

Extended Spectrum β -Lactamase (ESBL)-Producing Enterobacteriaceae

Considerations for Diagnosis, Prevention and Drug Treatment

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Abstract

Extended spectrum β -lactamase (ESBL)-producing organisms pose unique challenges to clinical microbiologists, clinicians, infection control professionals and antibacterial-discovery scientists. ESBLs are enzymes capable of hydrolysing penicillins, broad-spectrum cephalosporins and monobactams, and are generally derived from TEM and SHV-type enzymes. ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species. Although the prevalence of ESBLs is not known, it is clearly increasing, and in many parts of the world 10–40% of strains of *Escherichia coli* and *Klebsiella pneumoniae* express ESBLs.

ESBL-producing Enterobacteriaceae have been responsible for numerous outbreaks of infection throughout the world and pose challenging infection control issues. Clinical outcomes data indicate that ESBLs are clinically significant and, when detected, indicate the need for the use of appropriate antibacterial agents. Unfortunately, the laboratory detection of ESBLs can be complex and, at times, misleading.

Antibacterial choice is often complicated by multi-resistance. Many ESBL-producing organisms also express AmpC β -lactamases and may be co-transferred with plasmids mediating aminoglycoside resistance. In addition, there is an increasing association between ESBL production and fluoroquinolone resistance. Although in *in vitro* tests ESBLs are inhibited by β -lactamase inhibitors such as clavulanic acid, the activity of β -lactam/ β -lactamase inhibitor combination agents is influenced by the bacterial inoculum, dose administration regimen and specific type of ESBL present. Currently, carbapenems are regarded as the drugs of choice for treatment of infections caused by ESBL-producing organisms. Unfortunately, use of carbapenems has been associated with the emergence of carbapenem-resistant bacterial species such as *Stenotrophomonas* sp. or *Pseudomonas* sp.

A recurring theme in antimicrobial resistance has become readily apparent. Namely, the introduction of an antibacterial to the market is initially associated with exuberant use, at least partially as a result of fairly uniform susceptibility of targeted pathogens, which is followed by the emergence of resistance and rapid clonal spread. The rapidity of

the development and spread of resistance is a complex process that is influenced by selective pressure, pre-existence of resistance genes and use of infection control measures.

Extended spectrum cephalosporins (third generation cephalosporins such as ceftriaxone and cefotaxime) gained widespread clinical use in the

early 1980s and were developed because of the increasing prevalence of ampicillin-hydrolysing β -lactamases (TEM-1, TEM-2 and SHV-1) in Enterobacteriaceae, non-glucose fermenting Gram-negative bacilli, and some respiratory pathogens such as *Haemophilus influenzae* and *Moraxella catarrhalis*. In 1983, a β -lactamase capable of hydrolysing extended-spectrum cephalosporins was documented, based on genetic and functional characteristics, in strains of *Klebsiella pneumoniae* from Germany.^[1] Similar reports from elsewhere in Europe and the US quickly followed.^[2,3] Because of their spectrum of activity against oxyminocephalosporins, these enzymes became known as extended spectrum β -lactamases (ESBLs).

Currently, over 150 ESBLs have been described in a worldwide distribution.^[4] This paper summarises some of the biological characteristics of ESBLs, their prevalence and how they can be detected in the clinical microbiology laboratory. In addition, the clinical significance of ESBLs is discussed and recommendations for therapy are offered.

1. Biological Characteristics of Extended-Spectrum β -Lactamases (ESBLs)

A recent extensive review discussing the molecular characterisation of ESBLs has been published by Bradford and will not be reproduced here.^[4] Currently, β -lactamases are defined through the classification scheme proposed by Bush and colleagues that is based on molecular characteristics of the gene and enzyme rather than phenotypic hydrolysis characteristics alone.^[5] ESBLs, which have been isolated from a wide variety of Enterobacteriaceae,^[4] as well as *Pseudomonas aeruginosa*^[6,7] and *Capnocytophaga ochracea*,^[8] are strictly defined as β -lactamases capable of hydrolysing penicillins, broad- and extended-spectrum cephalosporins, and monobactams, and are inhibited by clavulanic acid (functional group 2be as defined by Bush-Jacoby-Medeiros). These phenotypic characteristics differentiate ESBLs from AmpC type β -lactamases, which are another group

of enzymes that are commonly isolated from extended-spectrum cephalosporin-resistant Gram-negative bacteria. AmpC β -lactamases are typically encoded on the chromosome of many Gram-negative bacteria including *Escherichia coli*, *Citrobacter freundii* and *Enterobacter* spp., but can also be found on plasmids.^[9] AmpC β -lactamases, in contrast to ESBLs, hydrolyse broad- and extended-spectrum cephalosporins but are not inhibited by clavulanic acid or other β -lactamase inhibitors.

1.1 TEM-Type ESBLs

The native TEM-1 β -lactamase confers resistance to ampicillin, penicillin and first-generation cephalosporins such as cephalothin. This enzyme, which is responsible for 90% of ampicillin-resistance in *E. coli* isolates,^[10] is also responsible for penicillin resistance in *H. influenzae* and *Neisseria gonorrhoeae*. Mutations within the *bla*_{tem-1} structural gene, presumably through antibacterial selection, have allowed the enzyme to expand the hydrolysis capabilities to particular extended-spectrum cephalosporins and aztreonam, while maintaining its original hydrolysis capabilities. TEM-2, the first variant described, differed from TEM-1 through the substitution of a lysine for a glutamine at position 39.^[11] However, TEM-2 is not considered an ESBL as the substrate profile is identical to TEM-1. Consequently, amino acid substitutions at 12 separate amino acid positions, acting alone or in concert with other structural gene mutations, have been defined in over 90 described TEM-1-or TEM-2-derived ESBLs.^[1] As expected, each TEM-derived ESBL has a slightly different substrate profile in which one ESBL may hydrolyse a specific extended-spectrum cephalosporin more efficiently than another ESBL. Although many ESBLs have subtle differences in substrate profile, these differences cannot be relied upon to differentiate between enzymes and discrimination requires analysis of the amino acid sequence. A complete listing of amino acid sequences of TEM- and SHV-derived (see section

1.2) ESBLs can be found at <http://www.lahey.org/studies/webt.htm>.

1.2 SHV-Type ESBLs

The native SHV-1 β -lactamase, found primarily in *K. pneumoniae*, is a plasmid or chromosomally encoded enzyme that confers resistance to penicillins and first-generation cephalosporins.^[10] As with TEM-1, specific mutations within the *bla*_{shv-1} structural gene expand the hydrolysis capabilities of SHV-1 to extended-spectrum cephalosporins and monobactams. Fewer ESBL variants have been described for SHV-1 than with TEM-1; currently 36 SHV-derived ESBLs are described at <http://www.lahey.org/studies/webt.htm>.

1.3 Other ESBLs

Other ESBLs have recently been described that are not closely related to TEM-1- or SHV-1-derived enzymes.^[4] These β -lactamases, which are found in a variety of different species within the family Enterobacteriaceae and *P. aeruginosa*, include OXA-type,^[4,12] CTX-M-type^[4,13] and PER-type^[4,14,15] β -lactamases among others. The preferred substrate of these ESBLs differs significantly ranging from cefotaxime (CTX-M-type) to ceftazidime (PER-type). Although not strictly defined as an ESBL, another group of β -lactamases, called the inhibitor resistant β -lactamases, have been isolated with increasing frequency.^[4,16] The inhibitor resistant β -lactamases are mostly TEM-derived, where 19 separate variants have been described.^[4] These TEM variants are resistant to the inhibition of clavulanic acid and sulbactam, but not tazobactam,^[17] and do not hydrolyse extended-spectrum cephalosporins.

2. Laboratory Detection of ESBLs

Detection of isolates expressing ESBLs in the clinical microbiology laboratory is not a trivial undertaking. Although a particular ESBL will typically confer resistance to at least one particular expanded-spectrum cephalosporin or aztreonam, the minimum inhibitory concentration (MIC) may

not be high enough for the strain to be called 'resistant' under current interpretations of the National Committee for Clinical Laboratory Standards (NCCLS).^[18] Because of the clinical significance of ESBLs, specific guidelines for the detection of ESBL-expressing organisms were proposed in 1999 by the NCCLS.^[19] The presence of an ESBL is suggested if bacterial growth is observed despite a concentration of 1 μ g/ml of at least one of three expanded-spectrum cephalosporins (ceftazidime, ceftriaxone or cefotaxime) or aztreonam, or growth occurs despite a concentration of 4 μ g/ml of cefpodoxime. The use of more than one antibacterial agent for screening improves the sensitivity of detection of ESBLs. Phenotypic confirmatory tests include the addition of clavulanic acid to both ceftazidime and cefotaxime. A ≥ 3 serial-dilution concentration decrease in a MIC for either antibacterial agent tested in combination with clavulanic acid versus its MIC when tested alone constitutes a positive phenotypic test for an ESBL. If disk diffusion is used by the laboratory, a ≥ 5 mm increase in zone diameter for either cefotaxime or ceftazidime tested with clavulanic acid versus its zone size when tested alone is considered a positive phenotypic ESBL test. It is important to note that the NCCLS confirmatory tests are only intended for the detection of ESBLs found in *E. coli*, *K. pneumoniae*, and *K. oxytoca*. Chromosomally encoded AmpC β -lactamases found in Enterobacteriaceae, such as *Enterobacter cloacae* and *C. freundii* can interfere with clavulanic acid inhibition of an ESBL. Because of the fact that *E. cloacae* and *C. freundii* are important nosocomial pathogens, specific NCCLS methodology guidelines are needed to detect ESBLs in species that produce chromosomally encoded inducible AmpC β -lactamases.^[20-23]

Although the methods described in the previous paragraph are those published by the NCCLS as ESBL confirmatory tests, there are other methods that investigators have used to phenotypically detect ESBLs in the clinical microbiology laboratory.

2.1 Double Disk Approximation Test

In this test, first described by Jarlier et al. in 1988, the organism to be tested is spread onto a Mueller-Hinton Agar plate.^[24] Next, two antimicrobial disks are placed 30mm apart (centre to centre). One of the disks contains amoxicillin/clavulanic acid and the other contains an expanded-spectrum cephalosporin (for example, ceftriaxone, cefotaxime or ceftazidime). The test is positive if, after 24-hour incubation, the zone of inhibition in between the disks is enhanced. The enhancement is due to the inhibition of the ESBL by clavulanic acid (provided by the amoxicillin/clavulanic acid disk) and the subsequent action of the expanded-spectrum cephalosporin. This test remains a reliable method to detect ESBLs in the clinical laboratory but at times can be difficult to read. It has been suggested that the sensitivity of the test can be increased if the disks are placed both 30mm and 20mm apart.^[25]

2.2 Three-Dimensional Test

In this test, the organism to be tested is spread onto a Mueller-Hinton agar plate and a slit is cut into the agar the length of the plate.^[25] The test organism is then inoculated into the slit and an expanded-spectrum cephalosporin is placed 3mm from the slit. A distorted zone on the side of the slit is considered a positive test. The test was shown to be as sensitive at detecting ESBLs as the double-disk approximation test but is more technically challenging.^[25,26]

2.3 E-Test

AB Biodisk (Solna, Sweden) has introduced a two-sided ESBL E-test strip that contains either a combination of ceftazidime and ceftazidime/clavulanic acid or cefotaxime and cefotaxime/clavulanic acid. Both strips have a decreasing gradient of ceftazidime or cefotaxime alone on one end and a decreasing gradient of ceftazidime or cefotaxime plus a fixed gradient of clavulanic acid on the other end. A $>3\log$ reduction in the MIC of cefotaxime or ceftazidime in the presence of

clavulanic acid is considered a positive test. The E-test was shown to be more sensitive in detecting ESBLs than the double-disk approximation test in one study^[27] and less sensitive in another.^[26] This discrepancy found between these two studies could be because only the ceftazidime/ceftazidime-clavulanic acid E-test strips were used in both studied instead of both the ceftazidime/ceftazidime-clavulanic acid strip and the cefotaxime/cefotaxime-clavulanic acid strip as suggested by the manufacturer.

2.4 Vitek

The Vitek automated susceptibility system (Biomérieux, Hazelwood, Missouri, USA) has introduced an ESBL test on their system whereby ceftazidime and cefotaxime are tested alone and in combination with clavulanic acid. Logarithmic reduction in growth within the well containing clavulanic acid compared to the well not containing clavulanic acid indicates expression of an ESBL. The ESBL test in combination with the Vitek 2 Advanced Expert System software represents a very sensitive methodology to detect ESBLs in clinical isolates.^[28-30]

If an ESBL is detected, all penicillins, aztreonam and cephalosporins including cefepime (but excluding cefoxitin and cefotetan as ESBLs typically do not hydrolyse cephamycins) are to be reported as resistant regardless of the original susceptibility report. For infection control purposes, a comment should be added to the final susceptibility report stating that the particular isolate is an ESBL producer so that appropriate isolation precautions can be employed (see section 5). Conflicting recommendations regarding the reporting of susceptibility tests for β -lactam/ β -lactamase inhibitor combinations (for example, piperacillin/tazobactam, ampicillin/sulbactam and amoxicillin/clavulanic acid) exist. Although successful treatment of infections caused by ESBL-producing isolates with β -lactam/ β -lactamase inhibitor combinations has been reported, *in vivo* susceptibility to these agents may be ESBL-specific. For example, Thomson and Moland demonstrated that TEM-

derived ESBLs were more susceptible to piperacillin/tazobactam than SHV-derived ESBLs; even when a 100-fold greater inoculum was used.^[31] Currently, the decision to report *in vitro* susceptibility results for β -lactam/ β -lactamase inhibitor combinations should be individualised and should be made after consultation with experts in infectious diseases, pharmacy and clinical microbiology.

It is important to note that the clinical microbiology laboratory is obviously the first line of defence in the detection and control of the spread of ESBLs. Therefore, it is imperative that laboratory personnel are well versed in the detection of these organisms as the consequence of not detecting them may lead to treatment failures.^[32-36] Unfortunately, many clinical laboratories have a fundamental lack of understanding regarding ESBLs and AmpC β -lactamases and their detection. This has been documented in a study in Connecticut, USA, where it was found that 21% of laboratories failed to detect extended-spectrum cephalosporin and aztreonam resistance in ESBL and AmpC-producing Enterobacteriaceae control strains.^[37] Whereas various methodologies will invariably unfold to detect both ESBLs and AmpC β -lactamases in a variety of Gram-negative organisms, it is important for both academia and the NCCLS to methodically educate clinical microbiology laboratorians in the appropriate detection of these antibiotic-resistant pathogens.

3. Prevalence of ESBLs

The true prevalence of ESBLs is not known and is probably underestimated because of difficulties encountered in their detection. However, it is clear that ESBL-producing organisms are distributed worldwide and their prevalence is increasing.

3.1 Europe

As noted in the introduction, ESBLs were first described in 1983 from Germany and England. The proliferation of ESBLs over the last two decades has been noteworthy. The prevalence of ESBL-producing *Klebsiella* spp. varies from country-

to-country.^[38] For example, in a survey of laboratories in the Netherlands less than 1% of *E. coli* and *K. pneumoniae* strains possessed an ESBL.^[39] While in France and Italy, ceftazidime resistance was observed in as many as 40% of strains of *K. pneumoniae*.^[40] It is not known why the prevalence varies so widely in closely related regions.

3.2 North America

The first reports of ESBL-producing organisms in the US appeared in 1988.^[41] The prevalence of ESBL production amongst Enterobacteriaceae in the US ranges from 0 to 25% with the national average being approximately 3%.^[4] The Centers for Disease Control and Prevention (CDC) reported that in US intensive care unit (ICU) patients, the rate of extended-spectrum cephalosporin resistance in strains of *E. coli* rose 48% when comparing the 1999 rate to the mean rate of resistance over the preceding 5 years (1994–1998).^[42] The rate of extended-spectrum cephalosporin resistance in isolates of *K. pneumoniae* recovered from US ICU patients was 10.4% in 1999.^[42]

3.3 Latin America

ESBL-producing strains of Enterobacteriaceae appear to be common in many Latin American countries.^[43-45] For example, the SENTRY antimicrobial surveillance programme noted, in approximately 10 000 bacterial isolates from ten centres widely distributed throughout South America, that 45% of *K. pneumoniae* and 8.5% of *E. coli* expressed an ESBL.^[43]

3.4 Asia and Australia

The prevalence of ESBL-producing strains of Enterobacteriaceae varies from country to country and from species to species in Asia.^[43,46-50] For example, in *E. coli* the rate of resistance varied from 5% in Korea to 23.3% in Indonesia.^[47] However, the same study noted the rate of ESBL-mediated resistance in *Klebsiella* spp. was 48.8% in Korea and ranged from 20–40% throughout Southeast Asia, China and Japan.^[47] Outbreaks of

infection due to ESBL-producing organisms have been described widely in Australia.

3.5 Africa and Middle East

Although national surveillance data are lacking, outbreaks of infection due to ESBL-producing organisms have been noted in some African nations.^[51]

4. Clinical Significance

It is generally recognised that patients infected with ESBL-producing organisms are at risk for poor outcome if they are treated with antibacterials to which the organism exhibits high-level resistance. The mortality rate in these 'susceptibility/treatment mismatched patients' has ranged from 42–100%.^[52–54] However, some confusion results when susceptibility testing reveals a cephalosporin MIC in the susceptible range for an organism producing an ESBL. There are reports of patients with urinary tract infections due to ESBL-producing bacteria successfully treated with cephalosporins.^[32,55] Such cases have been cited to preclude routine testing for ESBLs. However, Patterson and colleagues recently examined this issue by reviewing outcomes in 32 cephalosporin-treated patients infected with an ESBL-producing organism in which the *in vitro* susceptibility test did not indicate resistance.^[36] Four of four patients experienced clinical failure when the MIC was in the intermediate range and 15 of 28 (54%) were regarded as treatment failures even though the susceptibility test indicated the organism was fully susceptible. Therefore, it is currently recommended that any organism found to produce an ESBL be regarded as resistant to all extended spectrum β -lactam antibacterials regardless of the *in vitro* MIC result.^[19]

Some of these clinical failures may be due to large numbers of organisms in the local environment (inoculum effect). The MIC of most cephalosporins increases dramatically when the inoculum of organisms is increased 10- to 100-fold.^[31,56] This phenomenon has also been observed in *in vivo* models of infection.^[57,58] There are certainly infections in the human host in which the bacterial bur-

den can reach these levels (10^6 – 10^7 cfu/ml). Thus, the burden of evidence suggests that ESBLs are clinically significant and, when present, indicate the need to use appropriate antibacterials in the treatment of infection.

5. Epidemiology and Infection Control

5.1 Outbreaks

As previously discussed in section 3, ESBL-producing Enterobacteriaceae are distributed worldwide and are of increasing prevalence. A large number of outbreaks of infection due to ESBL-producing organisms have been described on every continent of the globe except Antarctica and a full review is beyond the scope of this paper. Table I summarises the major features of these outbreaks. Briefly, most outbreaks have occurred in debilitated, hospitalised patients located in ICUs. However, outbreaks have been described in out-of-hospital locations such as nursing homes, geriatric centres and rehabilitation units.^[59–63] Both adult and paediatric patients have been involved. Other specific patient populations have included solid organ transplant recipients,^[64,65] oncology patients,^[53,66] burn patients^[67] and neonates.^[68–71] Most commonly, *K. pneumoniae* and *E. coli* are the bacterial species incriminated, but outbreaks have been observed due to *Enterobacter* spp., *Pseudomonas* spp., *Citrobacter* spp., *Salmonella* spp., *Serratia* spp. and *Morganella* sp. The potential diversity and promiscuity of microbes was documented in one hospital outbreak in Poland in which seven different species of Enterobacteriaceae were involved.^[72]

Risk factors for acquisition of ESBL-producing Enterobacteriaceae are listed in table I, and generally are indicators of severity of illness and medical intervention. Reservoirs and vectors for infection have included thermometers,^[70] oxygen probes,^[70] liquid soap,^[66] cockroaches,^[51] ultrasound gel^[73] and healthcare workers.^[74] One outbreak was associated with severe cases of necrotising enterocolitis.^[75]

Table I. Outbreaks of infection due to extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae**Outbreak populations***Adults*

Intensive care units
Solid organ transplantation
Bone marrow transplantation
Long-term care units

Paediatrics

Neonatal intensive care units
Paediatric intensive care units
Solid organ transplantation

Risk factors for acquisition of ESBL-producing Enterobacteriaceae

Severity of illness

Length of hospital stay

Length of intensive care unit stay

Invasive procedures

Intravascular devices

arterial catheters

central venous catheters

Administration of total parenteral nutrition

Mechanical ventilatory assistance

Urinary catheters

Gastrostomy, jejunostomy or nasogastric tubes

Age

Haemodialysis

Decubitus ulcers

Poor nutritional status

Low birth weight

Antibacterial administration

extended-spectrum cephalosporins

aztreonam

fluoroquinolones

cotrimoxazole (trimethoprim/sulfamethoxazole)

aminoglycosides

metronidazole

Reservoirs/vectors

Healthcare worker hand colonisation

Contaminated ultrasonography gel

Thermometers

Cockroaches

ing, arbitrarily primed polymerase chain reaction (PCR) and random amplified polymorphic DNA (RAPD). Oftentimes, a single strain or a genetically related group of strains expand clonally in an institution. Endemic strains have been shown to persist in certain units for years.^[40] However, at other times the epidemiological situation may be quite complex. ESBLs are most often encountered on plasmids that can transfer from strain to strain. In an outbreak characterised by Weiner et al., a plasmid expressing a TEM-10 ESBL was documented in multiple strains of *E. coli* and *K. pneumoniae* in numerous patients in several different hospitals and nursing homes.^[76] Similarly, in a population of paediatric transplant recipients, an outbreak of ESBL-producing *K. pneumoniae* was shown to involve multiple strains of bacteria, a number of plasmids and different β -lactamases, even though it was observed in a small clinical population in a specific unit over a relatively short period of time.^[77] Similarly, in a report from France, a plasmid carrying a TEM-24 ESBL was found in four different species of Enterobacteriaceae from a single patient, clearly demonstrating the promiscuous nature of the plasmid.^[78]

5.3 Infection Control Issues

Several infection control factors should be emphasised when faced with combating the spread of ESBL-producing organisms. These issues include such things as isolation precautions, environmental decontamination and antibacterial usage patterns, and are summarised in table II. Antibacterial use protocols bear special mention, particularly if molecular epidemiological typing studies suggest polyclonal evolution of an outbreak, possibly indicating the effect of antibacterial selective pressure. As previously related, numerous studies have indicated that the use of extended-spectrum cephalosporins in particular, and other antibacterials in general, are associated with outbreaks of ESBL-producing Enterobacteriaceae. Consequently, restriction of the use of extended-spectrum cephalosporins is the most common antibacterial-restriction measure em-

5.2 Molecular Typing

A variety of molecular methods have been used to study the epidemiology of ESBL-producing bacteria. These methods include plasmid profiles, pulsed-field gel electrophoresis (PFGE), ribotyp-

Table II. Infection control recommendations to prevent the spread of extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae

Isolation measures

Private room, private bathroom
Contact isolation (gloves, gowns)
Hand hygiene compliance
Dedicated equipment (thermometers, stethoscopes, etc.)

Identification of patients

Patient charts should be 'flagged' so that contact isolation for ESBL-colonisation can be considered on subsequent admissions
Thorough decontamination of environment-of-care with routine low-level disinfectant (quaternary ammonium, phenolic)

Antibacterial use

Antibacterial use protocols should be instituted to eliminate indiscriminate use of antibacterials and decrease selective pressure for ESBL-producing strains of bacteria

ployed in controlling outbreaks of ESBL-producing organisms.^[79,80] Often, restriction of extended-spectrum cephalosporin use is accompanied by switching empirical therapy for serious infections to other classes of antibacterials. The two most studied alternatives are imipenem and piperacillin/ tazobactam.^[52,81] For example, Rice et al. reported successful control of an outbreak of ESBL-producing *K. pneumoniae* associated with a switch in therapy from ceftazidime to piperacillin/tazobactam.^[35] Interestingly, a decrease in the rate of resistance to piperacillin/tazobactam was also observed.

Other infection control measures that have been used in combating outbreaks have included the following: gut decontamination,^[65] povidone-iodine nasal spray,^[63] staff cohorting and reorganisation,^[35] and temporary ward closure.^[70]

6. Treatment of Infections Due to ESBL-Producing Organisms

ESBLs are clinically significant and patients infected with ESBL-producing Enterobacteriaceae experience a greater likelihood of poor outcome if they are treated with inappropriate antibacterials.^[36,41,52] The exception is uncomplicated urinary tract infections where a very high urinary concentration of β -lactam antibiotics can be

achieved.^[41] Unfortunately, other resistance determinants are often linked on the same resistance plasmid and ESBL-producing strains often exhibit multi-resistance. The following discussion summarises considerations in the treatment of ESBL-producing bacteria.

The cephamycins (e.g. cefoxitin, cefotetan) are structurally more stable than other cephalosporins to ESBL-mediated hydrolysis and many ESBL-producing Enterobacteriaceae remain susceptible to cephamycins in *in vitro* tests. However, there is very limited clinical information regarding treatment of serious infections due to ESBL-producing organisms with cephamycins. Clinical failure has been documented because of the emergence of resistance while a patient is receiving cephamycin therapy as a result of the development of porin-deficient mutants.^[82,83] In addition, increasing numbers of ESBL-producing strains express multiple β -lactamases including Amp C type enzymes that mediate resistance to cephamycins.^[84]

Cefepime, an oxyimino β -lactam with an amino thiazolyl side chain, that is often referred to as a fourth-generation cephalosporin, is active against most ESBL-producing organisms, particularly those with SHV derived enzymes.^[85,86] In addition, there are some data from *in vivo* models to support the use of cefepime in the treatment of infections due to ESBL-producing Enterobacteriaceae.^[87] However, cefepime susceptibility appears to decrease with increasing inoculum in *in vitro* susceptibility tests^[31,88] and in *in vivo* models.^[89] Extensive clinical experience with cefepime in the treatment of infections due to ESBL-producing microbes is lacking but clinical failures have been observed.^[36] In addition, use of cefepime has been associated with selection of ESBL-producing organisms and outbreaks of infection.^[90] Therefore, until more clinical data is available, clinicians should not regard cefepime as a first line therapy for ESBL-producing organisms and, if used, it should be given at high dose (≥ 2 g every 12 hours) usually in combination with other active agents (aminoglycosides, fluoroquinolones).

ESBLs are usually inhibited by β -lactamase inhibitors, such as clavulanic acid, sulbactam or tazobactam. Therefore, use of β -lactam/ β -lactamase inhibitor combinations has been considered for the treatment of infections due to ESBL-producing organisms. *In vitro* and *in vivo* models indicate potential efficacy of β -lactam/ β -lactamase inhibitor combinations.^[91-93] Unfortunately, as previously related, organisms expressing multiple β -lactamases as well as porin deficient mutants are being described with increasing frequency. In addition, the activity of these agents appears to be influenced by inoculum, dose administration regimen and the type of enzyme. Some TEM-derived β -lactamases are resistant to β -lactamase inhibitors.^[4,16,17] There is limited clinical experience with the use of β -lactam/ β -lactamase inhibitors in treating serious infections with ESBL-producing organisms and because of the variables cited in the previous two sentences, these agents should generally not be considered as first line therapy.

Because the β -lactamases do not influence the activity of non- β -lactam agents, fluoroquinolones have been considered as attractive alternatives in the treatment of infections due to ESBL-producing organisms. Unfortunately, there is an increasing association between ESBL production and fluoroquinolone resistance.^[94,95] Although fluoroquinolone resistance is usually chromosomally mediated, transferable plasmid-mediated resistance has been documented with an AmpC type β -lactamase.^[83] However, this appears to be a very rare event. In addition, a porin-deficient mutant with elevated MICs to fluoroquinolones has been observed in a strain of *K. pneumoniae* harbouring a plasmid-mediated ESBL.^[96]

Some of the same considerations regarding fluoroquinolones and ESBLs are also pertinent for the aminoglycosides. Although ESBLs have no intrinsic effect on the activity of the aminoglycosides, aminoglycoside-resistance is often co-transferred with plasmids mediating ESBLs.^[97] The relationship between ESBL expression and multi-resistance can be complex and is influenced by the location of resistance genes on integrons

that possess promoters that drive the coordinated expression of downstream resistance cassettes.^[98] Therefore, aminoglycosides are often not appropriate therapeutic choices for strains expressing an ESBL.

Currently, carbapenems are generally regarded as the preferred agent for treatment of infections due to ESBL-producing organisms. Carbapenems are resistant to ESBL-mediated hydrolysis^[10] and exhibit excellent *in vitro* activity against strains of Enterobacteriaceae expressing ESBLs.^[85,99,100] Clinical data supports the use of carbapenems for treatment of infections due to ESBL-producing organisms.^[101,102] For example, Meyer et al. related that during an outbreak of ESBL-producing *K. pneumoniae* the clinical outcome was most favourable amongst those patients treated with imipenem/cilastatin.^[52] Paterson and colleagues observed a similar difference in outcome between imipenem-treated patients and those receiving other active antibacterials in a group of patients with bacteremia due to ESBL-producing *K. pneumoniae*.^[103] Newer carbapenems such as ertapenem or faropenem also exhibit excellent activity against ESBL-producing organisms,^[104,105] and have various pharmacokinetic advantages over existing agents such as improved oral bioavailability or extended half-life. Although plasmid-mediated carbapenemases are unusual,^[106] chromosomally-mediated, extended-spectrum serine proteases (group 2F) and metallo- β -lactamases, active against carbapenems, are not uncommon.^[10] Carbapenem-resistance, due to alterations in porin proteins, has been observed to develop in *K. pneumoniae*. In addition, increased use of carbapenems to treat ESBL-producing organisms has been associated with the emergence of carbapenem-resistant organisms such as *Acinetobacter* spp., *Stenotrophomonas maltophilia* or *Pseudomonas* spp.

7. Conclusion

ESBLs are an example of the increasing number and diversity of enzymes that inactivate β -lactam-type antibacterials. The combination of these enzymes with other resistance traits give strong tes-

timony as to the resilience of microbes and their ability to adapt to their environment. Our ability to successfully treat infections due to these increasingly resistant organisms demands a multifactorial approach combining continued research and development of novel classes of antibacterials, more prudent use of existing agents and an increased emphasis on more effective infection control measures.

Acknowledgements

No sources of funding were used to assist in the preparation of this manuscript. The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

References

1. Knothe H, Shah P, Kremery V, et al. Transferable resistance to cefotaxime, ceftazidime, cefamandole and cefturoxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 1983; 11: 315-7
2. Jacoby GA, Medeiros AA, O'Brien TF, et al. Broad-spectrum, transmissible β -lactamases. *N Engl J Med* 1988; 319: 723-4
3. Quinn JR, Miyashiro D, Sahm D, et al. Novel plasmid-mediated β -lactamase (TEM-10) conferring selective resistance to ceftazidime and aztreonam in clinical isolates of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 1989; 33: 1451-6
4. Bradford PA. Extended-spectrum β -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 2001; 48: 933-51
5. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β -lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995; 39: 1211-33
6. Mugnier PI, Casin I, Casin G, et al. A TEM-derived extended-spectrum β -lactamase in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1996; 40: 2488-93
7. Naas T, Philippon L, Poirel L, et al. An SHV-derived extended-spectrum beta-lactamase in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999; 43: 1281-4
8. Rosenau A, Cattier B, Gousset N, et al. *Capnocytophaga ochracea*: characterization of a plasmid encoded extended-spectrum TEM-17 β -lactamase in the phylum *Flavobacter-Bacteroides*. *Antimicrob Agents Chemother* 2000; 44: 760-2
9. Philippon A, Arlet B, Jacoby GA. Plasmid-determined AmpC-type β -lactamases. *Antimicrob Agents Chemother* 2002; 46: 1-11
10. Livermore DM. β -lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8: 557-84
11. Matthew M, Hedges RW. Analytical isoelectric focusing of R factor determined β -lactamases: correlation with plasmid compatibility. *J Bacteriol* 1976; 125: 713-8
12. Huovinen PS, Huovinen S, Jacoby GA. Sequence of PSE-2 β -lactamase. *Antimicrob Agents Chemother* 1988; 32: 134-6
13. Bauernfeind A, Stemmlinger I, Jungwirth R, et al. Sequences of β -lactamase genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other β -lactamases. *Antimicrob Agents Chemother* 1996; 40: 509-13
14. Bauernfeind A, Stemmlinger I, Jungwirth R, et al. Characterization of β -lactamase gene blaPER-2, which encodes an extended-spectrum class A β -lactamase. *Antimicrob Agents Chemother* 1996; 40: 616-20
15. Nordman PE, Ronco T, Naas C, et al. Characterization of a novel extended-spectrum β -lactamase from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1993; 37: 962-9
16. Vedel GA, Belaouaj L, Gilly R, et al. Clinical isolates of *Escherichia coli* producing TRI β -lactamases: novel TEM-enzymes conferring resistance to β -lactamase inhibitors. *J Antimicrob Chemother* 1992; 30: 449-62
17. Chaibi EB, Sirot D, Paul G, et al. Inhibitor-resistant TEM- β -lactamases: phenotypic, genetic and biochemical characteristics. *J Antimicrob Chemother* 1999; 43: 447-58
18. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M7-A5 and informational supplement M100-S12. Wayne (PA): National Committee for Clinical Laboratory Standards, 2000
19. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: approved standard M100-S9. Wayne (PA): National Committee for Clinical Laboratory Standards, 1999
20. Thomson KS. Controversies about extended-spectrum and AmpC beta-lactamases. *Emerg Infect Dis* 2001; 7: 333-6
21. Steward CD, Rasheed JK, Hubert SK, et al. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 clinical laboratories using the national Committee for Clinical Laboratory Standards Extended-spectrum β -lactamase detection methods. *J Clin Microbiol* 2001; 39: 2864-72
22. Thomson KS, Sanders CC, Moland ES. Use of microdilution panels with and without β -lactamase inhibitors as a phenotypic test for β -lactamase production among *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, and *Serratia marcescens*. *Antimicrob Agents Chemother* 1999; 43: 1393-400
23. Tzelepi EP, Giakkoupi D, Sofianou V, et al. Detection of extended-spectrum beta-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. *J Clin Microbiol* 2000; 38: 542-6
24. Jarlier V, Nicolas MH, Fournier G, et al. Extended broad-spectrum β -lactamases conferring transferable resistance to newer β -lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10: 867-8
25. Thomson KS, Sanders CC. Detection of extended-spectrum β -lactamases in members of the family *Enterobacteriaceae*: comparison of the double-disk and three-dimensional tests. *Antimicrob Agents Chemother* 1992; 36: 1877-82
26. Vercauteren E, Descheemaeker P, Ieven M, et al. Comparison of screening methods for detection of extended-spectrum β -lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella* spp. in a Belgian Teaching hospital. *J Clin Microbiol* 1997; 35: 2191-7
27. Cormican MG, Marshall SA, Jones RN. Detection of extended-spectrum β -lactamase (ESBL)-producing strains by the E-test ESBL screen. *J Clin Microbiol* 1996; 34: 1880-4
28. Sanders CC, Peyret M, Moland ES, et al. Ability of VITEK 2 advanced expert system to identify β -lactam phenotypes in isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa*. *J Clin Microbiol* 2000; 38: 570-4
29. Sanders CC, Barry AL, Washington JA, et al. Detection of extended-spectrum β -lactamase -producing members of the

- family Enterobacteriaceae with the Vitek ESBL test. *J Clin Microbiol* 1996; 34: 2997-3001
30. Livermore DM, Struelens M, Amorim J, et al. Multicentre evaluation of Vitek 2 advanced expert system for interpretive reading of antimicrobial resistance tests. *J Antimicrob Chemother* 2002; 49: 289-300
31. Thomson KS, Moland ES. Cefepime, piperacillin-tazobactam, and the inoculum effect in tests with extended-spectrum beta-lactamase-producing Enterobacteriaceae. *Antimicrob Agents Chemother* 2001; 45: 3548-54
32. Brun-Buisson C, Legrand P, Philippon A, et al. Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. *Lancet* 1987; II: 302-6
33. Karas JA, Pillay DG, Muckart D, et al. Treatment failure due to extended spectrum beta-lactamase. *J Antimicrob Chemother* 1996; 37: 203-4
34. Venezia RA, Scarano FJ, Preston KE, et al. Molecular epidemiology of an SHV-5 extended-spectrum beta-lactamase in Enterobacteriaceae isolated from infants in a neonatal intensive care unit. *Clin Infect Dis* 1995; 21: 915-23
35. Rice LB, Eckstein EC, DeVente J, et al. Ceftazidime-resistant *Klebsiella pneumoniae* isolates recovered at the Cleveland Department of Veterans Affairs Medical Center. *Clin Infect Dis* 1996; 23: 118-24
36. Patterson DL, Ko WC, Von Gottberg A, et al. Outcome of cephalosporin treatment for serious infectious due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. *J Clin Microbiol* 2001; 39: 2206-12
37. Tenover FC, Mohammed MJ, Gorton TS, et al. Detection and reporting of organisms producing extended-spectrum beta-lactamases: survey of laboratories in Connecticut. *J Clin Microbiol* 1999; 37: 4065-70
38. Babini GS, Livermore DM. Antimicrobial resistance amongst *Klebsiella* spp collected from intensive care units in Southern and Western Europe in 1997-1998. *J Antimicrob Chemother* 2000; 45: 183-9
39. Stobberingh EE, Arends J, Hoogkamp-Korstanje JAA, et al. Occurrence of extended-spectrum β -lactamases in Dutch hospitals. *Infection* 1999; 27: 348-54
40. Branger C, Lesimple CA, Bruneu B, et al. I. Long-term investigation of the clonal dissemination of *Klebsiella pneumoniae* isolates producing extended-spectrum β -lactamases in a university hospital. *J Med Microbiol* 1998; 47: 210-09
41. Jacoby GA. Extended-spectrum β -lactamases and other enzymes providing resistance to oxymino β -lactams. *Infect Dis Clin North Am* 1997; 11: 875-87
42. Selected antimicrobial resistant pathogens associated with nosocomial infections in ICU patients, comparisons of resistance rates scan January-December 1999 with 1994-1998. Available from URL: <http://www.cdc.gov/ncidod/hip/NNIS/june2000sar> [Accessed 2002 Feb 4]
43. Winokur PL, Canton R, Casellas JM, et al. Variations in the prevalence of strains expressing an extended-spectrum β -lactamase phenotype and characterization of isolates from Europe, the Americas, and the western pacific region. *Clin Infect Dis* 2001; 32 Suppl. 2: S94-103
44. Lewis MT, Gales AC, Sader HS, et al. Frequency of occurrence and antimicrobial susceptibility patterns for pathogens isolated from Latin American patients with diagnosis of pneumonia: results from the SENTRY antimicrobial surveillance program (1998). *Diagn Microbiol Infect Dis* 2000; 37: 63-74
45. Pfaller MA, Jones RN, Doern GV, et al. Multicenter evaluation of antimicrobial resistance to six broad-spectrum β -lactams in Colombia: comparison of data from 1997 and 1998 using the E test method. *Diagn Microbiol Infect Dis* 1999; 35: 235-41
46. Bell JM, Turnidge JD, Gales AC, et al. Prevalence of extended spectrum β -lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY antimicrobial surveillance program (1998-99). *Diagn Microbiol Infect Dis* 2002; 42: 193-8
47. Jones RN. Summation: β -Lactam resistance surveillance in the Asia-western pacific region. *Diagn Microbiol Infect Dis* 1999; 35: 333-8
48. Biedenbach DJ, Lewis MT, Jones RN. In vitro evaluation of Cefepime and other broad-spectrum β -lactams for isolates in Malaysia and Singapore medical centers. *Diagn Microbiol Infect Dis* 1999; 35: 277-83
49. Johnson DM, Biedenbach DJ, Jones RN. In vitro evaluation of broad spectrum β -lactams in the Philippines medical centers: role of fourth-generation cephalosporins. *Diagn Microbiol Infect Dis* 1999; 35: 291-7
50. Lewis MT, Biedenbach DJ, Jones RN. In vitro evaluation of broad-spectrum β -lactams tested in medical centers in Korea: role of fourth-generation cephalosporins. *Diagn Microbiol Infect Dis* 1999; 35: 317-23
51. Cotton MF, Wasserman E, Pieper CH. Invasive disease due to extended-spectrum β -lactamase producing *Klebsiella pneumoniae* in a neonatal unit: the possible role of cockroaches. *J Hosp Infect* 2000; 44: 13-7
52. Meyer KS, Urban C, Eagan JA, et al. Nosocomial outbreak of *Klebsiella pneumoniae* resistant to late-generation cephalosporins. *Ann Intern Med* 1993; 119: 353-8
53. Naumovski L, Quinn JP, Miyashiro D, et al. Outbreak of ceftazidime resistance due to a novel extended-spectrum β -lactamase in isolates from cancer patients. *Antimicrob Agents Chemother* 1992; 36: 1991-6
54. Schiappa DA, Hayden MK, Matushek MG, et al. Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infection: a case-control and molecular epidemiologic investigation. *J Infect Dis* 1996; 174: 529-36
55. Emery CL, Weymouth LA. Detection and clinical significance of extended-spectrum β -lactamases in a tertiary-care medical center. *J Clin Microbiol* 1997; 35: 2061-7
56. Medeiros AA, Crelin J. Comparative susceptibility of clinical isolates producing extended-spectrum β -lactamases to ceftibuten: effect of a large inoculum. *Pediatr Infect Dis J* 1997; 16: S49-S55
57. Caron F, Gutmann L, Bure A, et al. Ceftriaxone-sulbactam combination in a rabbit endocarditis caused by a strain of *Klebsiella pneumoniae* producing extended broad spectrum TEM-3 β -lactamase. *Antimicrob Agents Chemother* 1990; 34: 2070-4
58. Rice LB, Yao JDC, Klimm K, et al. Efficacy of different β -lactams against an extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* strain in the rat intro-abdominal abscess model. *Antimicrob Agents Chemother* 1991; 35: 1243-4
59. Trick WE, Weinstein RA, DeMarais PL, et al. Colonization of skilled-care facility residents with antimicrobial-resistant pathogens. *J Am Geriatr Soc* 2001; 49: 270-6
60. Gouby A, Neuwirth C, Bourg G, et al. Epidemiological study by pulsed-field gel electrophoresis of an outbreak of extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* in a geriatric hospital. *J Clin Microbiol* 1994; 32: 301-5

61. Weller TM, MacKenzie FM, Forbes KJ. Molecular epidemiology of a large outbreak of multiresistant *Klebsiella pneumoniae*. *J Med Microbiol* 1997; 46: 921-6
62. Ma L, Matsuo H, Ishii Y, et al. Characterization of cefotaxime-resistant *Escherichia coli* isolates from a nosocomial outbreak at three geriatric hospitals. *J Infect Chemother* 2002; 8: 155-62
63. Hollander R, Ebke M, Barck H, et al. Asymptomatic carriage of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase by patients in a neurological early rehabilitation unit: management of an outbreak. *J Hosp Infect* 2001; 48: 207-13
64. Rebuck JA, Olsen KM, Fey PD, et al. Characterization of an outbreak due to extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* in a pediatric intensive care unit transplant population. *Clin Infect Dis* 2000; 31: 1368-72
65. Paterson DL, Singh N, Rihs JD, et al. Control of an outbreak of infection due to extended-spectrum beta-lactamase-producing *Escherichia coli* in a liver transplantation unit. *Clin Infect Dis* 2001; 33: 126-8
66. Parasakthi N, Vadivelu J, Ariffin H, et al. Epidemiology and molecular characterization of nosocomially transmitted multidrug-resistant *Klebsiella pneumoniae*. *Inf J Infect Dis* 2000; 4: 123-8
67. Revathi G, Shannon KP, Stapleton PD, et al. An outbreak of extended-spectrum, beta-lactamase-producing *Salmonella* seftenberg in a burns ward. *J Hosp Infect* 1998; 40: 295-302
68. Kartali G, Tzelepi E, Pournaras S, et al. Outbreak of infections caused by *Enterobacter cloacae* producing the intergron-associated beta-lactamase IBC-1 in a neonatal intensive care unit of a Greek hospital. *Antimicrob Agents Chemother* 2002; 46: 1577-80
69. Gonzalez-Vertiz A, Alcantar-Curiel D, Cuauhtli M, et al. Multiresistant extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* causing an outbreak of nosocomial bloodstream infection. *Infect Control Hosp Epidemiol* 2001; 22: 723-5
70. Macrae MB, Shannon KP, Rayner DM, et al. A simultaneous outbreak on a neonatal unit of two strains of multiply antibiotic resistant *Klebsiella pneumoniae* controllable only by ward closure. *J Hosp Infect* 2001; 49: 183-92
71. Royle J, Halasz S, Eagles G, et al. Outbreak of extended spectrum beta lactamase producing *Klebsiella pneumoniae* in a neonatal unit. *Arch Dis Child Fetal Neonatal Ed* 1999; 80: F64-8
72. Palucha A, Mikiewicz B, Hryniewicz W, et al. Concurrent outbreaks of extended-spectrum β -lactamase-producing organisms of the family Enterobacteriaceae in a Warsaw hospital. *J Antimicrob Chemother* 1999; 44: 489-99
73. Gaillot O, Maruejols C, Abachin E, et al. Nosocomial outbreak of *Klebsiella pneumoniae* producing SHV-5 extended spectrum beta-lactamase, originating from a contaminated ultrasoundography coupling gel. *J Clin Microbiol* 1998; 36: 1357-60
74. Coovadia YM, Johnson AP, Bhana RH, et al. Multiresistant *Klebsiella pneumoniae* in a neonatal nursery: importance of maintenance of infection control policies and procedures in the prevention of outbreaks. *J Hosp Infect* 1992; 22: 197-205
75. Gregersen N, VanNierop W, VonGottberg A, et al. *Klebsiella pneumoniae* with extended spectrum beta-lactamase activity associated with a necrotizing enterocolitis outbreak. *Pediatr Infect Dis J* 1999; 18: 963-7
76. Wiener J, Quinn JP, Bradford PA, et al. Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. *JAMA* 1999; 281: 517: 523
77. Fey PD, Moland, ES, Iwen PC, et al. Outbreak of extended-spectrum -lactamase-producing *Klebsiella pneumoniae* in pediatric liver transplant patients [abstract K-127]. 38th ICAAC; 1998 Sep 24-27; San Diego (CA)
78. Marchandin H, Carriere C, Siroit D, et al. TEM-24 produced by four different species of Enterobacteriaceae, including *Providencia rettgeri*, in a single patient. *Antimicrob Agents Chemother* 1999; 43: 2069-73
79. Pena C, Pujol M, Ardanuy C, et al. Epidemiology and successful control of a large outbreak due to *Klebsiella pneumoniae* producing extended-spectrum β -lactamases. *Antimicrob Agents Chemother* 1998; 42: 53-8
80. Rahal JJ, Urban C, Horn D. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella*. *JAMA* 1998; 280: 1233-7
81. Patterson JE, Hardin TC, Kelly CA, et al. Association of antibiotic utilization measures and control of multiple-drug resistance in *Klebsiella pneumoniae*. *Infect Control Hosp Epidemiol* 2000; 21: 455-8
82. Pangon B, Bizet C, Bure A, et al. In vivo selection of a cephamycin-resistant porin-deficient mutant of *Klebsiella pneumoniae* producing a TEM-3 B-lactamase. *J Infect Dis* 1989; 159: 1005-6
83. Martinez-Martinez L, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; 351: 797-9
84. Bradford PA, Urban C, Mariano N, et al. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC β -lactamase and the loss of an outer membrane protein. *Antimicrob Agents Chemother* 1997; 41: 563-9
85. Rebuck JA, Olsen KM, Fey PD, et al. In vitro activities of parenteral B-lactam antimicrobials against TEM-10-, TEM-26- and SHV-5-derived extended-spectrum β -lactamases expressed in an isogenic *Escherichia coli* host. *J Antimicrob Chemother* 2000; 46: 461-4
86. Ambrose RG, Grasela TH, Jones RN. Antimicrobial susceptibility and pharmacodynamic comparison of piperacillin-tazobactam (P-T) and cefepime (PIM) against Enterobacteriaceae with extended-spectrum β -lactamase (ESBL) [abstract E-2266]. 41st ICAAC; 2001 Dec 16-19; Chicago (IL). Washington (DC): American Society for Microbiology, 2001
87. Andes D, Craig WA. Impact of extended-spectrum β -lactamase (ESBL) production on the activity of cefepime in a murine thigh infection model [abstract A-1099]. 41st ICAAC; 2001 Dec 16-19; Chicago (IL). Washington (DC): American Society for Microbiology, 2001
88. Jacoby GA. Development of resistance in gram-negative pathogens: extended-spectrum -lactamases. In: Powers L (ed). *Emerging pathogens in infectious disease: a hospital practice special report*. Minneapolis: McGraw-Hill, 1999: 14-9
89. Thauvin-Elliopoulos C, Tripodi MF, Moellering Jr RC, et al. Efficacies of piperacillin-tazobactam and cefepime in rats with experimental intra-abdominal abscesses due to an extended-spectrum β -lactamase-producing strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 1997; 41: 1053-7
90. Pujol M, Pena C, Tubau F, et al. Emergence of an extended-spectrum β -lactamase producing *Klebsiella pneumoniae* following an increased use of cefepime in ICUs [abstract K-1180]. 41st ICAAC; 2001 Dec 16-19; Chicago (IL). Washington (DC): American Society for Microbiology, 2001
91. Mentec H, Vallois JM, Bure A, et al. Piperacillin, tazobactam, and gentamicin alone or combined in an endocarditis model of infection by a TEM-3-producing strain of *Klebsiella pneu-*

- moniae or its susceptible variant. *Antimicrob Agents Chemother* 1992; 36: 1883-9
92. Rice LB, Carias LL, Shlaes DM. In vivo efficacies of β -lactam- β -lactamase inhibitor combinations in *Klebsiella pneumoniae* and in vivo response to β -lactam therapy. *J Infect Dis* 1996; 173: 151-8
93. Philippon A, Labia R, Jacoby G. Extended-spectrum β -lactamases. *Antimicrob Agents Chemother* 1989; 33: 1131-6
94. Paterson DL, Mulazimoglu L, Casellas JM, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum β -lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000; 30: 473-8
95. Spanu T, Luzzaro F, Perilli M, et al. The Italian ESBL Study Group. Occurrence of extended-spectrum β -lactamases in members of the family Enterobacteriaceae in Italy: implications for resistance to β -lactams and other antimicrobial drugs. *Antimicrob Agents Chemother* 2002; 46: 196-202
96. Martinez-Martinez L, Hernandez-Alles S, Alberti S, et al. In vivo selection of porin-deficient mutants of *Klebsiella pneumoniae* with increased resistance to ceftaxime and expanded-spectrum cephalosporins. *Antimicrob Agents Chemother* 1996; 40: 342-8
97. Jacoby GA, Sutton L. Properties of plasmids responsible for production of extended-spectrum β -lactamases. *Antimicrob Agents Chemother* 1991; 35: 164-9
98. Poirel L, Le Thomas I, Naas T, et al. Biochemical sequence analyses of GES-1, a novel Class A extended-spectrum β -lactamase, and the Class 1 integron In52 from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2000; 44: 622-32
99. Moellering Jr RC, Eliopoulos GM, Sentochnik DE. The carbapenems: new broad spectrum β -lactam antibiotics. *J Antimicrob Chemother* 1989; 24 Suppl A: 1-7
100. Edwards JR, Turner PJ. Laboratory data which differentiate meropenem and imipenem. *Scand J Infect Dis* 1995; 96: 5-10
101. Paterson DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β -lactamases (ESBLs). *Clin Microbiol Infect* 2000; 6: 460-3
102. Wong-Beringer A. Therapeutic challenges associated with extended-spectrum β -lactams-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Pharmacotherapy* 2001; 21: 583-92
103. Paterson DL, Ko WC, Mohapatra S, et al. *Klebsiella pneumoniae* bacteremia: impact of extended spectrum β -lactamase (ESBL) production in a global study of 216 patients [abstract J-210]. 37th ICAAC; 1997 Sep 28-Oct 1; Toronto, Washington (DC): American Society for Microbiology, 1997
104. Black JA, Moland ES, Lockhart TJ, et al. Faropenem: Activity against ESBL, AMPC, and other β -lactamase-producing Enterobacteriaceae [abstract E-791]. 41st ICAAC; 2001 Dec 16-19; Chicago (IL). Washington (DC): American Society for Microbiology, 2001
105. Olsen KM, McNabb JC, Fey PD, et al. In vitro activities of MK-0826 and parenteral β -lactam antimicrobials against clinical isolates of ESBL-producing *Klebsiella pneumoniae* and in an isogenic host [abstract E-807]. 41st ICAAC; 2001 Dec 16-19; Chicago (IL). Washington (DC): American Society for Microbiology, 2001
106. Senda K, Arakawa Y, Ichiyama S, et al. PCR detection of metallo- β -lactamase gene (*bla_{imp}*) in gram-negative rods resistant to broad-spectrum β -lactams. *J Clin Microbiol* 1996; 34: 2909-13

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