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β-Lactamases in Laboratory and Clinical Resistance

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INTRODUCTION

β-Lactamases are the commonest cause of bacterial resistance to β-lactam antimicrobial agents. Their spread destroyed the utility of benzylpenicillin against staphylococci and has hugely undermined that of ampicillin against enterobacteria and *Haemophilus* and *Neisseria* spp. New enzymes and new modes of production of old enzymes now threaten the value of extended-spectrum cephalosporins against enterobacteria. Further β-lactamases, some of which will become important in the future, are described in most issues of *Antimicrobial Agents and Chemotherapy* and in other journals. In brief, the impact of

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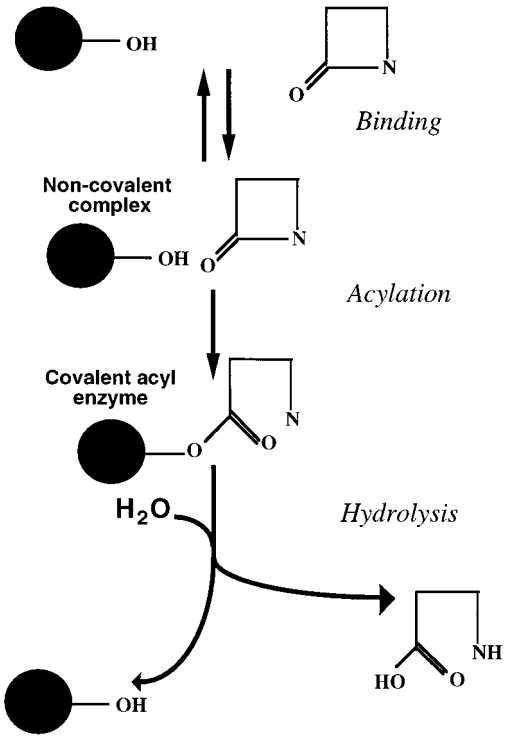

FIG. 1. Action of a serine β-lactamase. The enzyme first associates noncovalently with the antibiotic to yield the noncovalent Michaelis complex. The β-lactam ring is then attacked by the free hydroxyl on the side chain of a serine residue at the active site of the enzyme, yielding a covalent acyl ester. Hydrolysis of the ester finally liberates active enzyme and the hydrolyzed, inactive drug. This mechanism is followed by β-lactamases of molecular classes A, C, and D, but class B enzymes utilize a zinc ion to attack the β-lactam ring (256).

β-lactamases already has been huge, and their potential to challenge antimicrobial chemotherapy remains unexhausted.

Excellent reviews exist on the structure and function (64, 121, 151, 256) and regulation (20) of β-lactamases. Bush et al. (37) recently summarized the biochemical properties of over 190 enzyme types, and in 1992, Sanders and Sanders (225) reviewed the frequency of β-lactamase production worldwide. It would be pointless to recapitulate these reviews; instead, the present review aims primarily to aid readers who regularly assess susceptibility data for clinical isolates. It highlights the factors that determine whether a β-lactamase can cause resistance, which compounds are compromised by which enzymes, the extent to which routine tests reflect β-lactamase-mediated resistance, and how susceptibility tests for weak substrates should be interpreted. Finally, it considers the extent to which β-lactamase types can be predicted from antibiogram data and the value of this prediction. It emphasizes common microbial species and enzymes and does not aim to detail every obscure β-lactamase that has ever been described.

ACTION OF β-LACTAMASES

A few β-lactamases utilize zinc ions to disrupt the β-lactam ring, but a far greater number operate via the serine ester mechanism shown in Fig. 1 (256). Penicillin-binding proteins (PBPs) also react with β-lactams to give serine esters, but, unlike the similar esters formed by β-lactamases, these do not hydrolyze readily (72). This distinction blurs in a few cases: on one hand, some β-lactamase inhibitors form stable serine esters with β-lactamases (38); on the other, a few PBPs deacylate rapidly, and their weak β-lactam-hydrolyzing activity may protect the bacterial cell if drug entry is sufficiently restricted by impermeability (131).

CLASSIFICATION OF β-LACTAMASES

Until recently, β-lactamases have been classified by their hydrolytic spectrum, susceptibility to inhibitors, and whether they are encoded by the chromosome or by plasmids. Great weight has been placed on whether cephaloridine is hydrolyzed more rapidly than benzylpenicillin, or vice versa, and on whether an enzyme is inactivated by cloxacillin, clavulanate, aztreonam, or *p*-chloromercuribenzoate. The first such classification was proposed by Jack and Richmond in 1970 (94) and was expanded by Richmond and Sykes in 1973 (214). Deficiencies became apparent during the subsequent 15 years, and a major reorganization was proposed by Bush in 1989 (36), with further updating in 1995 (37). The revised Bush scheme classifies β-lactamases by their substrate preference among penicillin, oxacillin, carbenicillin, cephaloridine, expanded-spectrum cephalosporins, and imipenem and by their susceptibility to inhibition by clavulanate (Table 1).

Phenotypic classifications face the problem that point mutations can greatly alter substrate specificity (97, 191) and inhibitor susceptibility (253), changing the group to which an enzyme is assigned. Increasingly, therefore, β-lactamases are classified by sequence, as was first proposed by Ambler (4). Such classification is stable, as it reflects fundamental relationships and cannot be distorted by mutations. Moreover, at least for the present, sequence-based classification has the beauty of simplicity, recognizing only four classes, designated A to D. Classes A, C, and D comprise evolutionarily distinct groups of serine enzymes, and class B contains the zinc types (256). Subdivision within these classes can be conveniently illustrated by dendrograms based on sequence similarity, as in numerical taxonomy of bacteria. The most complete such dendrogram to date is to be found in reference 37. At present there is good correspondence between the classes recognized in Bush's phenotypic classification and those defined in the molecular scheme (Table 1), except that Bush's group 2d includes a few class A enzymes from actinomycetes as well as all the class D types from gram-negative rods. It is uncertain whether agreement will remain so good as more enzymes are sequenced.

Aside from their taxonomy, the nomenclature of β-lactamases often seems designed to confuse rather than help the uninitiated, and the Bush classification (36, 37) is bedeviled by unmemorable codes (2be, 2f, etc.). Worse, many of the three-letter, one-number monikers conventionally used to name plasmid-mediated β-lactamases have lost their historical meanings: for example, SHV stands for sulfhydryl variable and indicates that *p*-chloromercuribenzoate (which binds sulfhydryl groups) inhibits hydrolysis of cephaloridine but not of benzylpenicillin (153); PSE stands for *Pseudomonas*-specific enzyme (153); and SAR stands for Southern Africa (207). Since these names were proposed, it has been revealed that a serine hydroxyl, not a sulfhydryl, is the active site residue of SHV-1 enzyme and that PSE types occur in enterobacteria. SAR-2 was named because it resembled SAR-1, but it came from an isolate collected in India (279)!

DISTRIBUTION OF β-LACTAMASES

Chromosomal Enzymes

Sensitive tests detect slight β-lactam-hydrolyzing activities in virtually all bacteria, even beta-hemolytic streptococci (237). It

TABLE 1. Correspondence between Ambler's molecular classification of β-lactamases and the functional groups of Bush et al.[a]

Structural class (Ambler)	Functional group (Bush)	Activity[b]							Inhibition by clavulanate
		Penicillin	Carbenicillin	Oxacillin	Cephaloridine	Cefotaxime	Aztreonam	Imipenem	
Serine β-lactamases									
A	2a	+++	+	−	±	−	−	−	++
	2b	+++	+	+	++	−	−	−	++
	2be	+++	+	+	++	++	++	−	++
	2br	+++	+	+	+	−	−	−	−
	2c	++	+++	+	+	−	−	−	+
	2e	++	++	−	++	++	++	−	++
	2f	++	+	?	+	+	++	++	+
C	1	++	±	Inhibitor	+++	+	Inhibitor	−	−
D	2d	++	+	+++	+	−	−	−	±
Undetermined[c]	4[c]	++	++	++	V	V	−	−	−
Zinc β-lactamases									
B	3	++	++	++	++	++	−	++	−

[a] Data from Ambler's classification (4, 256) and the classification of Bush et al. (37). This table includes some simplifications. In particular, (i) group 2d includes molecular class A oxacillinases from *Actinomadura* and *Streptomyces* spp., as well as class D enzymes from gram-negative rods; (ii) hydrolytic activity varies within each group, and (iii) sequences remain to be determined for many enzymes included in Bush's scheme.

[b] +++, preferred substrate (highest V_{max}); ++, good substrate; +, hydrolyzed; ±, barely hydrolyzed; −, stable; V, variable within group; ?, uncertain.

[c] None of Bush's group 4 enzymes has yet been sequenced; they are assumed to be serine types because they lack carbapenemase activity.

is arguable, in some cases, whether these activities reflect true β-lactamases rather than the hydrolytic side reactions of PBPs, but it is certain that true β-lactamases, for example, AmpC cephalosporinases of enterobacteria, occur in organisms long pre-dating the antibiotic era. These enzymes may have some physiological role in peptidoglycan assembly or may have evolved to defend bacteria against β-lactams produced by environmental bacteria and fungi. What is certain is that the clinical use of β-lactams is now the major selective factor influencing β-lactamase production by pathogens.

Some species produce chromosomal β-lactamases copiously, whether constitutively or inducibly (36, 37, 237, 238). Examples include many *Bacteroides fragilis* group and klebsiella isolates, in which class A enzymes are constitutive; *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Morganella morganii*, *Serratia* spp., *Providencia* spp., *Pseudomonas aeruginosa* and other fluorescent pseudomonads, which have inducible class C enzymes; *Citrobacter diversus*, *Proteus vulgaris*, and *Burkholderia* (*Pseudomonas*) *cepacia*, which have inducible class A enzymes; *Stenotrophomonas* (*Xanthomonas*) *maltophilia*, which has an inducible class B carbapenem-hydrolyzing β-lactamase and an unsequenced Bush group 2e cephalosporinase; and *Bacillus cereus*, which has up to three inducible β-lactamases.

Plasmid-Mediated and Other Secondary β-Lactamases

During the past 20 to 50 years, plasmid-mediated β-lactamases have become common in staphylococci, enterobacteria, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. Over 75 enzymes now appear on lists of "plasmid-mediated β-lactamases," although many of these are sequence variants of a few prevalent types. In general, plasmid-mediated β-lactamases are distinct from the chromosomal types, but a few overlaps exist. In particular, SHV-1 β-lactamase, which is common as a plasmidic type (153), is also a typical chromosomal β-lactamase of *Klebsiella pneumoniae* (see below), and the BIL-1, CMY-1, CMY-2, CMY-3, FOX-1, LAT-1, MIR-1, and MOX-1 plasmid-mediated β-lactamases are AmpC enzymes encoded by genes that have escaped from the chromosomes of *Enterobacter* and *Citrobacter* spp. (37). It is likely that all "plasmid-mediated" β-lactamases have chromosomal origins, although the source organisms for many types remain unknown. The distribution of the plasmid-mediated enzymes reflects the transmissibility of the elements to which their genes have spread. Many of the enzyme genes are on transposons, facilitating dissemination among different plasmids and organisms. For example, TEM β-lactamases were first found encoded by enterobacterial plasmids in 1965 but had spread to *P. aeruginosa* by 1969, to *Vibrio cholerae* by 1973, and to *Haemophilus* and *Neisseria* species by 1974 (153, 201).

Confusingly, a few enzymes that appear in lists of plasmid-mediated types are rarely encoded by plasmids. The occurrence of SHV-1 β-lactamase as a chromosomal type in klebsiellae has been mentioned already. PSE-4 β-lactamase in *P. aeruginosa* is another particular example, almost always being encoded by chromosomally inserted transposons (141). The class A carbapenemases Imi-1, Sme-1, and NMC-A should also be mentioned in this context. These are chromosomally determined types, known from *Serratia marcescens* and *Enterobacter cloacae* isolates, but are absent from typical members of these species. The term "secondary β-lactamase" will be used in this review to describe enzymes such as these, which are encoded by chromosomal inserts and which are supplementary to the normal chromosomal β-lactamase of an organism.

Frequency of Enzyme Production

Later sections of this review include data on the frequency of β-lactamases. This is easy to define when an enzyme is chromosomally mediated and ubiquitous in a species but is far harder when expression demands mutation or plasmid acquisition. In these cases, frequencies vary hugely among countries, hospitals, unit types, and patient types. Resistance generally is most common where antibiotic usage is greatest (63, 225), notably in intensive care units, hematology departments, and burns units, as well as in developing countries, where medical and surgical advances often outpace infection control. Conversely, resistance rates often are very low in the general wards of community hospitals of developed countries. Such differences are easy to rationalize in terms of the degree of selection pressure, as are high resistance rates in recalcitrant infections (e.g., *P. aeruginosa* in cystic fibrosis), in which bacteria are repeatedly exposed to antimicrobial agents. Rates of β-lactamase production also vary hugely in community-acquired

pathogens from different geographic sources. Fewer than 5% of *H. influenzae* isolates from Austria and Germany have β-lactamase (106, 147), but upward of 30% of those from Spain (106, 147) and Taiwan (116) produce the enzyme. These differences may be explained by the high level of community use of antibiotics and the over-the-counter availability in Spain and Taiwan; however, a β-lactamase production rate of 29% among *H. influenzae* isolates from Canada (229) is harder to rationalize.

All frequencies cited for particular modes of β-lactamase-mediated resistance should be seen against this place-to-place variation. They apply when and where they were found but maybe not elsewhere. The relative frequency is more constant than the absolute frequency. Thus, for example, β-lactamase production is much rarer among *H. influenzae* isolates from Germany and Austria than among those from Taiwan, Spain, or Canada but TEM-1 enzyme remains the predominant β-lactamases from *Haemophilus* isolates worldwide (147, 196, 229). It is rare for one enzyme to predominate in one country and for others to predominate elsewhere. The exception comes when a new type of resistance is evolving: thus, extended-spectrum TEM β-lactamases became a clinical problem in France before they were recorded elsewhere (97, 233), and PER-1 enzyme, an unusual class A cephalosporinase, is now scattered in salmonellae and *P. aeruginosa* in Turkey (55, 137) while remaining virtually unknown elsewhere.

DETERMINANTS OF β-LACTAMASE FUNCTION IN RESISTANCE

The ability or inability of a β-lactamase to confer resistance depends on its location, kinetics, and quantity and on the physiochemical conditions.

Location

The β-lactamases of gram-positive species are largely extracellular, although, depending on the growth conditions, some enzyme may adhere to the cytoplasmic membrane. By contrast, the β-lactamases of gram-negative species are largely periplasmic, although some extracellular release may occur, mediated by leakage rather than secretion.

Kinetics

The activity of most β-lactamases against most substrates can be described by the Michaelis-Menten equation:

$$v = V_{max}S/(K_m + S) \quad (1)$$

where v is the hydrolysis rate, S is the β-lactam concentration, and V_{max} and K_m are the kinetic constants. A low K_m reflects high affinity and may be as critical to resistance as a high V_{max} (80). Occasionally, simple Michaelis-Menten kinetics are not obeyed, either because burst kinetics arise, with acylation much faster than deacylation, or because there is a branched reaction pathway, with either the acyl-enzyme or the Michaelis complex (Fig. 1) able to isomerize between two forms with differing stabilities. Behavior of this type is common with class D β-lactamases (121) but occasionally occurs with other types, for example, for class A and C enzymes with meropenem (117, 274). Its significance in the bacterial cell is uncertain but seems likely to reduce hydrolytic efficiency.

Enzyme Quantity

Higher levels of enzyme cause greater resistance (135, 140, 269). This relationship is simplest for constitutively expressed enzymes and is more complex when a β-lactamase is inducible, as the expression or not of resistance depends on the inducer activity of a compound as well as on its lability to hydrolysis (130, 144).

TABLE 2. Medium effects on β-lactamase-mediated resistance

Phenomenon	Explanation	Reference(s)
Enterobacteria with TEM and SHV enzymes are less susceptible to sulbactam or tazobactam combinations at pH 6.5–7.0 than at pH 7.5–8.0, and in 5% CO_2 than in air	TEM and SHV enzymes are less susceptible to inhibition by sulfones at lower pH because the enzyme-inhibitor complex partitions to a less stable form	108, 114, 138
"Nonspecific" induction of AmpC β-lactamases of some (not all) enterobacteria and *P. aeruginosa* by urine (not serum or ascites fluid), glycine, aromatic amino acids, and histidine		52, 54, 69, 70, 125
Increased imipenem resistance of *S. maltophilia* in zinc-rich media[a]	Availability of zinc to L-1 β-lactamase or nonenzymatic inactivation of imipenem by zinc ions	85

[a] *S. maltophilia* also is more resistant to many penicillins and cephalosporins on Mueller-Hinton agar than on Iso-Sensitest or DST agar, but this phenomenon, unlike that of zinc on imipenem, seems unrelated to β-lactamase (27, 29).

Physiochemical Conditions

Most β-lactamases protect producer strains against substrate drugs on standard media such as Mueller-Hinton, Iso-Sensitest, and DST agars or broths. Unusual media may give different results, and Table 2 provides a list of examples and, where available, their explanations. None of the phenomena listed in the table has proven clinical significance.

Interplay of Determinants

The location, kinetics and quantity of a β-lactamase determine its ability to reduce the drug concentration around the PBPs. The interactions of these factors can be modelled quantitatively for some species. The extracellular β-lactamases of gram-positive species serve to reduce the external drug concentration (S_0), with growth commencing only once this falls below a toxic threshold (S_+) that reflects PBP sensitivity. The period for this to be achieved (t) can be predicted, with reasonable accuracy (79, 82), from the integrated Michaelis-Menten equation:

$$t = [K_m (\ln S_0 - \ln S_+) + S_0 - S_+]/V_{max} \quad (2)$$

where V_{max} reflects both the amount of enzyme and the number of drug molecules hydrolyzed per unit time by each enzyme molecule (k_{cat}). Increasing the number of cells increases V_{max} and, because of the reciprocal relationship, reduces t. This explains the inoculum effects that notoriously occur when substrate penicillins are tested against β-lactamase-producing staphylococci (120, 220). Conversely, a high K_m (low affinity) extends t and reduces the ability of the enzyme to cause resistance. "β-Lactamase-stable" antistaphylococcal agents may owe their efficacy either to a low V_{max} (e.g., cephalothin) or to a high K_m (e.g., methicillin) (79).

By contrast, the periplasmic β-lactamases of gram-negative species function jointly with the permeability barrier provided by the outer membrane (254, 282). For enterobacteria, the rate of drug entry (v) across this membrane can be represented by Fick's law of diffusion:

$$v = C(S_0 - S_p) \quad (3)$$

where S_0 and S_p are the external and periplasmic drug concentrations, respectively, and C is a diffusion coefficient related to the permeability of the membrane and the surface area available for diffusion. An equilibrium is established, such that the rate of entry of drug equals the rate of hydrolysis within the periplasm (282). Under these circumstances, v in equation 3 equals v, the rate of drug hydrolysis in equation 1, and S_p is constant. Therefore,

$$C(S_0 - S_p) = V_{max}S_p/(K_m + S_p) \quad (4)$$

The MIC is the minimum S_0 level to give an S_p, termed I_p, just causing intolerable PBP inactivation. Substituting into equation 4 and rearranging, it is found that

$$MIC = I_p \{1 + [V_{max}/C(K_m + I_p)]\} \quad (5)$$

The MIC is raised when the drug is hydrolyzed rapidly (high V_{max}), when the enzyme has high affinity for the compound (low K_m), and/or when drug entry is slow (low C). Conversely, low affinity (high K_m) or rapid penetration of the antibiotic (high C) reduces the ability of the enzyme to protect, even when V_{max} is high. This model allowed Nikaido and Normark (169) to accurately predict the MICs of many β-lactams for *Escherichia coli* transconjugants with various β-lactamases. Other mathematical models, notably that of Waley (255), may provide a further refinement by taking into account the extent to which the periplasmic drug concentration is reduced by PBP binding as well as by β-lactamase-mediated drug hydrolysis.

Nevertheless, such models have limitations. They do not apply to *P. aeruginosa* (139), which apparently effluxes β-lactams (126). More generally, equation 5 treats the β-lactamase solely as protecting the individual cell. Doubling the number of cells doubles V_{max} but also doubles the cell surface area, thereby doubling C; since V_{max} and C appear as a ratio, there should be no effect on the MIC. Nevertheless, inoculum effects do occur for gram-negative bacteria, for example, with narrow-spectrum cephalosporins against enterobacteria with TEM-1 enzyme (155, 260). They may reflect leaked enzyme and do not fundamentally negate the model, but they do indicate a limitation.

DEFINITION OF RESISTANCE

The effects of β-lactamases on resistance sometimes are unequivocal. For example, MICs of ampicillin for *E. coli* isolates without significant β-lactamase activity are 1 to 8 μg/ml, whereas those for isolates with the TEM-1 enzyme almost always exceed 256 μg/ml (140, 269). Often, however, β-lactamases reduce susceptibility without raising MICs above the breakpoint and, similarly, β-lactamase inhibitors reduce MICs without restoring full susceptibility. This begs the question, "What degree of resistance predicts that clinical failure is likely?" The answer, never simple, is complicated further when MICs are inoculum dependent.

It should be appreciated, in this context, that susceptibility and resistance can be defined on the basis of either biological or pharmacological criteria. Biological breakpoints view an organism as resistant if the observed MIC or inhibition zone falls outside the normal distribution of MICs or zones for isolates without specific resistance mechanisms (173, 262); pharmacological breakpoints, favored by the National Committee for Clinical Laboratory Standards (NCCLS) (166) and the British Society for Antimicrobial Chemotherapy (267), define resistance relative to the drug concentration achievable in vivo. Biological analysis automatically gives significance to small reductions in susceptibility and allows emerging low-level resistance to be detected and monitored, whereas pharmacological breakpoints may demand tiny or huge MIC changes before an organism is deemed to be resistant. For example, the NCCLS (pharmacological) breakpoint for cefoxitin of 16 μg/ml is only double the modal MIC for typical *E. coli* or *Bacteroides* isolates, whereas the breakpoint for cefotaxime of 8 μg/ml exceeds the MICs for typical enterobacteria by 50- to 100-fold. Examples can be found to defend either basis of choosing breakpoints, and it is worth citing two contrasting examples. First, many extended-spectrum TEM β-lactamases raise the MICs of cefotaxime and other extended-spectrum cephalosporins for enterobacteria to only 1 to 4 μg/ml, compared with 0.03 μg/ml for isolates without these enzymes (103, 200). Although the MICs for the producers are below the NCCLS breakpoint, the organisms commonly prove resistant in vivo (33, 212). Such a situation is powerful ammunition to proponents of biological breakpoints, who can point out that MICs for the enzyme producers were 32- to 128-fold above the normal level. "If that isn't resistance what is. . . .?" On the other hand, *N. gonorrhoeae* isolates which, through impermeability and target modification, are biologically resistant to penicillin (MICs of 0.25 to 1 μg/ml compared with 0.008 μg/ml for fully susceptible isolates) remain susceptible to high-dose penicillin in vivo. Here, the pharmacological breakpoint of 2 μg/ml is defensible, particularly since the β-lactamase-producing strains, which are unresponsive in vivo, tend to be more highly resistant, with MICs being around 16 μg/ml.

This review will indicate several instances in which pharmacological breakpoints give a falsely optimistic picture of susceptibility for β-lactamase producers and for which biological analysis is more appropriate, on the basis of clinical experience. Biological rather than pharmacological breakpoints must also be considered by anyone attempting to use antibiogram data to predict the resistance mechanisms present in clinical isolates, and this aspect is considered at the end of this review. It should, however, be appreciated that biological breakpoints are easy to define when a susceptibility distribution is bimodal, with the MICs or zones for isolates with a β-lactamase (or other resistance mechanism) distinct from those for isolates without the enzyme, but are harder to set when the possessors of the mechanism merely form the tail of a skewed normal distribution (262). β-Lactamase–inhibitor combinations present a particular problem in this regard, as the inhibitors reduce the MIC of their partner penicillins for β-lactamase producers but generally fail to render the bacteria as susceptible as those without the enzyme (135, 222). Some β-lactamase hyperproducers are resistant to inhibitor combinations, but the zones or MICs for these organisms do not form a distinct distribution from those for organisms that have slightly less enzyme and that, consequently, are susceptible. Pharmacological analysis allows a breakpoint to be drawn in these circumstances, although its positioning may be highly arguable, as has been the case with ticarcillin-clavulanate (222).

EFFECTS OF DIFFERENT β-LACTAMASES ON RESISTANCE

Much of this review concerns the consequences of particular β-lactamases on resistance. Many of the tables detail the ef-

TABLE 3. Geometric mean MICs for *Staphylococcus aureus* strains with reference β-lactamases and their cured derivatives at two different inocula[a]

β-Lactamase type, (no.), and inoculum[a]	Mean MIC (μg/ml) of[b]:									
	Pip	Pip-tazo 8:1	Pip-tazo (4 μg/ml)[c]	Amox	Amox-clav (4 μg/ml)[c]	Meth	Pen G	Cld	Cfz	Clt
A (5), 10^4	**64**	3	1	**23**	0.4	3	**3**	0.17	1.12	0.5
A (5), 10^6	**512**	*11*	2	**512**	2	4	**128**	*3.17*	*11.3*	1.41
Cured A (5), 10^4	1.15	1.15	0.66	0.22	0.12	2.3	0.03	0.05	0.5	0.25
Cured A (5), 10^6	1.15	1.15	0.87	0.5	0.16	2.3	0.03	0.06	0.5	0.43
B (2), 10^4	**11**	3	1	*3*	0.4	3	**4**	0.17	0.17	0.35
B (2), 10^6	**512**	**23**	*8*	**256**	2	4	**128**	*4*	1.4	1.4
Cured B (2), 10^4	1	1	0.7	0.25	0.12	2	0.03	0.06	0.5	0.25
Cured B (2), 10^6	1	1	1	0.5	0.12	2	0.03	0.06	0.5	0.5
C (4), 10^4	**54**	*4*	1	*7*	1	3	**13**	0.21	0.6	0.5
C (4), 10^6	**512**	**27**	*13*	**512**	2	4	**128**	*4.8*	1.19	0.84
Cured C (4), 10^4	1.19	1.19	0.84	0.3	0.14	2.38	0.03	0.04	0.5	0.35
Cured C (4), 10^6	1.19	1.19	1	0.6	0.14	2.38	0.03	0.08	0.5	0.6
D (3), 10^4	*4*	2	1	*2*	0.3	3	**3**	0.09	0.5	0.31
D (3), 10^6	**512**	*8*	2	**323**	0.5	3	**128**	0.8	1.6	0.63
Cured D (3), 10^4	1	1	0.5	0.12	0.08	1.6	0.03	0.03	0.31	0.2
Cured D (3), 10^6	1	1	0.5	0.19	0.09	1.6	0.03	0.05	0.31	0.4

[a] Table is adapted from reference 30.
[b] Values are shown in boldface type when resistance is indisputable and in italic type when resistance is equivocal. Note that the designation varies with the inoculum and that debate continues as to the correct interpretation for cefazolin and piperacillin-tazobactam (30, 109, 219). Abbreviations: Pip, piperacillin; Pip-tazo, piperacillin-tazobactam; Amox, amoxicillin; Amox-clav, amoxicillin-clavulanate; Meth, methicillin; Pen G, penicillin G; Cld, cephaloridine; Cfz, cefazolin; Clt, cephalothin.
[c] Fixed inhibitor concentration.

fects of various enzymes, and in constructing these, I have tried to show representative data rather than to follow particular studies. Although the results of experiments by different researchers generally agree at a qualitative level, in terms of which enzymes affect which drugs, there is considerable variation in the MICs reported for producers of the same enzyme. This is a particular problem with plasmid-mediated β-lactamases of enterobacteria, which vary widely in amount between strains and which are present in species with differing permeability properties. For these enzymes, it has proved most appropriate to show qualitative data indicating whether enzyme production raises MICs and, if so, whether the effect usually is sufficient to give a clear resistance. MICs for producers of particular chromosomal β-lactamases are more tightly clustered, doubtless because these enzymes are produced by single species and because their quantity tends to be either very small or very large. It should, however, be stressed that not every isolate with a particular mechanism will show the precise MICs listed in the tables: what is more critical and more constant than the actual numbers is, anyway, the pattern of resistances and susceptibilities. To illustrate this, boldface type is used in the tables to indicate clear resistance, italic type is used for equivocal or intermediate behavior, and plain type is used when enzyme producers are as susceptible as those without the enzyme.

β-LACTAMASES OF GRAM-POSITIVE SPECIES

Staphylococcal Penicillinase

Staphylococci are the only common gram-positive pathogens in which β-lactamases have caused major resistance problems. Penicillinases occurred in only about 5% of *Staphylococcus aureus* isolates when benzylpenicillin was introduced but have since spread, through plasmid transfer and strain selection, to 80 to 90% of isolates, both of *S. aureus* and of the coagulase-negative isolates (118).

The staphylococcal enzymes are molecular class A types, placed in Bush's group 2a. Those from different strains resemble one another closely, but Richmond (213) produced antisera that allowed them to be subdivided into four types, designated A to D. Unfortunately, further batches of antisera could not be produced, and the typing method was lost when the original material was exhausted. Isoelectric focusing, which is so useful for typing β-lactamases of gram-negative species (see below), is unsatisfactory for those of staphylococci, which smear on the gels, probably because they are poorly soluble around their isoelectric points (283). Recently, β-lactamase typing has been reachieved by measuring the relative activities of cell suspensions of staphylococci against cefazolin, cephaloridine, and nitrocefin (110, 283). The types distinguished by this method correspond to Richmond's type A to D enzymes, although discrimination between types B and C is tenuous. The structural basis of the distinction between types is uncertain, and East and Dyke found no clear relationship between sequence and serotype (61). Types A and C enzymes are common both in Europe and the United States, and type D is rare everywhere (30, 76, 110, 242). Type B is well represented among isolates from United States (76, 110) and Scandinavian (242) studies but rare in the United Kingdom (30).

Staphylococcal β-lactamases are largely surface attached and serve to reduce the external drug level, with growth commencing only once this falls below a toxic threshold for PBP inactivation (equation 2). If more cells are present, they can destroy a greater amount of substrate in a given time; consequently, MICs of substrates rise as the inoculum is raised (Table 3) (28, 120, 220). Disk tests are even more sensitive (119), reflecting the dynamics of zone formation (28). The zones for strains without β-lactamase producers expand continuously from when they first become discernible, at 5 to 6 h

postinoculation, whereas the zones of substrate drugs for enzyme producers rapidly assume a constant diameter, as an equilibrium is established between diffusion of the drug to the cells and its destruction by them. When the zones are measured after overnight incubation, those for β-lactamase producers are consequently much larger than those for isolates without the enzyme, even for very weak substrates.

All four staphylococcal enzyme subtypes are strongly active against benzylpenicillin and amino- and carboxypenicillins, whereas the isoxazolyl penicillins, nafcillin, and methicillin are largely stable. Nevertheless, low-level resistance to methicillin and the isoxazolyl penicillins may be associated with β-lactamase hyperproduction (154). Unlike classical methicillin resistance, which is mediated by production of PBP-2′ (84), this lack of susceptibility is reversed by β-lactamase inhibitors. V_{max} rates for cephalosporins are very low, but stability is incomplete for some compounds, particularly cephaloridine and cefazolin (81, 283). Producers of each enzyme type are less susceptible to cephaloridine than are nonproducers, but the ability to hydrolyze cefazolin is more specific to the type A enzyme, and producers of this type are less susceptible than those with other enzyme types (Table 3) (30, 283). Conversely, type C enzyme is more active against cefamandole than is type A (109, 110) and is 10-fold less susceptible than type A to inhibition by tazobactam (30). Reflecting the last point, producers of type C enzyme are less susceptible than those of type A to combinations of tazobactam with piperacillin or amoxicillin (30). All these susceptibility differences become evident in MIC tests only if large inocula ($>10^6$ organisms) are used (28, 30). They are also apparent in disk tests: indeed, disk tests reveal susceptibility differences between β-lactamase producers and nonproducers for even the most stable agents, such as cephalothin and methicillin, and curing experiments confirm the role of the enzymes in these phenomena (30).

The relevance of slight penicillinase lability has long been debated for cephaloridine and cefazolin (219). Clinical failures have been reported with both compounds in endocarditis caused by β-lactamase producers, but these may reflect the recalcitrance of the infection rather than enzyme-mediated resistance. More tellingly, Kernodle et al. found that surgical wound infections were caused predominantly by producers of type A enzyme when patients received cefazolin prophylactically but were caused by producers of type C enzyme when prophylaxis was with cefamandole, mirroring the relative β-lactamase labilities of these two compounds (109).

Most laboratories test only benzylpenicillin and either methicillin or oxacillin against staphylococci. Isolates that are resistant to benzylpenicillin but susceptible to methicillin or oxacillin are viewed as methicillin-susceptible β-lactamase producers and are inferred to be susceptible to all "penicillinase-stable" penicillins, β-lactamase inhibitor combinations, and antistaphylococcal cephalosporins. Such categorization seems acceptable, provided that use of the more labile cephalosporins (and, by inference, piperacillin-tazobactam) is discouraged for serious staphylococcal infections.

Other Gram-Positive Bacteria

Plasmids encoding staphylococcal penicillinase have spread to *Enterococcus faecalis* isolates in the United States and Argentina (162, 163). In general, however, β-lactam resistance among enterococci is centered in *Enterococcus faecium*, where it is associated with production of a low-affinity penicillin-binding protein, PBP-5, and not with β-lactamase. β-Lactamase producers are resistant to amoxicillin, ampicillin, and piperacillin but susceptible to amoxicillin-clavulanate, piperacillin-tazobactam, and carbapenems, whereas *E. faecium* strains that hyperproduce PBP-5 are resistant to all β-lactams (162). As with staphylococci, the resistance of β-lactamase producers is often apparent only when high inocula are tested (162), and high-inoculum testing may become mandatory if these enzymes do disseminate in the species. Nitrocefin tests can be used in a confirmatory role when β-lactamase production is suspected. β-Lactamase-mediated resistance has yet to be found in any *Streptococcus* spp.

Many *Bacillus* spp. have chromosomal β-lactamases, which have received much attention from biochemists (256). Anthrax nevertheless generally remains treatable with penicillin (15); a few strains have multiple β-lactamases and are more resistant (159). Chromosomal β-lactamases are produced by some clostridia that may be pathogenic in compromised hosts, including *Clostridium butyricum* (111) and *C. clostridiforme* (10). The *C. clostridiforme* enzyme contributes to resistance to penicillins and cefotaxime, as demonstrated by the fact that these resistances are reversed by the penem β-lactamase inhibitor BRL42715 and, less effectively, by clavulanate or tazobactam (10). *C. perfringens* is occasionally resistant to β-lactams (148), but the mechanism entails changes to its PBPs, not to β-lactamase production (265).

Acid-fast bacteria also have chromosomal β-lactamases. The long-held view that β-lactam resistance in *Mycobacterium tuberculosis* involves β-lactamase (104) is supported by the recent observation that clavulanic acid potentiates the activity of amoxicillin against the organism (280). β-Lactamases also play a role in the resistance to β-lactams of *Mycobacterium fortuitum* (5) and various *Nocardia* species (235, 257), although their importance in the resistance of *Nocardia farcinica* to aminothiazolyl cephalosporins is probably secondary to impermeability (235).

CHROMOSOMAL β-LACTAMASES OF ENTEROBACTERIA

Chromosomal β-lactamases are almost ubiquitous in enterobacteria, except for salmonellae, but vary greatly in amount, mode of production, and, consequently, in their contribution to resistance (237, 238). Some species typically have molecular class A enzymes, but a greater number have class C types. Expression may be inducible, high-level constitutive, or low-level constitutive, according to the species and the strain (Table 4). Relationships between antibiogram and chromosomal enzymes are discussed below by species, and the effects of secondary β-lactamases, which are common in enterobacteria, are considered later.

E. coli and Shigellae

E. coli and shigellae present the simplest cases, as they usually have only insignificant levels of uninducible molecular class C enzymes, often called AmpC types (172, 238). Consequently, and unlike enterobacteria with more formidable β-lactamase systems, they are inherently susceptible to ampicillin and the narrow-spectrum cephalosporins, such as cephalothin and cephalexin. Resistance is typically apparent only to those agents that penetrate poorly, such as isoxazolyl penicillins and benzylpenicillin, and to cefsulodin, which fails to bind the PBPs of enterobacteria. Resistance to other β-lactams is mostly via acquisition of secondary β-lactamases (129, 225). Occasional *E. coli* isolates do, however, produce copious amounts of the AmpC enzyme and are more resistant (172). Like derepressed *Enterobacter* spp. (below), which they resemble in phenotype although not in genetic organization, these isolates have re-

TABLE 4. Chromosomal β-lactamases and their expression in enterobacteria

Organism	Name	Class	Bush group	Mode of expression[a]			
				Inducible	Constitutive		
					Minimal	Moderate	High
E. coli	AmpC	C	1	–	●	–	○
Shigellae	AmpC	C	1	–	●	–	○
Enterobacter spp.	AmpC	C	1	●	○	–	◍
C. freundii	AmpC	C	1	●	○	–	◍
M. morganii	AmpC	C	1	●	–	–	◍
Providencia spp.	AmpC	C	1	●	○	–	○
Serratia spp.	AmpC	C	1	●	–	–	◍
K. pneumoniae	SHV-1	A	2b	–	–	●	○[b]
K. oxytoca	K1	A	2be	–	–	●	◍
C. diversus		A[c]	2e	●	○	–	○
P. vulgaris	CXase[d]	A	2e	●	–	–	○
P. penneri	CXase	A	2e	●	–	–	○
P. mirabilis		?[e]	?[e]	–	●	–	–

[a] ●, Normal mode of production, typical of the species; ◍, frequently encountered, variable among countries, hospitals, and units, but seen in 10 to 50% of isolates in most recent surveys; ○, rare, seen in fewer than 10% of isolates; –, unknown or isolated reports only. Minimal production denotes that enzyme is detectable but causes no significant resistance; moderate denotes that the enzyme contributes to resistance to good substrates; high denotes huge levels of enzyme—up to 3% of total cell protein in some *Enterobacter*—able to confer resistance even to weak substrates.

[b] Frequent when SHV-1 enzyme is plasmid mediated.

[c] Production of class C enzymes has been seen in a few *C. diversus* isolates (102).

[d] CXase, cefuroximase belonging to Bush's (37) group 2e.

[e] No particular chromosomal β-lactamase has been defined as being typical of *P. mirabilis*.

duced susceptibility to all β-lactams except carbapenems, temocillin, and mecillinam (Table 5). Resistance to extended-spectrum cephalosporins is often only moderate, with MICs of ceftazidime, ceftriaxone, and cefotaxime being 1 to 16 μg/ml. Such values are considerably above those (0.008 to 0.03 μg/ml) typical for normal *E. coli* strains with negligible amounts of the β-lactamase and should be viewed as signifying resistance, but they remain below the corresponding MICs (>64 μg/ml) for β-lactamase-derepressed *Enterobacter* isolates (see below). This lower level of resistance probably reflects lesser enzyme quantity and greater permeability in *E. coli* than in *Enterobacter* spp. Neither clavulanate nor the sulfones can inhibit AmpC enzymes and reverse these resistances (3).

AmpC hyperproducers account for fewer than 2% of all *E. coli* isolates in most surveys (48, 91, 128, 129, 165, 208, 218, 268) but were commoner than this in Sweden in the late 1970s (22, 172) and may remain so. Their rarity reflects the fact that hyperproduction requires either two separate mutations in *E. coli* (20) or acquisition of more efficient promoter from shigellae (177), whereas a single mutation, to *ampD*, gives hyperproduction in *Enterobacter* spp. (20). Hyperproduction of AmpC β-lactamase can be obtained in laboratory mutants of shigellae, in which it gives resistance patterns similar to those described for *E. coli* (137).

Enterobacter spp., *Citrobacter freundii*, *Serratia* spp., *Morganella morganii*, *Providencia stuartii*, and *P. rettgeri*

Enterobacter spp., *C. freundii*, *Serratia* spp., *Morganella morganii*, *Providencia stuartii*, and *P. rettgeri* have AmpC β-lactamases resembling those of *E. coli* but, unlike *E. coli*, typically have inducible expression of these enzymes. Only trace amounts of β-lactamase are made in the absence of antibiotics, but transient high-level production can arise while β-lactams are present. Permanent hyperproduction (derepression or stable derepression) can arise by mutation, usually at the *ampD* locus, and is increasingly frequent among clinical isolates (225). The regulation of synthesis of these enzymes and the mutations that cause derepression have been reviewed recently (20).

Whether or not resistance is expressed depends on the lability of a compound to hydrolysis, its inducer power below its MIC, and the mode of β-lactamase expression (Table 5; 130, 272–274, 276). Ampicillin and narrow-spectrum cephalosporins are labile and generally induce strongly below MIC, with the result that both inducible and derepressed organisms are resistant (130). Extended-spectrum cephalosporins, ureidopenicillins, and carboxypenicillins also are labile to hydrolysis but fail to induce below their MICs. Consequently, β-lactamase-inducible strains appear susceptible whereas derepressed organisms are resistant (53, 130, 224). Carbapenems, which are strong inducers, are very stable and retain activity against both derepressed and inducible strains (130, 273, 274, 276), as does temocillin, which is a weak inducer and is very stable to hydrolysis by AmpC enzymes (272). Cefepime and cefpirome, which are rapid permeants and are more stable than other extended-spectrum cephalosporins (83), also retain reasonable activity against derepressed enterobacteria, with MICs of 1 to 4 μg/ml, compared with cefotaxime and ceftazidime MICs of over 64 μg/ml (Table 5) (42, 83). Clavulanate, sulbactam, and tazobactam generally do not have sufficient inhibitory activity to potentiate their partner penicillins against derepressed organisms (3, 115). Indeed, clavulanate is a stronger inducer than is ticarcillin, which can be hydrolyzed by the induced enzyme. Consequently, ticarcillin-clavulanate frequently is less active than unprotected ticarcillin against inducible isolates, especially of *Enterobacter* spp. and *M. morganii*. Whether this antagonism is of clinical significance is doubtful, since the concentrations of clavulanate required for induction (8 to 10 μg/ml) are only briefly attained in vivo. Sulfones are not prone to antagonize their partner drugs in this manner, being weaker inducers than is clavulanate.

Some species-to-species variation is superimposed on these general patterns. In particular, the levels of enzyme and resistance in derepressed *Serratia*, *Providencia*, and *Morganella* strains are about 10-fold below those in derepressed *Enterobacter* spp. and *C. freundii* (Table 5). Other differences are (i) that cefoxitin seems almost stable to the enzymes of *Serratia*, *Providencia*, and *Morganella* spp. and is moderately active against both β-lactamase-derepressed and -inducible strains of these species (MICs, 1 to 16 μg/ml), whereas it is a labile strong inducer for *Enterobacter* spp. and *C. freundii* and is inactive against both inducible and derepressed organisms (MICs, ≥256 μg/ml) (161, 273, 274, 276); (ii) that tazobactam inhibits the *M. morganii* enzyme sufficiently to potentiate piperacillin against derepressed strains, whereas it has little effect against the AmpC enzymes of the other species (3); and (iii) that ampicillin and amoxicillin, which are labile strong inducers for most species, are labile weak inducers for some *C. freundii* and *Providencia* isolates, which consequently appear susceptible (137).

Although the β-lactamase-inducible and -derepressed organisms are the most important phenotypes of these species, two other types merit mention. First, a few *Enterobacter* and *C. freundii* isolates have only basal expression of AmpC enzymes, similar to that typical in *E. coli* (130, 225, 276). Such isolates are susceptible to low concentrations (2 to 8 μg/ml) of ampicillin, cefoxitin, cephalothin, and cephalexin, as well as to extended-spectrum compounds, ureidopenicillins, and carbapen-

TABLE 5. Susceptibility and resistance of enterobacteria with common modes of expression of AmpC β-lactamases

Antimicrobial agent	MIC (μg/ml)[a] for:											
	E. coli		*E. cloacae*			*C. freundii*			*M. morganii*		*S. marcescens*	
	Basal	Hyp	Ind	Hyp	Basal	Ind	Hyp	Basal	Ind	Hyp	Ind	Hyp
Ampicillin[b]	2	**512**	512	**2,048**	4	**32**	**2,048**	8	**512**	**512**	**16**	**256**
Ticarcillin[c]	2	*16*	2	**256**	2	1	**128**	1	1	**32**	4	**32**
Ticarcillin-clavulanate	2	*16*	8	**256**	2	1	**128**	1	16	**32**	4	**32**
Piperacillin[d]	1	**32**	2	**256**	2	1	**64**	1	0.5	**32**	2	**32**
Piperacillin/tazobactam	1	8	2	**64**	2	1	**32**	1	0.25	2	2	**32**
Temocillin	2	2	1	16	1	2	8	2	2	2	16	16
Cephalothin[e]	8	**512**	**2,048**	**2,048**	**16**	**16**	**2,048**	8	**>2,048**	**>2,048**	**>2,048**	**>2,048**
Cefoperazone	0.25	**32**	0.25	**128**	0.25	0.25	**32**	0.25	1	**16**	**16**	**64**
Cefuroxime	4	**32**	*16*	**512**	8	8	**128**	2	**64**	**256**	**512**	**512**
Cefotaxime[f]	0.03	*4*	0.12	**256**	0.06	0.06	**32**	0.03	0.015	*4*	0.25	*8*
Ceftazidime	0.06	*4*	0.25	**64**	0.25	0.25	**32**	0.5	0.06	*8*	0.25	1
Cefpirome	0.03	0.12	0.06	*4*	0.06	0.03	1	0.06	0.03	0.03	0.06	0.25
Cefepime	0.03	0.12	0.12	*2*	0.12	0.03	0.5	0.06	0.03	0.03	0.12	0.25
Cefoxitin	4	**256**	**256**	**512**	2	**64**	**256**	4	8	8	*16*	*16*
Moxalactam	0.015	*2*	0.06	**32**	0.06	0.3	*8*	0.06	0.12	0.12	0.5	*4*
Aztreonam	0.03	*2*	0.06	**16**	0.06	0.015	*8*	0.015	0.06	0.12	0.06	*2*
Imipenem	0.12	0.12	0.25	0.25	0.25	0.25	0.25	0.12	2	2	0.25	0.25
Meropenem	0.03	0.03	0.06	0.06	0.015	0.03	0.03	0.03	0.03	0.03	0.03	0.03

[a] MICs are representative of the different phenotypes and are taken from references 42, 53, 137, 172, 224, 272 through 274, and 276. Values are shown in boldface type when resistance is indisputable and in italic type when resistance is equivocal. Ind, inducible for AmpC β-lactamase; Hyp, constitutively hyperproducing the AmpC β-lactamase; Basal, having only trace level of AmpC β-lactamase.
[b] Representative also of amoxicillin.
[c] Representative also of carbenicillin, which is one to two dilutions less active against all phenotypes.
[d] Representative also of azlocillin and mezlocillin, which are one to two dilutions less active against all phenotypes.
[e] Representative also of other narrow-spectrum cephalosporins, e.g., cephalexin and cefazolin.
[f] Representative also of ceftriaxone and ceftizoxime.

ems. Second, a few β-lactamase-derepressed *Enterobacter cloacae* and *Enterobacter aerogenes* strains lack major porins. Their combination of enzyme production and impermeability confers resistance to carbapenems, temocillin, cefepime, and cefpirome as well as to other β-lactams (122, 203). Fortunately, such resistance remains extremely rare among clinical isolates.

As has been widely reported, β-lactamase-derepressed mutants can be selected from inducible populations during therapy with labile weak inducers (130, 223, 224). Such compounds kill the β-lactamase-inducible cells but allow survival and overgrowth of derepressed mutants, which arise at frequencies as high as 10^{-5} in inducible populations. The likelihood of selection varies with the infection site, which determines the population density of the bacteria and the level of drug available to combat them, and with the particular β-lactam being used. Selection was estimated to occur in ca. 20% of *Enterobacter* bacteremia cases treated with extended-spectrum cephalosporins (45) and probably is even commoner when these compounds are used in *Enterobacter* pneumonia (225). Coadministration of aminoglycosides does not diminish this likelihood of selection but may be of clinical benefit to the patient if selection does occur (45). On the other hand, selection is a minimal risk when extended-spectrum cephalosporins are used in urinary tract infections, doubtless because the high drug levels attainable in this site exceed the MICs for the derepressed mutants (225). Piperacillin and ticarcillin are substantially less active in vitro against derepressed mutants (MICs, 32 to 128 μg/ml) than against inducible strains (MICs, 1 to 8 μg/ml) (53, 272) but, for uncertain reasons, have proved less selective in vivo for derepressed mutants than have extended-spectrum cephalosporins (45, 95). Carbapenems have equal activity against inducible and derepressed organisms and so do not select derepressed mutants in vitro or in vivo. They do cause induction of the β-lactamase, but this effect, unlike mutational derepression, is lost once the drug is removed and is not a concern except in the unlikely event that the carbapenems are used in combination with newer-generation cephalosporins (130).

Because of the risk of selecting derepressed mutants, the use of extended-spectrum cephalosporins in infections should be discouraged, except for urinary tract infections caused by β-lactamase-inducible species. Carbapenems, quinolones, or, if available, temocillin is preferred. High-risk organisms in clinical laboratories are most easily recognized by accurate identification to the species level. Some workers have advocated direct induction tests, usually by placing a disk containing a labile weak inducer such as cefotaxime close to one containing a strong inducer such as cefoxitin (223). Induction is then detected from the blunting of the cefotaxime zone adjacent to the cefoxitin disk. However, the success of such tests depends on precise positioning of the disks, and their sensitivity, compared with direct induction assays, is only about 80% (137). A simple alternative, useful for laboratories that do not routinely identify enterobacteria to a species level, is to screen with cefoxitin: β-lactamase-inducible *Enterobacter* spp. and *C. freundii* (and those already derepressed) typically grow to the disk edge, whereas β-lactamase-basal strains are susceptible (161). Unfortunately, this approach is inappropriate for β-lactamase-inducible *M. morganii*, *Providencia* spp., or *Serratia* spp., which are moderately susceptible to cefoxitin. These species are, however, less frequent pathogens than *Enterobacter* spp. and *C. freundii* and seem less prone to segregate highly resistant mutants in therapy.

Once selected, derepressed mutants are stable and can accumulate in the hospital microflora. The extent to which this happens varies with time, with the hospital or unit type, and with the extent of cephalosporin usage (225). Derepression was seen in approximately 70% of 90 *E. cloacae* isolates from 12

TABLE 6. Susceptibility and resistance of enterobacteria with various modes of expression of chromosomal class A β-lactamases

Antimicrobial agent	MIC (μg/ml)[a] for:							
	K. pneumoniae		*K. oxytoca*		*C. diversus*		*P. vulgaris*	
	β-ve[b]	SHV-1	K1-low	K1-Hyp	Ind	Hyp	Ind	Hyp
Ampicillin[c]	2	**128**	**256**	**2,048**	**>1,000**	**>1,000**	**512**	**1,024**
Amoxicillin-clavulanate	2	4	4	**64**	8	*16*	2	4
Ticarcillin[d]	2	**64**	**256**	**256**			4	**32**
Ticarcillin-clavulanate	2	2	4	**64**			2	2
Piperacillin[e]	1	*8*	*8*	**256**	4	**128**	1	**32**
Piperacillin-tazobactam	1	2	2	**64**	4	4	1	1
Temocillin	2	2	1	2	8	8	1	1
Cephalothin[f]	8	8	8	**2,048**			**512**	**512**
Cefoperazone	0.06	0.06	0.06	**128**	2	**32**	2	**128**
Cefuroxime	4	4	4	**256**			**512**	**1,024**
Cefotaxime[g]	0.03	0.03	0.03	**32**	0.12	*4*	0.06	*2*
Ceftazidime	0.06	0.06	0.06	0.03	0.25	0.5	0.06	0.25
Cefpirome	0.03	0.03	0.03	*2*			0.25	4
Cefepime	0.03	0.03	0.03	*2*			0.12	1
Cefoxitin	4	4	4	8	8	8	4	4
Moxalactam	0.015	0.03	0.03	0.06	0.12	0.25	0.12	0.12
Aztreonam	0.03	0.03	0.03	**32**			0.25	2
Imipenem	0.12	0.12	0.12	0.25	0.12	0.12	1	1
Meropenem	0.03	0.03	0.06	0.06			0.03	0.06

[a] MICs are representative of the different phenotypes and are taken from references 42, 137, 176, 273, and 274. Values are shown in boldface type when resistance is indisputable and in italic type when resistance is equivocal. Ind, β-lactamase inducible; Hyp, β-lactamase hyperproducing.
[b] β-Lactamase negative; extremely rare phenotype, shown for comparison only.
[c] Representative also of amoxicillin.
[d] Representative also of carbenicillin, which is one to two dilutions less active against all phenotypes.
[e] Representative of azlocillin and mezlocillin, which are one to two dilutions less active against all phenotypes.
[f] Representative also of other narrow-spectrum cephalosporins, e.g., cephalexin and cefazolin.
[g] Representative of ceftriaxone and ceftizoxime.

hospitals around Athens (246) but in only 5% of 233 isolates from a community hospital in Connecticut (63). The situation in most American and European hospitals lies between these extremes, with 15 to 50% of *E. cloacae* and *C. freundii* exhibiting derepression but with lower rates among *M. morganii* and *Serratia* spp. (35, 41, 129). These frequencies represent a dramatic increase over the rates of 0 to 5% prevalent when extended-spectrum cephalosporins entered use (225).

Klebsiellae

Klebsiellae generally have class A chromosomal β-lactamases, which differ greatly from the class C types discussed above. Most *K. pneumoniae* isolates have chromosomally mediated SHV-1 β-lactamases, but some have similar enzymes that focus at a scatter of pI values between 7.1 and 8.6 (129, 209). It is uncertain whether SHV-1 enzyme, which occurs in other enterobacteria as a plasmid-mediated type (153), was an historical chromosomal β-lactamase of *K. pneumoniae* or whether it has become prevalent only since the introduction of ampicillin. *K. oxytoca* isolates typically have K1 or KOXY type enzymes, which also vary in isoelectric point among different strains (11, 270, 271).

All these β-lactamases of klebsiellae are constitutive and usually are produced at low levels, which, nevertheless, are sufficient to protect against ampicillin, amoxicillin, carbenicillin, and ticarcillin. Thus, MICs of these compounds are around 32 to 64 μg/ml for enzyme producers compared with 1 to 2 μg/ml for the few klebsiellae that lack these β-lactamases (Table 6). The behavior of ureidopenicillins and piperacillin is more equivocal. MICs of these agents are only 2 to 4 μg/ml for many β-lactamase-producing isolates in low-inoculum tests (10^4 CFU per spot); likewise, the inhibition zones of high-content disks (75 or 100 μg) often are substantial. Consequently, it is common to see laboratory reports claiming that "70% of our klebsiella isolates are susceptible to piperacillin." Nevertheless, the MICs of piperacillin and other ureidopenicillins rise dramatically as the inoculum is raised (1, 66), and the inhibition zones of disks containing 75 μg of piperacillin plus 10 μg of tazobactam are larger than those of disks containing 75 μg piperacillin alone for virtually all *K. pneumoniae* and *K. oxytoca* isolates (Fig. 2) (41). These observations underscore a role for the β-lactamase against ureidopenicillins, and there seems every reason to discourage the use of any unprotected penicillin except temocillin against klebsiellae. Narrow-spectrum cephalosporins such as cephalothin and cephalexin also can be inactivated by the SHV-1 and K1 enzymes, although, as with piperacillin, their MICs are low (1 to 8 μg/ml) for isolates with normal low levels of these enzymes. Such cephalosporins have proved adequate against klebsiellae in urinary tract infections, probably because high drug concentrations are attainable at this site (168), but seem better avoided for infections elsewhere, because more stable drugs, including inhibitor combinations, extended-spectrum cephalosporins, cephamycins, monobactams, and carbapenems, are available (Table 6).

When greater levels and wider spectra of resistance to β-lactams are observed in *K. pneumoniae* isolates, they are usually attributable to plasmid-mediated β-lactamases, which are considered elsewhere in this review. Further resistance in *K. oxytoca* can, however, occur via mutational hyperproduction of the chromosomal K1 enzyme (11, 240). Hyperproducers have a very characteristic antibiogram, being highly resistant to all the penicillins (MICs, >64 μg/ml) except temocillin, resistant to cefuroxime and aztreonam (MICs, ≥32 μg/ml), and resistant or moderately so to cefotaxime and ceftriaxone (MICs, 4 to 32 μg/ml), but as susceptible as normal isolates to ceftazi-

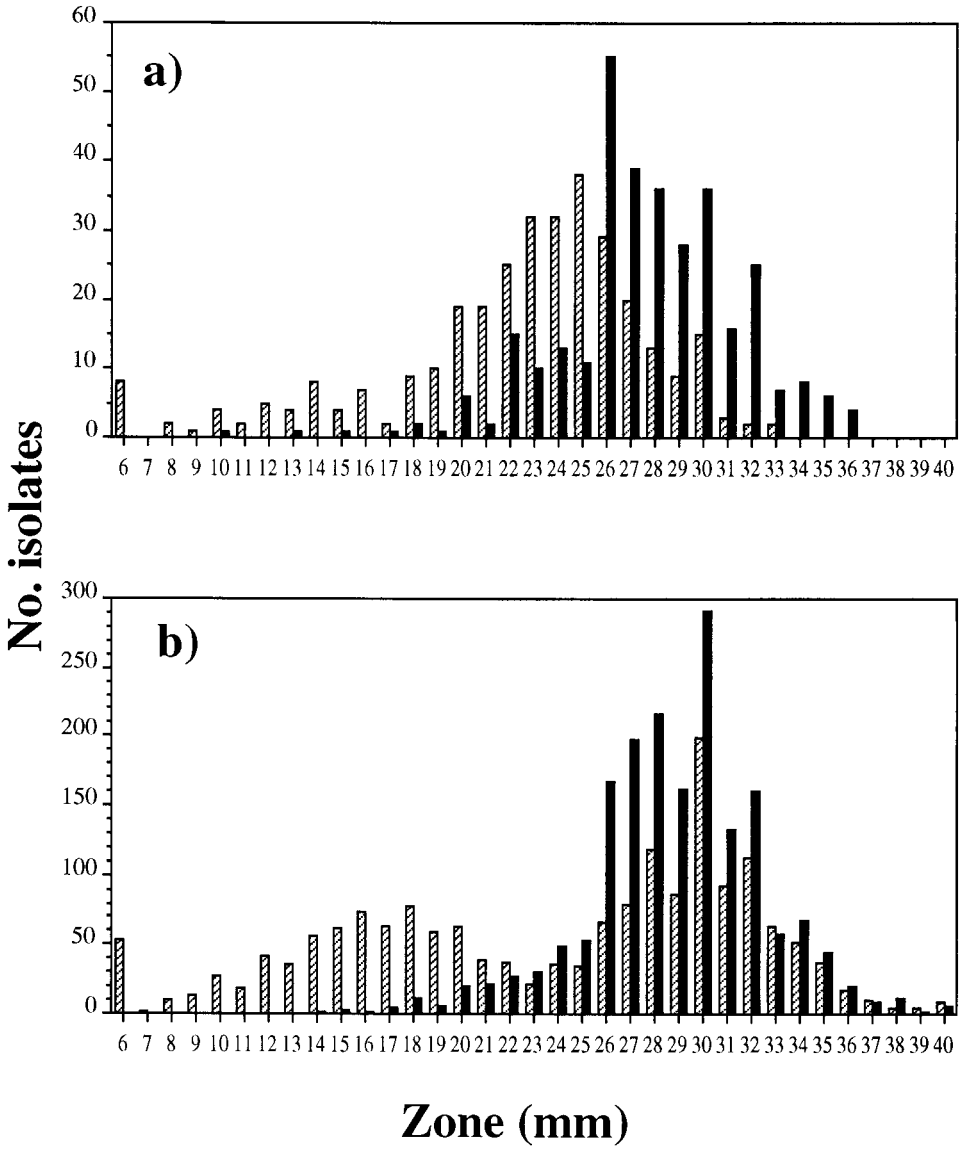

FIG. 2. Inhibition zones of 75 μg of piperacillin (▨) and 75 plus 10 μg of piperacillin-tazobactam (■) disks for 325 klebsiellae (a) and, for comparison, 1,788 *E. coli* isolates (b). Note that the zones of the piperacillin-tazobactam disks are larger than those of the piperacillin disks for virtually all the klebsiellae, reflecting the ubiquity of SHV-1 and K1 enzymes in *K. pneumoniae* and *K. oxytoca*, respectively, whereas tazobactam expands the zones of piperacillin for some but not all of the *E. coli* isolates—specifically for those that have secondary, plasmid-mediated β-lactamases. Reprinted from reference 41 with permission of the publisher.

dime (129, 270, 271). This last point distinguishes them from klebsiellae with extended-spectrum TEM and SHV enzymes (see below), which are usually resistant to ceftazidime. Hyperproducers commonly are resistant to all inhibitor combinations (Table 6), despite in vitro susceptibility of K1 enzyme to inhibition by clavulanate and the sulfones. This resistance to inhibitor combinations probably reflects the sheer amount of enzyme present.

At present, hyperproduction of K1 enzyme is seen in 10 to 20% of *K. oxytoca* isolates in Europe (129, 209, 270), and there are isolated reports of hyperproducing mutants being selected during cephalosporin therapy (240), but selection is much rarer than with *Enterobacter* spp.

Proteus vulgaris and *Citrobacter diversus*

Proteus vulgaris and *P. penneri* isolates have class A chromosomal β-lactamases, which are placed in group 2e by Bush et al. (37) and which are sometimes referred to as cefuroximase types. Similar enzymes occur in many *C. diversus* strains (6, 102, 176), although some isolates of this species apparently have class C enzymes akin to that of *C. freundii* (102). The hydrolytic properties of the chromosomal β-lactamases of *C. diversus* and these members of the *Proteeae* resemble those of the K1 type of *K. oxytoca*, in that all these enzymes are active against penicillins, cefuroxime, ceftriaxone, and cefotaxime but not against ceftazidime, cephamycins, and carbapenems (6, 176, 273). Unlike K1 enzyme, however, the *Proteus* and *C. diversus* enzymes are inducible and have minimal activity against aztreonam.

Ampicillin, amoxicillin, and narrow-spectrum cephalosporins are labile strong inducers (273) for the *Proteus* and *C. diversus* enzymes, so their MICs for inducible and derepressed strains are high (Table 6). Ureido- and carboxy-penicillins, cefotaxime, and ceftriaxone are labile weak inducers and so

TABLE 7. Resistance and chromosomal β-lactamase expression in *P. aeruginosa*

Uninduced β-lactamase activity (U[a]/mg of protein)	No. of isolates in group[b]	No. of these isolates remaining inducible	Geometric mean MIC[c] (μg/ml) of:							
			Carbenicillin	Azlocillin	Piperacillin	Pip-tazo[d]	Ceftazidime	Cefepime	Imipenem	Meropenem
<10[e]		Most or all	32–128	2–16	2–8	2–8	1–4	2–8	1–2	0.25–0.5
45–100	7	7	128	**86**	**24**	*13*	*8.8*	7.3	1.8	1.0
101–300	7	7	*141*	**156**	**35**	*15*	*13*	9.3	1.0	0.7
301–1,000	23	22	*121*	**128**	**34**	**21**	*19*	6.9	1.5	0.7
1,001–3,000	11	4	*189*	**226**	**60**	**53**	**27**	7.0	1.5	1.2
3,001–10,000	3	0	*161*	**256**	**161**	**161**	**51**	*13*	1.3	0.8
10,001–300,000	3	0	**406**	**406**	**322**	**323**	**101**	**20**	5.0	2.0

[a] 1 unit is 1 nmol of nitrocefin hydrolyzed per min at 37°C and pH 7.0.
[b] Inducibility was assessed with cefoxitin, 500 μg/ml, as inducer, and isolates giving less than twofold induction were counted as uninducible.
[c] Data are from references 44 and 137. Values are shown in boldface type when resistance is indisputable and in italic type when resistance is equivocal.
[d] Piperacillin plus tazobactam, 4 μg/ml.
[e] Behavior of typical isolates of the species (43, 263).

remain active against inducible strains, although resistance or decreased susceptibility is evident in derepressed mutants. Unlike derepressed *Enterobacter* spp. and *C. freundii*, however, derepressed *P. vulgaris* and *C. diversus* strains retain full susceptibility to ceftazidime, cephamycins, and aztreonam, as well as to carbapenems and temocillin; moreover, their resistances can be reversed by clavulanate, sulbactam, and tazobactam.

For unknown reasons, probably because they are less resistant, derepressed mutants of *P. vulgaris* and *C. diversus* rarely are selected during therapy; nevertheless, isolates with antibiograms suggesting derepression are occasionally encountered clinically and mutants with this phenotype are easy to select in vitro (176, 273).

Proteus mirabilis

Chromosomal β-lactamase expression is negligible in *P. mirabilis*, and the organism is inherently the most susceptible of all enterobacteria to β-lactams (41). Most of the resistance that does occur depends on secondary TEM-type β-lactamases, as in *E. coli* (129). A chromosomally mediated class A carbenicillinase from one strain (N-29) has been widely studied in Japan, but its gene was absent from more susceptible isolates, indicating that the organism was not a hyperproducer of a normal chromosomal enzyme (221).

CHROMOSOMAL β-LACTAMASES OF NUTRITIONALLY VERSATILE NONFERMENTERS

Chromosomal β-lactamases are ubiquitous in nonfermenters and, as in enterobacteria, are variable in amount and significance. The enzymes of *P. aeruginosa* and *Stenotrophomonas maltophilia* have been studied extensively, and their roles in resistance are well established; those of *Acinetobacter*, *Flavobacterium*, and *Burkholderia* spp. are less well understood.

Pseudomonas aeruginosa

P. aeruginosa has an inducible AmpC enzyme, similar to that of *Enterobacter* spp. (132). As with *Enterobacter* spp., ampicillin and narrow-spectrum cephalosporins are labile to hydrolysis and induce the enzyme strongly, destroying their own activity, whereas ureidopenicillins and extended-spectrum cephalosporins are labile but induce weakly and so are active against inducible strains but not against derepressed mutants (Table 7) (144). Carbapenems are strong inducers that are marginally labile (imipenem) (144) or are effectively stable (meropenem) (143) and so remain active irrespective of the mode of β-lactamase expression. Differences from the *Enterobacter* system are (i) that carbenicillin is less affected by derepression in *P. aeruginosa*, with its MICs increasing from 32 to 128 μg/ml for inducible isolates to 64 to 256 μg/ml for derepressed ones, as against from 1 to 2 μg/ml to 128 to 256 μg/ml, respectively, for β-lactamase-inducible and derepressed *Enterobacter* spp. (144, 276); (ii) that the *P. aeruginosa* enzyme, whether inducible or derepressed, gives slight protection against imipenem, with MICs for producers being around 1 to 2 μg/ml compared with 0.12 to 0.5 μg/ml for β-lactamase-deficient laboratory mutants (134); and (iii) that derepression in *P. aeruginosa* is often only partial, such that the uninduced enzyme level is higher than is normal for the species but substantial inducibility is retained (132, 225, 264), whereas derepression in *Enterobacter* spp. is almost always total, with the enzyme being manufactured constitutively. *P. aeruginosa* strains segregate partially derepressed mutants at rates of ca. 10^{-7}, but totally derepressed mutants occur at frequencies below 10^{-9}, whereas *Enterobacter* spp. segregate totally derepressed mutants at frequencies of 10^{-5} to 10^{-7}. The resistance of partially derepressed isolates mirrors the amount of enzyme produced without induction (Table 7); even a small degree of derepression compromises ureidopenicillins, whereas only total derepression noticeably compromises cefepime and carbenicillin (42, 264).

Selection of totally or partially derepressed mutants can occur during antipseudomonal therapy with labile weak inducers. Ureidopenicillins and piperacillin, as well as extended-spectrum cephalosporins, have been widely reported to select for these mutants in *P. aeruginosa* infections (130, 231), whereas selection of derepressed enterobacteria is predominantly by cephalosporins. Overall, however, selection of derepressed mutants is rarer with *P. aeruginosa* than with *Enterobacter* spp. and *C. freundii*, except under the specialized conditions of the lungs of patients with cystic fibrosis. Evolution during therapy from inducible through partially derepressed to totally derepressed has been reported (231), but it is unclear whether this is the invariable pattern or whether total derepression can also arise directly from inducibility. The risk of selecting stably derepressed mutants suggests that caution be exercised in the use of extended-spectrum cephalosporins and ureidopenicillins against *P. aeruginosa* infections, but the argument is more finely balanced than for *Enterobacter* spp. and *C. freundii*. Unlike these enterobacterial species, *P. aeruginosa* is prone to mutate to resistance to most alternative chemotherapeutic agents: carboxypenicillin resistance arises via mutations that decrease permeability or increase efflux (126); imipenem resistance arises via mutational loss of D2 porin;

quinolone resistance arises via mutations that increase efflux or alter the target DNA gyrase; and aminoglycoside resistance arises via impermeability. In the future, it may be reasonable to prefer meropenem, which has potent antipseudomonal activity and to which substantial single-step resistance seems unlikely (143).

The frequency of *P. aeruginosa* strains that are already derepressed varies with the country and source (225). In a 24-center survey in the United Kingdom 1993 (44), we found some degree of derepression in 10 of 134 *P. aeruginosa* isolates from patients in intensive care units, in 34 of 1,041 isolates from other inpatients, and in 10 of 797 isolates from outpatients. Overall, 54 of 1,991 isolates were derepressed, compared with 17 of 1,866 collected in a similar survey in 1982 (264), and this increase was significant ($P < 0.01$, χ^2 test). Higher rates of derepression are often seen in isolates from patients with cystic fibrosis.

Stenotrophomonas maltophilia

Stenotrophomonas maltophilia is an opportunistic pathogen which is becoming increasingly common in some centers; it is notoriously resistant to β-lactams. It produces two inducible chromosomal β-lactamases, L-1 and L-2, both of which are regulated by the same induction system. L-1 is a class B zinc enzyme, broadly active against penicillins, carbapenems, and many cephalosporins although not against cefsulodin or aztreonam; L-2 is an unusual cephalosporinase that can hydrolyze those cephalosporins and monobactams that escape L-1 (24, 157). L-2 falls into group 2e of Bush's scheme, as a clavulanate-inhibited cephalosporinase (36, 37), but there is little reason to suppose that it is closely related to the other members of this group, such as the chromosomal cefuroximases of *P. vulgaris*, *C. diversus*, and the *Bacteroides fragilis* group.

Laboratory mutants that lack L-1 become moderately susceptible to carbapenems (imipenem MICs, 8 to 16 μg/ml, compared with >128 μg/ml for L-1 producers) but are still resistant to cefsulodin and aztreonam; mutants that lack both enzymes also have increased susceptibility to these last two compounds (2). These observations demonstrate that the β-lactamases contribute to resistance, but so do other factors, as is apparent from the fact that laboratory mutants lacking both enzymes remain resistant to many β-lactams at breakpoint (2). A further complexity is that the susceptibility test results for *S. maltophilia* strains vary grossly with the test medium and method used (27, 182). The reasons for this behavior are uncertain but do not relate to β-lactamase expression or its absence (27, 29). It is uncertain which conditions give the most clinically relevant results.

S. maltophilia often appears susceptible to ticarcillin-clavulanate or aztreonam-clavulanate in vitro, even on Mueller-Hinton agar. Ticarcillin and aztreonam apparently are only weak substrates for L-1 β-lactamase or are stable to this enzyme, and clavulanate protects these agents against L-2 (107, 182, 244). However, there are few clinical data to support clinical use of such combinations against *S. maltophilia* infections (182), and sulfamethoxazole-trimethoprim remains the preferred therapy.

Acinetobacter spp.

Acinetobacter spp. have β-lactamases and are resistant to many β-lactams (21), but the relationship between these bare facts is unclear. The problem is exacerbated by recent reclassification, which splits the former biotypes *A. calcoaceticus* subsp. *anitratus* and *A. calcoaceticus* subsp. *lwoffii* into 17 DNA relatedness groups, of which *A. baumannii* is the one most commonly isolated from clinical material (77). Members of this species are consistently more resistant to β-lactams than are members of *A. lwoffii*, which is the next most frequent species isolated from clinical specimens.

The resistance of some isolates to penicillins and narrow-spectrum cephalosporins is attributable to TEM-1- and CARB-type β-lactamases (101), but the presence of these enzymes cannot explain why increasing numbers of isolates are resistant to extended-spectrum cephalosporins or why a few are resistant to carbapenems. Cephalosporin resistance in individual isolates has been attributed to hyperproduced chromosomal cephalosporinases (87), to extremely large (>1-MDa!) difficult-to-detect cephalosporinases (89), and to extended-spectrum TEM enzymes (22); carbapenem resistance in one isolate entailed a plasmid-mediated β-lactamase, ARI-1 (185). In general, however, no convincing relationship has been established between the antibiogram and production of any of these enzymes, and early claims that *Acinetobacter* spp. have inducible AmpC β-lactamases are discounted (89). Susceptibility to combinations of penicillins and β-lactamase inhibitors is frequent but cannot be taken as proof of a role for β-lactamase in resistance, as most isolates are susceptible to the inhibitors themselves, particularly sulbactam (112, 248, 249). Perhaps the most striking point is that resistance to several β-lactams including imipenem can arise independently of β-lactamase, via PBP modification or impermeability (71, 249). These mechanisms, rather than β-lactamase, may be the major causes of β-lactam resistance in the genus, but proof of their general importance remains lacking.

Other Nonfastidious Nonfermenters

Chromosomal β-lactamases are present in many uncommonly encountered nonfermenters (206), but their roles in resistance remain uncertain, as does the extent of variation of these enzymes within species. *Burkholderia cepacia* and *B. pseudomallei* are among the more important and resistant species. Both commonly have inducible chromosomal cefuroximases (88, 136), resembling the class A enzyme of *Proteus vulgaris*, but other enzymes occur in some isolates. Thus, Prince et al. (199) found a strict penicillinase in one *B. cepacia* strain and Simpson et al. (232) suggested that all isolates of this species have a weak carbapenemase, which was inhibited by the penem BRL42715 although not by tazobactam. This latter activity seems unlikely to have depended on the class A cefuroximase, and it is possible that multiple β-lactamases are present. In the case of *B. pseudomallei*, Godfrey et al. (74) found that various changes in hydrolytic profile and inhibitor susceptibility of the chromosomal β-lactamase accompanied the development of resistance to cephalosporins or to amoxicillin-clavulanate. The mechanisms underlying this behavior are uncertain. β-Lactamases also are important in flavobacteria, and Raimondi et al. (202) and Fujii et al. (67) found broad-spectrum enzymes in *Flavobacterium meningosepticum* isolates. The enzymes protected against most penicillins and cephalosporins, including extended-spectrum compounds, but not against imipenem. Resistance was reversed by clavulanate. On the other hand, *Flavobacterium odoratum* is reported to have a class B (zinc) β-lactamase, which is active against carbapenems as well as penicillins and cephalosporins and is resistant to clavulanate (227). Resistance to imipenem reflected the amount of the β-lactamase produced, and varied greatly among isolates.

TABLE 8. Occurrence of secondary β-lactamases in *E. coli* isolates

Yr(s)	Country or city[a]	% Ampicillin resistant	%[b] of ampicillin-resistant isolates with:					Reference
			TEM-1	TEM-2	OXA-1	SHV-1	TEM + other	
1983	Germany	NS[c]	86	4	0	0	0	179
1983–1984	United States	NS	86	1	<1	0	0	48
1983–1986	Spain	50	94	0	2	3	1	218
1983–1987	Finland	55	61	0	7	1	0	91
1984	Scotland	NS	73	2	0	0	0	208
1984	India	76	66	1	0	1	0	165
1984–1988	Hong Kong	NS	89	0	3	0	3	128
1986–1987	Spain	NS	83	0	1	2	14	216
1988–1990	Portugal	54	78	1	2	8	6	234
1990	Sweden	21	86	0	7	0	0	34
1990	London	46	90	<1	6	1	2	129
1991	London	NS	78	7	5	3	2	268

[a] The country is stated for multicenter surveys; otherwise the city is given.
[b] These do not total 100%, since a few isolates had other enzymes besides those listed here.
[c] NS, not stated, because some studies collected and examined only ampicillin-resistant isolates.

PLASMID-MEDIATED AND OTHER SECONDARY β-LACTAMASES OF NONFASTIDIOUS GRAM-NEGATIVE BACTERIA

Distribution and Diversity

Over 75 different plasmid-mediated β-lactamases have been recorded in gram-negative bacilli (37), and numerous surveys of their frequency have been undertaken. The commonest of these enzymes in enterobacteria is TEM-1, which is responsible for most of the ampicillin resistance now seen in about 50% of *E. coli* isolates (Table 8) (225). TEM-2, SHV-1, and OXA-1 β-lactamases also are widespread in enterobacteria, although they are much rarer than TEM-1 (Table 8), and numerous other types have been seen in occasional isolates.

Secondary β-lactamases in *P. aeruginosa* have been reported widely but are much rarer than in enterobacteria. Thus, multicenter surveys in the United Kingdom found secondary β-lactamases in only 2.5% of 1,866 *P. aeruginosa* isolates collected in 1982 (264) and 0.7% of 1,991 isolates collected in 1993 (44); incidence rates of 13 and 7% have been reported from France (239) and Spain (243), respectively, but these last studies are over 10 years old. Aside from their scarcity, the other characteristic of secondary β-lactamases in *P. aeruginosa* is their diversity (132). PSE-1 and PSE-4 enzymes predominate in *P. aeruginosa* (44, 239, 243, 264), largely because of the clonal selection of producers rather than because of plasmid spread (141, 193). In addition, however, numerous OXA types have been recorded in *P. aeruginosa*, as have various more obscure types such as NPS-1 and LCR-1. TEM and SHV types do occur but are rare, in contrast to their predominance in enterobacteria. Data on secondary β-lactamases in other nonfermenters are sketchy, but TEM and CARB enzymes have been found in *Acinetobacter* spp. (101).

The classical TEM-1, TEM-2, SHV-1, OXA-1, PSE-1, PSE-4 enzymes have minimal activity against newer cephalosporins, other than cefamandole and cefoperazone (174). In the past 10 years, however, there has been increasing emergence of "extended-spectrum" β-lactamases (ESBLs), which attack many newer cephems and monobactams as well as narrow-spectrum cephalosporins and anti-gram-negative-bacterium penicillins (97, 191). Most ESBLs are mutants of TEM-1, TEM-2, and SHV-1, with 1- to 4-amino-acid sequence substitutions. These changes, amounting to less than 2% of the protein sequence, sufficiently remodel the enzyme active site to allow attack on most or all aminothiazolyl cephalosporins. Over 25 different TEM and SHV variants have been claimed and are numbered TEM-3 to TEM-27 and SHV-2 to SHV-7. They are commonest in klebsiellae but also occur in other enterobacteria. The first major outbreak due to producers, specifically isolates with TEM-3 β-lactamase, occurred around Clermont-Ferrand in 1985 to 1987 (189, 233) and was soon followed by outbreaks elsewhere in France (97). Currently, ESBLs occur in about 20 to 25% of klebsiellae from patients in intensive care units in Europe, although they have been found in up to 30 to 40% of those from France (137). Strains with these enzymes began to be reported in the United States around 1989 to 1990, but major outbreaks of producers have since occurred in Chicago (32), New York (158), San Francisco (167), and Boston (210, 211). By 1994, the Center for Disease Control and Prevention National Nosocomial Infections Surveillance Scheme was reporting that 8% of klebsiellae in the United States had ESBLs, although producers were predominantly from a few large centers and in the peripheral hospitals that exchanged patients with these establishments (35, 160). In a fast-changing situation, it is difficult to define which enzyme types are most important, but TEM-3 seems commonest in France (189, 191) whereas TEM-10, TEM-12, and TEM-26 predominate in the United States (32, 167, 200, 210, 211, 250). SHV variants are also important worldwide. SHV-2 and SHV-5 enzymes have each been recorded in at least five countries (97), with the latter type widespread in Greece (73, 252). A single serotype K25 *K. pneumoniae* strain with SHV-4 β-lactamase has been transferred among many hospitals in France (13).

The predilection of ESBLs for klebsiellae is partly explained by cross-infection, with clonal spread of producers (13, 189, 233), and this is facilitated by the fact that klebsiellae survive longer than most other gram-negative rods on skin (39). Cross-infection does not, however, explain why so many different enzyme types have been found in klebsiellae compared with other genera or why some hospitals have recorded dissemination of single ESBLs among klebsiellae of diverse serotypes (129). Maybe klebsiellae are particularly good vectors for plasmids or allow evolution of ESBL genes more readily than do other enterobacteria. It is notable, in this context, that many ESBL genes are on large plasmids with low copy numbers and that large multiresistance plasmids have long been more common in klebsiellae than in *E. coli* (97, 191). With the passage of time, ESBLs are disseminating to other enterobacterial genera besides *Klebsiella* (57, 73, 97, 191).

TABLE 9. Effects of common secondary β-lactamases on antimicrobial resistance in *E. coli* and other enterobacteria

Antimicrobial agent	Effect of β-lactamase[a]:					
	TEM-1, TEM-2, or SHV-1	OXA-1	TEM-3	TEM-12	TEM-10 (=23) or 26	SHV-2
Ampicillin[b]	●	●	●	●	●	●
Amoxicillin-clavulanate	◍	●/◍	◍	◍	◍	◍
Ticarcillin[c]	●	●	●	●	●	●
Ticarcillin-clavulanate	◍	●/◍	◍	◍	◍	◍
Piperacillin[d]	●/◍	◍	●	●	●	●
Piperacillin-tazobactam	○/◍	○/◍	○/◍	○/◍	○/◍	●[e]
Temocillin	○	○	○	○	○	○
Cephalothin[f]	●/◍	○	●	○	●	●
Cefoperazone	◍	◍	●	◓	◓	●
Cefuroxime	○	○	●	●	◓	●
Cefotaxime[g]	○	○	●	◓	◓	●
Ceftazidime	○	○	●	◓	●	●
Cefpirome	○	○	◓	◓	◓	◓
Cefepime	○	○	◓	◓	◓	◓
Cefoxitin	○	○	○	○	○	○
Moxalactam	○	○	○/◍	○	○/◍	○/◍
Aztreonam	○	○	●	◓	●	●
Imipenem	○	○	○	○	○	○
Meropenem	○	○	○	○	○	○

[a] Data from references 32, 96 to 98, 105, 115, 129, 140, 142, 191, 230, and 269. Patterns are shown qualitatively, as MICs vary with enzyme quantity (Fig. 3). Symbols: ●, unequivocal resistance, with MICs above all commonly used breakpoints; ●/◍, resistance obvious except in isolates with only low levels of the enzyme: nevertheless, these should be viewed as resistant; ◍, behavior depends on amount of enzyme, and susceptibility tests should be accepted at face value; ◓, biologically resistant, with MICs greatly above those for nonproducers and with clinical failures reported (should be reported as resistant, but may appear susceptible at current breakpoints); ○/◍, normal levels of enzyme may raise MICs two- or fourfold, but clinical susceptibility is retained unless exceptional levels of enzyme are present; ○, as susceptible as isolates without the enzyme.

[b] Representative also of amoxicillin.

[c] Representative also of carbenicillin, which is one to two dilutions less active against all phenotypes.

[d] Representative of azlocillin and mezlocillin, which are one to two dilutions less active against all phenotypes.

[e] Uncertain. Producers of SHV derivatives were reported resistant to sulfone combinations, including piperacillin-tazobactam, by Jacoby and Carreras (96) and Bauernfiend (17) but susceptible by Goldstein et al. (75) and Philippon et al. (190).

[f] Representative also of other narrow-spectrum cephalosporins, e.g., cephalexin and cefazolin.

[g] Representative of ceftriaxone and ceftizoxime.

Other ESBLs besides TEM and SHV derivatives are emerging, but these presently are very rare. They include representatives of all four molecular classes. Yet another recent development is the emergence of mutants of TEM and SHV β-lactamases that lack ESBL activity but are resistant to inhibition by clavulanate and penicillanic acid sulfones.

It should be emphasized that the dissemination of plasmid-mediated β-lactamases in gram-negative bacteria is very recent. TEM-1 enzyme was first reported from a single *E. coli* isolate in 1965, and the earliest known ESBLs date from 1982 to 1983 (113). There can be few examples of faster evolution than the spread of these enzymes.

Effects of Secondary β-Lactamases on Resistance

TEM-1, TEM-2, and SHV-1 enzymes. TEM-1, TEM-2, and SHV-1 are widespread enzymes that attack narrow-spectrum cephalosporins, cefamandole, and cefoperazone and all the anti-gram-negative-bacterium penicillins except temocillin. Aminothiazolyl cephalosporins, cephamycins, monobactams, and carbapenems are considerably more stable (96, 98), although SHV-1 has been suggested to possess slight activity against the aminothiazolyl cephalosporins (188) and slight inoculum effects were noted with aztreonam and cefotaxime, although not with ceftazidime, for TEM-1, TEM-2, and SHV-1 producers (236). The degree of resistance conferred on substrates depends on the amount of TEM or SHV enzyme, which varies at least 150-fold among isolates (140, 230, 269), reflecting gene dosage and, maybe, promoter efficiency. Resistance is also modulated by permeability and is enhanced in enterobacteria that lack porins (equation 5) (96).

Even a low level of a TEM or SHV enzyme confers unequivocal resistance to ampicillin, amoxicillin, ticarcillin, and carbenicillin, with MICs exceeding 256 μg/ml compared with 1 to 4 μg/ml for *E. coli* isolates without the enzyme (140, 230, 269). Resistance to these agents likewise is obvious in disk tests. Susceptibility test results for weaker substrates are harder to interpret. Thus, for example, MICs of piperacillin and mezlocillin for *E. coli* with TEM-1 β-lactamase range from 8 to 512 μg/ml, according to enzyme quantity, compared with 0.5 to 4 μg/ml for nonproducers; those of cephalothin range from 16 to 256 μg/ml compared with 4 to 8 μg/ml; and those of cefoperazone range from 0.5 to 64 μg/ml compared with 0.03 μg/ml (Table 9) (98, 140, 269). Analogously, isolates with low levels of TEM and SHV enzymes often give large zones to weak substrates in diffusion tests, particularly in response to high-content (75 or 100 μg) ureidopenicillin disks. Consequently, hospitals' susceptibility summaries often indicate that "60% of *E. coli* isolates are resistant to ampicillin; 30% to mezlocillin, piperacillin, and cephalexin; and 10% to cefoperazone." A Centers for Disease Control and Prevention survey published in 1990 (48) provides a classic example of this type of reporting. Such analysis seems to embody a false optimism when it is reflected that TEM and SHV enzymes do attack these ureidopenicillins and cephalosporins and that the MICs of the compounds for enzyme producers rise dramatically as the inoculum is increased (155). Moreover, and crucially, narrow-spectrum cephalosporins have shown poor efficacy against

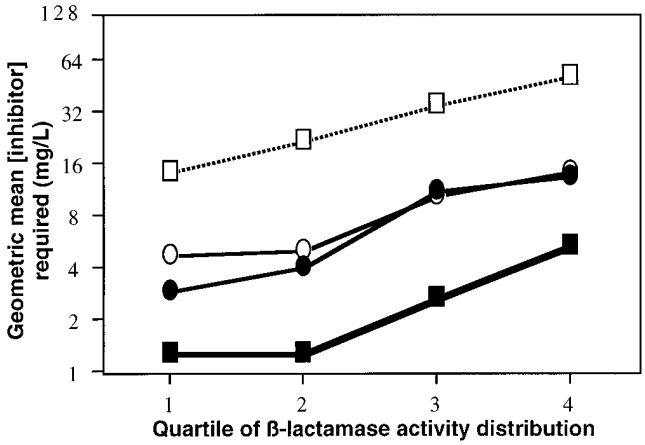

FIG. 3. Effects of β-lactamase quantity on the concentrations of inhibitors required to potentiate their partner penicillins to breakpoint against 36 *E. coli* isolates with TEM-1 enzyme. The amounts of TEM enzyme produced by the isolates were measured by spectrophotometric assay against benzylpenicillin and graded: thus, quartile 1 comprised the nine isolates with the least enzyme, quartile 2 comprised the nine isolates with the next least, and so on to quartile 4, which comprised the nine isolates with the most enzyme. □, Concentration of sulbactam needed to reduce ampicillin MICs to 8 μg/ml; ○, concentration of clavulanate needed to reduce amoxicillin MICs to 8 μg/ml; ●, concentration of clavulanate needed to reduce ticarcillin MICs to 16 μg/ml; ■, concentration of tazobactam needed to reduce piperacillin MICs to 16 μg/ml. Data are from references 133 and 142.

infections caused by ampicillin-resistant *E. coli* (168, 260, 269). A cautious but reasonable approach is to view all *E. coli* and *Proteus mirabilis* isolates that are resistant to ampicillin and all other enterobacteria that are resistant to ticarcillin as likely producers of TEM enzymes and to consider them resistant to all ureidopenicillins and narrow-spectrum cephalosporins. The cases of cefamandole and cefoperazone are more equivocal (168), but it seems prudent to avoid these agents against isolates suspected of harboring TEM or SHV enzymes unless the infection is at a site, such as the urinary tract, where a high level of drug can be ensured.

Interpretation of susceptibility data is also difficult for β-lactamase–inhibitor combinations, which are rarely as active against β-lactamase producers as against those without the enzyme. Their activity depends, inter alia, on the sensitivity of the enzyme to the inhibitor, the activity of the enzyme against the substrate to be protected, and the amount of enzyme produced (Fig. 3) (135). Clavulanate and tazobactam are similarly good inhibitors of TEM and SHV enzymes, but sulbactam is a much poorer inhibitor. Ampicillin, amoxicillin, and ticarcillin are good substrates to which the TEM and SHV enzymes give high-level resistance, and so they are difficult to protect, whereas piperacillin and cefoperazone are weaker substrates and are easier to protect (96, 135). Adding these factors together, only about 25% of *E. coli* isolates with TEM-1 β-lactamase, specifically those with the smallest amounts of enzyme, are susceptible to ampicillin-sulbactam (8 plus 4 μg/ml) (133); about 60 to 70% are susceptible to the amoxicillin-clavulanate (8 plus 4 μg/ml) and ticarcillin-clavulanate (16 plus 2 μg/ml) combinations, and over 90% are susceptible to piperacillin-tazobactam (16 plus 4 μg/ml) or cefoperazone-sulbactam (16 plus 2 μg/ml) (Fig. 3). Only isolates with exceptional levels of enzyme are resistant to the last two combinations (133, 142, 216).

Classical OXA and PSE enzymes, and rarer narrow-spectrum enzymes. The classical OXA enzymes including OXA-1, which is the most common type in enterobacteria (Table 8), generally give low levels of resistance to penicillins, with MICs of ampicillin for *E. coli* remaining around 128 to 256 μg/ml and those of ureidopenicillins being around 4 to 16 μg/ml (Table 9). Potentiation by clavulanate is often poor, and many OXA-1 producers are resistant to combinations incorporating this inhibitor, although they are susceptible to piperacillin-tazobactam (137, 271). Narrow-spectrum cephalosporins are only slightly compromised, and extended-spectrum agents are spared. PSE-1 and PSE-4 β-lactamases give resistance patterns similar to the classical TEM types, compromising all anti-gram-negative penicillins, together with cefoperazone and cefsulodin (Table 10) (98, 132, 264). In addition, *P. aeruginosa* isolates with these enzymes often are resistant to inhibitor combinations (190). This does not seem to reflect resistance of the enzyme to inhibition but may depend on the impermeability of the organism or on its ability to efflux β-lactams.

Antibiograms similar to those for producers of classical TEM, SHV, PSE-1, and PSE-4 enzymes have been reported for many isolates with rarer secondary β-lactamases, such as SAR-1, SAR-2, PSE-3, NPS-1, and TLE-1. These enzymes are, however, known from only a few isolates apiece and are primarily of academic interest.

Extended-spectrum TEM and SHV β-lactamases. The most notable feature of these enzymes, distinguishing them from their TEM-1, TEM-2, and SHV parent types, is their ability to attack extended-spectrum cephalosporins and monobactams, as well as narrow-spectrum cephalosporins and anti-gram-negative-bacterium penicillins (97, 191). Carbapenems and cephamycins are stable, as is temocillin. Ceftibuten is also stable to most types, except to a few SHV derivatives (96).

All the TEM- and SHV-derived ESBLs confer antibiograms that reflect this general pattern of activity, but individual enzymes vary in the levels of resistance they cause to different compounds. Some types, including TEM-3 and SHV-2, give clear resistance (MIC, >16 μg/ml) to all extended-spectrum cephalosporins and to aztreonam (Table 9) (96); others, including the TEM-10 and TEM-26 types that currently predom-

TABLE 10. Effects of common secondary β-lactamases on antimicrobial resistance in *P. aeruginosa*

Antimicrobial agent	MIC (μg/ml) for[a]:			
	No enzyme[b]	PSE-1 or PSE-4	TEM-1 or TEM-2	OXA-3[c]
Ticarcillin	16	**1,024**	**2,048**	**512**
Ticarcillin-clavulanate	32[d]	**64**	**32**	**512**
Piperacillin	4	**64**	**128**	**64**
Piperacillin-tazobactam	4	*16*	**32**	**64**
Cefoperazone	4	**32**	**32**	**64**
Ceftazidime	1	1	1	1
Cefpirome	4	4	4	4
Cefepime	2	2	2	2
Moxalactam	8	8	8	8
Aztreonam	4	4	4	4
Imipenem	2	2	2	2
Meropenem	0.25	0.25	0.25	0.25

[a] Data are from references 44, 132, 137, and 264. MICs are representative of those found for isolates with "typical" levels of enzyme; hyperproducers are more resistant, especially to β-lactamase–inhibitor combinations and weak substrates. Values are shown in boldface type when resistance is indisputable and in italic type when resistance is equivocal.

[b] Inducible expression of AmpC enzyme only.

[c] Data are for a strain with OXA-3 enzyme; similar results are obtained for producers of other OXA types, except OXA-10 and OXA-11. Data for producers of the latter enzymes are shown in Table 11.

[d] Slight antagonism compared with ticarcillin alone, because of β-lactamase induction.

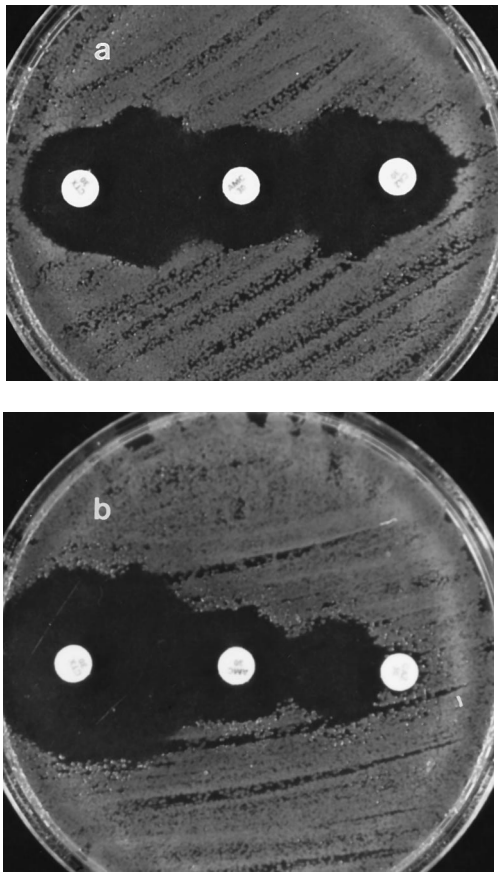

FIG. 4. Double-disk tests to detect ESBLs. (a) Isolate with TEM-3 enzyme; (b) isolate with TEM-10. The left-hand disks contain 30 μg of cefotaxime, the central disks contain 20 plus 10 μg of amoxicillin-clavulanate, and the right-hand disks contain 30 μg of ceftazidime. Note the potentiation of the cephalosporins by the clavulanate. Note also that although the producer of TEM-3 enzyme is clearly resistant to both cephalosporins, the producer of TEM-10 β-lactamase is obviously resistant only to ceftazidime, not to cefotaxime. Nevertheless, cefotaxime and similar drugs have proved unsatisfactory against isolates with TEM-10 and similar enzymes, and should be avoided in the clinic (33, 212).

inate in the United States, give clear resistance to ceftazidime (MIC, ≥128 μg/ml) but raise the MICs of cefotaxime, ceftriaxone, cefpirome, and ceftizoxime to only around 0.5 to 4 μg/ml (96, 105, 129, 200, 210–212). TEM-12, which is the evolutionary ancestor of TEM-10 and TEM-26, is even feebler, generally raising the MICs of ceftazidime for *E. coli* and klebsiella isolates to only 4 to 8 μg/ml and leaving those of cefotaxime and ceftriaxone at around 0.06 to 0.25 μg/ml (Table 9) (97, 105, 210, 211) although giving greater resistance, especially to ceftazidime, in a porin-deficient strain (259). Disk tests give results that parallel the MICs just described (Fig. 4). Producers of TEM-10, TEM-12, and TEM-26 enzyme types thus have biological resistance to the many newer cephalosporins, being up to 100-fold less susceptible than are strains without enzyme, but remain apparently susceptible at the breakpoints advocated by the NCCLS (8 to 16 μg/ml) (105, 200, 212). This can lead to serious interpretive problems. Animal and clinical data support the view that ESBL producers are resistant to aminothiazolyl cephalosporins, even when MICs of these compounds are raised only to 1 to 2 μg/ml. Such modest MICs appear predictive of failure in vivo, except in urinary tract infections (33, 212). It follows that organisms suspected of harboring ESBLs should be reported as resistant to all extended-spectrum cephalosporins, regardless of the actual susceptibilities found. This begs the question of the best way to detect ESBL producers. Various more or less complicated tests have been advocated, but the most practicable is simply to screen with ceftazidime, since virtually all ESBLs give clear resistance to this compound (105). When isolates have reduced susceptibility to ceftazidime, double-disk tests can be used to screen for synergy between this compound and clavulanate, which is most conveniently available in amoxicillin-clavulanate (20 plus 10 μg) disks. When the ceftazidime zone is expanded by the clavulanate, production of an ESBL is inferred (Fig. 4). Such testing would be facilitated if disks combining ceftazidime and clavulanate were available, as one could simply compare the zones with and without inhibitor. E-test ellipseometers and Vitek cards with this combination are under development, and early results indicate that they allow accurate detection of ESBL producers (226).

Carbapenems are stable to ESBLs, and imipenem has been used successfully in vivo against many enzyme producers. Inhibitor combinations and cephamycins may also overcome these enzymes, but their efficacy is more controversial. Despite sensitivity of the enzymes to inhibition, MICs of clavulanate combinations and ampicillin-sulbactam often are high for ESBL producers (96, 97), presumably because high levels of enzyme are present. Piperacillin-tazobactam seems a better prospect than other inhibitor combinations, at least against isolates with TEM derivatives, most of which are susceptible to the combination at 16 plus 4 μg/ml (96, 190). Moreover, piperacillin-tazobactam was effective against a *K. pneumoniae* strain with TEM-3 enzyme in a rabbit endocarditis model, whereas unprotected piperacillin was (unsurprisingly) inadequate (124). Whether or not piperacillin-tazobactam is a viable option against producers of SHV-derived ESBLs is less certain: two groups (17, 96) have reported that *E. coli* and *K. pneumoniae* strains and transconjugants with SHV-2, SHV-3, SHV-4, and SHV-5 enzymes typically were resistant to piperacillin-tazobactam, with MICs ranging upwards from 64 plus 4 μg/ml, whereas another group (190) reported that producers of SHV derivatives were very susceptible to the combination, with MICs under 4 plus 4 μg/ml, as did a final group (75), who tested tazobactam in combination with apalcillin rather than piperacillin.

Cephamycins are stable to ESBLs, and the continued activity of these compounds, but not of other cephalosporins, facilitates laboratory recognition of ESBL producers (96, 104). Nevertheless, failures were reported when cefoxitin was used against ESBL producers and were associated with selection of porin-deficient mutants (181). It is unclear whether this risk would arise with cefotetan or moxalactam, which share the stability of cefoxitin to ESBLs but have 10- to 100-fold-greater antienterobacterial activity. Perhaps it is significant that there have been few reports of ESBL producers in Japan, where moxalactam has long been a favored drug, and many reports from France and the United States, where cefotaxime, ceftriaxone, and ceftazidime have been preferred.

Inhibitor-resistant TEM mutants. In addition to their ESBL derivatives, TEM-1 and TEM-2 β-lactamases segregate mutants that have reduced affinity for clavulanate and the sulfones. Ten variants have been described (25, 241, 253, 281) and, to add confusion, have been numbered variously as IRT (inhibitor-resistant-TEM) types 1, 2, 3, etc.; as TRI (TEM, resistant to inhibitors) types 1, 2, 3, etc.; and as TEM types 30, 31, 32, etc.! Producer strains are resistant to all β-lactamase–inhibitor combinations but are less resistant than isolates with classical TEM-1 enzyme to narrow-spectrum cephalosporins and remain fully susceptible to extended-spectrum cephalospo-

TABLE 11. Effects of "exotic" secondary β-lactamases on the MICs of β-lactams for producer strains

Antimicrobial agent	MIC[a] (μg/ml) for:					
	PER-1[b] (55) (class A), *P. aeruginosa*	Sme-1[b,c] (275) (class A), *S. marcescens*	Carbapenemase[b,d] (258) (class B), *P. aeruginosa*	MIR-1[b,e] (183) (class C), *K. pneumoniae*	OXA-10[b,f] (78) (class D), *P. aeruginosa*	OXA-11[b,g] (78) (class D), *P. aeruginosa*
Ampicillin	—[h]	**32**	—	**1,024**	—	—
Amoxicillin-clavulanate	—	**32**	—	**256**	—	—
Ticarcillin	**256**	16	**1,024**	—	**256**	**256**
Ticarcillin-clavulanate	16	16	**1,024**	—	**128**	**128**
Piperacillin	*8*	—	**32**	—	**64**	**64**
Piperacillin-tazobactam	*8*	—	**32**	—	**64**	**64**
Temocillin	—	16	—	16	—	—
Cephalothin	—	**512**	—	—	—	—
Cefoperazone	—	—	—	—	**256**	**128**
Cefotaxime	**64**	0.25	—	**64**	**16**	**32**
Ceftazidime	**128**	0.12	512	**128**	4	**1,024**
Cefpirome	**32**	—	—	1	16	**128**
Cefepime	**32**	—	—	1	8	**64**
Cefoxitin	—	*16*	—	**256**	—	—
Moxalactam	16	—	512	**64**	*16*	**128**
Aztreonam	128	*4*	4	**128**	*16*	**32**
Imipenem	2	**16**	16	1	2	2
Meropenem	1	*1*[i]	128	0.12	2	2

[a] Few representatives are known with each enzyme types (except for those with plasmid-borne AmpC enzymes), and it is uncertain whether these have "normal" or "abnormal" levels of enzyme. Consequently, although values are shown in boldface type when resistance is indisputable and in italic type when resistance appears equivocal, these categorizations carry less weight than elsewhere in this review. References and molecular class are given in parentheses.

[b] Comparative MIC data for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. marcescens* strains without these enzymes are shown in Tables 5, 6, and 7. It should be noted that these comparators are different strains and that other factors besides β-lactamase may cause susceptibility differences.

[c] Similar results were reported for NMC-A enzyme in *E. cloacae* (170), to which Sme-1 is closely related (164).

[d] Similar results were reported for IMP-1 in *S. marcescens* (180).

[e] Similar results were reported for other plasmid-mediated AmpC enzymes in *E. coli* and *K. pneumoniae*, e.g., BIL-1, CMY-1, CMY-2, CMY-3, FOX-1, LAT-1, and MOX-1 (19, 90, 99, 123, 152, 186, 247).

[f] Classical type, with slight activity against cefotaxime and aztreonam, not ceftazidime.

[g] ESBL mutant of OXA-10.

[h] —, no data available.

[i] Equivocal on the grounds that it constitutes biological resistance.

rins. At present, resistance to inhibitor combinations is more often caused by high-level production of TEM-1 enzymes (230, 269) than by these mutants, but this situation may change as more potent inhibitor combinations, such as piperacillin-tazobactam and cefoperazone-sulbactam, are increasingly used.

Extended-spectrum secondary β-lactamases not related to TEM and SHV. The enzymes described in this section include examples from each of the four molecular classes. They are rare, but they merit inclusion because they are some of the most potent β-lactamases yet known and because they may become more important in the future.

Among extended-spectrum class A β-lactamases that are not TEM and SHV derivatives are PER-1 (55, 171), its close relative CTI-1 (18), and MEN-1 (23). Each gives resistance to all cephalosporins, aztreonam, and penicillins but spares carbapenems and cephamycins. CTI-1 and MEN-1 have been detected only in single isolates, but PER-1 appears well established in Turkey, having been found in 14 *P. aeruginosa* isolates collected over an 18-month period at an Ankara teaching hospital and in salmonellae from Istanbul (55, 137). It has not been seen elsewhere, except in a *P. aeruginosa* isolate from a Turkish patient who was transferred to Paris (171). Its gene has been recorded on at least three different plasmids (55, 137), suggesting transposition. Isolates with PER-1 enzyme have a characteristic antibiogram (Table 11), being highly resistant to ceftazidime (MIC, >256 μg/ml) but very susceptible to ceftazidime-clavulanate (MIC, 1 to 4 μg/ml) and having only slightly reduced susceptibility to piperacillin (MIC, 8 to 16 μg/ml) as compared with typical *P. aeruginosa* isolates.

Other exotic class A enzymes include three related carbapenemases, Sme-1, Imi-1, and NMC-A, all of which are encoded by nontransferable chromosomal inserts. Sme-1 was from two *Serratia marcescens* isolates obtained in London in 1982 (164, 275); Imi-1 was from *Enterobacter cloacae* isolates collected at a California hospital in 1984 (205), and NMC-A was from a single *E. cloacae* isolate obtained in Paris in 1990 (170). Each attacks and gives resistance to penicillins, aztreonam, and carbapenems, with imipenem being compromised more than meropenem (Table 11). Aminothiazolyl cephalosporins are largely spared, and the enzymes are subject to inhibition by clavulanate.

The molecular class B β-lactamases are most notable for their carbapenemase activity. Closely similar members of this class, one of which has been dubbed IMP-1, have been recorded in at least three *P. aeruginosa* (258) and five *S. marcescens* (12, 180) isolates from Japan although in none from elsewhere. Production was plasmid mediated and transferable in some isolates of each of these species. Resistance was conferred to carbapenems and to all other β-lactams except aztreonam (Table 11) and was not reversed by any available inhibitor. Producers could be distinguished from those with class A carbapenemases, such as Sme-1, on the grounds that they were equally resistant to imipenem and meropenem, remained susceptible to aztreonam, and did not show susceptibility to imipenem if clavulanate was added. Moreover, the extracted class B enzymes were inhibited by EDTA and heavy metal ions whereas the class A carbapenemases were little affected by these substances.

Plasmid-encoded class C enzymes have been reported from klebsiellae and *E. coli* isolates and represent cases where the

chromosomal β-lactamase genes of *Enterobacter* spp. and *C. freundii* have escaped on extrachromosomal elements. The first claim of a plasmid-mediated AmpC enzyme dates from 1976 (26), but the strain and its putative transconjugant were subsequently lost. Since 1989, however, several plasmid-encoded AmpC enzymes have been documented, including MIR-1 from U.S. isolates (183), CMY-2 from South Korea (19), BIL-1 from Pakistan (186), FOX-1 from Argentina (123), LAT-1 from Greece (247), FEC-1 (152) and MOX-1 (90) from Japan, and CMY-3 from the United Kingdom (99). Producers have antibiograms resembling those of derepressed *Enterobacter* strains, with resistance to all β-lactams except carbapenems, temocillin, and mecillinam (Table 11). Resistance usually is not reversed by clavulanate and sulbactam, although FEC-1 and MOX-1 provide exceptions to this generalization. The lack of susceptibility to cephamycins and inhibitor combinations distinguishes such producers from isolates with TEM- and SHV-derived ESBLs. The fact that producers of these enzymes have been reported from many different countries in a very brief period suggests that a real problem may be developing.

Finally, extended-spectrum activity has been found in mutants of a class D enzyme, OXA-10 (PSE-2). One such mutant, OXA-11, has been reported (78), and others are under study (137). All are from *P. aeruginosa* isolates obtained at one hospital in Ankara, Turkey. OXA-10 itself has a broader spectrum than other OXA types, giving high-level resistance to cefoperazone and, if hyperproduced, causing small reductions in susceptibility to aztreonam, cefotaxime, and ceftriaxone, (Table 11). Its extended-spectrum mutants give greater resistance to cefotaxime, ceftriaxone, and aztreonam and cause high-level resistance to ceftazidime (MIC, 256 to 512 μg/ml), which is spared by OXA-10 itself (Table 11). OXA-10 and its derivatives are poorly inhibited by clavulanate or sulfones. Carbapenems are stable to their activity.

FASTIDIOUS GRAM-NEGATIVE ORGANISMS AND *MORAXELLA CATARRHALIS*

Until the mid-1970s, all isolates of *Haemophilus* spp., *Neisseria* spp. and *Moraxella catarrhalis* had negligible amounts of chromosomal β-lactamases, lacked secondary enzymes, and were susceptible to clinically achievable concentrations of ampicillin and, for *Neisseria* spp., to benzylpenicillin. The only problem for chemotherapy was accumulation of impermeability- or PBP-mediated penicillin resistance in *Neisseria gonorrhoeae*. Since then, TEM-1 enzymes have reached and disseminated in *N. gonorrhoeae*, *Haemophilus ducreyi*, and *H. influenzae* (153, 238) and BRO-1 and BRO-2 types have become almost ubiquitous in *M. catarrhalis* (46, 68). Other β-lactamases occur in a few isolates: specifically, ROB-1 is found in up to 8% of β-lactamase-producing *H. influenzae* isolates from North America (56, 229) but has been seen in only a few strains elsewhere (100), and an uncharacterized pI 5.5 β-lactamase has been recorded together with TEM-1 enzymes in highly penicillin-resistant *N. gonorrhoeae* isolates from Spain (86, 251). Unidentified β-lactamases, probably TEM-1, have been claimed to exist in tiny numbers of *N. meningitidis* isolates (31, 65). In addition, increasing numbers of *Neisseria* and *H. influenzae* isolates have intrinsic resistance to β-lactams, as a result of impermeability or PBP modification (156, 196, 198).

The frequencies of β-lactamase production in *H. influenzae* and *N. gonorrhoeae* vary widely between and within countries, and this is exemplified for *H. influenzae* in Table 12. A similar table could be drawn for *N. gonorrhoeae*; indeed, the rate of β-lactamase production in this latter species varies widely within the United States, from 1.2% in Albequerque, N.M., to 31.2% in Long Beach, Calif. (40)!

TABLE 12. Geographic and temporal variation in β-lactamase production by *H. influenzae*

Country	Yr isolated	No. of isolates examined	% with β-lactamase	Reference
United States	1984	3,356	15.2	58
United States	1986	2,811	20.0	59
United States	1989	564	16.4	103
Canada	1989	2,503	16.9	245
Canada	1992–1993	1,688	28.4	229
United Kingdom	1977	952	1.7	92
United Kingdom	1981	1,841	5.8	192
United Kingdom	1986	2,434	6.2	196
United Kingdom	1991	2,212	8.6	198
Austria	1986	187	5.9	147
Austria	1988–1989	209	1.5	106
Belgium	1986	202	26.7	147
Belgium	1988–1989	189	15.8	106
France	1986	238	10.9	147
France	1988–1989	314	16.6	106
Germany (West)	1986	248	1.6	147
Germany (West)	1988–1989	397	2.5	106
The Netherlands	1986	177	6.8	147
The Netherlands	1988–1989	287	3.9	106
Spain	1986	177	30.6	147
Spain	1988–1989	175	34.9	106
Sweden	1986	368	7.3	147
Switzerland	1986	167	6.0	147
Switzerland	1988–1989	358	5.9	106
Hong Kong	1986	292	20.0	116
Hong Kong	1993	134	25.0	127
South Korea	1986	200	9.0	116
Philippines	1986	200	6.3	116
Taiwan	1986	200	35.7	116
Thailand	1986	200	14.6	116

Haemophilus and *Neisseria* spp.

TEM β-lactamase gives lower-level resistance to ampicillin and benzylpenicillin in *Haemophilus* and *Neisseria* spp. than in enterobacteria, doubtless because the fastidious species are more permeable and produce less enzyme. Thus, ampicillin MICs for β-lactamase producers typically are in the range of 16 to 64 μg/ml, and residual zones are apparent around 10-μg ampicillin disks. MICs of weak-substrate cephalosporins, including cefaclor, may be elevated by one to two dilutions, at most (197), but other expanded-spectrum oral cephalosporins such as cefixime, cefpodoxime, and ceftibuten retain full activity (16). ROB-1 enzyme gives weaker resistance to ampicillin and amoxicillin than does TEM-1 (56).

It is important for the clinical laboratory to distinguish β-lactamase producers from isolates with intrinsic resistance, and this is better done by direct β-lactamase tests than by inference from the antibiogram. A caution is that nitrocefin, which generally is the most sensitive β-lactamase indicator, is only weakly labile to ROB-1 enzyme, which consequently may be missed (217). If the resistance mechanism is to be inferred from the antibiogram, it is most useful to compare the zones around ampicillin and amoxicillin-clavulanate disks and—at least for *H. influenzae*—to test cefaclor. MICs and disk zones of this last compound for hemophili are minimally affected by TEM enzyme, but are grossly affected by intrinsic resistance (197). The use of 2-μg ampicillin and 2- plus 1-μg amoxicillin-clavulanate disks, rather than those with 10 μg of ampicillin and 20 plus 10 μg of amoxicillin-clavulanate increases the accuracy of detec-

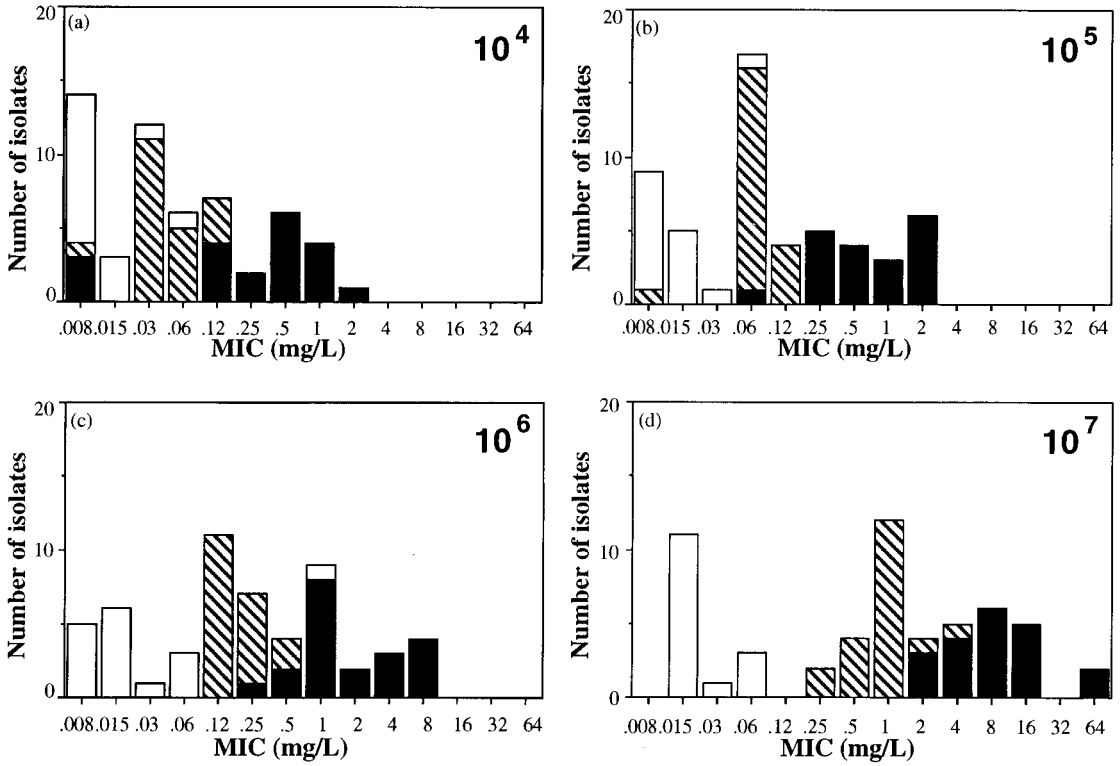

FIG. 5. Inoculum effects on agar dilution MICs of ampicillin for *M. catarrhalis*. We tested 60 isolates, of which 20 had BRO-1 enzyme (■), 20 had BRO-2 (▨), and 20 had no β-lactamase (□). Shown are the MIC distributions with inocula of 10^4 (a), 10^5 (b), 10^6 (c), and 10^7 (d) per spot, respectively. Note that the MICs for enzyme producers are distinct from those for nonproducers at high inocula but overlap at low inocula; also note that isolates with BRO-1 enzyme generally are more resistant than those with BRO-2. Reprinted from reference 277 with permission of the publisher.

tion of both β-lactamase- and non β-lactamase-mediated resistance in the *H. influenzae* isolates (156).

Moraxella catarrhalis

β-Lactamases were unknown in *Moraxella catarrhalis* until the mid-1970s. Since then, BRO-1, a class A enzyme, has spread to over 75% of isolates in the United States and Western Europe and its close relative, BRO-2, has spread to a further 10% (46, 68). Another enzyme variant, BRO-3, has been claimed but was demonstrable only after bacterial extracts had been treated with papain (47). These β-lactamases attack ampicillin and narrow-spectrum cephalosporins but spare cefaclor, cefuroxime, cefixime, and loracarbef and are readily inactivated by inhibitors (46, 277). Producers are clinically resistant to labile drugs, particularly ampicillin (187), but this is not always obvious in MIC tests with standard inocula (10^4 to 10^5), which often show enzyme producers to be as susceptible as those without β-lactamase (Fig. 5). Low MICs are particularly likely with producers of BRO-2, which tends to be expressed at a lower level than BRO-1 and so to give less resistance (46, 68). Resistance is readily demonstrable in disk tests (146) and in high-inoculum (10^7 CFU per spot) MIC determinations (Fig. 5) (60, 277). These methods provide a reasonable means of detecting enzyme producers in the clinical laboratory. Alternatively, direct β-lactamase tests can be performed or, more simply, one can reasonably assume all *M. catarrhalis* isolates to have β-lactamase and avoid unprotected labile drugs whenever the organism is thought to be a significant pathogen.

BACTEROIDES SPP. AND OTHER GRAM-NEGATIVE ANAEROBES

Chromosomal class A β-lactamases with predominantly cephalosporinase activity are virtually ubiquitous in *Bacteroides fragilis* group isolates (215, 266), although their amount varies greatly among isolates. Like most class A enzymes, they are inhibited by clavulanate, tazobactam, and sulbactam (8). These enzymes determine the natural resistance of *B. fragilis* isolates to many penicillins and cephalosporins, and their importance is underscored by the fact that most *B. fragilis* group isolates are more susceptible to piperacillin-tazobactam, ticarcillin-clavulanate, amoxicillin-clavulanate, and ampicillin-sulbactam than to piperacillin, ticarcillin, amoxicillin, or ampicillin (7, 8). Moreover, Maskell et al. (149) found that clavulanate, 2 μg/ml, reduced the MICs of cephaloridine, cefotaxime, and ceftriaxone by 16-fold or more for most *B. fragilis*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus* isolates and also reduced the MICs of cefsulodin, cefoperazone, and ceftazidime for *B. fragilis* species. On the other hand, clavulanate caused only two- to fourfold reductions in the MICs of cefsulodin, cefoperazone, and ceftazidime for resistant isolates of *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus*, and it appears that factors other than β-lactamase contributed to resistance in these species.

The extended-spectrum cephalosporins cefotaxime, and ceftizoxime appear active at ≤16 μg/ml against some *B. fragilis* strains, probably those with the smallest amount of chromosomal enzyme. Nevertheless, it seems prudent to avoid these agents in therapy and to use β-lactamase–inhibitor combina-

TABLE 13. Antimicrobial susceptibility of *B. fragilis* in relation to β-lactamase type

Antimicrobial agent	MIC (μg/ml)[a] for:	
	Strains producing chromosomal cephalosporinase only[b]	Carbapenemase (*ccrA*) producers
Ampicillin	≥**256**	**256**
Amoxicillin-clavulanate	4	**256**
Ticarcillin	>**128**	ND
Ticarcillin-clavulanate	8	ND
Piperacillin	**64**	**256**
Piperacillin-tazobactam	4	**256**
Cefoperazone	≥**128**	**256**
Cefoperazone-sulbactam	8	**256**
Cefotaxime	**32**	**256**
Cefoxitin	8	**128**
Moxalactam	8	**256**
Imipenem	0.25	**16**
Meropenem	0.25	**32**

[a] Values are shown in boldface type when resistance is indisputable and in italic type when resistance is equivocal. Data are drawn from references 7, 50, and 278.

[b] Enzyme and susceptibility pattern typically seen in this species (215).

tions or compounds, such as cephamycins or carbapenems, that have genuine stability to the β-lactamase (261). These caveats against weak-substrate cephalosporins also apply to piperacillin, which was reported to be active, relative to a breakpoint of 64 μg/ml, against 70 to 90% of *B. fragilis* isolates, with the exact proportion varying with the species and geographic source (51). Although such activity appears adequate, it is apparent both that the breakpoint is very high and that virtually all *B. fragilis* isolates are more susceptible to piperacillin-tazobactam than to unprotected piperacillin (Table 13) (7, 8).

Various other β-lactamases occur in a few *B. fragilis* strains and may replace or accompany the normal chromosomal enzyme (204). Some are plasmid determined but are distinct from the plasmid-mediated enzymes of enterobacteria and other gram-negative aerobes. They can conveniently be divided into two groups. The first comprises zinc-independent, inhibitor-sensitive enzymes, mostly of molecular class A, with activity against cephamycins (62, 93, 178, 184, 204). A few of these have marginal activity against carbapenems. They are diverse, and no individual type is common. In some cases, resistance depends not only on the enzyme but also on impermeability, as was proposed to account for imipenem resistance in *B. distasonis* TAL7860, which had an enzyme with scanty ability to hydrolyze the drug (93). The second group of unusual β-lactamases in *Bacteroides* spp. comprises zinc-dependent types belonging to molecular class B. These are both more homogeneous and more disturbing than the class A types. Closely similar zinc enzymes dubbed CerA or CfiA have been found in isolates from France, Sweden, the United Kingdom, the United States, and Japan (204). The first example was described by Yotsuji et al. (278). Like other class B enzymes, they are strongly active against carbapenems and cephamycins and are resistant to all inhibitors. All these drugs consequently lack activity against enzyme producers (Table 13). Only the monobactams escape hydrolysis, and they cannot bind the PBPs of *Bacteroides* spp.! Expression of these enzymes is unusual in that their gene commonly is silent; hence, Podglajen et al. (195) reported that although 8 of 550 *B. fragilis* isolates from 21 French hospitals had the carbapenemase gene, only 4 produced the enzyme. Expression and resistance demanded migration of an insertion sequence upstream of the β-lactamase gene, and this rearrangement occurred spontaneously at a frequency of ca. 10^{-7} (194, 195). There appears to be a risk of enzyme-expressing variants being selected during therapy, but no case reports have appeared as yet. On the other hand, the risk of producers spreading seems small, allowing that most infection with *Bacteroides* spp. is endogenous in its origin. The gene for the zinc carbapenemase was reported to be plasmid borne in one strain (14), but this situation seems unusual.

β-Lactamases also occur and cause resistance to penicillins and cephalosporins in many non-*B. fragilis Bacteroides* spp., *Prevotella* spp., *Porphyromonas* spp., and fusobacteria (266), as confirmed by the fact that these organisms typically are resistant to ampicillin, piperacillin, and cefoperazone but are susceptible to ampicillin-sulbactam, amoxicillin-clavulanate, ticarcillin-clavulanate, and piperacillin-tazobactam (7–9). Besides their susceptibility to β-lactamase–inhibitor combinations, these species tend also to be susceptible to cephamycins and carbapenems, as are members of the *B. fragilis* group (7). For many non-*B. fragilis Bacteroides* spp., at least, this behavior correlates with detection of cefuroximase-type (Bush 2e) enzymes resembling those of the *B. fragilis* group (9).

TESTS FOR β-LACTAMASE PRODUCTION

Many tests for β-lactamase production, entailing either chromogenic reactions or detection of the destruction of antibiotic activity have been proposed (145, 238). The chromogenic methods are faster and more convenient, and they divide into those in which the hydrolysis of the β-lactam itself engenders a color change and those in which this change depends on a linked reaction. In the former group are nitrocefin (175), which changes from yellow to pink/red on hydrolysis, and 7-(thienyl-2-acetamido)-3-[2-(4-*N*,*N*-dimethylaminophenylazo) pyridinium methyl]-3-cephem-4-carboxylic acid (PADAC) (228), which changes from violet to yellow. Of these two, nitrocefin is the more readily available (albeit expensively) and can be used as a solution or as disks upon which test cultures are smeared. It is highly sensitive to most β-lactamases, although false-negative results are a risk for *H. influenzae* isolates with ROB-1 β-lactamase and for staphylococci, in which uninduced penicillinase levels are often inadequate to give a color reaction. These problems are minor, since ROB-1 enzyme is rare and β-lactamase tests are rarely performed on staphylococci. Linked detection systems include the iodometric and acidimetric methods. The former depends on the fact that the hydrolysis products of β-lactams reduce iodine to iodide; consequently, decolorization of starch-iodine complex occurs if an isolate is a β-lactamase producer but not if the enzyme is absent (238). Acidimetric tests depend on the fact that opening the β-lactam ring generates a free carboxyl and that this acidity can turn bromocresol purple from violet to yellow in an unbuffered system (238). Acidimetric and iodometric tests can be performed with bacterial suspensions or on paper strips impregnated with the appropriate reagents. These methods are cheaper than nitrocefin and, given care, almost as sensitive, but they are more prone to false-positive results. With iodometric tests, such errors probably reflect nonspecific reaction of iodine with bacterial proteins; for acidimetric tests, false-positive results arise if the inoculum or the distilled water used to moisten the test strip is slightly acidic. I have found it better to moisten acidimetric strips with tap water rather than distilled water, but this tactic may not help those without London's calcareous water supply! More generally, these problems underscore the importance of performing parallel controls with known enzyme producers and nonproducers.

Such β-lactamase detection tests do not provide any infor-

mation about what type of β-lactamase is present. Preliminary typing can, however, be achieved by running parallel tests in the presence and absence of 0.1 mM clavulanate and 0.1 mM cloxacillin (44, 264): most class A enzymes are inhibited by clavulanate but not cloxacillin, whereas class C enzymes give the opposite pattern; class B and most class D enzymes are inhibited by neither compound. These methods are, however, most useful for preliminary β-lactamase typing in surveys rather than routine use. Their main limitation is that increasing numbers of isolates have multiple β-lactamases and that these, obviously, can distort the inhibition patterns observed.

Aside from chromogenic methods, β-lactamase production may be detected by various biological methods, which depend on the β-lactamase produced by one organism allowing an indicator strain to grow. Examples include "clover leaf plates" (238) and Masuda double-disk tests (150). These tests are extremely sensitive but are slow and tedious. More useful biological methods are the double-disk tests used to examine for synergy between clavulanate and ceftazidime and, thereby, to detect ESBLs. These were discussed in an earlier section, as were double-disk tests to detect β-lactamase induction.

At present, most hospitals perform direct β-lactamase tests only on *Haemophilus* and *Neisseria* spp. and *M. catarrhalis*. They are desirable for the first two species because their β-lactamases often give only low level resistance, which may pass undetected. This argument is true also for *M. catarrhalis*, although β-lactamase production is now so widespread in this species that it seems simpler to assume its presence and to avoid labile drugs. There is little reason why a clinical laboratory should perform β-lactamase production tests on enterobacteria or pseudomonads. What is more useful with these species is to try to predict the type of enzyme present from the antibiogram, and this aspect is covered in the next and final section of this review.

PREDICTING ENZYME TYPE FROM THE ANTIBIOGRAM

If particular resistance patterns are associated with particular β-lactamases, it follows that it should be possible to predict an isolate's enzyme type from its antibiogram. This is an aspect of what Courvalin (49) dubbed "interpretive reading of susceptibility tests." Its potential value is twofold. First, once an isolate's resistance mechanism is inferred, one can usefully edit the antibiogram reported to the clinician, and second, one can predict which further antimicrobial agents might merit testing. If, for example, a *Klebsiella* isolate is resistant to penicillins, narrow-spectrum cephalosporins, ceftazidime, and aztreonam but appears susceptible to cefotaxime and cefoxitin, one can reasonably guess that it had a ceftazidime-preferring ESBL, such as TEM-10 or TEM-26. Experience suggests that producers of these enzymes fail to respond to cefotaxime, and the result for this antibiotic should be reported as resistant. Furthermore, the inference of this mechanism suggests that it is futile to test further extended-spectrum cephalosporins but that carbapenems and inhibitor combinations may merit testing. If, on the other hand, the *Klebsiella* isolate was also resistant to cefoxitin and cefotaxime, its likely mechanism would have been a plasmid-mediated AmpC enzyme and there would be little point in testing inhibitor combinations. Such interpretive reading can save time, materials, and money and, more important, can help to ensure that the patient receives appropriate therapy.

Certain prerequisites are necessary to allow such prediction. First and foremost, bacteria must be identified to the species level. One can devise schemes for predicting the resistance mechanisms from antibiogram in, e.g., *Klebsiella*, *E. coli*, or *Enterobacter* isolates, but no scheme can predict the mechanisms present from antibiograms for unidentified "coliforms." Second, it is necessary to test a wide range of β-lactams, some of which would not be obvious therapeutic choices. Of particular importance are (i) the comparison of susceptibility to penicillins with and without inhibitors; (ii) ceftazidime, which is the best indicator for ESBLs and which helps distinguish these enzymes from hyperproduced K1 β-lactamase in *K. oxytoca*; (iii) cefoxitin, which is a good indicator of β-lactamase inducibility in *Enterobacter* spp. and *C. freundii* and which helps test whether a *Klebsiella* isolate has an ESBL or an AmpC enzyme; and (iv) carbapenems, which escape most β-lactamase-mediated resistance. Third, it is preferable to record susceptibility and resistance quantitatively, whether as inhibition zone diameters or MICs, rather than solely in categorized (susceptible/intermediate/resistant) form. This allows biological resistance to be noted and given significance. Finally, one must have a good memory for patterns or, better, a computer that can compare antibiogram data for current isolates with those for reference strains with known resistance mechanisms. Several automated or semiautomated susceptibility testing systems already offer "expert rules" based on this type of analysis. These may not actually tell the user which mechanism an isolate has, but they work by predicting the mechanism and then reviewing the susceptibility data accordingly. For those who prefer to analyze their data manually or to program their own computers, the resistance patterns associated with the more important β-lactamases have been summarized, by the use of bold and italic fonts or differing symbols, in Tables 3, 5, 6, 7, 9, 10, 12, and 13 of this review.

Prediction has limits. First, it is inappropriate for organisms in which relationships between antibiogram and mechanism are poorly established, such as *Acinetobacter* spp. Second, isolates with exceptionally large or small amounts of enzyme may behave anomalously, especially with β-lactamase inhibitor combinations. Third, prediction fails when isolates have multiple mechanisms of resistance or more than one type of β-lactamase. Such organisms are an increasing problem, particularly in developing countries and intensive care units. Finally, β-lactamases continue to evolve and to be recognized, and some isolates with unusual antibiograms really do have novel enzymes!

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