Cycles of development and resistance

β-Lactams – penicillins, cephalosporins, carbapenems and monobactams – represent 60% of all antimicrobial use by weight. They are preferred because of their efficacy and safety and because their activity can be extended or restored by chemical manipulation. No other antibiotic class has such chemical malleability and versatility. Inevitably, however, their heavy usage has selected strongly for resistance. Among Gram-positive bacteria, resistance largely arises by penicillin-binding protein (PBP) modification or substitution. PBP modification is also important in Haemophilus and Neisseria but, in general terms, resistance among Gram-negative bacteria depends mostly on β-lactamases and efflux, with their effects ‘geared up’ by impermeability, which might be increased by porin loss (Box 1) [1,2].

Based on sequence data, β-lactamases divide into four classes, each including types that are usually plasmid-mediated or chromosomal (Table 1). However, these distinctions are blurred because it is increasingly appreciated that most ‘plasmid-mediated’ types are in fact genetic escapes from the chromosomes of other species.

β-Lactamases probably have a natural role in cell-wall metabolism, as evidenced by the fact that expression of inducible AmpC β-lactamases is intimately linked to that of peptidoglycan recycling enzymes [3]. Their role in protecting against clinically used β-lactam drugs might, therefore, be gratuitous; nevertheless, it is in this role that they now have the greatest clinical importance and are under great selection pressure.

The spread of β-lactamases has driven β-lactam development for 60 years. The first analogue, benzylpenicillin, penetrated Gram-negative bacteria poorly and was destroyed by penicillinases, which spread rapidly in Staphylococcus aureus. These problems were overcome in the early 1960s with the development of semi-synthetic penicillins (e.g. ampicillin and carbenicillin) that could penetrate Gram-negative bacteria, and those that were stable to staphylococcal penicillinase (methicillin and oxacillins). The anti-Gram-negative analogues were compromised, in turn, by the spread of plasmid-mediated penicillinases (notably TEM-1) among Enterobacteriaceae. From the 1970s, this drove the development of (a) second-, third- and fourth- generation oximino-cephalosporins (e.g. cefuroxime, cefotaxime, ceftriaxone, cefazidime and ceftazidime); and (b) of β-lactamase inhibitors such as clavulanic acid.

In the subsequent 25 years, oximino-cephalosporins have become workhorse antibiotics worldwide, used as primary therapy in many clinical settings from pneumonia to intra-abdominal sepsis. Once again, clinical use has selected for resistance and this cephalosporin resistance, along with dramatically rising enterobacterial resistance to fluoroquinolones [4] (http://www.earss.rivm.nl), is now driving the use of carbapenems. Unfortunately – but predictably – carbapenem resistance is now emerging too, especially in Acinetobacter spp. This review outlines the nature of these evolving resistance problems to cephalosporins and carbapenems, considering both the epidemiology and the molecular biology, and what might be done to mitigate the situation.

Cephalosporin resistance in Enterobacteriaceae

Enterobacteriaceae are important opportunist pathogens and account for ~35–40% of all bacteraemia isolates in the UK [Health Protection Agency (HPA); http://www.hpa.org.uk] and for the majority of urinary tract infections. When oximino-cephalosporins were introduced, virtually all Enterobacteriaceae were susceptible, but resistance
A β-lactam must diffuse across the outer membrane of the Gram-negative cell, using pores formed by porin proteins, and then cross the periplasm (which can contain any type of β-lactamase) before reaching its PBP targets, which lie on the outer surface of the cytoplasmic membrane (Figure I). Resistance is often attributed to impermeability or the presence of a β-lactamase alone but in reality, these factors work together so that for any given external β-lactam concentrations, the periplasmic β-lactam [1] concentration maintains a steady-state level, the magnitude of which determines the extent of PBP poisoning. Reducing permeability through porin loss or increased β-lactamase activity (by a raised enzyme quantity, raised K_m or a lowered K_m) activity (by a raised enzyme quantity, raised K_m or a lowered K_m) reduces the steady-state periplasmic drug concentrations and thereby reduces PBP inactivation. For Escherichia coli it is possible to model this interplay mathematically; for Pseudomonas aeruginosa these models break down because efflux substantially contributes to periplasmic drug clearance [2]. In some cases, β-lactamases that have only feeble activity in vitro can confer resistance in a suitably impermeable host strain. Thus, for example, ESBLs or AmpC enzymes can confer carbapenem resistance in porin-deficient Enterobacteriaceae but not in normal strains.

**Box 1. How impermeability augments resistance**

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**Derepression of AmpC**

Soon after their introduction, it became apparent that AmpC β-lactamases of Enterobacter spp., Citrobacter freundii, Serratia spp. and Morganella morganii, and only remained active against typical isolates of these species (which have inducible ampC) because they are weak inducers of β-lactamase expression. Cephalosporin resistance ensues if ampC becomes derepressed, a process that arises through the mutation inactivation of ampD, which encodes a cell-wall-recycling enzyme. In the worst case, derepressed mutants which arise at a high frequency of $10^{-7}$ (and occasionally higher) in inducible populations can be selected during oximino-cephalosporin treatment and cause clinical failure. There is a 20–30% risk of clinical failure through resistance selection when an Enterobacter bacteraemia is treated with a third-generation cephalosporin, although the hazard is much lower in urinary infections owing to the high local cephalosporin concentrations [5]. This risk is reduced with fourth-generation cephalosporins such as ceftazime, which have weaker affinity for AmpC and so are inactivated less efficiently.

Compared with control patients, the mortality risk associated with selection of derepressed Enterobacter in one study rose from 13% to 26%, while mean hospital stay increased from 19 to 29.5 days and hospitalization cost increased from US$40,000 to US$79,000 [6]. Once selected in one patient, derepressed mutants are stable (despite diverting up to 4% of total protein production into β-lactamase) and can spread to others, with ~30–40% of initial Enterobacter cloacae isolates from inpatients in the UK now having this mechanism. AmpC-mediated resistance cannot be overcome with available inhibitors; monobactam and penem-based inhibitors have been investigated [7,8] but have proved unsuitable for pharmaceutical development. As already noted, AmpC-mediated resistance is partly overcome by the fourth-generation cephalosporins cephaloridine and ceftazidime, which are more stable, but resistance to these derivatives can arise either by reduced permeability in AmpC-derepressed strains or by mutations that change the enzyme sequence and hydrolytic profile [9,10].

**Plasmid-mediated AmpC enzymes**

Multiple AmpC genes (Table 2) have been mobilized by transfer to plasmid DNA and are increasingly prevalent as plasmid-mediated types in Escherichia coli, Klebsiella spp. and Salmonella spp. [11], although they remain less

---

**Table 1. Classification of β-lactamases**

<table>
<thead>
<tr>
<th>Molecular class and functional mechanism</th>
<th>Types that are normally chromosomal and ubiquitous in species or group</th>
<th>Types that are normally plasmid-, transposon- or integron-mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class A Serine β-lactamases</td>
<td>SHV-1, LEN-1 and K1 in Klebsiella spp.; chromosomal cefuroximases of Proteus vulgaris; chromosomal β-lactamases of Bacteroides spp.</td>
<td>Staphylococcal penicillinase; TEM, SHV, VEB, PER and CTX-M penicillinases and ESBLs; KPC, IMI/NMC and SME carbapenemases</td>
</tr>
<tr>
<td>Class B Metallo-β-lactamases</td>
<td>L1 enzyme of Stenotrophomonas maltophilia; chromosomal enzymes of some Chryseobacterium spp. and Aeromonas spp.; CcrA enzyme found in 1-3% of Bacteroides fragilis isolates</td>
<td>IMP, VIM and SPM types</td>
</tr>
<tr>
<td>Class C Serine β-lactamases</td>
<td>Chromosomal AmpC enzymes of Escherichia coli, Shigella spp., Enterobacter spp., Citrobacter freundii, Morganella morganii, Providencia spp. and Serratia spp.</td>
<td>CMY-1, LAT-1, BIL, MOX, ACC, FOX and DHA types*</td>
</tr>
<tr>
<td>Class D Serine β-lactamases</td>
<td>Chromosomal (along with other β-lactamases) in Acinetobacter spp. (OXA-51-like); P. aeruginosa (OXA-50) and some Aeromonas spp. (e.g. OXA-12).</td>
<td>Most OXA types, excluding those detailed here as chromosomal</td>
</tr>
</tbody>
</table>

*See Table 2.
Table 2. Sources of AmpC β-lactamases that have escaped to mobile DNA

<table>
<thead>
<tr>
<th>Class</th>
<th>Source</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIT</td>
<td>Citrobacter freundii</td>
<td>CMY-2 to 7; LAT-1,3,4</td>
</tr>
<tr>
<td>ENT</td>
<td>Enterobacter spp.</td>
<td>ACT-1; MIR-1</td>
</tr>
<tr>
<td>FOX</td>
<td>Aeromonas spp.</td>
<td>FOX-1 to -5</td>
</tr>
<tr>
<td>MOX</td>
<td>Aeromonas spp.</td>
<td>MOX-1,2; CMY-1 and 8</td>
</tr>
<tr>
<td>DHA</td>
<td>Morganella morganii</td>
<td>DHA-1, -2</td>
</tr>
<tr>
<td>ACC</td>
<td>Hafnia alvei</td>
<td>ACC-1</td>
</tr>
</tbody>
</table>

frequent than the extended-spectrum enzymes discussed later. The USA has reported significant problems with CMY-2-producing Salmonella Newport in cattle and zoonotic infections [12], and a Proteus mirabilis clone with CMY-16 caused infections in four Italian cities [13]. High occurrence rates of plasmid-encoded AmpC enzymes have been reported in India (J. Child, MD thesis, University of London, 2001) and some early UK cases seem to represent importations from the Indian subcontinent (HPA, unpublished), although comprehensive surveillance is not available.

Extended-spectrum β-lactamases

ESBLs were first described in the mid-1980s. Most early examples were mutants of the TEM and SHV plasmid-mediated penicillinases with one or more amino acid substitutions. The mutations enlarge the active site, which enables deflection of the oxyimino group and attack on the β-lactam ring. Such mutants – there are now >200 known (http://www.lahey.org/studies) – attack all oxyimino-cephalosporins but not α-methoxy-cephalosporins (cephemycins) or carbapenems. They are most prevalent in Klebsiella spp., often from specialist clinical units, and their epidemiology reflects a mixture of clonal expansion, plasmid transfer and repeated mutational events. Some producer clones have spread among hospitals, including a serotype K25 Klebsiella pneumoniae with SHV-4 [14] and a strain of Enterobacter aerogenes that contains a TEM-24 enzyme [15]; both clones are widespread in France and Belgium.

The epidemiology of ESBLs is now undergoing rapid change with the spread of CTX-M types. Comprising >50 enzymes in five subgroups [16], these evolved by the escape of chromosomal β-lactamase genes from Kluyvera spp., a genus of little clinical importance, with their mobilization facilitated by the insertion sequence ISEc1 [17] or related insertion sequences [18]. CTX-M β-lactamases hydrolyze cefotaxime more rapidly than ceftazidime, reversing the pattern of many TEM types. One type, CTX-M-2, spread hugely in Argentina in the early 1990s [19] and is now also frequent in Israel [20]. Other types are spreading elsewhere, predominantly CTX-M-9 and -14 in East Asia and Iberia [21,22] and CTX-M-3 and M-15 in Europe and, anecdotally, in India and the Middle East. Unknown in the UK before 2001, CTX-M-15 is now the dominant ESBL in E. coli and Klebsiella spp. [23].

The accumulation of CTX-M enzymes involves a mixture of clonal expansion and plasmid spread, giving an epidemiology that varies with time and place. In the UK, one major clone of E. coli with a CTX-M-15 enzyme (strain ‘A’) dominates in Lancashire, Shropshire, Hampshire and Ulster and is related with a similarity of ~78% to four further clones (B–E), which are locally prevalent [24]. All of these groups probably share a common ancestor. In addition, the CTX-M-15 enzyme is found in diverse clones of E. coli and, in some regions (for example, around London), most producers are diverse. The CTX-M-15 enzymes of UK isolates are encoded by large multiresistance plasmids (E. Karisik et al., unpublished). Clonal CTX-M-15 producers have been reported in France, Canada and Italy [16,25,26]. In Spain, most producer E. coli with CTX-M-9 enzymes are diverse [27,28]; in Poland, the main story is the spread of promiscuous CTX-M-3 plasmids among diverse Enterobacteriaceae [29].

Despite geographic differences in enzyme type and strain epidemiology, two themes emerge. First, CTX-M enzymes have recently and sharply accumulated in E. coli and in Klebsiella species; second, producers are often isolated outside the hospital environment. Risk factors for infection outside hospitals in Israel (where CTX-M-2 predominates) include diabetes, hospitalization in the preceding three months, treatment with a cephalosporin, quinolone and/or penicillin. All of these risk factors were at least threefold more likely to be present among patients with community infections due to ESBL producers compared with those with ESBL non-producers [30]. Similar risk factors were identified in Spain, where enzymes related to CTX-M-9 and CTX-M-14 predominate [31].

Carbapenem resistance in Enterobacteriaceae

Growing cephalosporin resistance is causing increased reliance on carbapenems, which have good stability to both AmpC and ESBL enzymes. This reliance is increased by the fact that many ESBL producers (less so the AmpC hyper-producers) are also multiresistant to aminoglycosides, trimethoprim, tetracycline and, especially, fluoroquinolones.

Impermeability plus ESBL or AmpC

Carbapenem resistance was extremely slow to appear in Enterobacteriaceae, with rates of under 1% even after 20 years of imipenem use [32]. However, carbapenem resistance can arise by three routes: (i) permeability lesions in organisms with AmpC enzymes or ESBLs; (ii) acquisition of IMP or VIM metallo-β-lactamases; or (iii) acquisition of non-metallocarbapenemases of the KPC, IMP/IMI/NMC, SME or OXA families. The first of these mechanisms is increasingly seen among K. pneumoniae in the UK, with >200 such isolates received at the national reference laboratory in the past 18 months. These came from around one-third of UK diagnostic laboratories. Such isolates lack the OprK-35 and -36 porins and produce CTX-M-15 β-lactamase (usually along with TEM-1 and OXA-1, although these seem to be of little relevance) and show what is rapidly becoming a diagnostic profile, with an ertapenem MIC (minimum inhibitory concentration, the lowest drug level needed to inhibit bacterial growth) > meropenem MIC > imipenem MIC. E. cloacae isolates that have impermeability along with an AmpC β-lactamase (rather than a CTX-M type) have a similar profile but with the ertapenem MIC > imipenem MIC > meropenem MIC*

* D.G. Pillay et al., abstract 1621, 16th European Congress of Clinical Microbiology and Infectious Diseases, Nice, France, April 2006.
In vivo selection of porin-deficient mutants is sometimes — albeit rarely — seen from susceptible, AmpC- or ESBL-producer populations during carbapenem therapy [34]. It remains unclear whether the porin-deficient organisms are biologically fit and whether this mode of resistance is increasing or if it is just increasingly recognized following the introduction of ertapenem, which is the best indicator compound.

**Metallo-β-lactamases**

Metallo-β-lactamases of the IMP and VIM families are a greater concern in non-fermenters (see later) but have been found repeatedly in Enterobacteriaceae, predominantly *Klebsiella* and *Enterobacter* spp. There have been sizeable outbreaks of *K. pneumoniae* with VIM-1 or -2 enzymes in France [35] and Greece [36], and of IMP-8-producing *K. pneumoniae* [37] in Taiwan, along with a wide scattering of reports of small numbers of isolates from elsewhere around the world. At least one representative of a Greek VIM-1 *K. pneumoniae* strain was imported to the UK by a patient who was repatriated following a road-traffic accident, which illustrates how spread can occur (HPA, unpublished). Surprisingly, despite strong hydrolytic activity, metallo-β-lactamases often only raise imipenem MICs for Enterobacteriaceae from 0.12 or 0.25 mg L\(^{-1}\) to 1–4 mg L\(^{-1}\) [38] (values around the upper limit define clinical susceptibility). Substantial resistance arises only if the organism becomes impermeable through porin loss. The clinical significance of borderline resistance without porin loss remains unclear; it could be that the weakness of this resistance militates against its selection by carbapenems or that it is just increasingly recognized following the introduction of ertapenem, which is the best indicator compound.

**Non-metallo-carbapenemases in Enterobacteriaceae**

Non-metallo-carbapenemases that belong to the SME and NMC families are known from tiny numbers of *Serratia* and *Enterobacter* isolates, many of which were collected before the introduction of imipenem in 1985. They confer resistance to carbapenems, aztreonam and penicillins but not oxyimino-cephalosporins [39]. None is transmissible and there is no evidence of spread, although curiously, plasmid-mediated IMI-2 has recently been found in clonal *Enterobacter asburiae* isolates from multiple rivers in the USA [40].

*KPC* enzymes are of greater concern. Unlike IMI/NMC and SME types, these confer resistance to all β-lactams, including cephalosporins, monobactams and carbapenems. Clones of *K. pneumoniae* and *E. cloacae* with KPC enzymes have spread in multiple hospitals around New York since 2003, where they have presented severe treatment problems, causing up to 47% mortality [41]. KPC producers have also been found in Europe, South America and China on a few occasions. The potential for wider spread is disturbing, with extreme infection control measures warranted wherever producers are encountered.

A few carbapenem-resistant *K. pneumoniae* from Turkey have OXA-48, which is reported to hydrolyze carbapenems and penicillins but not oxyimino-cephalosporins [42]; otherwise OXA carbapenemases are more important in *Acinetobacter* species (see later).

**Resistance in Pseudomonas aeruginosa**

Like *Enterobacter*, *P. aeruginosa* has a chromosomal AmpC β-lactamase. It also has a recently discovered chromosomal class D enzyme OXA-50, although this seems of little significance in resistance [43]. AmpC might become derepressed by mutation and confer resistance to oxyimino-cephalosporins as in *Enterobacter* spp; however, derepression is rarer than in *Enterobacter* spp and is only often partial [44]. Upregulated MexAB–OprM-mediated efflux is a more common mode of resistance and affects penicillins, cephalosporins and meropenem (but not imipenem), along with non-β-lactam agents including fluoroquinolones. As with AmpC derepression, mutants with upregulated efflux could be selected during therapy, with both β-lactams and fluoroquinolones acting selectively [45].
seem most prevalent, producers account for <2% of *P. aeruginosa* isolates and are greatly outnumbered by isolates that owe multi-resistance to combinations of AmpC expression, impermeability and efflux [53].

**Resistance in Acinetobacter spp.**

Acinetobacter spp. – principally *A. baumannii* – are opportunistic pathogens of greatest concern in nosocomial pneumonias, especially in intensive care and as invaders of burn wounds. *A. baumannii* is notoriously associated with outbreaks, facilitated by resistance to disinfectants and desiccation. Until the 1970s, most isolates were susceptible to a wide range of antibiotics [54]; subsequently, *A. baumannii* has shown a remarkable propensity to develop resistance to virtually every antibiotic class [55].

Classical penicillinas (e.g. TEM-1) are widespread and a few isolates have ESBLs, notably a clone with a VEB-1 enzyme that was recovered in north-eastern France in 2003–2004 [56,57]. ESBLs do not, however, account for the oxyimino-cephalosporin resistance that is now nearly universal in clinical strains of *A. baumannii*. Rather, such resistance depends on a chromosomal AmpC β-lactamase that is intrinsic to the species. This is normally expressed at only a low level and is not inducible; nevertheless, it can be overexpressed as a result of the upstream insertion of ISAba1 sequences, which provide an efficient promoter [58]. ISAba1 is widespread in *A. baumannii*, with up to 13 copies per cell, and is believed to serve as a ‘moving switch’ to turn on those genes with which it is juxtaposed.

**Inherent and acquired carbapenemases in Acinetobacter spp.**

By the late 1990s, carbapenems were the only remaining useful agents that could combat many severe *Acinetobacter* infections. Now, however, carbapenem resistance is accumulating, largely through clonal spread. In the Far East, some outbreaks involve clones with IMP or VIM metallo-β-lactamases [48,59]. Isolates with non-β-lactamase-mediated resistance are also reported, although these assertions should be viewed with scepticism, allowing for the difficulty of detecting what is the main cause of carbapenem resistance in *A. baumannii* – class D (OXA) carbapenemases.

All *A. baumannii* isolates (including type strains from the 1950s [60]) have the gene for OXA-51-like enzyme, an intrinsic class D carbapenemase. Several sequence variants are known (e.g. OXA-69), although their importance is unclear and comparative kinetics are yet to be determined [60]. As with the AmpC enzyme, OXA-51-like enzymes are expressed poorly in most strains, which explains the general susceptibility to carbapenems but, once again, expression can be activated by migration of ISAba1. Studies by Turton et al. [61] on a prevalent UK lineage designated the SE clone (Figure 1) reveal that carbapenem-resistant representatives (now the majority) of this clone consistently have ISAba1 upstream of *bla*OXA-51-like, whereas carbapenem-susceptible representatives lack this insertion. Similar results were obtained for the T strain, a lineage that is prevalent in one UK hospital, where it is associated both with casualties repatriated from Iraq and with local transmission at the hospital [62].

Other OXA carbapenemases that are not intrinsic to the species occur in several successful *A. baumannii* clones. These belong to at least three clusters, termed OXA-23-like, OXA-(24)-40-like and OXA-58, with the first two groups encompassing several sequence variants. OXA-23-like has been found repeatedly in the species from 1985 onwards, including outbreak strains collected in the UK, East Asia and South America. It is present in one multi-resistant clone that is now prevalent in London and south-east England (OXA-23 clone 1; Figure 1) [63], whereas OXA-40 occurs in a clone that is prevalent in

![Figure 1](https://www.sciencedirect.com)
Iberia and in another clone increasingly identified around Chicago [64]. OXA-58-like was first described only recently in isolates from France but was subsequently recognized as having occurred worldwide over the preceding eight to ten years [65].

Unlike the class A and B carbapenemases (e.g. KPC, VIM, IMP), OXA enzymes have only feebly carbapenemase activity when extracted, although laboratory transfer and deletion experiments confirm their role in resistance (Table 3) [60]. They might be more active in the bacterial periplasm because other OXA enzymes can convert between monomeric (less active) and dimeric (more active) forms, with the latter favoured at the high enzyme concentrations present in the periplasm [66]. It is plausible (but speculative) that similar effects occur with the OXA carbapenemases. Some isolates with OXA carbapenemases could have additional co-determinants of resistance; in particular, some lack outer-membrane proteins, although these were not formally shown to be porins [67].

The erosion of carbapenemase activity against Acinetobacter spp. is disturbing given the paucity of alternative agents. Treatment is coming to depend on intravenous polymyxins – agents that were discarded long ago owing to their toxicity and poor efficacy in pulmonary infections. There is debate whether tigecycline, a novel tetracycline derivative, is an alternative [68] and whether adding nebulated polymyxins is of value in pneumonias to give high local level concentrations [69]. In any event, we are closer to the much-threatened ‘end of antibiotics’ for A. baumannii more than for any other common pathogen.

**Concluding remarks and future perspectives**

From the 1940s to the 1980s there was a succession of β-lactam generations that each overcome resistance to earlier generations. The most important trend that now affects β-lactam utility is the spread of CTX-M ESBLs in Enterobacteriaceae. This shift, along with rapidly increasing quinolone resistance, will drive earlier and wider use of carbapenems, previously the ‘last reserve’ β-lactams. Carbapenem resistance remains rare in Enterobacteriaceae, although outbreaks of Klebsiella spp. with KPC enzymes in the north-east USA are disturbing, as is the growing scatter of isolates with resistance caused by combinations of impermeability and CTX-M or AmpC enzymes. Metallo-carbapenemases have been recognized in Enterobacteriaceae for longer than the KPC types and have been slower to spread but there is no certainty that they will not accumulate more rapidly in the future. Carbapenem resistance is a greater immediate concern in non-fermenters, especially Acinetobacter spp., in which clones with acquired OXA carbapenemases or upregulated chromosomal OXA-51-like enzymes are becoming widespread.

Although most carbapenem resistance in P. aeruginosa remains as a result of porin loss, there must be concern about the growing number of outbreaks, some of them large and protracted, caused by strains with IMP, VIM and SPM metallo-β-lactamases.

Disturbingly, there are few new β-lactams to overcome this accumulating resistance in Gram-negative pathogens, although there are major innovations on other aspects, notably the development of compounds that can inhibit PBP-2’ of methicillin-resistant Staphylococcus aureus [e.g. ceftobiprole and ceftaroline (PPI-0903)] [70]. These compounds, however, remain liable to ESBLs. New carbapenems such as ertapenem and doripenem could offer pharmacological advantages over imipenem and meropenem but do little to overcome resistance to these older carbapenems [49]. Various inhibitors of carbapenemases are in the early stages of investigation but all are far from the clinic; moreover, the task of finding good inhibitors is complicated by the fact that many of the carbapenemase producer strains partly owe their resistance to impermeability or efflux, both of which are likely to exclude any inhibitor. It should be easier to develop useful inhibitor combinations against ESBL producers, which are far more numerous than those with carbapenemases. Combinations of those cephalosporins that are least compromised by ESBLs (e.g. cefepime or ceftizoxime) with available inhibitors such as clavulanate or talozobactam should be effective, although patent considerations make it unlikely that they will be evaluated in Europe or the USA. For the future, there is a clear need to revitalize research into anti-Gram-negative β-lactams because no other antibiotic class has such a reputation for safety and efficacy.

**References**


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**Table 3. Contribution of acquired OXA carbapenemases to resistance in Acinetobacter species**

<table>
<thead>
<tr>
<th>β-lactam</th>
<th>R-recipient</th>
<th>Transconjugant with pFER encoding OXA-23</th>
<th>Transconjugant with pMAD encoding OXA-58</th>
<th>Wild type with OXA-40 β-lactamase</th>
<th>Wild type ∆OXA-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>0.25</td>
<td>&gt;32</td>
<td>2</td>
<td>&gt;32</td>
<td>2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.25</td>
<td>&gt;32</td>
<td>2</td>
<td>&gt;32</td>
<td>4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>2</td>
<td>&gt;32</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

*Data are from Heritier et al. [60].*


14 Arlet, G. et al. (1994) Molecular epidemiology of *Klebsiella pneumoniae* strains that produce SHV-4 \( \beta \)-lactamase and which were isolated in 14 French hospitals. *J. Clin. Microbiol.* 32, 2553–2558


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