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# OXA β-lactamases in *Acinetobacter*: the story so far

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**The emergence of carbapenem resistance in *Acinetobacter baumannii* has become a global concern since these β-lactams are often the only effective treatment left against many multiresistant strains. A recent development has been the discovery of a novel group of narrow-spectrum OXA β-lactamases in carbapenem-resistant strains, some of which have acquired the ability to hydrolyse the carbapenems. The first of these was found in a strain isolated in Edinburgh before imipenem was in use in the hospital. Whether these carbapenemases have been acquired or are part of the genetic make-up of this species has yet to be determined. More importantly, however, they represent an important stage in the evolution of antibiotic resistance in *Acinetobacter*. This paper discusses the emergence of these unusual enzymes over the past decade.**

Keywords: *Acinetobacter*, OXA-type β-lactamases, carbapenem resistance

Antibiotic resistance in Gram-positive bacteria, notably methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci, has been the focus of attention both in the clinical setting and the press, however, there has been a slow but steady emergence of resistant Gram-negative pathogens. One such organism, *Acinetobacter*, and in particular *Acinetobacter baumannii*, is recognized as playing a significant role in the colonization and infection of patients, especially those in intensive care units.[1] A major problem facing clinicians is how to effectively treat infections caused by this organism. The carbapenems have been relied upon for treating infections caused by multidrug-resistant *A. baumannii*; however, resistance to this β-lactam class is now a common occurrence, and pan-drug-resistant strains are beginning to emerge.[2]

The first reports of imipenem resistance in *A. baumannii* started to emerge over a decade ago.[3,4] In 1993, the first of a novel group of narrow-spectrum OXA-type β-lactamases was discovered in an imipenem-resistant *A. baumannii* strain from a patient in the Royal Infirmary of Edinburgh that was found to possess carbapenem-hydrolysing activity.[5] The strain itself was isolated in 1985, before the use of imipenem in the hospital. Imipenem resistance was subsequently demonstrated to be transferable[6] and sequence analysis of the gene discovered that it encoded an unusual OXA-type enzyme (designated OXA-23) of Ambler molecular class D.[7] This raised concern at the time but appeared to be an isolated case. However, in 1997, another OXA β-lactamase was found in an imipenem-resistant strain isolated in France.[8] Although the encoding gene was never sequenced, the purified enzyme was shown to hydrolyse imipenem.

Three oxacillinases lacking carbapenemase properties were subsequently discovered. OXA-21 was found in an imipenem-susceptible *A. baumannii* strain in Spain but did not appear to be widely disseminated in *Acinetobacter* although the encoding gene was located on an integron.[9] However, it was subsequently found in carbapenem-susceptible and -resistant *Pseudomonas aeruginosa* in France.[10] The integron-borne enzyme OXA-37 was then discovered in a multidrug-resistant (but imipenem-susceptible) *A. baumannii* strain in Spain,[11] which differed by one amino acid from the restricted-spectrum oxacillinase OXA-20, originally identified in *P. aeruginosa*.[12] OXA-20 was subsequently found on class 1 integrons in multiresistant *A. baumannii* strains from France[13] and Spain,[14] and in an imipenem-resistant *A. baumannii* clone from Italy, although the observed carbapenem resistance was not attributed to the presence of this enzyme.[15] There are some similarities in the integrons associated with these enzymes; however, it is uncertain which genus they have originated from.

The incidence of carbapenem-resistant strains and the OXA-type β-lactamases associated with them has continued to increase (Figure 1). These strains have been associated with infection outbreaks and have contributed to patient mortality. Between the years 2000 and 2004, six novel OXA-type enzymes were characterized from carbapenem-resistant strains collected worldwide from 1995 onwards. OXA-24 was found in a highly carbapenem-resistant strain from Spain[16] and represented a second subgroup of these enzymes since it shared <60% amino acid identity with OXA-23. A further three OXA-24-related β-lactamases (OXA-25, OXA-26 and OXA-40) were subsequently identified in strains from Spain, Belgium and Portugal,[17–19] and two OXA-23 variants, OXA-27[17] and OXA-49 (AY288523), were found in resistant strains from Singapore and China, respectively.

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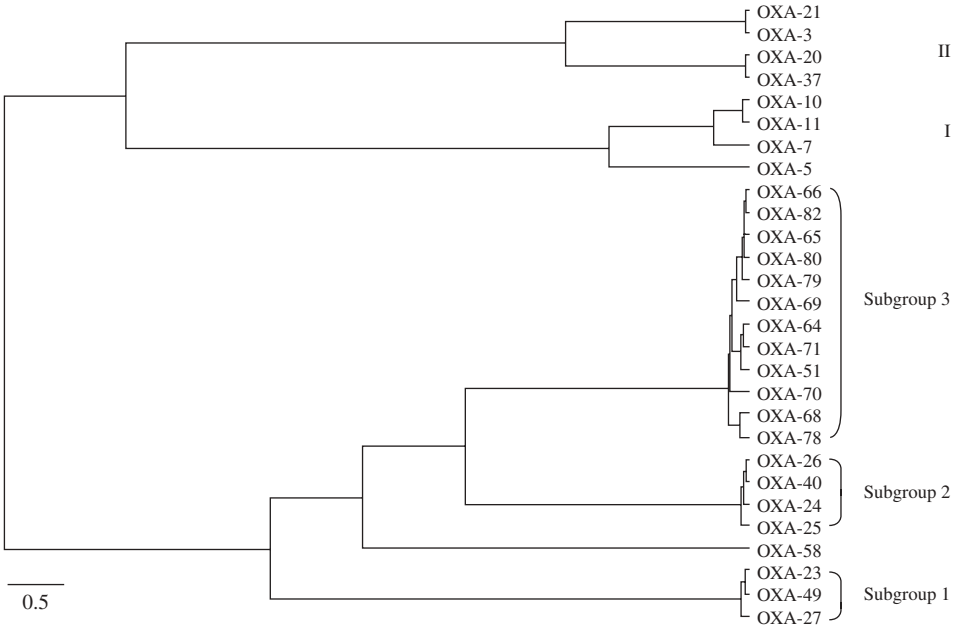


**Figure 1.** UPGMA dendrogram of OXA β-lactamases in *Acinetobacter* and most closely related OXAs from groups I and II.[35] A gap penalty of 3 was applied.

Within the last couple of years, OXA-23 has been found in *Acinetobacter* strains from Brazil,[20] China (accession number AY554200)[21] and Singapore (accession number AY795964), and is now endemic in numerous hospitals in Southern England.[22] It has also been found in related strains of *Proteus mirabilis* in France;[23] however, its presence was insufficient to render them resistant to the carbapenems. Indeed, a common feature of these enzymes is their narrow-spectrum profiles and relative weak hydrolytic activity, compared with the metallo-carbapenemases, against the carbapenems, although the MIC values of the strains expressing them vary (typically from 16 to 256 mg/L),[7,16–19] indicating the presence of additional resistance factors notably reduced permeability.[24]

More recently, a third subgroup of OXA β-lactamases sharing <63% amino acid identity with subgroups 1 and 2 has been identified in *A. baumannii*. OXA-51 was characterized from two imipenem-resistant *A. baumannii* clones isolated in Argentina.[25] A further seven enzymes were subsequently found in carbapenem-resistant strains collected worldwide, which share 98–99% identity with OXA-51,[26] and at least another four derivatives have recently been identified (S. Brown and S. Amyes, unpublished results). A common feature of this subgroup is retention of the class D Y-G-N motif at positions 144–146 according to the numbering system of Couture *et al.*,[27] in contrast to subgroups 1 and 2 OXA-type carbapenemases which possess a Phe-144.[7,16–19] However, this Phe change is not thought to be associated with carbapenem-hydrolysing activity.[19] An unrelated OXA β-lactamase (OXA-58) has recently been found in unrelated carbapenem-resistant *A. baumannii* isolates in France.[28,29] OXA-58 shares <50% amino acid identity to the other oxacillinases, and retains the Y-G-N motif. An OXA-58-like enzyme has also been found in strains from Argentina, Kuwait and Southern England.[30]

Unlike the majority of oxacillinases in other genera, none of the *Acinetobacter* OXA genes from carbapenem-resistant strains have been found on integrons. With the exception of the plasmid-encoded OXA-23 and OXA-58, the others have either been demonstrated or assumed to be chromosomally-mediated. At least four enzymes of subgroup 3 have been found in strains from more than one country, suggesting that dissemination between strains may have taken place.[26] It also raises the question as to whether these enzymes always confer carbapenem insusceptibility. For instance, the subgroup 3 OXA-69 first reported by us in strains from Singapore and Turkey was associated with an intermediate resistance to carbapenems in the absence of other apparent resistance factors.[26] A subsequent identification of a small number of *A. baumannii* strains harbouring this β-lactamase was associated with carbapenem susceptibility despite the fact that the enzyme was shown to hydrolyse the carbapenems, albeit at a low level.[31] This is further supported by recent findings which show that expression of these genes is not always closely related to carbapenem insusceptibility.[32] It remains to be seen whether these genes are naturally occurring or have been acquired, although the known enzymes do not appear to be ubiquitous in this species (K. Towner, University Hospital, Nottingham, UK, personal communication).

What is certain is that carbapenem resistance in *A. baumannii* is becoming more prevalent, and resistant strains are now emerging in regions that have up to now managed to avoid the problem. In addition, strains are now emerging that harbour more than one OXA-encoding gene (S. Brown and S. Amyes, unpublished results). Further studies are needed to determine the exact roles that these enzymes play in the evolution of carbapenem resistance in this genus. Does *Acinetobacter* possess its own group of naturally-occurring oxacillinases, as has been found with other 'environmental' bacteria?[33,34] Furthermore, will some become the progenitors of future derivatives that possess carbapenem-hydrolysing capabilities in response to prolonged antibiotic exposure, as seen with the extended-spectrum β-lactamases and cephalosporin resistance in Enterobacteriaceae? The carbapenems are the drugs of choice for treating infections caused by ESBL-producing bacteria. An alternative therapeutic strategy for

carbapenem-resistant *A. baumannii* is proving a bigger problem to solve and may only succeed if future research includes the development of inhibitors of class D carbapenemases.

## Transparency declarations

None to declare.

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