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Review

Resistance to *β*-lactam antibiotics

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Abstract. *β*-lactams have a long history in the treatment of infectious diseases, though their use has been and continues to be confounded by the development of resistance in target organisms. *β*-lactamases, particularly in Gram-negative pathogens, are a major determinant of this resistance, although alterations in the *β*-lactam targets, the penicillin-binding proteins (PBPs), are also important, especially in Gram-positive pathogens. Mechanisms for the efflux and/or exclusion of these agents also contribute, though often in conjunction these other two. Approaches for overcoming these resistance mechanisms include the development of novel *β*-lactamase-stable *β*-lactams, *β*-lactamase inhibitors to be employed with existing *β*-lactams, *β*-lactam compounds that bind strongly to low-affinity PBPs and agents that potentiate the activity of existing *β*-lactams against low-affinity PBP-producing organisms.

Key words. *β*-lactam; *β*-lactamase; penicillin-binding protein; efflux; impermeability.

Introduction

β-lactam antibiotics are an important component of the antimicrobial armamentarium of the infectious disease specialist, used in the treatment of a variety of Gram-negative and Gram-positive infections [1]. These agents represent >65% of the world antibiotic market with >50 marketed drugs of this class which include the penicillins, cephalosporins, carbapenems, monobactams (reviewed in [2, 3]) and more recently the penicillin-cephalosporin hybrids, the penems (e.g. faropenem) [4]). Characterized by a four-membered *β*-lactam ring, these agents target the bacterial enzymes of cell wall biosynthesis (the so-called penicillin-binding proteins, PBPs) [2, 3], although the actual mechanism of killing is as yet unresolved [5]. Resistance to these agents is, unfortunately, all too common in Gram-positive [6, 7] and Gram-negative [8–11] bacterial pathogens and occurs as a result of drug inactivation by *β*-lactamases, target site (i.e. PBP) alterations, diminished permeability and efflux [2].

β-lactamases

A major mechanism of *β*-lactam resistance, particularly amongst Gram-negative bacteria, is the production of *β*-lactamases, hydrolytic enzymes that disrupt the amide bond of the characteristic four-membered *β*-lactam ring, rendering the antimicrobial ineffective [12, 13]. Intriguingly, *β*-lactamases are structurally related to PBPs [14] and may have evolved from these *β*-lactam-binding enzymes of cell wall biosynthesis. First reported in *Escherichia coli* isolates prior to the clinical release of the first *β*-lactam, penicillin, these enzymes have since been described in a myriad of Gram-negative and Gram-positive organisms and in the mycobacteria [12, 15], where they are variably chromosomally or plasmid encoded, often associated with mobile genetic elements such as transposons and integrons (see [16] for a review on integrons). Four molecular classes of *β*-lactamases are known, dubbed A–D (table 1), and include both metal-dependent (Zn2+-requiring; class B) and metal-independent (active site serine; classes A, C and D) enzymes [12, 13]. Numerous kinetic, mutagensis and structural studies

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Table 1. Classification and properties of β-lactamases

Ambler classification	Type of enzyme	Preferred substrates	Inhibited by:		Representative enzymes
			CA[a]	EDTA	
A	penicillinase	penicillins	+	–	penicillinases from Gram-positive bacteria
	restricted-spectrum β-lactamase	penicillins, cephalosporins	+	–	TEM-1, TEM-2, SHV-1
	extended-spectrum β-lactamase	penicillins, narrow-spectrum and extended-spectrum cephalosporins, monobactams	+	–	numerous SHV and TEM variants, CTX-M-1 to -28, PER-1 & -2, VEB-1, GES-1, IBC-1, several chromosomal enzymes in Gram-negative bacteria
	inhibitor-resistant β-lactamase	penicillins, cephalosporins	–	–	TEM-30 to -41, -44, -45, -51, -54
	inhibitor-resistant extended-spectrum β-lactamase	penicillins, narrow-spectrum cephalosporins, extended-spectrum cephalosporins (low level)	–	–	TEM-50, -68, -80
	carbapenemase	penicillins, cephalosporins, carbapenems, monobactams; sometimes extended-spectrum β-lactams	+	–	NMC-A, SME-1 to -3, IMI-1, KPC-1 to –3, GES-2, SHV-38
B	carbapenemase	most β-lactams, including carbapenems and extended-spectrum β-lactams and 4th generation cephalosporins	–	+	IMP-1 to -13, VIM-1 to -7, SPM-1, several chromosomal enzymes of Gram-negative bacteria
C	expanded-spectrum cephalosporinase	penicillins, narrow and extended-spectrum cephalosporins, cephamycins, monobactams	–	–	CMY-2 to -13, LAT-1, MOX-1 and -2, FOX-1 to -6, ACT-1, MIR-1, DHA-1 and -2, ACC-1, CFE-1, several chromosomal enzymes of Gram-negative bacteria
D	narrow-spectrum penicillinase	penicillins, cloxacillin	±	–	numerous OXA variants
	extended-spectrum β-lactamase	penicillins, cloxacillin, extended-spectrum β-lactams, sometimes monobactams or 4th generation cephalosporins	±	–	several OXA-2 and -10 derivatives, OXA-18, -29, -30, -31, -32, -45
	carbapenemase	penicillins, oxacillin, carbapenems	+	–	OXA-23 to -27, -40, -48, -54

[a] Clavulanic acid.

have been performed on these enzymes, providing important details of their catalytic mechanisms and substrate specificities (reviewed in [12, 13, 17]). Of particular concern are enzymes able to target the expanded spectrum β-lactams, including the AmpC (class C cephalosporinases) enzymes [18, 19], the so-called extended spectrum β-lactamases (ESBL) (classes A and D) [20–22] and the carbapenemases that hydrolyze most β-lactams, including the carbapenems (classes A, B and D) [23, 24].

Extended spectrum β-lactamases

First appearing following the widespread use of broad-spectrum β-lactams in the early 1980s, ESBLs constitute a major problem in the use of β-lactams to treat infectious disease given their broad substrate specificity and ability to hydrolyze many of the extended-spectrum, third-generation cephalosporins (reviewed in [20–22]). Typically plasmid encoded but also present on chromosomes, often in association with integrons, these enzymes are derivatives, predominantly, of class A and class D β-lactamases and are generally inhibited by available β-lactamase inhibitors (table 1). Found in a range of Gram-negative or-

ganisms [25], particularly members of the Enterobacteriaceae [26–34], these enzymes are most commonly reported in *E. coli* and *Klebsiella pneumoniae* [28, 34–37]. ESBL-producing organisms are typically resistant to penicillins, first- and second-generation cephalosporins as well as the third-generation oxyimino cephalosporins (e.g. cefotaxime, ceftazidime, ceftriaxone) and monobactams (aztreonam), retaining susceptibility only to cephamycins, fourth-generation cephalosporins (cefepime, cefpirome) and carbapenems [38]. Still, the presence of an AmpC enzyme [22, 27, 39] or loss of porins [38] in ESBL producers will compromise cephamycin use. Moreover, cefepime effectiveness in treating ESBL producers is uneven and often less effective than anticipated [40–42], shows adverse inncolum effects [43, 44] and its use in treating ESBL producers can lead to clinical failure [45]. In some instances, porin loss in ESBL-producing strains increases resistance to fourth-generation cephalosporins and/or carbapenems (e. g. [46, 47]). The description, too, of in vitro-selected TEM-1 variants providing resistance to cefepime [48] suggests that ESBL variants resistant to fourth-generation cephalosporins may occur in clinical strains in the future, under appropriate antibiotic selection.

ESBL families

Classical ESBLs evolved from class A TEM (from TEM-1 or TEM-2) and SHV (from SHV-1) enzymes, and these remain the most prevalent types of ESBLs, though class D ESBLs (i. e. of the OXA family) have also been known for some time [20, 21]. Still, over the past several years a plethora of non-TEM, non-SHV, non-OXA ESBLs have been reported in several organism all over the world (e.g. families BES, GES, PER, TLA, VEB and CTX-M) [20, 21], with CTX-M-type ESBLs, in particular, increasingly prevalent (reviewed in [49]). In contrast to the TEM-, SHV- and OXA-derived ESBLs, which result from mutation of their narrow-spectrum counterparts, many of these other ESBLs, which are typically plasmid encoded or otherwise mobile, originated from naturally occurring (i.e. chromosomal) enzymes that are innately broad spectrum.

TEM-/SHV-derived ESBLs

More than 150 TEM/SHV-derived ESBLs have been reported (http://www.lahey.org.studies/), most commonly in *E. coli* and *Klebsiella* spp. but also in other members of the Enterobacteriaceae, including *Serratia marcescens*, *Shigella dysenteriae*, *Morganella morganii*, *Citrobacter* spp., *Enterobacter* spp. , *Proteus* spp., *Providencia* spp., *Salmonella* spp. and *Leclercia adecarboxylata* [26, 27, 30, 31, 50–55] (and several references in [20]), as well as in *Burkholderia cepacia*, *Capnocytophaga*

ochracea, *Aeromonas* spp., *A. baumannii* and *Pseudomonas aeruginosa*, [56–63] (and several references in [20]). Amino acid substitutions responsible for the ESBL phenotype occur at a limited number of positions within theTEM and SHV enzymes, with mutations at Glu104, Arg164, Gly238 and Glu240 in TEM [20] and at Gly238 and Glu240 in SHV-1 of particular importance [20, 64]. Substitutions at Arg164 in TEM are typically associated with resistance to ceftazidime and substitutions at Gly238 with resistance to cefotaxime, while substitutions at the Gly238 and Glu 240 residues of SHV-1 are important for resistance to ceftazidme and cefotaxime, respectively [12, 20]. Avalable crystal structures for TEM [65, 66] and SHV [67]-derived ESBLs reveal enzymes with expanded/altered binding cavities that are apparently able to accommodate the bulky oxyimino substituent of extended spectrum agents such as cefotaxime and ceftazidime, a finding common to many ESBLs (e.g. [68–70]). Still, ESBL structures lacking an expanded binding cavity have been reported [71], which suggests that alternative ways of accommodating extended-spectrum β-lactams are possible.

A few inhibitor-resistant enzymes, derivatives mostly of TEM, have been described and though most of these are not ESBLs, a few rare instances of inhibitor-resistant enzymes that confer some resistance to extended spectrum β-lactams have been noted recently (e.g. TEM-50, TEM-68 and TEM-80) [21, 72]. Inhibitor-resistant TEM variants remain susceptible to inhibition by tazobactam and have been reported mostly in *E. coli* but also in *Klebsiella* spp., *Proteus. mirabilis*, *Citrobacter freundii*, *Shigella sonnei* and *E. cloacae* [13, 20, 72–74]. Inhibitor-resistant TEM (IRT) enzymes, like their ESBL counterparts, carry amino acid substitutions at a limited number of positions, with changes at Met69 most commonly encountered [20]. Intriguingly, the crystal structure of one such IRT reveals no alterations in the enzyme's three-dimensional (3-D) structure, reflecting a very modest impact of the Met69Leu change on the enzyme, in keeping, perhaps, with the need to maintain activity against its β-lactam substrates [75].

OXA-type ESBLs

ESBL-type members of the OXA family (OXA-18 and derivatives of OXA-2 and OXA-10) are comparatively rare, and found mostly in *P. aeruginosa* [20, 21, 63, 76], with the majority providing resistance to oxyiminocephalosporins such as ceftazidime, although a recently described variant, OXA-45, provides resistance to several third-generation cephalosporins as well as to aztreonam and a fourth-generation cephalosporin, cefepime [77]. OXA-29, a chromosome-encoded enzyme from *Legionella gormanii* also exhibited activity against oxyimino cephalorporins and aztreonam [78]. An un-

usual OXA ESBL from *P. aeruginosa*, OXA-31, provides resistance to cefepime but not to ceftazidime [79], and a similar enzyme (OXA-30) showing preference for fourth-generation cephalosporins has been described in *E. coli* [80]. OXA-type ESBLs typically carry multiple mutations, with OXA-10-derived ESBLs often carrying substitutions at Gly167 that are responsible for resistance to ceftazidime [12]. OXA-type ESBLs can be plasmid or chromosomal but apparently are not associated with classical integrons, in contrast to *oxa* determinants for narrow-spectrum enzymes.

CTX-M ESBLs
Members of the plasmid-encoded CTX-M series, of which at least 37 variants have been described [49], are geographically widespread [21, 32, 47, 81–87] and reported in several members of the Enterobacteriaceae, including *E. coli* [32, 83, 84, 87], *Enterobacter* spp. [32, 88], *S. marcescens* [32, 89], *Proteus* spp. [32, 84, 85, 90], *K. pneumoniae* [47, 83, 85, 86], *Salmonella* spp. [91–94], *C. freundii* [88], *Vibrio cholerae* [95], *Providencia stuartii* [32] and *S. sonnei* [96]. CTX-M enzymes preferentially hydrolyze cefotaxime over ceftazidime and do not generally confer resistance to the latter drug.

Interestingly, a chromosomal *β*-lactamase of *Kluyvera ascorbata* (KLUA-1) also demonstrates activity against extended spectrum cephalosporins, especially cefotaxime, reminiscent of the CTX-M series of ESBLs. Moreover, KLUA-1 and several CTX-M enzymes show a high degree of sequence similarity consistent with KLUA-1 being the progenitor of at least some of the plasmid-borne CTX-M family ESBLs [97, 98], particularly CTX-M-2 [91, 99]. Still, other studies indicate that the CTX-M-8 enzymes originated with the chromosomal *β*-lactamases of *Kluyvera georgiana* [100].

Other ESBLs
PER-1, first identified in *P. aeruginosa*, is also present in *S. enterica* serovar Typhimurium, *K. pneumoniae*, *A. baumannii* and *P. mirabilis* [20, 81, 101–103], and a highly related enzyme (86% amino acid homology), PER-2, has been identified in *S. enterica* serovar Typhimurium, *Enterobacter* spp., *K. pneumoniae* and *V. cholerae* [20, 32, 47, 95]. Related enzymes, which like PER preferentially hydrolyze and promote resistance to both oxy-iminocephalosporins (e.g. ceftazidime) and aztreonam, include VEB-1, found in *E. coli* [85, 104, 105], *P. aeruginosa* [56, 106, 107], *P. putida* [56], *Acinetobacter* spp. [108], *P. mirabilis* [85, 109], *Enterobacter* spp. [104], *C. freundii* [56] and *K. pneumoniae* [85, 104] (see also several references in [20]), and the CME-1 and TLA-21 enzymes reported in *Chryseobacterium meningospeticum* and *E. coli*, respectively [20]. The VEB-1 enzyme is usu-

ally integron and/or transposon associated [104, 105, 107–109] and typically plasmid-borne in Enterobacteriaceae but chromosomal in *P. aeruginosa* and *A. baumannii*. Other uncommon ESBLs include the SFO-1 enzyme of *E. cloacae* (related to the class A enzyme of *Serratia fonticola*) [110]), the BES-1 enzyme of *S. marcescens* related to a penicillinase of *Yersinia enterocolitica* [111], the DES-1 ESBL found in *Desulfovibrio desulfuricans* and related to the PenA *β*-lactamase of *Burkholderia pseudomallei* [112], and the FEC-1 enzyme found in a single isolate of *E. coli* [20]. Another ESBL related to the *Y. enterocolitica* enzyme, IBC-1, is encoded by an integron-associated gene first reported in *E. cloacae* [113], although it is also seen in *E. coli* and *K. pneumoniae* [114–116]. A related enzyme, IBC-2, has also been identified in *P. aeruginosa* [117]. Finally, a GES-1 enzyme first reported in *K. pneumoniae* [118–120] but also seen in *P. aeruginosa* [121] is an ESBL possibly related to a *P. mirabilis* *β*-lactamase [119]. A similar ESBL, GES-2, has been identified in *P. aeruginosa* [122], and like GES-1 [120, 121], its gene is integron associated [122].

ESBLs have not been reported in *Haemophilus influenzae*, though in vitro-derived mutants of the ROB-2 *β*-lactamase, an important determinant of cefaclor resistance in this organism, have been generated that provide resistance to extended spectrum *β*-lactams (e.g. cefotaxime) [123]. Interestingly, inhibitor (i.e. clavulanate)-resistant variants of this enzyme have also been isolated in vitro [123]. Similarly, clinical isolates of *B. pseudomallei* showing resistance to ceftazidime and clavulanate have been described as a result of mutation of this organism's chromosomal class A *β*-lactamase [124]. Expression of a class D *β*-lactamase in clinical isolates of *B. pseudomallei* has also been implicated in resistance to ceftazidime [125]. Finally, a *Klebsiella oxytoca* chromosomal enzyme, previously dubbed K1 and now called OXY-2, is also an ESBL [126], and a chromosomally encoded variant of this enzyme, OXY-2-5, has been reported in a clinical strain of this organism displaying resistance to ceftazidime [127].

Multidrug resistance in ESBL producers
ESBL-producing strains sometimes demonstrate resistance to an even broader range of *β*-lactams, as a result of coproduction of imported AmpC [22, 27, 39], their presence in strains stably derepressed for the chromosomal AmpC [128] or porin deficiency (the latter providing for pan-*β*-lactam resistance in some instances [47]). Co-resistance to non-*β*-lactams is also frequently seen in ESBL-producing Enterobacteriaceae [22, 129, 130], particularly to fluoroquinolones and aminoglycosides (e.g. [52, 129, 131–133]), with such multidrug resistance severely limiting therapeutic options in the treatment of these strains. The association of some ESBL genes with

integrons carrying additional antibiotic resistance genes, especially in non-fermentative Gram-negative organisms [107, 108, 121, 122] but also in the Enterobacteriaceae [91, 104, 113, 114, 118, 120] is one explanation for this.

AmpC

The class C cephalosporinase, AmpC, is a significant contributor to multiple β-lactam resistance in Gram-negative pathogens, particularly the Enterobacteriaceae [25, 29, 31, 134], and can be chromosomal or plasmid encoded. AmpC β-lactamases demonstrate activity against most penicillins and cephalosporins, including in many cases the oxyiminocephalosporins (cefotaxime, ceftazidime and cefpodoxime), cephamycins (e.g. cefoxitin, cefotetan) and monobactams (aztreonam), usually retaining susceptibility to carbapenems and, in some instances, fourth-generation cephalosporins (e.g. cefepime, cefpirome). Still, the susceptibility of AmpC-producing organisms to cefepime can be compromised by high innocula [135], and in vitro-derived AmpC variants active against cefepime have been reported [136, 137]. Production of mutant AmpC enzymes has also been described in clinical isolates resistant to fourth-generation cephalosporins [138, 139]. Finally, concomitant porin loss in AmpC-producing strains can provide resistance to carbapenems (e.g. [140–142]). Unlike the class A β-lactamases, including most ESBLs, AmpC β-lactamases are not readily inhibited by approved β-lactamase inhibitors.

Chromosomal AmpC β-lactamases

Genes encoding AmpC enzymes are present in the chromosomes of several Enterobacteriaceae (C. freundii, E. coli, Enterobacter spp., P. stuartii, M. morganii, S. marcescens (and other Serratia spp.), Y. enterocolitica (and other related Yersinia spp.), Hafnia alvei, Buttiauxella spp. and Ewingella americana [19, 143–146] and other Gram-negative organisms (P. aeruginosa, Aeromonas spp., A. baumannii and Ochrobactrum anthropi) [19, 143, 147] and, with the exception of E. coli, are inducible by a number of β-lactam antibiotics, though apparently poorly if at all by many of the newer cephalosporins. And while carbapenems are, in fact, good inducers of AmpC enzymes, their rapid bactericidal activity and stability to hydrolysis nonetheless renders them effective against AmpC-producing organisms [148]. A number of chomosomal wild-type AmpC enzymes are not effective at hydrolyzing extended-spectrum β-lactams such as the oxyiminocephalosporins (e.g. enzymes from E. coli, C. freundii and E. cloacae), owing to an inability to bind these agents in a catalysis-competent conformation [149]. Still, mutant variants capable of accommodating oxy-

iminocephalosporins have been described (e.g. the GC1 variant of the E. cloacae P99 AmpC) whose structures now permit efficient hydrolysis of these agents [150].

Expression of chromosomal ampC genes are typically influenced by the AmpR regulator (negatively in the absence of inducer, positively in the presence of inducer) and the ampD-encoded cytosolic amidase whose activity (in the absence of inducer) negatively impacts ampC expression (reviewed in [151]). AmpC-mediated resistance, especially to many of the newer-generation β-lactam, typically results from mutational expression of the ampC gene [134], often owing to mutations in ampR [152] or ampD [151, 153, 154], though promoter mutations may also contribute [155]. Overexpression of a two-component system response regulator gene, fimZ, has also been shown to promote AmpC-dependent β-lactam resistance in E. coli, though how is unclear [156]. ampC derepression in the absence of any of these has also been reported, indicating that other means for upregulating this enzyme exist [154]. Finally, recent reports on AmpC-mediated β-lactam resistance in A. baumanni show that ampC hyperexpression can occur as a result of upstream insertion of an insertion sequence (IS) element, possibly due to an IS-provided strong promoter [157, 158].

Stable depression of chromosomal AmpC enzymes is a significant determinant of resistance to β-lactams, particularly the newer, broad-spectrum cephalosporins, in several Enterobacteriaceae [134, 148], particularly Enterobacter spp., Citrobacter spp. and S. marcescens [133, 159–161], and is seen, also, in clinical strains of P. aeruginosa [134, 162–164] and Acinetobacter spp. [157, 165]. A chromosomal AmpC enzyme (OCH-1) also explains the general resistance of O. anthropi to most β-lactams with the exception of the carbapenems [147].

Plasmid-encoded AmpC β-lactamases

Of increasing importance as regards resistance to expanded-spectrum β-lactams are plasmid-encoded AmpC β-lactamases whose spread to historically AmpC⁻ organisms, particularly Klebsiella spp. [166, 167] and E. coli (although it carries a chromosomal ampC gene, it is generally expressed at only very low levels), threatens to compromise the use of these important therapeutic agents (see [18, 19] for recent reviews). Most plasmid-borne ampC genes are not inducible, owing to the absence of a corresponding ampR regulatory gene, and overproduction of the AmpC in resistant strains likely results from promoter alterations/mutations rather than, for example, plasmid copy number effects or loss of AmpR repression [168]. Plasmid-borne ampC genes originate with the chromosomes of certain naturally AmpC⁺ organisms (see below) from which they have been mobilized. Consistent with this, many of these are associated with integrons and transposable elements, though unlike plasmid-borne β-

lactamases of classes A, B and D, they are not contained in characteristic gene cassettes [19]. AmpC plasmids can carry additional resistance determinants for non-β-lactams such that their acquisition can promote multidrug resistance [169–172]. On the basis of amino acid sequence homologies, plasmid-encoded AmpC β-lactamases are divided into five families, C-1 to -5 [19].

Families of plasmid-borne AmpC β-lactamases

Family C-1, whose members are all closely related and are among the most disseminated geographically appear to be derived from the chromosomal AmpC enzyme of *C. freundi* [173, 174]. Members include CMY-2 (= BIL-1 = LAT-2; [18]), CMY-3, CMY-4, CMY-5, CMY-6 (= LAT-3; [18]), LAT-1 (= LAT-4; [18]) (reviewed previously in [19]), three recently identified CMY variants, CMY-7 [175], CMY-12 [176] and CMY-13 (GenBank Accession number AAQ16660), and the recently reported CFE-1 [177]. CMY-2 has been reported in a variety of *Salmonella* spp. [19, 178-180], *E. coli* [19, 179-182], *Klebsiella* spp. [19, 176, 179, 180] and *Proteus* spp. [19, 179]. CMY-4 has been reported in *S. enterica* servar Wein [140], *E. coli* [19] as well as *K. pneumoniae* and *P. mirabilis* [19, 176]. CMY-5 and CMY-6 have been described in *K. oxytoca* and *E. coli*, respectively [19], and CMY-7 in both *E. coli* [19] and *S. enterica* serovar Typhimurium [175]. The lone examples of CMY-12 and CMY-13 reported to date are found in *P. mirabilis* and *E. coli*, respectively, with CMY-12 being chromosomal owing, presumably, to transposon/plasmid mobilization [176]. Finally, the LAT series has been described in both *E. coli* and *K. pneumoniae* [19].

Family C-2 includes CMY-1, CMY-8 through-11, MOX-1, MOX-2 and FOX-1 through-6 [19] and appear to be derived from the chromosomal AmpC β-lactamases of *Aeromonas* spp. [183–185]. CMY-1, -9 and -11 are found in *E. coli* [184, 186, 187] with CMY-1 and CMY-8 also found in *K. pneumoniae* [19, 186]. Intriguingly, the CMY-10 enzyme was described in a strain of *E. aerogenes* [188], which is unusual given the natural occurrence of a chromosomal AmpC enzyme in this organism. MOX-1 and MOX-2 have both been described only in *K. pneumoniae* [19, 189]. Organisms harbouring the FOX series include *K. pneumoniae* (FOX-1 through -6) [19, 135, 185], *E. coli* (FOX-2 and FOX-4) [19] and *K. oxytoca* [19].

The C-3 family includes the ACT-1 and MIR-1 enzymes which, despite earlier suggestions that they originated from the chromosomal enzyme of *E. cloacae*, appear in fact to be most closely related to the chromosomal AmpC of *Enterobacter asburiae* [190]. ACT-1 has been described in *K. pneumoniae* [19, 135, 191] and MIR-1 in *E. coli* [19], with the former occurring, in some instances, in the chromosome, again suggestive of horizontal transfer

from another organism. Unlike most plasmid-borne AmpC enzymes, plasmid-encoded ACT-1 in *K. pneumoniae* isolates was shown to be inducible [191].

The C-4 family includes DHA-1 (found in *Salmonella enteriditis* and *K. pneumoniae*; [192]] and DHA-2 (found in *K. pneumoniae*; [19]) and appear to have originated from *M. morganii* [193, 194]. The DHA enzymes, like some plasmid-borne ACT-1, are inducible [19, 195].

The lone example of the C-5 family is the ACC-1 enzyme found in *K. pneumoniae* [19], *S. enterica* serovar Mbandaka [196], *E. coli* [197] and *P. mirabilis* [197] and apparently related to the chromosomal AmpC of *H. alvei* [198, 199].

Carbapenemases

Carbapenems (e.g. imipenem, meropenem, biapenem, panipenem, ertapenem) are an important class of β-lactams owing to their stability to most β-lactamases (reviewed in [200]) and are of particular use in treating infections associated with ESBL- and AmpC-producers. β-lactamases capable of hydrolyzing carbapenems are known, though comparatively rare and are found amongst three of the four molecular classes of β-lactamases, A, B and D (reviewed in [23, 24, 201]). An apparently novel carbapenem-hydrolyzing β-lactamase not inhibited by either EDTA or a serine β-lactamase inhibitor has been reported in an imipenem-resistant isolate of *Aeromonas veronii* biovar sobria, AVS-1 [202]. Carbapenemases can occur naturally, often in environmental strains of limited clinical significance, and can be acquired by clinically relevant organisms where they can be plasmid or chromosomally encoded.

Class A carbapenemases

Class A β-lactamases with activity against carbapenems are uncommon and are active site serine enzymes that are inhibitable by available β-lactamase inhibitors (e.g. clavulanate). These enzymes are able to hydrolyze some penicillins, early (first- and second-)generation cephalosporins, imipenem (and sometimes meropenem) and aztreonam. Examples include the chromosomal NMC-A enzyme from *E. cloacae*, the SME-1 through -3 enzymes from *S. marcescens* and IMI-1 from *E. cloacae*, and the plasmid-encoded GES-2 from *P. aeruginosa* and KPC-1 from *K. pneumoniae* [24]. The crystal structure of NMC-A reveals an enzyme with several structural differences in the vicinity of the active site relative to other class A (non-carbapenemase) enzymes, including additional space in the region where e.g. carbapenems would need to be accommodated [203]. Recently, a second plasmid-encoded KPC enzyme, KPC-2, was reported in *K. oxytoca* [204], *K. pneumoniae* [36, 205] and *S. enterica*

serovar Cubana [206]. A KPC-3 enzyme has also been described, also in *K. pneumoniae* (GenBank accession number AF395881). The KPC enzymes are active against most β-lactams, including oxyiminocephalosporins and imipenem. GES-2 is derived from the ESBL GES-1 from which it differs by a single amino acid, and while it hydrolyzes imipenem much more effectively as a result, it is still much less active than other enzymes of this class, producing reduced but not insusceptibility in strains harbouring it [23, 24, 122]. Not surprisingly, the KPC (transposon) and GES-2 (integron) enzymes are associated with mobile genetic elements, with GES-2 one of only two class A β-lactamases known to be integron associated (the VEB-1 ESBL is the other) [24].

Class B metallo-β-lactamases
Class B metal-requiring enzymes are divided into three subclasses, B1-3 [207], all exhibiting resistance to commercially available β-lactamase inhibitors but inhibitable by metal ion chelators such as EDTA. This class of enzymes is of particular interest and concern owing to the ability of many of these to hydrolyze and, thus, provide resistance to virtually all classes of β-lactams, including the carbapenems. Despite the lack of activity against monobactams, the presence of additional β-lactamases (e.g. chromosomal or acquired AmpC) tends to compromise the use of these agents in metallo-β-lactamase-producing strains. Naturally occurring, generally chromosomal class B metallo-β-lactamases have been described in a number of environmental species, most of which are of limited clinical significance [23, 24, 208]. A number of clinical *Burkholderia cepacia* isolates producing an inducible metalloenzyme (PCM-I) that also shows preferential hydrolysis of carbapenems/imipenem have also been described [208]. A limited number of *B. fragilis* isolates have been shown to produce a chromosomal metalloenzyme, CcrA (a.k.a. CfiA) that provides resistance to e.g. imipenem [209].
Acquired carbapenem-hydrolyzing metallo-β-lactamases are generally of two types, IMP and VIM, are plasmid or chromosome encoded and usually associated with integrons carrying addition resistance genes. Enzymes of the IMP series hydrolyze most β-lactams except monobactams and were first described in a clinical strain of *S. marcescens* from Japan [24]. There are currently 13 IMP-type metallo-β-lactamases described in the literature (IMP1-9 [23, 24], IMP-10 [210], IMP-11 (GenBank accession number AB07437), IMP-12 [211] and IMP-13 [212]). These have been described in a number of organisms, particularly *P. aeruginosa* [210, 212–217] and *A. baumanii* [214, 215, 217–219], but also *P. putida* [211, 217], *K. pneumoniae* [221], *E. cloacae* [222], *Alcaligenes xylosoxidans* [210, 217], *Shigella flexneri* [23, 24], *S. marcescens* [217] and *Citrobacter youngae* [23, 24],

mostly in Europe and the Far East, though IMP-7 has been described in North America (Canada) [224].
Enzymes of the VIM series were first described in a *P. aeruginosa* isolated from Italy and like the IMP enzymes hydrolyze most β-lactams very well, again with the exception of monobactams [24]. Seven VIM-type enzymes have been described to date (VIM-1 and -2 [23], VIM-3 [223], VIM-4 [224], VIM-5 (GenBank accession number AY144612), VIM-6 (GenBank accession number AY165025) and VIM-7 [225] mostly in Europe and the Far East, though a VIM-7-carrying *P. aeruginosa* isolate was recently reported in the US [225]. VIM type enzymes have been described in several organisms including *P. aeruginosa* [214, 217, 223–231], *P. putida* [217, 229, 232], *Acinetobacter* spp. [233], *A. xylosoxidans* [23, 24], *E. cloacae* [234, 235], *S. marcescens* [236], *K. pneumoniae* [235, 237], *E. coli* [238] and *C. fruendii* [222] (see additional references in [23, 24]).
Recently, a third class of acquired metallo-β-lactamase, SPM-1, was found in *P. aeruginosa* [239–241]. A chromosomally encoded SHV-type enzyme (SHV-38) providing reduced susceptibility to ceftazidime and imipenem in *K. pneumoniae* was also recently described [242]. This is the first report of an SHV enzyme with imipenem hydrolytic activity.

Class D oxacillinases with carbapenemase activity
A limited number of class D enzymes with some activity against carbapenems have been reported, mostly in *A. baumannii* (e. g. OXA-23 through -27 [24, 201]) found in Europe, South America and the Middle and Far East. Chromosomal OXA-23 has also been reported in *P. mirabilis* [243], and a chromosome-encoded novel OXA variant with activity against carbapenems, OXA-40, has recently been described in *A. baumannii* [244, 245]. Plasmid-encoded OXA-48 with activity against imipenem has also been described in *K. pneumoniae* [246]. A related (98% identity) carbapenem-hydrolyzing class D enzyme, OXA-54, was shown recently to be chromosome-encoded in *Shewanella oneidensis* and is proposed to be the progenitor for carbapenem-hydrolyzing oxacillinases [247]. These enzymes do not generally hydrolyze extended-spectrum β-lactams (OXA-40 has some activity against ceftazidime) or aztreonam but are active against oxacillin (OXA-27 being an exception). While class D carbapenemases typically display weak activity against carbapenems in vitro they are, nonetheless, associated with carbapenem resistance in vivo, possibly owing to the limited permeability of host strains. In contrast to most class D oxacillinases, those with carbapenemase activity are inhibited by one or more of the available β-lactamase inhibitors.

Gram-positive and mycobacterial *β*-lactamases

β-lactamases are generally uncommon in Gram-positive organisms of clinical significance (e.g. *S. pneumoniae* and *Enterococcus* spp. [248, 249]), although chromosomal and plasmid-encoded (i.e. BlaZ) penicillinases are prevalent in *Staphylococcus aureus* [250]. A plasmid-encoded enzyme of presumed staphylococcal origin, BlaZ, has, however, been described in *Enterococcus faecalis* [251]. While these enzymes are generally only active against older-generation *β*-lactams, borderline or low-level resistance to newer agents such as methicillin in *S. aureus* has been attributed to hyperproduction of native *β*-lactamases and/or production of a novel enzyme with activity against methicillin [252]. Recent studies on *Bacillus anthracis* reveal the presence of two chromosomal *β*-lactamase genes, *bla1* and *bla2* [253], encoding a class A penicillinase and class B cephalosporinase with activity against carbapenems, respectively [254]. Still, these genes are expressed weakly if at all in most strains [253], although *β*-lactamase-mediated resistance to penicillins in this organism has been reported [255]. Mycobacterial resistance to *β*-lactams is commonplace and generally attributed to the production of *β*-lactamases by these organisms [256, 257]. In *Mycobacterium tuberculosis* the major enzyme is a class A penicillinase [258], although an enzyme with cephalosporinase activity has also been reported in this organism and, indeed, other mycobacteria [257, 258].

Target site mutations

Gram-negative organisms

Although *β*-lactamases are associated with resistance to *β*-lactams in organisms such as *H. influenzae* and *Neisseria* spp., increasingly, non-*β*-lactamase resistance to these agents is seen in *H. influenzae* [259], *Neisseria gonnorhoeae* [260] and *Neisseria meningitidis* [261], possibly due to alterations in PBPs. Indeed, mutations in PBP genes have been reported in *β*-lactam-resistant strains of these organisms (*H. influenzae* [262−265], *N. gonorrhoeae* [266−268] and *N. meningitidis* [269−271]), including strains resistant to extended-spectrum cephalosporins [263, 267, 268]. Interestingly, the PBP genes (i.e. *penA*) of resistant *Neisseria* spp. often show a mosaic-like structure reminiscent of *β*-lactam-resistant *S. pneumoniae*, consistent with horizontal transfer of *penA* sequences from e.g. other resistant *Neisseria* [267, 269]. Resistance to carbapenems owing to changes in PBPs has been seen in *A. baumanni* [272] and *P. mirabilis* [273]. PBP changes responsible for *β*-lactam resistance in anaerobes (e.g. *B. fragilis* group [274, 275] and *Veillonella* spp. [276]), including resistance to extended-spectrum *β*-lactams [275], have also been reported. Pro-

duction of altered PBPs with reduced affinity for *β*-lactams is seen, too, in *β*-lactam-resistant *S. dysenteriae* [277], *P. aeruginosa* [278] and *Helicobacter pylori* [279, 280].

Enterococci

The enterococci are intrinsically resistant to most *β*-lactams, including the newer cephalosporins as a result of production of a low-affinity PBP, PBP5 [281], although high-level resistance to e.g. ampicillin in clinical isolates of *Enterococcus* spp. is typically achieved by overproduction (e.g. [282]) or mutation (e.g. [283, 284]) of this PBP. Resistance to imipenem owing to hyperproduction of an altered PBP5 with reduced affinity for this agent has also been seen in *E. faecium* [285]. A *β*-lactam-responsive two-component sensor kinase-response regulator, CroRS, was recently described in *E. faecalis* and shown to be essential for PBP5-mediated *β*-lactam resistance, although it was not required for PBP5 production [286]. Recently, too, PBP5-independent high-level resistance to ampicillin was confirmed in *E. faecium* as involving a bypass of this otherwise essential PBP, as a result of its transpeptidase function being replaced by a *β*-lactam-insensitive transpeptidase [287].

S. pneumoniae

β-lactamases are virtually unheard of in *S. pneumoniae*, with resistance to these agents almost exclusively resulting from changes to the PBP targets of the *β*-lactams [288–293], such changes decreasing the affinities of the PBPs for *β*-lactams [292]. While point mutations in PBP genes are associated with *β*-lactam resistance in lab isolates, clinical isolates typically express PBPs whose genes vary substantially from wild-type *pbp* genes, and these so-called mosaic genes have been taken as evidence of horizontal transfer of ready-made resistant genes that have evolved in other *Streptoccus* spp. (reviewed in [248, 294]). Despite the substantial variation in sequence between the PBPs of susceptible and resistant isolates, recent in vitro studies suggest that only a few mutations are important for resistance [295–297]. This was confirmed by the crystal structure of one of these, PBP 2X, bound to cefuroxime, which highlighetd only a limited number of residues as being directly related to resistance [298]. Unlike *S. aureus* where production of a single, low-affinity PBP is sufficient for resistance to virtually all *β*-lactams, significant *β*-lactam resistance in *S. pneumoniae* is associated with alterations to several (e.g. PBPs 1A, 2B and 2X, [289, 290]), and sometimes all of the organism's six endogenous PBPs [248, 294]. A specific mutation in PBP2x, Met339Phe, has been reported in several highly resistant strains that carry, as well, multiple mutations in this and other PBPs. This mutation, alone and with a sec-

ond mutation common in resistant strains, Thr338Ala, has been shown to reduce susceptibility of the PBP to the action of β-lactams, apparently owing to their alteration of the stucture of the PBP2X active (i.e. β-lactam binding) site [297].

Resistance associated with expression of low-affinity PBPs in *S. pneumoniae* is dependent upon functional MurM, a muropeptide branching enzyme that works together with a similar enzyme, MurN, and whose inactivation compromises such resistance [299, 300]. The significance of peptide branching in the context of low-affinity PBP production and β-lactam resistance is, however, still unclear. As with *E. faecium*, β-lactam resistance associated with mutations in a two-component system, CiaHR, has been noted, with resistance attributable to constitutive expression of a functional CiaR response regulator [301]. *ciaH* mutations also have a negative impact on transformability, a phenotype shared by another non-PBP mutation associated with β-lactam resistance in *S. pneumoniae*, *cpoA* [248].

S. aureus

Although β-lactamases have been an important determinant of β-lactam resistance in *S. aureus*, resistance to now important β-lactams like methicillin results from the production of a low-affinity PBP, PBP 2a (a.k.a. PBP 2′), whose low affinity for virtually all β-lactams renders methicillin-resistant *S. aureus* (MRSA) pan-β-lactam resistant. PBP 2a is encoded by the *mecA* gene found on a novel mobile element, the 21- to 60-kb staphylococcal cassette chromosome *mec* (SCC*mec*) that is found at a unique site in the chromosome of MRSA (reviewed in [302, 303]). SSC*mec* also carries the *mecA* regulator genes, *mecI* and *mecR1* (though these can be completely or partially deleted), and the cassette chromosome recombinase (*ccr*) genes, *ccrA* and *ccrB*, responsible for site-specific integration and excision of SSCmec, as well as a number of open reading frames and pseudogenes of unknown function and/or benefit. Significantly, some SSC*mec* elements carry additional antibiotic (non-β-lactam) resistance genes, which likely contributes to the well-known multidrug resistance of MRSA [7, 34, 304, 305]. While horizontal transfer of SSC*mec* does occur among staphylococci [305, 306], there appear to be host-specific restrictions as regards stability and maintenance of *mecA* in *Staphylococcus* spp. [307], which might explain the rather limited number of closely related MRSA clonal complexes observed worldwide [304, 308].

The acquisition of SSC*mec* alone is, however, insufficient to provide for the level and spectrum of β-lactam resistance seen in MRSA strains, such resistance typically relying on mutations in the staphylococcal genome (but not in SSC*mec*) [302, 303]. Several genes impacting methicillin resistance in MRSA have been identified to date,

many of which are either regulatory or play a role (direct or indirect) in peptidoglycan biosynthesis and turnover and whose loss negatively impacts resistance (e.g. the *fem*/*fmt* genes [303], *pbpB* [309], *murE* [310] and *murF* [311]). Consistent with the apparent importance of peptidoglycan structure for expression of methicillin resistance in MRSA, alterations in the terminal stem peptide amino acid achieved in feeding studies markedly reduced the methicillin resistance of a *mecA*-containing *S. aureus* [312]. Recently, a two-component putative positive regulator of cell-wall peptidoglycan synthesis, VraSR, was shown to contribute the β-lactam resistance of MRSA (and methicillin susceptible *S. aureus*), though methicillin was not specifically examined – inactivation of these genes decreased β-lactam resistance in both instances [313]. Methicillin resistance independent of *mecA* is also seen in *S. aureus* [7, 314] and may be explained by alterations in [252] or overproduction of [252, 315] other PBPs in this organism.

Impermeability

The entry of hydrophilic antimicrobials such as β-lactams into Gram-negative bacteria occurs via channels in the outer membrane formed by porins [316]. Reduced outer membrane permeability to β-lactams, then, as a result of porin loss of or changes in porin structure can promote resistance to these agents. Indeed, porin deficiency is a contributing factor to β-lactam (including newer generation cephalosporin) resistance in a number of organisms, including *E. coli*, *Proteus* spp., *P. aeruginosa*, *A. baumannii*, *S. dysentariae*, *N. gonorrhoeae*, *S. marcescens* and *H. pylori* (see [142, 280, 317–319] and several references in [320]), though it is most often seen in *K. pneumoniae* [46, 47, 320–322] and *Enterobacter* spp. [320, 323–325], usually in conjunction with expression of a β-lactamase. Reduced permeability is also a factor in the β-lactam resistance seen in some clinical strains of *M. smegmatis* [326] and possibly *Bacteroides* spp. [274, 275]. Porin deficiency is an important determinant, too, of carbapenem resistance/reduced susceptibility, particularly in *P. aeruginosa* where loss or mutation of the OprD porin is common in carbapenem-, especially imipenem-resistant strains (see [327–329] and several references in [320]), but also in *E. aerogenes* [324, 325], *A. baumannii* [142, 272, 330], *K. pneumoniae* [46, 47, 321], *S. enterica* serovar Wien [140], *S. dysentariae* [320] and *P. mirabilis* [320]. Intriguingly, recent observations that eluates from siliconized latex urinary catheters enhance resistance of *P. aeruginosa* to carbapenems are explainable by the loss of OprD expression in the presence of these eluates [331, 332]. Moreover, it has been shown that it is the zinc present in these eluates which downregulates OprD expression [332, 333], such downregulation being related some-

how to the activity of the CzcRS two-component system that controls expression of the CzcCBA heavy metal efflux system in this organism [333]. Indeed, 20% of in vitro-selected imipenem-resistant strains in one study were coresistant to zinc and carried a mutation in the CzcS protein, and this mutation was responsible for increased expression of the efflux system and decreased production of OprD [333]. This is reminiscent of *nfxC*-type multidrug resistant strains of *P. aeruginosa*, which simultaneously overproduce the MexEF-OprN multidrug efflux system and shut down OprD production, the latter being responsible for the imipenem resistance of these mutants as well [334].

Efflux

Efflux has long been appreciated as a mechanism of antimicrobial resistance, with five families of bacterial efflux systems described to date: the major facilitator superfamily (MFS); the ATP-binding cassette (ABC) family; the resistance-nodulation-division (RND) family; the multidrug and toxic compound extrusion (MATE) family; and the small multidrug resistance (SMR) family (reviewed in [334, 335]). Members of the RND family, which are almost exclusively chromosomal and widely distributed in Gram-negative bacteria, appear to be the most significant as regards export of and, thus, resistance to clinically important antimicrobials [334]. Moreover, several of these in *Campylobacter jejuni*, *E. coli*, *N. gonorrhoeae*, *P. aeruginosa*, *P. putida*, *S. enterica* serovar Typhimurium and *S. maltophilia* accommodate β-lactams [334], including third (e.g. cefotaxime; MexAB-OprM (*P. aeruginosa*) [336]) and fourth (e.g. cefepime and cefpirome; MexCD-OprJ (*P. aeruginosa*) [336]) generation cephalosporins and carbapenems (e.g. several Mex systems of *P. aeruginosa* [337]). While MexAB-OprM, MexCD-OprJ and MexXY-OprM all demonstrated some ability to promote resistance to carbapenems, none of these had any effect on imipenem or biapenem resistance [337], and only MexAB-OprM has been implicated in carbapenem resistance in clinical strains [329]. The recent observation that in vitro selected imipenem-resistant isolates of *E. aerogenes* demonstrate increased production of the AcrA component of this organism's AcrAB-TolC efflux system has been taken as evidence for an efflux contribution to resistance [338], though this still needs to be verified. An observed increase in OprM in several ticarcillin-resistant isolates of *P. aeruginosa* also supports a contribution by MexAB-OprM and/or MexXY-OprM to resistance in these strains [164]. Similarly, the recent demonstration that the cefuroxime resistance of a clinical *E. coli* isolate was modestly reduced by the efflux inhibitor MC-201,110 suggested that efflux was a contributing factor for this resistance [339]. Efflux

in the form of MexAB-OprM also contributes substantially to the noted intrinsic resistance of *P. aeruginosa* to penems, though both the chromosomal AmpC β-lactamase and the outer membrane barrier contribute as well [340, 341].

Overcoming β-lactam resistance

One response to the continuing problem of β-lactam resistance in pathogenic bacteria, particularly when it is coupled with multidrug resistance, is to develop entirely new drugs active against entirely novel targets or to investigate novel therapies unrelated to antimicrobials (see [342] for a review of novel approaches to tackling multidrug resistance). A number of agents have, e.g. been/are being developed for treating MRSA, including oxazolidinones (e.g. linezolid), glycopeptides (e.g. oritavancin), streptogramins (e.g. quinupristin/dalfopristin), glycycyclines (e.g. tigecycline) and lipopeptides (e.g. daptomycin) (reviewed in [343, 344]). Still, given their proven safety and efficacy, β-lactams remain a popular and useful class of agents whose continued use will, however, necessitate changes to overcome existing resistance mechanisms, particularly β-lactamases in Gram-negative bacteria and low-affinity PBPs in Gram-positive [345].

β-lactamases

Much of the history of β-lactam development has been a response to β-lactamase-mediated resistance, with currently effective agents like carbapanems (imipenem, meropenem, ertapenem, biapenem, panipenem) and penems (e.g. faropenem) useful specifically because of their stability to most β-lactamases [4, 200]. Inactivation of β-lactamases is also a proven approach, with β-lactam/β-lactamase inhibitor combinations often effective in treating infections caused by β-lactamase-producing organisms [346]. Thus, targeting these enzymes, particularly the broad-spectrum class C serine and class B metalloenzymes, will remain an active area of investigation [342, 347]. Indeed, recent reports highlight a number of novel inhibitors, some of which are themselves β-lactams [348–351], of class C [348–356] and class B [357–363] enzymes (see also several references in [342]). Several of the class C inhibitors have demonstrated activity against class A enzymes, including ESBLs, [342, 349–351, 355, 356] and class D enzymes [350, 356], and potentiate the activity of β-lactams against β-lactamase-producing organisms [342, 349, 350, 353, 354, 364]. The current availability of a number of β-lactamase crystal structures bound to inhibitors (e.g. [149, 361, 365, 366]) should also assist the rational structure-based design of inhibitors. The use of so-called dual action hybrid antimicrobials in which β-lactams are fused to another antimicrobial such

that β-lactamase cleavage of the β-lactam component releases the latter (reviewed in [367]) continue to be exploited to provide for a means of not just overcoming but actually exploiting β-lactamase production. A recent paper highlights the utility of two such dual action 'prodrugs' in which the biocide triclosan is fused to a cephalosporin, and β-lactamase-dependent killing of Gram-positive and Gram-negative bacteria expressing class A or class C enzymes is seen [368].

PBPs

Given the importance of PBP changes for β-lactam resistance in Gram-positive organism, particularly methicillin-resistance in *S. aureus*, much of the focus in overcoming β-lactam resistance in these organisms relates to developing agents with improved binding to the low-affinity PBPs. Several novel cephalosporins with anti-MRSA activity (due to increased PBP binding) (reviewed in [343, 345, 369]) have been reported to date (e.g. BAL-9141 [370], RWJ-54428 [371, 372], S-3578 [373, 374], BMS-247243 [375], CP6679 [376], LB 11058 [377] and several anti-MRSA cephems being developed by Bristol-Meyers Squibb [378, 379]) many of which show activity against enterococci and/or penicillin-resistant *S. pneumoniae* [345, 374, 375, 377, 380]. Significantly, since the majority of staphylococcal clinical isolates produce β-lactamase, these are stable to hydrolysis by this enzyme. Novel carbapenems with anti-MRSA activity [369, 381, 382] have also been described with many, again, active against enterococci and penicillin-resistant pneumococci [382]. Still, the observation that resistance to an experimental carbapenem with high affinity to PBP 2a could be selected in vitro as a result of mutation in PBP 2a [383] is worrying, and suggests that PBP 2a-mediated resistance to β-lactams that bind with high affinity may be a problem in the future. Significantly, the available crystal structures of low-affinity PBPs such as PBP5 of *E. faecium* [384] and PBP 2a from MRSA [385] should provide insights to the features of any newly developed β-lactams that will be important for high-affinity binding and, thus, assist the development of agents that overcome low-affinity PBP-mediated resistance. Finally, given the therapeutic success of β-lactams, the search is now on for novel agents that also target PBPs (e.g. [386–389]).

Others

A number of novel oxapenems lacking intrinsic antimicrobial activity have been shown to potentiate β-lactam activity in enterococci and *S. aureus*, including MRSA, via an as yet unknown mechanism [350]. Similarly, flavones have been shown to intensify MRSA's susceptibility to β-lactams via an as yet unknown effect that is, however, independent of any direct impact on β-lacta-

mase or PBP2a and presumably involves an action on some mechanism essential for expression of the MRSA phenotype [390, 391]. Epigallocatechin gallate [392–394] and corilagin [395], both tea extracts, also potentiate the activity of β-lactams against MRSA although, again, the mechanism(s) are not yet fully elucidated. Intriguingly, phenothiazines reduce methicillin (oxacillin) MICs of MRSA, and while these compounds have been shown to adversely impact drug efflux systems, it is not entirely clear whether the reduced methicillin resistance was related directly or indirectly to efflux inhibition [396].

Conclusions

Bacterial resistance to β-lactams has historically compromised use of these agents in treating infectious disease and continues to do so, necessitating the continued development of new β-lactams capable of overcoming this resistance. Clearly, however, the development of new agents must be informed by strategies for their use in ways that will minimize the selection, first, and spread, second, of resistance. The observation, for example, that use of extended-spectrum cephalosporins selects for ESBL-producing Enterobacteriaceae [397] and that reduced use of such agents (replaced by equally effective fourth-generation cephalosporins) can correspondingly reduce the incidence of ESBL producers [398, 399] highlights the importance of informed use of β-lactams in limiting resistance. Because of their broad spectrum of activity and their potential for spread, plasmid and/or integron-associated ESBL, AmpC and metallo-β-lactamases are of particular concern, with methods for accurately screening/detecting these (e.g. [21, 22, 400]) increasingly vital to inform appropriate antimicrobial therapy and infection control measures. Similarly, given the increased mortality associated with serious infections due to MRSA (vs. MSSA) (e.g. [401]), accurate and rapid identification of MRSA (e.g. [402–404]) is also needed to inform appropriate drug therapy and control measures. With appropriate use and monitoring of resistance β-lactams will continue to play an essential role in antimicrobial chemotherapy.

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