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The β-lactamase threat in Enterobacteriaceae, *Pseudomonas* and *Acinetobacter*

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Over the past 60 years, the use of successive generations of β-lactam antibiotics has selected successive generations of β-lactamase enzymes, each more potent than the last. Currently, rising problems include CTX-M extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases and KPC carbapenemases in Enterobacteriaceae, while OXA- and metallo-carbapenemases are of growing importance in *Acinetobacter* spp. and (less so) in other non-fermenters. *Escherichia coli* isolates with CTX-M ESBLs are spreading multiresistance in the community and in hospitals, while carbapenemase-producing *Acinetobacter* spp., mostly from intensive care, are among the most multiresistant nosocomial bacteria known and are often susceptible only to polymyxins and, potentially, tigecycline. This review discusses the epidemiology and microbiology of these resistance problems, along with possible solutions.

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 oxyimino-cephalosporins (e.g. cefuroxime, cefotaxime, ceftriaxone, ceftazidime and cefepime); and (b) of β-lactamase inhibitors such as clavulanic acid.

In the subsequent 25 years, oxyimino-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 oxyimino-cephalosporins were introduced, virtually all Enterobacteriaceae were susceptible, but resistance

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Box 1. How impermeability augments 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_{cat} 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.

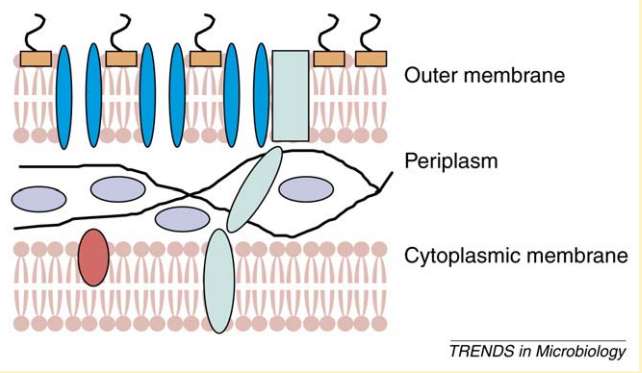

Figure I. Diagram of the Gram-negative cell envelope across which β-lactams must diffuse to reach PBPs. The outer membrane is traversed by porin channels (blue) and exit portals for efflux systems (light green); the periplasm contains β-lactamases (light purple) and linker proteins for efflux systems. The cytoplasmic membrane contains efflux pumps and the PBPs (red) that are targeted by β-lactams.

has accumulated through the selection of strains with hyperproduced AmpC enzymes, acquired AmpC enzymes and, most importantly, extended-spectrum β-lactamases (ESBLs).

Derepression of AmpC

Soon after their introduction, it became apparent that oxyimino-cephalosporins were labile to the chromosomal 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 $\sim 10^{-7}$ (and occasionally higher) in inducible populations] can be selected during oxyimino-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 cefepime, 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 cefepime and cefpirome, 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

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

[a]See Table 2.

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

Class	Source	Examples
CIT	*Citrobacter freundii*	CMY-2 to 7; LAT-1,3,4
ENT	*Enterobacter* spp.	ACT-1; MIR-1
FOX	*Aeromonas* spp.	FOX-1 to -5
MOX	*Aeromonas* spp.	MOX-1,-2; CMY-1 and 8
DHA	*Morganella morganii*	DHA-1, -2
ACC	*Hafnia alvei*	ACC-1

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 (cephamycins) 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 IS*Ecp1* [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 hyperproducers) 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, 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.

[33]. *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 facilitates a hidden spread, which is unlikely to be detected by routine clinical microbiology tests.

Non-metallo-carbapenemases in Enterobacteriaceae

Non-metallo-carbapenemases that belong to the SME and IMI/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 often only 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].

β-Lactamases in Pseudomonas aeruginosa

Many β-lactamases encoded by plasmids or chromosomally-integrated transposons have been reported in *P. aeruginosa* but, in contrast to the situation in Enterobacteriaceae, none has become internationally frequent. Most are penicillinases that lack activity against oxyimino-cephalosporins or carbapenems; ESBLs are rare. Nevertheless, an acquired ceftazidimase ESBL called PER-1 is widespread in *P. aeruginosa* in Turkey [46] with scattered reports from Europe, some of which represent importations. Another class A ESBL, VEB-1, is scattered in *P. aeruginosa* (and Enterobacteriaceae) in East Asia [47]. Extended-spectrum cephalosporin-hydrolyzing mutants of the class D penicillinases OXA-10 and OXA-2 have been found in isolates from Turkey and France and are so far unique to *P. aeruginosa*.

IMP, VIM and SPM metallo-carbapenemases (class B) are increasingly scattered in *P. aeruginosa* and are slowly but steadily increasing in prevalence [48]; SPM types are confined to Brazil whereas IMP and VIM types have been reported worldwide. *P. aeruginosa* isolates with metallo-carbapenemases are more consistently resistant to carbapenems than are Enterobacteriaceae with the same enzymes, perhaps owing to greater concurrent impermeability and efflux activity. Producers are almost universally cephalosporin resistant whereas aztreonam, which is stable, remains active unless other mechanisms are also present [49]. IMP and VIM enzymes are integron-associated but SPM is not; all three types can be plasmid-mediated and are transferable, although chromosomal gene integration is common.

Major clinical problems that have arisen with metallo-β-lactamase-producing *P. aeruginosa* have involved the clonal spread of producer strains, often of serotype O12; gene transfer seems rare. In Thessaloniki, a strain with VIM-2 enzyme persisted for over three years and >200 isolates were obtained [50]; in Cali (Colombia), another persisted for four years, infecting patients and surviving in sink traps and fittings [51]; in Brazil, a single strain with SPM-1 enzyme has spread to multiple hospitals [52]. Although such outbreaks are disturbing, this concern should not be overplayed; even in the Far East where they

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 penicillinases (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 IS*Aba1* sequences, which provide an efficient promoter [58]. IS*Aba1* 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 IS*Aba1*. 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 IS*Aba1* upstream of $bla_{\text{OXA-51}}$-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

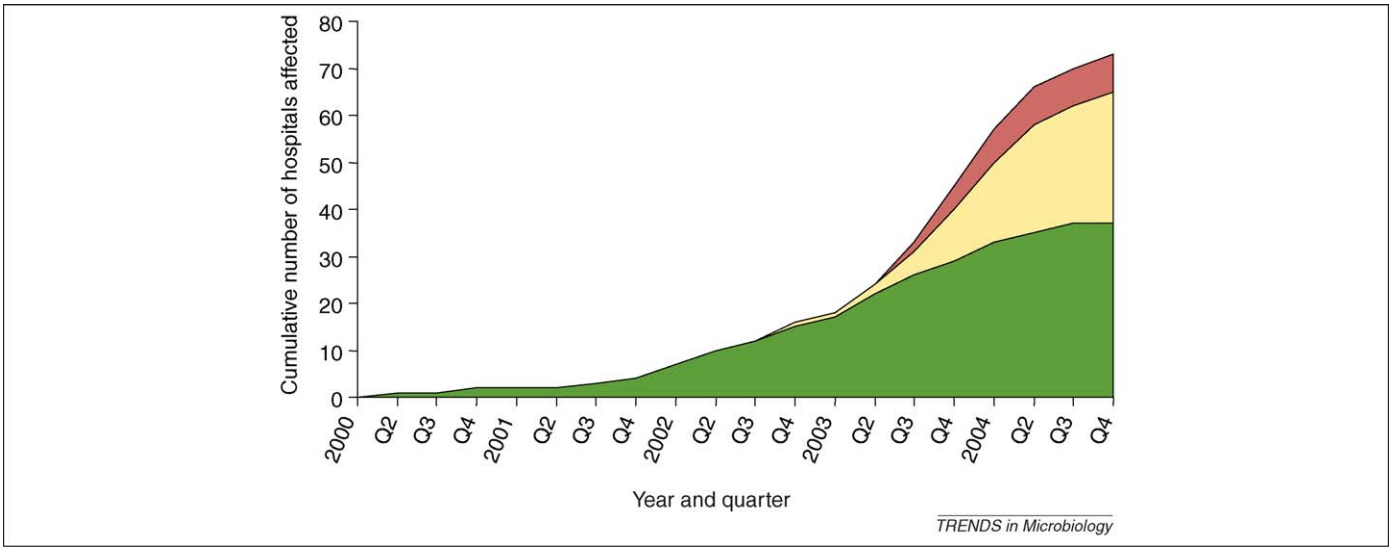

Figure 1. The rise of carbapenem-resistant *Acinetobacter* clones in the UK. In a survey undertaken in 2000, it was found that <2% of *Acinetobacter* isolates from UK hospitals were carbapenem resistant [55] with no evidence of widely disseminated resistant clones. Subsequently, the SE clone (green), which often has an upregulated chromosomal OXA-51-like enzyme, and the OXA-23 clone 1 (yellow), which has the OXA-23 enzymes and the OXA-51-like enzyme (not upregulated), have become prevalent, with each clone affecting ~40 hospitals. Another OXA-23-producing clone, the OXA-23 clone 2 (red), affected eight sites by 2004 but has since declined. The two OXA-23 clones are consistently resistant to carbapenems, other β-lactams, quinolones and aminoglycosides (except amikacin in the case of clone 2) and are consistently susceptible only to polymyxins and perhaps tigecycline. The SE clone shows more variable but frequent carbapenem resistance, dependent on whether or not $bla_{\text{OXA-51}}$-like is upregulated by IS*Aba1*, otherwise it is resistant to all agents except polymyxins and tigecycline.

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

β-lactam	MIC (mg L^{-1}) for:				
	R-recipient	Transconjugant with pFER encoding OXA-23	Transconjugant with pMAD encoding OXA-58	Wild type with OXA-40 β-lactamase	Wild type ΔOXA-40
Imipenem	0.25	>32	2	>32	2
Meropenem	0.25	>32	2	>32	4
Ceftazidime	2	>32	2	>32	>32

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

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 feeble 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 carbapenem 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 nebulized 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 overcame 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 labile 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 cefpirome) with available inhibitors such as clavulanate or tazobactam 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.

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