REVIEW ARTICLE

MECHANISMS OF DISEASE

The New β-Lactamases

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N Engl J Med 2005;352:380-91.

The β-lactamases are the major defense of gram-negative bacteria against β-lactam antibiotics. β-Lactamases can be broadly divided into enzymes with a serine residue at the active site, similar to bacterial penicillin-binding proteins, from which they probably evolved,[1] and metalloenzymes with zinc ion as a cofactor and with a separate heritage.[2] Both are ancient enzymes. The serine group is estimated from current sequence diversity to have evolved with bacteria over the past 2 billion years.[3]

Since β-lactam antibiotics came into clinical use, β-lactamases have coevolved with them.[4] Early events were an increase in their prevalence in organisms in which the enzyme was known but uncommon (such as *Staphylococcus aureus*) and spread to pathogens that previously lacked β-lactamase (namely, *Haemophilus influenzae* and *Neisseria gonorrhoeae*). Beginning about 20 years ago, agents that shared the property of resistance to the then-common β-lactamases were introduced; they included cephamycins, cephalosporins with an oxyimino side chain, carbapenems, and the monobactam aztreonam. Bacteria responded with a plethora of "new" β-lactamases — including extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC enzymes, and carbapenem-hydrolyzing β-lactamases (carbapenemases) — that, with variable success, can confer resistance to the latest β-lactam antibiotics (Table 1). The properties of these new β-lactamases, the ways in which they can be detected, their origins, and options for treating the associated infections are considered in this article; aspects of these topics have been the subject of other recent reviews.[5-8]

Classification of β-Lactamases

Understanding the new enzymes requires a brief review of β-lactamase classification. Hundreds of β-lactamases have been described and have been given a bewildering variety of names (see Glossary). Fortunately, the enzymes can be classified on the basis of their primary structure into four molecular classes (A through D),[9] or on the basis of their substrate spectrum and responses to inhibitors into a larger number of functional groups.[10] Class A and class C β-lactamases are the most common and have a serine residue at the active site, as do class D β-lactamases. Class B comprises the metallo-β-lactamases. Twenty years ago, plasmids mediating resistance to β-lactam antibiotics in *Escherichia coli* and other Enterobacteriaceae most often carried genes encoding class A enzymes such as TEM-1 or SHV-1 or class D enzymes such as OXA-1.[11] Class B and C enzymes had a broader spectrum of activity but were almost always encoded by chromosomal genes and hence were confined to particular bacterial species.

The New β-Lactamases

TEM-type ESBLs (Class A)

Amino acid substitutions at many sites in TEM-1 β-lactamases can be created in the laboratory without loss of activity.[12] Those responsible for the ESBL phenotype change the

Glossary

AmpC β-lactamase: This type of broad-spectrum enzyme, usually encoded on the bacterial chromosome, is active on cephamycins as well as oxyimino-β-lactams.

β-Lactam–β-lactamase inhibitor combinations: Clavulanic acid, sulbactam, and tazobactam are inhibitory β-lactams that bind to and block the action of class A, and to a lesser extent, class D β-lactamases. The inhibitors are available in combinations with otherwise β-lactamase–susceptible antibiotics, such as ticarcillin–clavulanic acid, ampicillin–sulbactam, and piperacillin–tazobactam.

Carbapenems: Compounds with a fused β-lactam system in which the sulfur atom of the five-member ring is replaced by carbon. Examples include imipenem, meropenem, and ertapenem.

Cephamycins: Cephalosporins with a 7α-methoxy side chain that blocks hydrolysis by class A and class D β-lactamases. Examples include cefoxitin, cefotetan, and cefmetazole.

Extended-spectrum β-lactamase (ESBL): This name was originally coined to reflect the expanded substrate spectrum of enzymes derived from narrower-spectrum TEM, SHV, or OXA β-lactamases. The term now also refers to β-lactamases, such as those in the CTX-M family, with a similar phenotype but a separate heritage.

Inhibitor-resistant β-lactamase: Enzyme variants in the TEM family (and, less often, the SHV family) with reduced sensitivity to clavulanic acid, sulbactam, and tazobactam inhibitors as a result of amino acid substitutions.

Inoculum effect: Increased resistance with increasing numbers of test bacteria. One possible mechanism is increased hydrolysis with larger inocula of β-lactamase–producing organisms.

Integron: A unit of DNA containing a gene for a site-specific integrase (*intI*) and a recombination site (*attI*), into which gene cassettes made up of an antibiotic-resistance gene linked to a 59-base element (or *attC* site) can be integrated. A strong promoter adjacent to the *attI* site ensures that the integrated genes will be efficiently expressed. Integrons can be part of a transposon or a defective transposon and thus have an additional potential for mobility.

Monobactam: A monocyclic β-lactam. The single commercially available example is aztreonam, which has an oxyimino side chain and is therefore also an oxyimino-β-lactam.

Oxyimino-β-lactams: β-Lactams with an oxyiminoside chain designed to block the action of β-lactamase. Sometimes referred to as "third-generation cephalosporins," they include cefotaxime, ceftriaxone, ceftazidime, and cefepime (a "fourth-generation" derivative).

Plasmid: An extrachromosomal segment of DNA, usually circular, varying in size from a few kilobases to a 10th or more of the size of the bacterial chromosome. Plasmids larger than 20 kb are often conjugative and can promote their transfer between bacterial hosts. Resistance plasmids carry resistance genes, often organized into integrons or carried on transposons. Other plasmids carry metabolic genes or act as sex factors to promote transfer of the bacterial chromosome.

SHV, TEM, OXA, IMP, VIM, and KPC: β-Lactamase families with members (denoted by numerals, as in SHV-1) that are related by a few amino acid substitutions. β-Lactamase nomenclature is not standardized. SHV denotes a variable response to sulfhydryl inhibitors; TEM was named after the patient (Temoneira) from whom the first sample was obtained; CTX-M, OXA, and IMP reflect an ability to hydrolyze cefotaxime, oxacillin, and imipenem, respectively; VIM denotes Verona integron-encoded metallo-β-lactamase; and KPC is derived from *Klebsiella pneumoniae* carbapenemase. The origin of names for other β-lactamases is just as variable and arcane.

Transposon: A mobile unit of DNA that can jump, or transpose, from one DNA molecule to another — for example, from a plasmid to a chromosome or from a plasmid to a plasmid, usually without site specificity. In class I transposons, a pair of insertion sequences (segments of DNA that can replicate and insert more or less randomly at other sites) flank a resistance gene. In class II transposons, terminal inverted-repeat segments enclose the genes for a transposase (*tnpA*), a resolvase (*tnpR*), and one or more antibiotic-resistance genes. Some transposons are conjugative.

configuration of the active site of the enzyme, allowing access to oxyimino-β-lactams (Fig. 1).[14,18]

Opening the active site to β-lactam substrates also typically enhances the susceptibility of the enzyme to β-lactamase inhibitors, such as clavulanic acid. Amino acid substitutions distinct from those leading to the ESBL phenotype can confer resistance to inhibitors, but the combination of inhibitor resistance and an extended spectrum of activity seems to be, with rare exceptions,[19] incompatible. More than 130 TEM enzymes are currently recognized, and their variety provides a useful way to follow the spread of individual resistance genes.[20] TEM-10, TEM-12, and TEM-26 are among the most common in North and South America.[21]

SHV-type ESBLs (Class A)

SHV-1 shares 68 percent of its amino acids with TEM-1 and has a similar overall structure (Fig. 1).[22] As with TEM, SHV-type ESBLs have one or more amino acid substitutions around the active site. More than 50 varieties of SHV are currently recognized on the basis of unique combinations of amino acid replacements.[20] SHV-type ESBLs currently predominate in surveys of resistant clinical isolates in Europe and America.[21,23] SHV-5 and SHV-12 are among the most common members of this family.[21]

CTX-M–type ESBLs (Class A)

The most common group of ESBLs not belonging to the TEM or SHV families was termed CTX-M to

Table 1. Selected β-Lactamases of Gram-Negative Bacteria.

β-Lactamase	Examples	Substrates	Inhibition by Clavulanic Acid*	Molecular Class
Broad-spectrum	TEM-1, TEM-2, SHV-1	Benzylpenicillin (penicillin G), aminopenicillins (amoxicillin and ampicillin), carboxypenicillins (carbenicillin and ticarcillin), ureidopenicillin (piperacillin), narrow-spectrum cephalosporins (cefazolin, cephalothin, cefamandole, cefuroxime, and others)	+++	A
	OXA family	Substrates of the broad-spectrum group plus cloxacillin, methicillin, and oxacillin	+	D
Expanded-spectrum	TEM family and SHV family	Substrates of the broad-spectrum group plus oxyimino-cephalosporins (cefotaxime, cefpodoxime, ceftazidime, and ceftriaxone) and monobactam (aztreonam)	++++	A
	Others (BES-1, GES/IBC family, PER-1, PER-2, SFO-1, TLA-1, VEB-1, and VEB-2)	Same as for TEM family and SHV family	++++	A
	CTX-M family	Substrates of the expanded-spectrum group plus, for some enzymes, cefepime	++++	A
	OXA family	Same as for CTX-M family	+	D
AmpC	ACC-1, ACT-1, CFE-1, CMY family, DHA-1, DHA-2, FOX family, LAT family, MIR-1, MOX-1, and MOX-2	Substrates of expanded-spectrum group plus cephamycins (cefotetan, cefoxitin, and others)	0	C
Carbapenemase	IMP family, VIM family, GIM-1, and SPM-1	Substrates of the expanded-spectrum group plus cephamycins and carbapenems (ertapenem, imipenem, and meropenem)	0	B
	KPC-1, KPC-2, and KPC-3	Same as for IMP family, VIM family, GIM-1, and SPM-1	+++	A
	OXA-23, OXA-24, OXA-25, OXA-26, OXA-27, OXA-40, and OXA-48	Same as for IMP family, VIM family, GIM-1, and SPM-1	+	D

* Plus signs denote relative sensitivity to inhibition.

highlight their greater activity against cefotaxime than against ceftazidime. More than 40 CTX-M enzymes are currently known.[6] Belying their name, some hydrolyze ceftazidime more rapidly than they do cefotaxime. CTX-M-14, CTX-M-3, and CTX-M-2 are the most widespread.[6]

OTHER CLASS A ESBLs

Other class A ESBLs are uncommon and have been found mainly in *Pseudomonas aeruginosa* and at a limited number of geographic sites: PER-1 in isolates in Turkey, France, and Italy; VEB-1 and VEB-2 in strains from Southeast Asia; and GES-1, GES-2, and IBC-2 in isolates from South Africa, France, and Greece.[24] PER-1 is also common in multiresistant acinetobacter species in Korea and Turkey.[25] Some of these enzymes are found in Enterobacteriaceae as well, whereas other uncommon ESBLs (such as BES-1, IBC-1, SFO-1, and TLA-1) have been found only in Enterobacteriaceae.[26-29]

OXA-TYPE ESBLs (CLASS D)

Twelve ESBLs derived from OXA-10, OXA-1, or OXA-2 by amino acid substitutions are currently

known.[20] They have been found mainly in *P. aeruginosa* in specimens from Turkey and France.[5,30] Most OXA-type ESBLs are relatively resistant to inhibition by clavulanic acid. Some confer resistance predominantly to ceftazidime, but OXA-17 confers greater resistance to cefotaxime and cefepime than it does resistance to ceftazidime.[31]

PLASMID-MEDIATED AmpC ENZYMES (CLASS C)

AmpC β-lactamases, usually inducible by β-lactams, are encoded by chromosomal genes in many gram-negative bacilli. Mutations that increase their expression are responsible for the ready emergence of broad-spectrum cephalosporin resistance in *Enterobacter cloacae*.[32] The AmpC enzyme in *E. coli* is poorly expressed and the AmpC gene is missing from the chromosome of klebsiella and salmonella species, but plasmid-mediated AmpC enzymes can give these organisms the same resistance profile as a β-lactam–resistant enterobacter isolate. More than 20 different AmpC β-lactamases have been found to be mediated by plasmids.[7] Some, like the parental chromosomal enzymes, are accompanied by regulatory genes and are inducible, but most are not. Characteristically, AmpC β-lactamases provide resistance to cephamycins as well as to oxyimino-β-lactams and are resistant to inhibition by clavulanic acid.

CARBAPENEMASES (CLASSES A, B, AND D)

Carbapenemases are a diverse group of enzymes. They are currently uncommon but are a source of considerable concern because they are active not only against oxyimino-cephalosporins and cephamycins but also against carbapenems.[8] Plasmid-mediated IMP-type carbapenemases, 17 varieties of which are currently known, became established in Japan in the 1990s in both enteric gram-negative organisms and in pseudomonas and acinetobacter species. IMP enzymes spread slowly to other countries in the Far East, were reported from Europe in 1997, and have been found in Canada and Brazil.

A second growing family of carbapenemases, the VIM family, was reported from Italy in 1999 and now includes 10 members, which have a wide geographic distribution in Europe, South America, and the Far East and have been found in the United States.[33] A few class A enzymes, notably the plas-

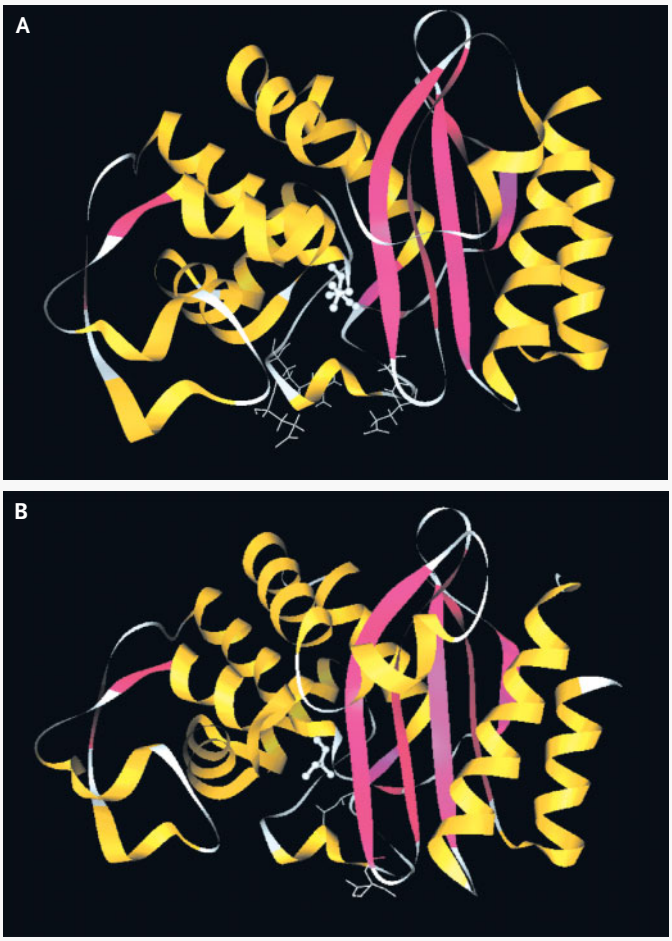

Figure 1. Schematic Diagrams of TEM and SHV β-Lactamases.

In these ribbon diagrams of TEM β-lactamases[13] (Panel A) and SHV β-lactamases (Panel B),[14] the critical serine residue at position 70 is shown in ball-and-stick mode (at the center of each molecule) and the atoms of residues in which amino acid substitutions yield an extended-spectrum β-lactamase (ESBL) phenotype are shown in stick mode. Colors are used to highlight the molecule's secondary structure: yellow indicates α-helixes, pink β-strands, and gray turns. Amino acid substitutions at positions 104, 164, 238, and 240 in TEM β-lactamases lead to the ESBL phenotype, but ESBLs with the broadest spectrum of activity usually have more than a single substitution. Many TEM ESBLs confer greater resistance to ceftazidime and aztreonam than to cefotaxime, but those with a serine substitution at position 238 may enhance resistance to cefotaxime as well. In the SHV family, substitutions at position 238 or at positions 238 and 240 are the most common and are associated with resistance to ceftazidime, cefotaxime, and aztreonam.[15] Less commonly, an alteration at position 146 or 179 provides selective ceftazidime resistance; the change at position 146 causes a moderate decrease in susceptibility to imipenem as well.[16,17]

mid-mediated KPC enzymes, are effective carbapenemases as well. Finally, some OXA-type β-lactamases have carbapenemase activity, augmented in clinical isolates by additional resistance mechanisms, such as impermeability or efflux.[8,34]

FACTORS INFLUENCING β-LACTAMASE EXPRESSION

As if the variety of enzymes were not enough, further complications arise because expression of resistance is affected by additional factors. The same enzyme may express different resistance phenotypes, depending on the bacterial host and the test conditions. For ESBLs of the TEM and SHV families, the expanded spectrum is accompanied by a loss of intrinsic hydrolytic activity.[35,36] This loss can be compensated for by an increase in gene dosage (through gene duplication or carriage on a multicopy plasmid) or the presence of a promoter with increased activity (through a mutation or insertion-sequence substitution).

In some organisms (*P. aeruginosa* in particular), an active efflux system can reduce the intracellular accumulation of antibiotic and allow an enzyme with only limited hydrolytic capacity to inactivate the drug before it can reach its target; in other organisms, this effect is achieved by diminished expression of an outer-membrane porin required for β-lactam uptake. In *Klebsiella pneumoniae*, decreased expression of outer-membrane porins often accompanies ESBL production and may allow a TEM- or SHV-type ESBL to express resistance to cefepime or allow an AmpC β-lactamase to express resistance to imipenem.[37,38]

GENETICS OF β-LACTAMASES

Plasmids are responsible for the spread of most of the new β-lactamases, but the genes encoding these enzymes may also be located on the bacterial chromosome. The genes encoding some β-lactamases are carried by transposons.[39] Genes for many of the new β-lactamases are found in integrons, which often include genes conferring resistance to other antibiotics. For this reason, the new β-lactamases are usually produced by organisms that are resistant to multiple antimicrobial agents.

Occasionally, the ESBL phenotype emerges in an organism isolated from a patient treated for multiple episodes of bacteremia,[40] but much more often an ESBL-producing plasmid or strain disseminates to multiple patients, so that in hospital outbreaks one type of ESBL often predominates. Particular TEM-type ESBL varieties seem to have a fixed geographic distribution, whereas at least some SHV types have been found all over the world, suggesting that they have a multifocal origin. For example, TEM-3 is common in France and has been reported in a few other European countries but has not been reported in the United States, whereas SHV-5 and SHV-12 have been detected worldwide.

The genes encoding the TEM-1 and TEM-2 β-lactamases are carried by transposons, as are the genes encoding some TEM-type ESBLs (Fig. 2).[44] The gene encoding SHV-1 is found on the chromosome of most strains of *K. pneumoniae*.[45-47] SHV genes also occur on transmissible plasmids; for example, one has been found on a 7.5-kb block of DNA apparently captured from the klebsiella chromosome.[48] Genes encoding the remaining types of β-lactamase are often found incorporated into integrons (Fig. 2) but have their origin elsewhere. For example, the genes for CTX-M–type enzymes are found on the chromosome of kluyvera, a genus of rarely pathogenic commensal organisms. Rather than evolving from a progenitor with a more limited spectrum of activity, the CTX-M group appears to have emerged in multiple places by plasmid acquisition of β-lactamase genes from such a widespread environmental reservoir.[6]

Integrons are also involved in the acquisition of AmpC-type β-lactamases by plasmids. Many of these plasmid-mediated enzymes can be related to chromosomal AmpC enzymes of particular species: thus, ACC-1 is related to the enzyme produced by *Hafnia alvei*; ACT-1 and MIR-1 to enzymes of enterobacter species; some CMY enzymes as well as LAT-1 and LAT-3 to enzymes of citrobacter species; other CMY enzymes and the FOX and MOX families to enzymes of aeromonas species; and DHA-1 to the enzyme of *Morganella morganii*.[7] Carbapenemases of the IMP and VIM families are also found within integrons (Fig. 2), but the origin of their genes is not yet known.

PREVALENCE

Despite worldwide use of β-lactam antibiotics, the distribution of the enzymes responsible for resistance to oxyimino-cephalosporins and carbapenems is far from uniform. Some hospitals in the United States seem to have no ESBLs, whereas in other hospitals as many as 40 percent of *K. pneumoniae*

isolates have been reported to be ceftazidime-resistant as a result of ESBL production.[49] ESBLs are most likely to be found in *K. pneumoniae*, *K. oxytoca*, and *E. coli* but have been reported in citrobacter, enterobacter, proteus, salmonella, serratia, and other genera of enteric organisms[50] and in such nonenteric organisms as *Acinetobacter baumannii*[51,52] and *P. aeruginosa*.[24] Their prevalence is higher in isolates from intensive care units than in isolates from other hospital sites.

In a sample of more than 4700 *K. pneumoniae* isolates obtained during the period from 1997 through 1999, the percentage expressing an ESBL phenotype was highest in isolates from Latin America (45.4 percent), the Western Pacific (24.6 percent), and Europe (22.6 percent) and lowest in strains from the United States (7.6 percent) and Canada (4.9 percent).[53] In more than 13,000 isolates of *E. coli*, the percentages expressing the ESBL phenotype were as follows: in Latin America, 8.5 percent; in the Western Pacific, 7.9 percent; in Europe, 5.3 percent; in the United States, 3.3 percent; and in Canada, 4.2 percent.[53]

In another large data set from the United States collected from 1998 through 2001, ceftazidime resistance was present in 9.6 percent of *K. pneumoniae* isolates from intensive care units and 6.6 percent of isolates from other hospital locations.[54] The higher the apparent frequency in a particular hospital, the more likely a single ESBL is involved. Outbreaks have been due both to a single ESBL-producing strain and to a single ESBL plasmid carried by unrelated strains. A resistant strain or plasmid may cause problems in several hospitals locally or involve a large geographic area.[23,55,56] Community clinics and nursing homes have also been identified as potential reservoirs for ESBL-producing *K. pneumoniae* and *E. coli*.[57,58]

In 1989, nontyphoid salmonella strains producing CTX-M-2 began to spread among neonatal units in Argentina and to neighboring South American countries, and by 2002 this enzyme was present in about 75 percent of ESBL-producing Enterobacteriaceae in Buenos Aires.[59] CTX-M enzymes, which are also common in Japan, China, Korea, Taiwan, Vietnam, and India, are a rapidly emerging problem in the United Kingdom[60] and have been reported in Eastern Europe, Germany, France, and Spain and recently in the United States.[6,61]

It is estimated that in the United States, 3 to 4 percent of clinical *K. pneumoniae* and *K. oxytoca* isolates carry plasmid-mediated AmpC enzymes.[62]

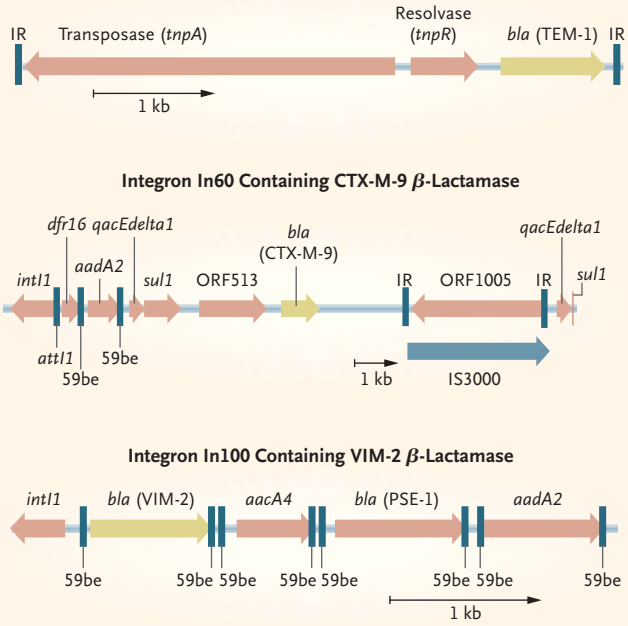

Figure 2. Schematic Diagrams of Genetic Units Encoding Various β-Lactamases.

Diagrams of transposons and integrons encoding TEM-1,[41] CTX-M-9,[42] and VIM-2[43] β-lactamases are shown. IR denotes inverted repeat, *bla* β-lactamase gene, *dfr* dihydrofolate reductase gene, *qac* gene conferring resistance to quaternary ammonium compounds, *delta* deletion derivative, *intI* site-specific integrase gene, *aad* aminoglycoside adenylyltransferase gene, *sul* dihydropteroate synthetase gene, ORF open-reading frame, *attI* recombination site, 59be 59-base element, *aac* aminoglycoside acetyltransferase gene, and IS insertion sequence.

One particular plasmid-mediated AmpC enzyme, CMY-2, has been responsible for increasing resistance to ceftriaxone and other oxyimino-β-lactams in salmonella isolates from the United States.[63,64]

In Japan, IMP-type carbapenemases, first detected in *Serratia marcescens* and *P. aeruginosa*, have spread to other gram-negative bacilli,[65] but the prevalence of this resistance mechanism is surprisingly low: 1.3 percent in *P. aeruginosa* and less than 0.5 percent in *E. coli* and *K. pneumoniae*.[66,67] Considering the broad resistance to β-lactam antibiotics that is conferred by carbapenemases and considering their presence in Japan for more than a decade, their limited occurrence is surprising and somewhat reassuring considering the potential for future spread. Worldwide, 99.9 percent of Enterobacteriaceae remain susceptible to carbapenems.[68] Carbapenemases can, however, be associated with lethal in-

fections. In Greece and Italy, outbreaks due to carbapenem-resistant *P. aeruginosa* producing VIM-1 carbapenemase were identified in separate hospitals and associated with a high mortality rate.[69,70] In Brazil, a strain of *A. baumannii* resistant to imipenem and meropenem due to an OXA-type carbapenemase infected eight patients in two hospitals; five of the patients died, despite therapy with multiple antibiotics, including polymyxin B.[71] *K. pneumoniae* strains with reduced susceptibility to carbapenems due to KPC-2 or KPC-3 has been found recently in several hospitals in New York City.[72]

DETECTION

Detection of the new β-lactamases is less straightforward than implied by the properties listed in Table 1 because of the heterogeneity of the enzymes, their variable activity against potential substrates, their coexistence with other β-lactamases, and the confounding factors that modify their expression. The procedure currently recommended by the Clinical and Laboratory Standards Institute (CLSI) to detect ESBL-producing *K. pneumoniae*, *K. oxytoca*, and *E. coli* involves an initial disk-diffusion or broth-dilution screening test with one or more oxyimino-β-lactams, followed by a confirmatory test to measure susceptibility to ceftazidime and to cefotaxime alone and in combination with clavulanic acid. Automated procedures have also been developed.

Currently there are no CLSI-recommended tests for detecting AmpC β-lactamases or carbapenemases, nor are there recommended tests for detecting ESBLs in *P. aeruginosa* or in enteric bacteria other than *E. coli* and klebsiella species. Cefoxitin or cefotetan resistance along with oxyimino-β-lactam resistance raises the suspicion of an AmpC-type enzyme, although there are other possibilities.[7] Carbapenem resistance in an enteric gram-negative organism is currently rare enough to ensure that such an isolate would receive special attention. Unfortunately, with so many different ESBLs and other new β-lactamases, no test is completely reliable[73]; better tests continue to be proposed, and recommendations continue to evolve.

Success in identifying these mechanisms of resistance in clinical laboratories is rather poor, suggesting that patients are at risk for receiving inappropriate treatment and that the prevalence of ESBLs and AmpC β-lactamases is underreported. In a study published in 1999, before the current CLSI detection criteria were widely known, ESBL- and AmpC-producing *K. pneumoniae* and *E. coli* were sent as "unknown" specimens to 38 hospital-affiliated and commercial clinical laboratories in Connecticut. Six laboratories failed to test for resistance to any oxyimino-β-lactam, and only nine included both ceftazidime and cefotaxime in their evaluation. Depending on the strain tested, between 24 and 32 percent of laboratories incorrectly reported it as susceptible.[74] In a recent evaluation of the ability of rural laboratories in the United States to identify specific resistance mechanisms, only 5 of 60 laboratories screened *K. pneumoniae* isolates for ESBL production.[75] In a proficiency test of 129 laboratories outside the United States, 7 misreported a highly resistant ESBL-producing *K. pneumoniae* strain as susceptible to all cephalosporins, and only 2 specifically reported the strain as an ESBL producer.[76]

RISK FACTORS FOR INFECTION

Risk factors for colonization or infection by ESBL-producing organisms are little different from the risk factors for other nosocomial infections.[77] Reported risks, many of which are linked, include an increased length of stay in the hospital,[78,79] an increased length of stay in the intensive care unit,[80,81] increased severity of illness,[82-84] the use of a central venous or arterial catheter,[80-82,84,85] the use of a urinary catheter,[78,80-84] ventilatory assistance,[81,82,86] hemodialysis,[87] emergency abdominal surgery,[81] the use of a gastrostomy or jejunostomy tube,[84] gut colonization,[80,88] prior administration of an oxyimino-β-lactam antibiotic,[84,88-92] and prior administration of any antibiotic.[84,85,93] Similar risk factors are emerging for infection with *P. aeruginosa* producing IMP-type carbapenemases.[94]

TREATMENT

IN VITRO DATA

ESBL-producing organisms vary in their susceptibility to different oxyimino-β-lactams, and despite resistance to some they may appear sensitive to others. For organisms producing TEM and SHV-type ESBLs, apparent in vitro sensitivity to cefepime and to piperacillin–tazobactam is common, but both drugs show an inoculum effect, with diminished susceptibility as the size of the inoculum is increased from 10^5 to 10^7 organisms.[95-97]

Strains with some CTX-M–type and OXA-type ESBLs are resistant to cefepime on testing, despite

the use of a standard inoculum.[6,30] Strains producing only ESBLs are susceptible to cephamycins and carbapenems in vitro and show little if any inoculum effect with these agents.[96-98] AmpC-producing strains are typically resistant to oxyimino-β-lactams and to cephamycins and are susceptible to carbapenems; however, diminished porin expression can make such a strain carbapenem-resistant as well.[38] Strains with IMP-, VIM-, and OXA-type carbapenemases usually remain susceptible to aztreonam.[8] Resistance to non–β-lactam antibiotics is common in strains making any of these enzymes, such that alternative options for non–β-lactam therapy need to be determined by direct susceptibility testing. Resistance to fluoroquinolones and aminoglycosides is especially high.[99,100]

STUDIES IN HUMANS

No randomized, controlled trials have evaluated various treatments for infections caused by organisms producing the new β-lactamases. Most reports present a compilation of a small number of cases in the setting of an outbreak, with treatment consisting of a particular antibiotic, often given in combination with other agents and followed by other infections. Furthermore, the outcome may be specific to the particular enzyme involved, suggesting that caution is warranted in generalizing the results.

For infections caused by ESBL-producing *E. coli* or klebsiella species, treatment with imipenem or meropenem has been associated with the best outcomes in terms of survival and bacteriologic clearance.[101-106] Cefepime and piperacillin–tazobactam have been less successful. Ceftriaxone, cefotaxime, and ceftazidime have failed even more often, despite the organism's susceptibility to the antibiotic in vitro.[107] Several reports have documented failure of cephamycin therapy as a result of resistance due to porin loss.[108,109] Some patients have responded to aminoglycoside or quinolone therapy, but in a recent comparison of ciprofloxacin and imipenem for bacteremia involving an ESBL-producing *K. pneumoniae*, imipenem produced the better outcome.[106]

There have been few clinical studies to define the optimal therapy for infections caused by ESBL-producing *P. aeruginosa* strains.[24] There are also insufficient data to evaluate the benefit of combination therapy with a β-lactam plus a quinolone or aminoglycoside for infections due to ESBL-positive organisms. The data that are available concerning the treatment of infections caused by AmpC-type β-lactamase–producing *K. pneumoniae* indicate a much better response to carbapenem than to cephalosporin therapy.[110] Data on treatment for carbapenemase-producing organisms are also very limited. Although these enzymes may fail to hydrolyze aztreonam, some clinical isolates have been aztreonam-resistant, presumably because of porin loss, suggesting that caution should be exercised in assuming that the antibiotic can be used successfully for treatment.

OUTBREAK CONTROL

In outbreak situations, successful control has usually involved both restriction of the use of oxyimino-β-lactams and the institution of barrier precautions (hand washing, gloves, and gowns) for patients with infection or colonization.[88,111,112] Successful control with the use of strict isolation procedures without limitations on antibiotic use has also been reported.[113] Substitution of imipenem,[112] piperacillin–tazobactam,[114] or cefepime–amikacin[115] as the antibiotic of choice for empirical therapy has been followed by decreased isolation of ESBL-producing organisms.

Antibiotic substitutions can, however, have unintended consequences. In an outbreak of infection with *K. pneumoniae* resistant to other β-lactam antibiotics, increased use of imipenem was followed by the emergence of imipenem-resistant *K. pneumoniae* that produced an AmpC enzyme (ACT-1) and was missing an outer-membrane porin.[37,116] At the same hospital, increased use of imipenem also led to the emergence of imipenem-resistant *A. baumannii*.[117]

CONCLUSIONS

Gram-negative bacteria have adapted to broad-spectrum β-lactam antibiotics by modifying the substrate spectrum of common plasmid-mediated β-lactamases and by mobilizing resistance-promoting chromosomal β-lactamase genes into plasmids, allowing their spread to new hosts. Currently, the most common new β-lactamases are ESBLs in the TEM, SHV, and CTX-M families. These enzymes confer resistance to ceftazidime, cefotaxime, ceftriaxone, aztreonam, and other oxyimino-β-lactams and are found most often in klebsiella species and *E. coli*, although they also have been detected in many other gram-negative pathogens. Their prevalence is probably underestimated because detection

in clinical laboratories is imperfect. Carbapenems are the surest agents for therapy, but the variety of β-lactamases that confer resistance to carbapenems is increasing, and overuse of any single class of antibiotic is likely to be followed by the selection of pathogens resistant to that agent. There are no β-lactams in development that can treat infections with organisms producing some of the new β-lactamases. Available agents need to be used judiciously and infection-control measures implemented in outbreak situations to prevent the further spread of pathogens with these all-too-successful mechanisms of resistance.

Dr. Jacoby reports having received consultation or lecture fees from Bayer and Ortho-McNeil and grant support from Merck. Dr. Munoz-Price reports having received grant support from Merck.

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