marcescens, Citrobacter freundii, Providencia spp. and Morganella morganii all contain inducible AmpC enzymes. Plasmid-mediated AmpC increasing in E. coli, Klebsiella spp.

Cephalosporinase: CMY, DHA, ACT

Class D

Group 2d

Contain serine residues at active site. Oxacillinases that may have carbapenemase activity. Only weakly inhibited by clavulanate

May be acquired or naturally occurring chromosomal genes. May be co-located on plasmids with other β-lactamases (e.g., OXA-48 and CTX-M-15)

Increasingly described in Enterobacteriaceae (e.g., K. pneumoniae and OXA-48)

Carbapenemase: OXA-types

Abbreviation: ESBLs, extended-spectrum β-lactamases.

term can be useful in encapsulating a complex issue in shorthand, but one should be mindful of its limitations.

Chromosomally encoded AmpC enzymes in the species listed earlier render them intrinsically resistant to some narrow spectrum β-lactams and early generation cephalosporins. Under exposure to β-lactam antibiotics, the action of regulatory elements (particularly AmpR, which represses AmpC expression in the absence of an inducer) is altered and ampC expression can occur at significant levels.23 For instance, the AmpR protein found in C. freundii downregulates the expression of AmpC by 2.5-fold in the absence of an inducing agent, but when exposed to a β-lactam inducer, AmpC expression increases to 10- to 200-fold.22 This process is now understood to be linked to cell-wall recycling involving a complex interaction of peptidoglycan breakdown products, penicillin-binding proteins, the ampC gene and its regulators (such as AmpR), enzymes involved in recycling muropeptides (such as AmpD), and other modulating elements such as the permease AmpG (see Fig. 2).23-25 This phenomenon is of key importance to antibiotics such as ampicillin, amoxicillin-clavulanate, and first-generation cephalosporins. The “ESCPM” species are intrinsically resistant to these agents—to the extent that susceptibility may call the species identification into question. However, once β-lactam exposure ceases, AmpC levels usually return to baseline. If mutations occur in genes that contribute to the regulation of ampC transcription, AmpC can become constitutively hyper-expressed.6,26 Such AmpC hyper-producers (sometimes termed “de-repressed mutants”) demonstrate additional resistance to third-generation cephalosporins, cephamycins (e.g., cefotixin), new anti-staphylococcal cephalosporins such as ceftaroline,9 anti-pseudomonal penicillins (such as piperacillin and ticarcillin), and their β-lactamase inhibitor combinations.6,27 These variants occur spontaneously at a frequency of ~10⁻⁶ to 10⁻⁸ of the bacterial
population and may be selected rapidly following β-lactam therapy and predispose to clinical failure.

Plasmid-Mediated AmpC

While AmpC is usually chromosomally encoded, we now increasingly see ampC genes mobilized on plasmids, which can easily transfer between species. The first transmissible cephemycinase (CMY-1) was identified in a K. pneumoniae isolate from a patient in South Korea in 1989. Plasmid-mediated AmpC is now becoming increasingly common as a cause of resistance in Klebsiella and E. coli. Such isolates can be identified from nosocomial, community onset, and healthcare-associated infections and may be associated with high mortality. Like ESBL producers, isolates with plasmid AmpC may also frequently be resistant to other agents such as quinolones or trimethoprim–sulphamethoxazole. In the laboratory, they can give a phenotype similar to an ESBL producer (with resistance to third-generation cephalosporins) but fail to demonstrate synergy with clavulanate (the standard test to phenotypically confirm an ESBL) and
also show resistance to cefoxitin.\textsuperscript{31} However, other mechanisms (such as outer membrane protein permeability changes) may confer cefoxitin resistance.\textsuperscript{32} Several inhibitors have been proposed to help confirm AmpC production (such as boronic acid\textsuperscript{36} or cloxacillin\textsuperscript{33}), but the sensitivity and specificity of such tests have been variable and are not routinely used. This can cause substantial difficulty for the clinical microbiologist in knowing which agents to recommend to clinicians for isolates with resistance to key β-lactams, such as third-generation cephalosporins, without immediately defaulting to carbapenems (given the implications for antimicrobial stewardship). Furthermore, plasmid-mediated AmpC can coexist with ESBL enzymes in the same host, making phenotypic interpretation even less reliable.\textsuperscript{29}

Plasmid AmpC genes are usually noninducible, as they lack the genetic apparatus to regulate expression, but there have been reports of plasmid-mediated inducible AmpC spreading into new hosts\textsuperscript{35}—raising the alarming prospect of making it impossible to predict emergent AmpC-mediated resistance by species identification alone. Furthermore, there have been increasing reports of extended-spectrum AmpC β-lactamases, which have developed the ability to inactivate cefepime.\textsuperscript{38}

**Current Epidemiology**

The incidence of infection or colonization with ESBL-producing organisms has dramatically increased in recent years.\textsuperscript{39} A recent WHO report on Antimicrobial Resistance Surveillance reported high levels of resistance to third-generation cephalosporins; rates >50\% for \textit{E. coli} were reported from at least one country in five of six regions and in six of six regions for \textit{K. pneumoniae}.\textsuperscript{40} In some areas, ESBL-positive strains have not simply replaced wild-type ESBL-negative strains, but have added to the overall burden of \textit{E. coli} infection.\textsuperscript{41} There is significant geographical variation in the burden of ESBL-producing Enterobacteriaceae across the world, although limited data prevent forming an accurate and comprehensive assessment, a key weakness identified by the WHO.\textsuperscript{40} The Centers for Disease Control and Prevention have estimated that 23\% of \textit{K. pneumoniae} and 14\% of \textit{E. coli} are ESBL producers and have been associated with 26,000 infections and 1,700 deaths annually in the United States.\textsuperscript{42} In Australia, rates of resistance are relatively low; a national survey in 2012 suggested that 4.2\% of community \textit{E. coli} isolates were resistant to third-generation cephalosporins, but prevalence is increasing.\textsuperscript{43} A recent review of published literature reported the rate of \textit{E. coli} non-susceptibility to third-generation cephalosporins in Latin America to range between 11 to 25\% and 45 to 52\% for \textit{K. pneumoniae}.\textsuperscript{44} The Study for Monitoring Antimicrobial Resistance Trends (SMART) has tracked the susceptibility patterns of gram-negative bacteria identified from intra-abdominal infections since 2002. Of >1,700 isolates from patients with appendicitis in 39 countries from 2008 to 2010, the rates of ESBL positivity were highest for countries in the Asia-Pacific region (but excluding India) at 28\%, lowest in Europe (4.4\%) compared with a global average of 16.3\%.\textsuperscript{45} In a study of >3,000 \textit{E. coli} isolated from intra-abdominal infections across Europe from 2008 to 2009, 11\% were found to be ESBL producers.\textsuperscript{46} ESBL rates as high as 67.1\% in \textit{E. coli} have been reported from the SMART program in India.\textsuperscript{47}

Although a large number of acquired genes can confer antibiotic resistance in gram-negative bacteria, only a relatively small number of these tend to dominate. Across the Asian-Pacific region and the United States, \textit{E. coli} or \textit{Klebsiella} spp. with resistance to third-generation cephalosporins have most frequently acquired \textit{bla}_{CTX-M} type ESBLs.\textsuperscript{48,49} A highly successful pathogenic clone of \textit{E. coli}, known as sequence type 131 (ST131), which also frequently harbors CTX-M-type ESBL\textsuperscript{50,51} has rapidly disseminated globally following a relatively recent evolutionary divergence and demonstrates numerous adaptive responses (including point mutations, recombination events, and acquisition of mobile genetic elements) that have contributed to its prevalence.\textsuperscript{52}

\textit{E. coli} containing ESBLs, particularly CTX-M types, have been increasingly seen from community isolates.\textsuperscript{39,53–56} Residence of a long-term care facility has been recognized as a key risk factor for community acquisition of ESBL producers.\textsuperscript{57,58} In low-prevalence countries, such as Australia, travel to a region with high endemicity for resistance, especially with healthcare exposure, has emerged as a key risk factor for subsequent infection with an ESBL producer.\textsuperscript{59} Travel to India has been associated with a high prevalence of colonization; 37.4\% of returned travelers in Spain presenting with diarrhea symptoms tested positive for ESBL-producing \textit{E. coli}.\textsuperscript{60} Gut colonization with CTX-M-producing \textit{E. coli} was seen in 6\% of >3,000 individuals tested from the community in Germany, highlighting the reservoir of resistance that may exist in the population.\textsuperscript{61} Following infection, fecal carriage of ESBL-producing \textit{E. coli} can frequently persist for up to 12 months,\textsuperscript{62} with a median duration of 6.6 months in one study.\textsuperscript{63} The risk for subsequent infection with an expanded-spectrum cephalosporin-resistant \textit{E. coli} appears to be significantly increased for up to 6 months following exposure.\textsuperscript{59}

**Clinical Risk Factors and Outcomes**

Several studies have attempted to define risk factors for infection with ESBL or AmpC producers, primarily to aid selection of appropriate empirical therapy. Prior antibiotic use has emerged as a key influence upon the risk for infection by an ESBL producer. An international multicenter prospective observational study examined 455 consecutive bacteremia events caused by \textit{K. pneumoniae}, 18.7\% of which were ESBL producers.\textsuperscript{64} Prior use of a β-lactam containing an oxyimino group (particularly third-generation cephalosporins), even after adjusting for confounders, was associated with bacteremia caused by an ESBL-producing \textit{K. pneumoniae} (relative risk (RR), 3.9; 95\% confidence interval [CI], 1.1–13.8).\textsuperscript{64} It is worth noting that the great majority (96.5\%) of infections by ESBL-producing \textit{Klebsiella} were nosocomially acquired in this study, contrasting the current increasing incidence of community-acquired ESBL-producing \textit{E. coli}.\textsuperscript{65,66}

Kang et al examined factors associated with bacteremia caused by ESBL-producing \textit{K. pneumoniae}; risks included the...
presence of a catheter, a recent invasive procedure, or broad-
spectrum antibiotic use. Similar findings were reported by
Tumbarello et al, where prior antibiotic use, increasing age, and
length of hospitalization were risk factors for bloodstream
infection with ESBL-producing K. pneumoniae. In a
Korean study, by examining risk factors for community-
acquired ESBL E. coli bacteremia, a decision-tree analysis
suggested that empirical coverage for ESBL producers should be
used in patients with septic shock, hepatobiliary infection, and
healthcare-associated infections. Among ICU patients, risk of
subsequent infection in patients with prior colonization with
ESBL-producing Enterobacteriaceae was associated with
referral from a medical ward, nursing home, or rehabilita-
tion center; fluoroquinolone use; and the use of extracorpo-
real membrane oxygenation. Prior use of third-
generation cephalosporins appears to be a consistent risk
factor for subsequent infection by an ESBL producer. There is
equal risk with other antibiotic exposures, including
cephalosporins, β-lactam/β-lactamase inhibitors (BLBLs) and
quinolones. Risk-prediction models have been de-
veloped and can prove useful in predicting community-onset
ESBL infections, especially when combined with local epidemi-
ology. However, such scoring systems require careful
validation, as their performance can vary between hospital
locations.

Infections caused by ESBL producers have had a significant
clinical impact and may be associated with adverse outcomes
such as increased mortality, length of hospital stay, and
healthcare-associated costs, particularly when associated with inadequate initial antimicrobial
therapy.

Treatment
There is a significant evidence gap between our understand-
ing of basic biology of ESBL- and AmpC-producing bacteria
and the clinical application of this information. Despite many
hundreds of studies reporting on resistant gram negatives,
the majority of studies focus on laboratory, epidemiological,
or infection-control aspects of these bacteria—only a handful
provide reliable insight into optimal therapy. There have been
several notable but relatively small observational studies
reporting treatment outcomes for ESBL or AmpC producers.
While these have been invaluable to our limited evidence-
base, we have been lacking adequately powered, well-de-
designed, international prospective studies in this area. Particu-
larly, there has never been a randomized controlled trial
reported that specifically addresses these questions, which is
unfortunate given the significance and scale of the problem,
but not a surprise given the realities of clinical research.

It would seem intuitive that selecting appropriate initial
empirical therapy is important in patients with bacteremia,
and becomes increasingly difficult when the incidence of
resistance is high. Choosing inappropriate empirical antibiot-
ic therapy for bacteremia caused by ESBL-producing E. coli or
Klebsiella has been associated with increased mortality in
some studies, especially in nonurinary infections or with
multidrug resistant isolates. However, this has not been a
universal finding, with several studies showing no significant
impact of inappropriate empirical therapy on mortality.
A meta-analysis of 16 studies suggested increased
mortality in bacteremia caused by ESBL producers (RR, 1.85;
95% CI, 1.39–2.47), which increased with delayed therapy (RR,
5.56; 95% CI, 2.9–10.5), although only 1 study controlled for
confounders.

Expanded-Spectrum Cephalosporins for ESBL and
AmpC Producers
Soon after the recognition of ESBLs emerging as a concern,
clinical failures in patients treated with third-generation
cephalosporins for infections caused by ESBL producers
were reported, even when breakpoints used at that time
suggested susceptibility. This phenomenon was also
supported by animal studies. Observational studies sug-
gested that treatment with cephalosporins of bloodstream
infection caused by ESBL producers was associated with
poorer outcome when compared with non-ESBL strains in
children and adults. Empirical therapy with ceftriaxone in
patients with pyelonephritis, found subsequently to be
carried by ESBL-producing E. coli, was associated with delayed
resolution of symptoms, less likelihood of microbiological
resolution at 5 days and longer hospital admissions.

Given the concern that bacteria could harbor ESBLs that
would not be detected by the higher breakpoints for cepha-
losporins used at the time, the use of third-generation
cephalosporins for ESBL producers (even if susceptible)
was discouraged. Regulatory authorities issued guidance
that laboratories should report all ESBL-containing E. coli
and Klebsiella spp. as resistant to all penicillins, all cephalosporins,
and aztreonam, regardless of susceptibility results. Although
less supported by evidence, most laboratories extended
such guidance to include all other non-carbapenem
β-lactams, including inhibitor combination agents. Although
this was an understandable response to the challenges faced
at the time, it had the unintended consequence of directing
clinicians to use carbapenems increasingly frequently for
ESBL-related infections, even in relatively uncomplicated
disease like cystitis.

In recent years, the Clinical and Laboratory Standards
Institute (CLSI) and European Committee on Antimicrobial
Susceptibility Testing (EUCAST) have lowered the suscepti-
bility breakpoints for third- and fourth-generation cepha-
losporins against Enterobacteriaceae, without the need for
additional testing. For ESBL production, unless for infection
control or surveillance purposes. This still begs the
question of whether any known ESBL producer which tests
susceptible to an agent, against which the enzyme has
potential activity, can be safely used clinically. For instance,
CTX-M producers may retain susceptibility to cefazidime;
yet whether this would be safe drug to use for a serious
infection remains unclear, largely due to the presence of
pronounced inoculum effects and limited clinical data. In
theory (at least), given the revised standards, susceptibility
should be read as reported and therapeutic options provided
as such. In this way, the current guidance has the implicit
message that clinicians should worry less about the
underlying resistance mechanism when selecting therapy. However, there remains concern that drugs that may act as substrates for ESBLs should still be avoided for therapy, even if susceptibility is demonstrated.102

Emergent resistance during therapy with third-generation cephalosporins for AmpC-producing Enterobacteriaceae has been a major concern. A key study from 1991 by Chow et al reported outcomes for patients with bloodstream infections caused by Enterobacter spp. In those treated with a third-generation cephalosporin, 19% experienced relapsed bacteremia and resistance mediated by high level of AmpC, despite initial susceptibility.103 This phenomenon has been replicated in larger cohorts, although a lower risk of clinical failure has been reported with other AmpC-producing species.104,105 When emergent resistance occurs, it has been associated with higher mortality and healthcare-associated costs.106 As a result, the use of third-generation cephalosporins for the treatment of significant infections caused by AmpC producers such as Enterobacter spp. has been strongly discouraged, except perhaps in simple infections (such as uncomplicated urinary tract infection [UTI]), where a rapid bactericidal effect can be achieved before selection for hyper-producing mutants can occur.107 Poor outcomes have also been reported for plasmid AmpC-producing K. pneumoniae treated with third-generation cephalosporins; although such studies often are small, retrospective and report mortality rates unadjusted for comorbidity.108

Cefepime for AmpC and ESBL Producers
Cefepime is the only expanded spectrum cephalosporin with stability to AmpC β-lactamase and retains in vitro activity to species such as E. cloacae, including constitutively AmpC derepressed strains.109 Recent retrospective studies would suggest that cefepime is effective for infections caused by AmpC-producing Enterobacteriaceae. Comparing patients paired by propensity score matching given either meropenem or cefepime, there were no differences in 30-day mortality (odds ratio [OR], 0.63; 95% CI, 0.23–2.11) or length of hospital stay (RR, 0.96; 95% CI, 0.79–1.26), although this study included only 64 patients.110 In a large series of over 300 patients with Enterobacter bacteremia, mortality was similar for patients treated with meropenem or cefepime after adjustment for comorbidity and propensity score matching.111

However, the picture is complicated by the fact that Enterobacter, Citrobacter, and Serratia spp. can frequently acquire additional ESBLs,112 to which cefepime is not stable, thus elevating minimum inhibitory concentrations (MICs).113 Clinical failures from isolates with MICs at or above the previous CLSI breakpoint of 8 μg/mL treated with cefepime have been shown to be associated with an increased risk of mortality, especially with a dosing regimen of 1 g 12 hourly.114 ESBLs expressed in AmpC producers may be difficult to reliably detect and discriminate from chromosomal AmpC with routine laboratory methods. Cefepime may also be subject to significant inoculum effects with ESBL producers.115 Eightfold or greater increases in MIC values were observed with several cephalosporins, including cefepime, when tested against a variety of Enterobacteriaceae at inocula 100-fold higher than standard—a phenomenon not seen with carbapenems.116 Similarly, cefepime was prone to significant inoculum effects when tested against K. pneumoniae containing plasmid-mediated AmpC β-lactamase.117 Resistance to cefepime in Enterobacter has also been described to develop by the overexpression of an altered AmpC enzyme or porin mutations.118

Treatment of ESBL-producing Klebsiella or E. coli with cefepime is controversial. Cefepime, like other cephalosporins, demonstrates marked inoculum effects in vitro when tested against ESBL producers.109 Some small case series have reported a role for cefepime, although clinical failures were observed.119 In a retrospective study that compared cefepime to a carbapenem for the treatment of bacteremia caused by susceptible ESBL producers, cefepime was independently associated with an increased 30-day mortality on multivariate analysis (OR, 9.9; 95% CI, 2.8–31.9).120 A nonsignificant trend toward increased mortality was also seen for cefepime when used as empirical therapy for bacteremia caused by ESBL producers (OR, 1.66; 95% CI, 0.71–3.87).121

It has been suggested that standard dosing of cefepime should be effective for ESBL producers that demonstrate an MIC for cefepime of ≤2 mg/L (CLSI) or ≤1 mg/L (EUCAST), but higher or more frequent dosing would be required for an MIC between 4 and 8 mg/L.122 It should be noted that the method of susceptibility testing for cefepime against ESBL producers may provide variable results; lack of concordance between gold standard agar dilution and Vitek2 microbroth dilution methods have been reported and could lead to major interpretative errors, especially when lowered breakpoints are used.123

Cephamycins
Although rarely used in many countries, cephamycins (such as cefoxitin, flomoxef, and cefmetazole) remain stable to hydrolysis by ESBLs, but are susceptible to AmpC enzymes. Cefoxitin was effective in a murine model of UTI caused by CTX-M–15–producing E. coli, when compared with a carbapenem.124 A small study from Japan compared cefmetazole to meropenem for the treatment UTI caused by ESBL-producing Enterobacteriaceae and showed no differences in clinical or microbiological cure rates or adverse events.125 However, for dialysis patients with ESBL K. pneumoniae bacteremia and high acuity of illness, use of flomoxef was independently associated with mortality (OR, 3.52; 95% CI, 1.19–58.17).126

Carbapenems
Carbapenems have long been considered the first-line treatment option for significant infections caused by ESBL or AmpC producers. Carbapenems are generally stable to hydrolysis by ESBLs or AmpC. They are less affected by inoculum effects in vitro117 and in animal models.39 They demonstrate excellent pharmacodynamic exposure in vitro. Monte Carlo simulation of carbapenems against 133 ESBL-producing isolates showed that the bactericidal cumulative fraction of response (defined as ≥40% of the proportion of the dosing interval for which free drug levels were above the MIC) was...
achieved for 96.3% of isolates against ertapenem and >99% for imipenem and meropenem. 

Several observational studies have demonstrated that carbapenems are associated with improved outcome when compared with cephalosporins or other alternatives for bloodstream infections caused by ESBL producers. However, superiority has never been demonstrated in a randomized trial.

Ertapenem, a carbapenem lacking activity against *Pseudomonas*, has been increasingly used for directed therapy against ESBL and AmpC producers. Testing both ertapenem and meropenem against ESBL-producing *E. coli* or *Klebsiella* with a range of MICs in an animal model showed that both drugs had similar efficacy when MICs were low, but meropenem had greater efficacy against isolates with tertapenem MICs ≥ 2 μg/mL. Ertapenem achieved clinical success in 80% of patients with ventilator-associated pneumonia caused by ESBL producers, although this study only enrolled 20 patients and lacked any control group. Similarly, a clinical success rate of 78% and microbiological cure rate of 92% were seen in a series of patients treated with ertapenem for a variety of infections caused by ESBL producers, although this study also had only 50 evaluable patients and no comparison group. Favorable clinical response rates of up to 96% have been reported in its use against ESBL bacteremia. When ertapenem was compared with carbapenems (such as meropenem) for the treatment of bacteremia caused by ESBL-producing Enterobacteriaceae in a cohort of 261 patients, no difference in mortality was seen even after controlling for the propensity to receive ertapenem (OR, 0.50; 95% CI, 0.12–2.1). Other studies of ESBL bacteremia have also shown an equivalent mortality between ertapenem and other carbapenems.

Although most studies have concentrated on *Klebsiella* and *E. coli*, being the most common ESBL producers, some studies have examined treatment options for other ESBL-producing species. Huang et al. assessed the 14-day survival of 54 adult patients with bacteremia caused by ESBL producers other than *E. coli* or *Klebsiella* spp. (including intrinsic AmpC producers such as *E. cloacae* or *C. freundii*) and compared carbapenem to noncarbapenem therapy. Although improved survival (90.9%, 20/22) was seen with carbapenems compared with noncarbapenems (71.9%, 23/32), with ciprofloxacin as the main alternative choice, this difference was not statistically significant. As with many small retrospective cohorts, such studies may be underpowered to detect true differences in treatment regimens.

The treatment options for inducible AmpC-producing Enterobacteriaceae that also express ESBLs are limited. Among 31 patients with ESBL-producing *E. cloacae*, all (8/8) patients who received a carbapenem survived, whereas 38.5% died when given a noncarbapenem (p = 0.06). In a study that compared patients treated for bacteremia caused by ceftriaxone nonsusceptible *E. cloacae*, with or without ESBL production, carbapenems were associated with lower mortality in the ESBL group when compared with those treated by noncarbapenem β-lactam (5/53, 9.4% vs. 13/44, 29.5%; p = 0.01), although the difference was not significant in a multivariate analysis; breakthrough bacteremia was more common in the noncarbapenem β-lactam group (18/31, 58% vs. 3/31, 9.6%; p < 0.001).

However, emergent carbapenem resistance has been described during carbapenem therapy, leading to clinical failure. In a patient with pneumonia caused by a CTX-M–producing *K. pneumoniae* treated with ertapenem, carbapenem resistance developed via the loss of a porin. Carbapenem resistance in Enterobacteriaceae may occur either by the acquisition of a carbapenemase, hyperproduction of AmpC, or an ESBL combined with porin mutations or via efflux pumps. Resistance to ertapenem has also been described by chromosomal AmpC mutations that allow carbapenemase activity, especially when combined with loss of outer membrane proteins.

**β-Lactam/β-Lactamase Inhibitor Combinations**

By definition, ESBLs (Ambler class A enzymes) are inhibited by clavulanate and tazobactam. Indeed, phenotypic confirmation of an ESBL in *E. coli*, *Klebsiella* spp., and *Proteus mirabilis* relies upon this phenomenon. These inhibitors act as suicide substrates by irreversibly binding to β-lactamase enzymes. Despite this inhibition, the currently available BLBLIs (such as amoxicillin–clavulanate, piperacillin–tazobactam, ampicillin–sulbactam, cefoperazone–sulbactam, and ticarcillin–clavulanate) have generally been avoided for infections caused by ESBL producers in favor of carbapenems.

In general, piperacillin–tazobactam has retained good in vitro activity against ESBL producers, especially for *E. coli*, although *K. pneumoniae* are often less susceptible. MICs for BLBLIs tested against ESBL producers may tend to cluster around susceptibility breakpoints, so a single dilution change (within the margin of error) can alter the categorization. BLBLIs, especially piperacillin–tazobactam, may also be subject to significant inoculum effects in vitro. Although piperacillin–tazobactam exhibits significant MIC elevations against ESBL producers tested using a high inoculum, this phenomenon is less marked than that observed with expanded-spectrum cephalosporins. Inoculum effects are not universal to all BLBLIs. In time–kill studies of amoxicillin–clavulanate, bactericidal killing of ESBL-producing *E. coli* was maintained over 24 hours in the presence of a high inoculum, in contrast to piperacillin–tazobactam. However, an inoculum effect was also seen for piperacillin–tazobactam against non-ESBL strains, which suggests that the effect is more likely a property of the drug rather than related to β-lactamase activity alone. The significance of the inoculum effect has been debated and has been argued to represent a laboratory phenomenon of limited clinical significance. However, some animal models appear to reproduce the effect. In a murine model of pneumonia caused by ESBL-producing *K. pneumoniae* at higher inoculum, 100% of mice died with piperacillin–tazobactam treatment, in contrast to 100% survival with meropenem. However, in animal models at standard inocula, piperacillin–tazobactam appeared to be efficacious against ESBL-producing *K. pneumoniae*, whereas ceftazidime was not; although imipenem was the most effective agent.
Another theoretical concern relating to the use of BLBLIs for ESBL producers is the co-location of other β-lactamase types on acquired plasmids, some of which may be poorly inhibited (such as plasmid AmpC or OXA-1). Bacteria may overexpress other non-ESBL “parent” enzymes that can overcome β-lactamase activity.156,157 Resistance may also occur by the development of inhibitor-resistant enzymes, porin mutations, or efflux pumps.158 It should be noted that BLBLIs have been used for many years against isolates with narrow spectrum β-lactamasas, even in critical infections, without clear concerns over loss of efficacy.

However, there were early reports of clinical failure with piperacillin–tazobactam against ESBL producers.159,160 There were concerns over the reliability of tazobactam to inhibit some ESBL variants or if expression occurs at high levels156,161 and limited experience with the use of BLBLIs for this indication. As a result, a view was formed that these agents could not be relied upon.148

In recent years, clinical evidence has accumulated that may support the use of BLBLIs in the treatment of infections caused by ESBL producers. Piperacillin–tazobactam was effective in treating a small series of patients with UTI caused by ESBL producers, as well as 90% of infections from other sites, provided the MIC was ≤16 μg/ml.162 In a small study from Thailand, a predictor of mortality in patients with bloodstream infection caused by ESBL-producing E. coli or Klebsiella was failure to receive either a carbapenem or BLBLI for empirical therapy (93 vs. 43%; p = 0.002), although all patients switched to carbapenem therapy once susceptibility was determined.163 After adjustment for confounders, no association between empirical use of piperacillin–tazobactam and increased mortality was found in a study of 114 patients from Korea with bacteremia caused by ESBL-producing E. coli or K. pneumoniae (OR, 0.55; 95% CI, 0.16–1.88).164 In a large study of 387 ESBL E. coli bacteremia cases, piperacillin–tazobactam was associated with lower mortality when compared with carbapenems, provided treatment was adequate.165

Much of the current evidence to support the use of BLBLIs has been derived from a large Spanish cohort of patients with bacteremia caused by ESBL-producing E. coli. A post hoc analysis of six prospective studies compared BLBLI treatment with carbapenems and found no differences in mortality for empirical (hazard ratio [HR], 1.14; 95% CI, 0.29–4.40) or definitive therapy (HR, 0.76; 95% CI, 0.28–2.07).165 However, for nonurinary infection, an MIC ≤2 mg/L to piperacillin–tazobactam appears to be predictive of better outcome.167 A larger international observational study, including 656 patients, has recently been reported and also suggests non-inferiority for BLBLIs used for ESBL bloodstream infection in comparison to carbapenems, with an adjusted HR for 30-day mortality of 0.97 (95% CI, 0.48–2.03).168

Optimized dosing of piperacillin–tazobactam to reach therapeutic drug targets may be necessary in critically ill patients,169 who frequently demonstrate altered pharmacokinetics through variations in key variables such as renal clearance, increased capillary permeability, hypoalbuminemia and increased volumes of distribution.170 Continuous infusions of β-lactams may improve outcomes in critically ill patients.171

BLBLIs such as piperacillin–tazobactam may offer a carbapenem-sparing “step-down” option once susceptibility is proven—especially if the MIC is low and the burden of infection has been reduced.150 This seems most reliable for urinary infections. However, further evidence is required to allow confidence in efficacy for a wider set of clinical circumstances. An international randomized-controlled trial registered with www.clinicaltrials.gov that compares piperacillin–tazobactam with meropenem for the definitive treatment of bloodstream infections caused by ceftriaxone nonsusceptible E. coli or Klebsiella spp. is currently recruiting (Trial registration number NCT02176122).

**BLBLIs for AmpC Producers**

AmpC enzymes are generally poorly inhibited by clavulananate or tazobactam, although the concentration of tazobactam needed to inhibit AmpC β-lactamase is much lower than for clavulananate.172 Clavulananate is a powerful inducer of AmpC and poorly inhibits its activity, and so may antagonize the activity of ticarcillin when used in combination against isolates with inducible β-lactamase.173 Conversely, tazobactam is much less potent inducer of AmpC.174 However, once again, the clinical efficacy of drugs such as piperacillin–tazobactam against AmpC producers is controversial. Isolates with derepressed AmpC frequently demonstrate high MIC values to piperacillin–tazobactam. It is a curiosity that the AmpC enzyme produced by M. morganii is well inhibited by tazobactam, even when highly expressed.173,174 However, no clinical studies exist to corroborate efficacy in significant M. morganii infections.

Clinical studies to assess the efficacy of piperacillin–tazobactam in serious infections caused by AmpC producers are limited. Many laboratories do not report piperacillin–tazobactam susceptibility results for AmpC producers such as Enterobacter spp., over concerns of clinical failure and emergent resistance. This practice is somewhat extrapolated from the poor outcomes seen with third-generation cephalosporins.103 However, piperacillin–tazobactam was not associated with the emergence of cephalosporin resistance in the treatment of Enterobacter bacteremia (RR, 1.1; 95% CI, 0.4–2.7) in contrast to third-generation cephalosporins (RR, 3.3; 95% CI, 1.8–6.0).104 In another study that examined 377 Enterobacter bacteremia events in adults, the only factor independently associated with a reduction in 30-day mortality was the early use of piperacillin–tazobactam.175 However, piperacillin–tazobactam use may still cause selection pressure for isolates with derepressed AmpC. The risk of isolating a resistant Enterobacter following piperacillin–tazobactam or broad-spectrum cephalosporin was equal in one study (2% in both groups, RR = 1.02; p = 0.95).176

**Fosfomycin**

Fosfomycin has been used for many years in some countries as a single-dose treatment for uncomplicated UTIs caused by E. coli. It has a bactericidal effect by inhibiting cell wall
synthesis. There has been renewed interest in its use against urinary infections caused by ESBL- or plasmid AmpC-producing E. coli or K. pneumoniae, as it demonstrates excellent in vitro activity against such strains. Although only a handful of clinical studies have examined fosfomycin for the treatment of UTI caused by ESBL-producing E. coli, clinical response rates of >78% have been reported. In a prospective observational study from Turkey, oral fosfomycin (given alternate days for 3 doses) was compared with carbapenems (given for 14 days) for ESBL E. coli causing lower UTIs in 47 patients, with complicating factors such as catheterization or urological surgery, but no signs of pyelonephritis. Clinical and microbiological success rates were similar in both the fosfomycin and carbapenem groups, with significant cost savings seen and no adverse effects reported in those given fosfomycin. It achieves high concentrations in prostate tissue and may be a useful prophylactic antibiotic before transrectal prostate biopsy or for treatment of prostatitis caused by resistant gram-negative bacteria. Increased use of fosfomycin has been associated with a rising burden of resistance. In Spain, the incidence of fosfomycin resistance in ESBL-producing E. coli has increased from 0% in 2005 to 14.4% in 2011. Rates of resistance remain low, but high-level resistance can occur via single-step mutations, or may be acquired on plasmids. It should be noted that studies of fosfomycin resistance require attention to the methods used. Resistance can be overestimated by disk diffusion or microbroth dilution susceptibility testing, when compared with a reference agar dilution.

Data regarding the use of fosfomycin outside the urinary tract are sparse. In a murine model of ESBL-producing E. coli implant infections that compared combinations of fosfomycin, tigecycline, gentamicin, and colistin, fosfomycin was the only single agent able to eradicate biofilm in a small number of cases (17%) and, when combined with colistin, had the highest cure rate (8/12, 67%) and was superior to fosfomycin alone. Intravenous formulations of fosfomycin are available in some countries. It has been used successfully as salvage therapy in combination with meropenem for refractory Lemierre syndrome, bacteremia and cerebral abscesses caused by ESBL-producing K. pneumoniae. In a literature review of available evidence that examined 62 studies involving 1,604 patients with various infections treated with fosfomycin alone or in combination, an overall cure rate of 81.1% was reported.

Tigecycline
Tigecycline, a first in class glyccyclcline, has activity against most ESBL- and AmpC-producing Enterobacteriaceae. It should be noted that tigecycline has limited penetration into the urinary tract and may not be effective at this location, although successful treatment has been reported. It may also achieve poor serum levels because of a very large volume of distribution, which may limit effectiveness in bacteremia. Breakthrough infections have been reported. However, it has been used successfully as salvage therapy for a complex infection caused by a carbapenem-resistant, ESBL-producing K. pneumoniae. A recent meta-analysis has suggested excess mortality for tigecycline, limiting enthusiasm for its use in serious infections when alternatives exist.

Mecillinam and Pivmecillinam
Mecillinam is an amidopenicillin with a wide spectrum of gram-negative activity. There has been interest in its use against resistant Enterobacteriaceae since the 1970s. It appears to act by binding penicillin-binding protein-2 to inhibit cell wall synthesis. However, given the poor oral bioavailability of this drug, the prodrug pivmecillinam has been developed and is approved for the use against uncomplicated UTIs. It is now included in the Infectious Disease Society of America (IDSA) guidelines for the treatment of cystitis, although there are concerns that it has inferior efficacy for pyelonephritis when compared with other agents. However, (piv)meccillinam has the advantage of retaining activity against ESBL and plasmid AmpC-producing E. coli, even if expressing multiple β-lactamase types. In a series of 100 ESBL-producing E. coli isolated from patients with (predominantly community acquired) UTI, 85% demonstrated susceptibility to pivmecillinam. Against E. coli strains that expressed various β-lactamase types, it showed excellent activity when compared with other penicillins against isolates that contained TEM, IRT, and AmpC producers. The combination with clavulanate may also enhance its activity and mitigate against inoculum effects. In a small study of patients with lower UTI caused by ESBL-producing E. coli or K. pneumoniae, patients treated with pivmecillinam achieved good clinical responses (8/8) but low bacteriological cure rates (defined as <10^5 CFU/mL at 30-day follow-up). The drug is not approved for use in the United States and has not been widely used outside European countries, but deserves greater attention in an era of increasing community-acquired gram-negative resistance. It has also been suggested that, although resistance to pivmecillinam may develop by mutations in the genes affecting the bacterial elongation process, the risk for clonal spread is low and may be associated with limited epidemiological fitness.

Temocillin
Temocillin is a carboxypenicillin derivative of ticarcillin, which has been modified to improve stability to AmpC and ESBL enzymes, although it has less activity against Pseudomonas spp., gram positives, and anaerobes. Having initially received little market interest, it was withdrawn from the United Kingdom, but continued to be used in Belgium for infections caused by resistant Enterobacteriaceae, and it has now been relaunched. It demonstrated in vitro efficacy against 88% of 846 isolates with ESBL or AmpC phenotypes or K. oxytoca K1 hyperproducers, and >90% of multiresistant ESBL-producing E. coli. However, temocillin efficacy may be affected by porin mutations and is not stable to OXA-48 and NDM-1 carbapenemases. It achieves excellent levels in the urine and may be a useful agent for infections at this site, although clinical data are limited. One study reported outcome for 92 adults treated with temocillin, mainly for urinary or bloodstream infections caused by ESBL or derepressed AmpC producers. Good clinical (86%) and microbiological
variants. It has been shown to retain its effect against extend-spectrum AmpC enzymes. Coformulations with ceftazidime, ceftaroline, and tazobactam against ESBL producers is Avibactam, a new non-beta-lactam inhibitor of AmpC producers. However, overuse of carbapenems is likely to be driving increased selection pressure for carbapenem resistance—a new threat that is rapidly emerging on a global scale. Carbapenem-sparing options should be increasingly considered in less critical infections and in targeted circumstances. BLBLIs such as piperacillin–tazobactam are probably effective treatment for ESBL producers when susceptibility is proven, especially in urinary or biliary tract infections or when the MIC is low. Efficacy in critical or complex infections remains uncertain—a proposed trial may go some way to answering this question. Amoxicillin–clavulanate is also likely to be a reasonable choice for urinary infections caused by susceptible ESBL producers. Although theoretical concerns have limited the use of piperacillin–tazobactam against AmpC producers, several observational studies have not indicated a strong signal for high failure rates. Cefepime is stable to AmpC derepressed isolates and is increasingly considered in less critical infections and in targeted circumstances. BLBLIs such as piperacillin–tazobactam against AmpC producers, several observational studies suggest that it is effective against AmpC producers causing bacteremia, but efficacy against ESBL producers is unreliable. There also remains a reasonable selection of uncommon antibiotics to treat less critical infections, such as fosfomycin, pivmecillinam, or temocillin, but clinical experience is limited and some of these drugs are not widely available outside specific countries. Some novel BLBLI agents are in development, and we await larger clinical trials with interest.

Conflict of Interest
None.

References
Mark BL, Vocadlo DJ, Oliver A. Providing β-lactams a helping hand: targetting the AmpC β-lactamase induction pathway. Future Microbiol 2011;6(12):1415–1427


Hanson ND. AmpC β-lactamases: what do we need to know for the future? J Antimicrob Chemother 2003;52(1):2–4


Lob SH, Badal RE, Bouchillon SK, Hawser SP, Hackel MA, Hoban DJ. Epidemiology and susceptibility of Gram-negative appendicitis
Clinical Management of Infections Caused by Enterobacteriaceae

Harris

Valenza G, Nickel S, Pfeifer Y, et al. Extended-spectrum-

Rogers BA, Ingram PR, Runnegar N, et al; Australasian Society for

Stuart RL, Kotsanas D, Webb B, et al. Prevalence of antimicrobial-

Doi Y, Park YS, Rivera JI, et al. Community-associated extended-

Chen LF, Freeman JT, Nicholson B, et al. Widespread dissemination of


Skelovec C, Bertrand X, Leroy J, Faller JP, Talon D, Hocquet D. Identifying patients harboring extended-spectrum β-lactamase-producing Enterobacteriaceae on hospital admission is not that


Clinical Management of Infections Caused by Enterobacteriaceae

Harris


