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OXA-type carbapenemases

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In recent years, the number of class D β-lactamases with carbapenem-hydrolysing properties has increased substantially. Based on amino acid sequence identities, these class D or OXA-type carbapenemases are divided into eight distantly related groups, and they are only remotely related to other class D β-lactamases. A putative ancestor to one of the plasmid-encoded OXA-type carbapenemases has been found.

OXA-type carbapenemases are not integrated into integrons as gene cassettes like many class D oxacillinases, but most of the OXA-type carbapenemases are instead encoded by chromosomal genes. Some of these OXA-type carbapenemases are widely dispersed in *Pseudomonas aeruginosa* and especially in *Acinetobacter baumannii*.

Although most of the OXA-type carbapenemases show only weak carbapenemase activity, carbapenem resistance may result from a combined action an OXA-type carbapenemase and a secondary resistance mechanism such as porin deficiencies or overexpressed efflux pumps. This article reviews the phylogeny and the genetic environments of the encoding genes and kinetic properties of the OXA-type carbapenemases.

Keywords: class D β-lactamases, carbapenem resistance, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*

Introduction

β-Lactam antibiotics, including penicillins, cephalosporins and carbapenems, act cytostatically on bacteria by inactivating peptidoglycan transpeptidases irreversibly. The transpeptidases catalyse the cross-linking of the peptidoglycan polymers in the bacterial cell wall,[1,2] and inhibition of this polymerization leads to cell death. The transpeptidases are members of the family of penicillin binding proteins (PBPs) from which β-lactamases are likely to have evolved.[3]

β-Lactamases are enzymes of entirely bacterial origin that degrade β-lactam antibiotics into antimicrobially inert compounds. The enzymes thus protect the organisms against the lethal actions of β-lactam antibiotics,[4] and the enzymes are the primary cause of bacterial resistance to these drugs.[5]

The β-lactamases can be classified into four different molecular groups, A, B, C and D, according to amino acid sequence identities.[6–8] Class A, C (AmpC) and D β-lactamases use a catalytically active serine residue for inactivation of the β-lactam drug.[9] The enzymes assigned to the molecular class B are metallo-enzymes requiring zinc for their catalytic activity, and they operate through a completely different mechanism.[10,11]

Carbapenems, such as imipenem and meropenem, have a very broad spectrum of activity, and the drugs resist hydrolysis by most of the β-lactamases, including extended-spectrum β-lactamases and derepressed chromosomal AmpC β-lactamases.[12] Most of the metallo-β-lactamases together with some class A and D β-lactamases are able to hydrolyse these compounds.[13,14]

MIC (minimal inhibitory concentration) breakpoints for imipenem and meropenem resistance in non-fastidious Gram-negative pathogens, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and in members of the Enterobacteriaceae have been defined by the NCCLS as ≥16 mg/L. The NCCLS susceptibility breakpoints were established as ≤4 mg/L.

Acquired resistance to carbapenems is mediated by different mechanisms. β-Lactamases, especially the class B enzymes, account for most of the resistance to carbapenems in bacteria.[13,14] Other resistance mechanisms are attributed to reduced affinity of PBPs for carbapenems,[15–17] increased efflux of the β-lactam antibiotics,[18,19] decreased permeability of the outer membrane[20–22] or to a combination of reduced permeability and high-level production of a β-lactamase, typically an AmpC β-lactamase.[23–26]

The focus of this review is on the class D or OXA-type carbapenemases.

Class D β-lactamases

Historically, the first characterized class D β-lactamases were also referred to as oxacillinases because they commonly hydrolyse the isoxazolylpenicillin oxacillin much faster than classical penicillins, i.e. benzylpenicillin.[27] The designation, OXA, of the class D β-lactamases, thus, refers to their preferred penicillin substrate.

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Currently, 121 different variants of class D β-lactamases have been identified on the protein level and 45 of these exhibit carbapenem-hydrolysing activities, which is in contrast to other class D β-lactamases.

From multiple sequence alignment of the class D β-lactamases, it appears that the enzymes have three highly conserved active-site elements in common. The first element is the tetrad, Ser^{70}-X-X-Lys, where X represents a variable residue, containing the active-site serine [Ser^{70} according to the DBL (class D β-lactamase) numbering].[28] The second element, Ser^{118}-X-Val/Ile, is equivalent to the invariable Ser-Asp-Asn motif in class A β-lactamases and Tyr-Ala/Ser-Asn in AmpC β-lactamases, while the Lys^{216}-Thr/Ser-Gly element is common to the vast majority of serine-active β-lactamases. Other conserved motifs in class D β-lactamases are the triad Tyr/Phe^{144}-Gly-Asn and the tetrad Trp^{232}-X-X-Gly that have no analogues in either class A or AmpC β-lactamases.

Subgroups of OXA-type carbapenemases

The carbapenem-hydrolysing OXA enzymes can be subclassified into eight distinct branches or subgroups as shown in Figure 1. The sequence identities between members of each group are ≥92.5% whereas the identities between enzymes that belong to different clusters range from ~40 to 70%. These eight groups are only remotely related to class D oxacillinases that do not possess carbapenem-hydrolysing properties. In Table 1, the OXA-type carbapenemases are summarized together with their geographical distribution.

Four of the eight clusters have been identified in *A. baumannii*, and the isolates were collected in Europe, South America, Asia and French Polynesia (Table 1). The first cluster is formed by OXA-23,[30] also named ARI-1 (an acronym of *Acinetobacter* resistant to imipenem), together with OXA-27[40] and OXA-49 (AY288523). These enzymes differ by two to five amino acid substitutions.

The second family encompasses the OXA-24, -25, -26, -40[39–41] and -72 β-lactamases (AY739646). The sequence divergences between the members of this cluster vary from 1 to 5 amino acids.

The third group consists of the OXA-51 family enzymes,[45,51,52] while only a single member, namely OXA-58,[48] represents the fourth group. The recently identified OXA-51 group of β-lactamases comprises a novel phylum among the OXA-type carbapenemases, and the cluster includes also OXA-64 to -66, OXA-68 to -71 and OXA-75 to -78

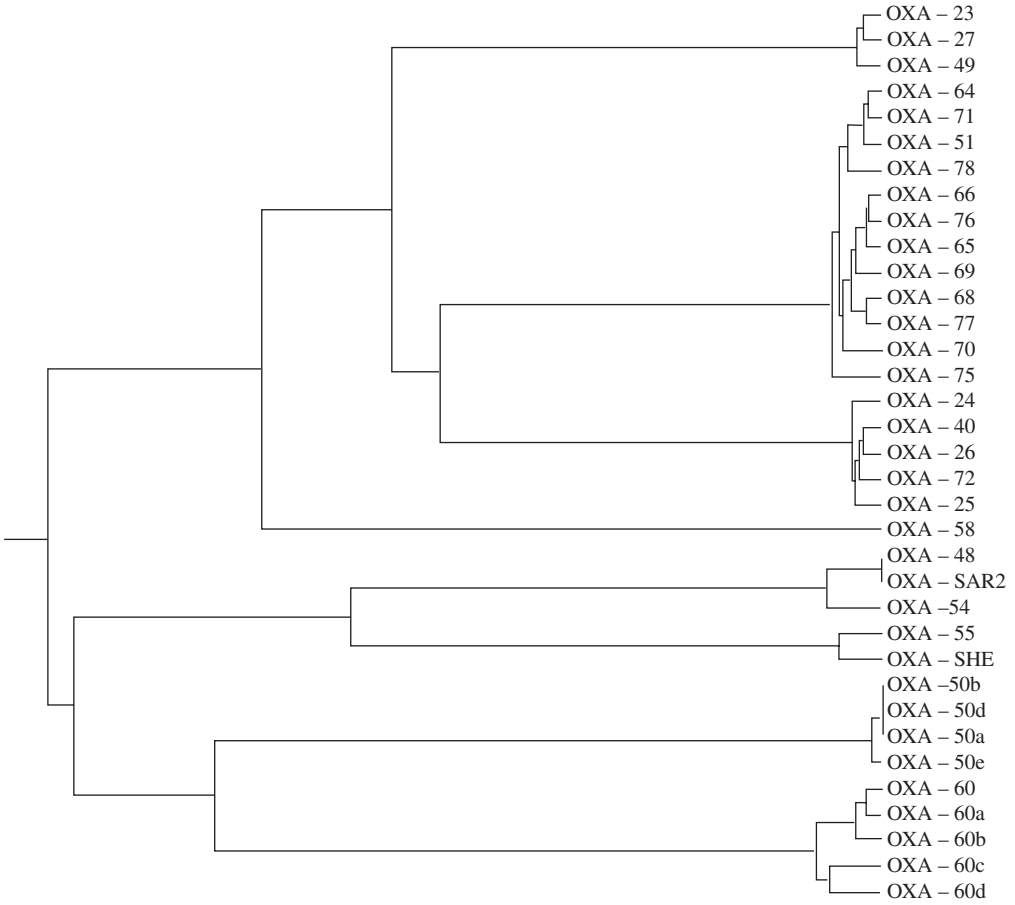

Figure 1. Dendrogram of precursor OXA-type carbapenemases. The tree was constructed by the use of the www-based services, ClustalW algorithm (http://www.genebee.msu.su/clustal) and the Phylogenetic tree printer (http://www.iubio.bio.indiana.edu/treeapp/treeprint-sample2.html). The PoxB enzymes related to the OXA-50 enzymes of *P. aeruginosa* have been omitted from the dendrogram. References are given in Table 1.

Table 1. Compilation of the OXA-type carbapenemase-producing bacteria, the pI values of the enzymes, and the geographical distribution and year of isolation

Enzyme	Loc[a]	Host	Strain	pI	Year	Country	Acc. no.[b]	Reference
OXA-23[c]	P	*Acinetobacter baumannii*	6B92	6.65	1985	UK	AJ132105	(29,30)
OXA-23		*A. baumannii*			1999	Brazil		(33)
OXA-23	C	*Proteus mirabilis*			1996–99	France		(34)
OXA-23		*A. baumannii*	25547/96		1996[d]	Singapore	AY795964	
OXA-23[e]	(C)	*A. baumannii*			1999–2001	China		(35)
OXA-23		*Acinetobacter junii*	ZN3858			China	AY554200	
OXA-23	C	*A. baumannii*		6.65	2003	Rep. of Korea		(37)
OXA-23	P	*A. baumannii*		6.9	2004	Tahiti		(38)
OXA-24	(C)	*A. baumannii*	RYC 52763/97	9.0	1997	Spain	AJ239129	(39)
OXA-25	(C)	*A. baumannii*	327009	8.0		Spain	AF201826	(40)
OXA-26	(C)	*A. baumannii*	04737	7.9		Belgium	AF201827	(40)
OXA-27	(C)	*A. baumannii*	I–16	6.8		Singapore	AF201828	(40)
OXA-40[f]	(C)	*A. baumannii*	CLA-1	8.6		France	AF509241	(41)
OXA-48	P	*Klebsiella pneumoniae*	11978	7.2	2001	Turkey	AY236073	(42)
OXA-49[g]		*A. baumannii*		6.8		China	AY288523	
OXA-50a	C	*Pseudomonas aeruginosa*	GW-1	8.6	2000	Rep. of South Africa	AY306130	(43)
OXA-50b[h]	C	*P. aeruginosa*	COL-1		1999	France	AY306131	(43)
OXA-50c	C	*P. aeruginosa*	Ka.209			Spain	AY306133	(43)
OXA-50d[h]	C	*P. aeruginosa*	1		1999	Thailand	AY306135	(43)
OXA-51[i]	C	*A baumannii*	788	7.0	1994	Argentina	AJ309734	(45)
OXA-54	C	*Shewanella oneidensis*	MR-1	6.8			AY500137	(46)
OXA-55[j]	C	*Shewanella algae*	KB-1	8.6	2001	France	AY343493	(47)
OXA-SHE	C	*S. algae*	N511				AY066004	
OXA-58[k]	C	*A. baumannii*	MAD	7.2	2003	France	AY570763	(48)
OXA-60[l]	C	*Ralstonia pickettii*	PIC-1	5.1		France	AF535203	(50)
OXA-64	C	*A. baumannii*			1996–2000	Rep. of South Africa	AY750907	(51)
OXA-65	C	*A. baumannii*			1996–2000	Argentina	AY750908	(51)

Table 1. *(Continued)*

Enzyme	Loc[a]	Host	Strain	pI	Year	Country	Acc. no.[b]	Reference
OXA-66[m]	C	*A. baumannii*			1996–2000	Spain	AY750909	(51)
OXA-68	C	*A. baumannii*			1996–2000	Spain	AY750910	(51)
OXA-69[n]	C	*A. baumannii*		8.4	1996–2000	Turkey	AY750911	(51)
OXA-70	C	*A. baumannii*			1996–2000	Hong Kong	AY750912	(51)
OXA-71[o]	C	*A. baumannii*			1996–2000	Rep. of South Africa	AY750912	(51)
OXA-72		*A. baumannii*				Thailand	AY739646	
OXA-75	C	*A. baumannii*	SDF		1997	France	AY750914	(52)
OXA-76	C	*A. baumannii*	AMA-1		1999	France	AY949203	(52)
OXA-77	C	*A. baumannii*	CIP7034				AY949202	(52)
OXA-78	(C)	*A. baumannii*	AO14			Turkey ?	DQ149247	
OXA-type	(C)	*A. baumannii*	148	6.3	1989	France	–	(53)
OXA-type	(C)	*A. baumannii*	BA HCT15	6.9	1995	Argentina	–	(54)
OXA-type		*A. baumannii*	A-15	7.0	1995–1997	Kuwait	–	(40)

[a]Loc, genetic location: C, chromosome; (C), probably a chromosome; P, plasmid.
[b]Acc. no., GenBank accession number.
[c]The isolate 6B52 was recovered in 1985 in Scotland. In 2003, *A. baumannii* isolates producing OXA-23 have been collected from 15 hospitals in southeast England.[31] Plasmid-encoded OXA-23 has also been identified in *A. baumanni* and *A. junii* recovered in Romania.[32]
[d]Sng Li Hiew (2005) Department of Pathology, Singapore General Hospital, 1 Hospital Drive, Singapore 169608, Singapore, personal communication.
[e]In 2000-02, an OXA-23-producing *A. baumannii* isolate was collected from a hospital in another part of China.[36]
[f]The gene encoding OXA-33 (GenBank accession no. AY082394) is 100% identical to the bla_{OXA-40} gene. The OXA-33-producing *A. baumannii* isolate was recovered in Portugal. A bla_{OXA-40} gene has also been found on a plasmid from *A. baumannii* (AY228470).
[g]OXA-49 was identified in an *A. baumannii* isolate not included among those described by Yu *et al*.[35] Yun-song Yu (2005) Department of Infectious Diseases, First Affiliated Hospital, College of Medicine, Zheijang University, Hangzhou 310003, China, personal communication.
[h]Variant enzymes have also been identified in *P. aeruginosa* collected in India in 2000 (variant b) and in France in 1998 (variant d).[43] The highly related enzymes, PoxB, have been described in *P. aeruginosa* from clinical and environmental specimens collected mainly in different European countries.[44]
[i]The OXA-51 enzyme has been identified in other clonally unrelated isolates of *A. baumannii* collected in Buenos Aires between Oct. 1993 and Nov. 1994.[45]
[j]Chromosomally encoded β-lactamases that were at least 98% identical to OXA-55 were found in two other strains of *S. algae*.[47]
[k]The OXA-58 enzyme was identified in several genetically related isolates of *A. baumannii*.[49] OXA-58 enzyme was also plasmid-encoded in the majority of isolates of *A. baumannii* collected from France, Italy, Spain, Turkey and Romania.[32] An *A. junii* isolate from Romania also produced a plasmid-encoded OXA-58.[32]
[l]OXA-60 variant enzymes have been recorded in clonally unrelated isolates of *R. pickettii*.[50] OXA-60a had a pI of 5.1, OXA-60b 5.2, and OXA-60c and OXA-60d both 5.3.[50]
[m]The bla_{OXA-66} gene has also been reported in *A. baumannii* isolates from Hong Kong and Singapore[51] and in other *A. baumannii* isolates recovered in Poland (1999) and France (2001).[52]
[n]The bla_{OXA-69} gene has also been recorded in an *A. baumannii* isolate from Singapore[51] and in other *A. baumannii* isolates collected in Italy (1997), Turkey (1998) and France (2001).[52]
[o]The bla_{OXA-71} gene has also been reported in another *A. baumannii* isolate from Spain.[52]

(Figure 1). The members of this family diverge by 1–15 amino acid modifications, and the enzymes may be naturally occurring in *A. baumannii*.[51,52]

The fifth group is represented by the chromosomally encoded enzymes OXA-55[47] and OXA-SHE (AY066004) from *Shewanella algae*. The enzymes deviate by five substitutions.

The plasmid-mediated OXA-48 from *Klebsiella pneumoniae*[42] groups with the chromosomally mediated OXA-54 from *Shewanella oneidensis*[46] and form the sixth branch, and the enzymes differ by 20 substitutions.

In a whole genome shotgun (WGS) sequence of *Shewanella* SAR-2 (CH004480), an environmental microbe collected from the Sargasso Sea,[55] a putative bla_{OXA} and downstream located *lysR* gene have been identified by an *in silico* analysis. The OXA-SAR2 enzyme deviates from OXA-48 by a single amino acid substitution located in the N-terminal part of the mature enzyme (residue no. 43 in the precursor sequence according to DBL numbering) implying that OXA-SAR2 may possess carbapenemase activity.

The plasmid-encoded bla_{OXA-48} and putative *lysR* genes and their flanking nucleotide sequences from *K. pneumoniae*[42] were compared with the corresponding sequence from *Shewanella* SAR-2 (nucleotides 42659-44883 of CH004480). The two sequences are 99.2% identical, owing to 8 transitions, 6 transversions and 4 deletions in the *Shewanella* sequence. One deletion in the *Shewanella* sequence had occurred in the region between the bla_{OXA} and *lysR* genes while the remaining three deletions were located in the 3′-end of the *lysR* gene resulting in truncation of the penultimate amino acid residue of the LysR regulator. This high identity documents that OXA-48 most likely has descended from an OXA β-lactamase of the genus *Shewanella*.

Genes encoding OXA-type β-lactamases may possibly be normal constituents of the genome of some species of *Shewanella.* The bla_{OXA-54} gene has been described in the genome of *S. oneidensis*,[46,56] while putative bla_{OXA}-type genes have been identified in WGS sequences of *Shewanella baltica* (AAIO01000124), *Shewanella putrefaciens* (NZ_AALB01000094), *Shewanella* sp. ANA-3 (AALH01000006), *Shewanella* sp. MR-7 (AALI01000021), and *Shewanella* SAR-1 (CH004559) and SAR-2 (CH004480). Apart from OXA-54, it is not known whether any of these gene products possess carbapenemase activity. Similar genes have not been found in *Shewanella frigidimarina* (AAIV01000000), *Shewanella denitrificans* (AAIU01000000) or *Shewanella amazonensis* (NZ_AAIN00000000).

The seventh and eighth branches are represented by the OXA-50 enzymes in *P. aeruginosa*[43] and the OXA-60 enzymes in *Ralstonia pickettii*,[50] respectively, and each group holds several variant enzymes. The members of the OXA-50 cluster differ by 1–5 substitutions, while the OXA-60 variant enzymes deviate from each other by 1–21 amino acid modifications.

The PoxB enzymes identified in various strains of *P. aeruginosa*[44] are also members of the OXA-50 family as the enzymes differ from the OXA-50s by 1–8 substitutions, corresponding to identities from 99.6 to 97.0%.

However, the PoxB enzyme from *P. aeruginosa* DSM 1128[44] deviates from this level of identity by having identities of 86.3–87.4% to the other members of the OXA-50 family. *P. aeruginosa* DSM 1128 could be another but closely related *Pseudomonas* sp.; however, DSM 1128 has been identified both phenotypically and genotypically as a *P. aeruginosa* strain. Kiewitz and Tümmler[57] have also found unusually high sequence variations in other loci of DSM 1128 which may be caused by a defective replication or DNA repair system.

Descriptions of putative OXA-type carbapenemases have been published, but their sequences are at present unknown. Afzal-Shah *et al*.[54] have characterized an OXA-type carbapenemase from *A. baumannii* that is not genetically related to either the OXA-23 or the OXA-24 families.[40] A presumptively chromosomally encoded OXA-type carbapenemase has been described in *A. baumannii*.[53]

Genetic environment of genes encoding OXA-type carbapenemases

In clinical strains, the genes encoding class D oxacillinases have commonly been found on plasmids incorporated as gene cassettes in integrons.[58] Among the genes encoding OXA-type carbapenemases, only bla_{OXA-23}, bla_{OXA-40} and bla_{OXA-58} in *A. baumannii* and bla_{OXA-48} in *K. pneumoniae* are plasmid borne (Table 1). None of the genes have, however, been found as gene cassettes, owing to the lack of the characteristic features of the 59-base element. Instead, bla_{OXA-23}, bla_{OXA-48} and bla_{OXA-58}, together with bla_{OXA-27}, are associated with insertion sequences (IS). Upstream of the bla_{OXA-48} gene IS*1999* is found;[42] whereas, two slightly divergent copies of IS*Aba3* bracket the bla_{OXA-58} gene forming a composite transposon.[48] Mobile elements were not linked to the plasmid-borne bla_{OXA-40} gene (AY228470).

The association of the bla_{OXA-48} gene with the reversely oriented IS*1999* may cause an increased expression of the *bla* gene, as shown for the bla_{VEB-1} gene in *P. aeruginosa.*[59]

The genetic context of the bla_{OXA-23} gene characterized by Donald and co-workers[30] was investigated by an *in silico* analysis of the DNA sequences flanking the gene. The examination showed that the upstream sequence from the bla_{OXA-23} gene was 99.3% identical to the insertion sequence, IS*Aba1*, described by Segal *et al*.[60] and Poirel (http://www-is.biotoul.fr; 21 June 2005, date last accessed). This IS*Aba1* isoform diverged slightly from IS*Aba1* in the *tnp* gene and inverted-repeat-left (IR-L), and therefore the variant was designated IS*Aba1-B*. The nucleotide sequence of IS*Aba1-B* contained a deletion and five changes compared with that of IS*Aba1*, and the former occurred in the 5′-end of the *tnp* gene resulting in a premature arrest of translation of the gene. As the orientation of IS*Aba1-B* was reversed compared with the bla_{OXA-23} gene, IR-L was contiguous to the 5′-end of the *bla* gene. The IR-L of IS*Aba1-B* was also identified in the upstream sequences of other bla_{OXA-23} genes (AY554200, AY795964, DQ029069) and the bla_{OXA-27} gene (AF201828). IS*Aba1* is related to IS*4*, a heterogeneous family of insertion sequences.

The sequences, including IR-L of IS*Aba1-B*, upstream of the bla_{OXA-23} (AY554200, AY795964, DQ029069) and bla_{OXA-27} genes[40] in isolates recovered from Asia were completely identical. In the equivalent sequence from the UK isolate,[30] a heptamer was truncated from the outer boundary to IR-L, but apart from this truncation the remaining sequences in the region between IR-L and the *bla* locus were identical. The first 75 nucleotides of downstream sequences from the bla_{OXA-23} and bla_{OXA-27} genes were completely identical; however, *A. baumannii* strain 25547/96 (AY795964) contained three transversions in the most distant part (>75 bp) of the downstream sequence. These findings may imply that the genes from Asia and the gene from the UK have been mobilized from the same unknown ancestor.

Most of the OXA-type carbapenemase genes in *A. baumannii* seem to be chromosomally located (Table 1), but these genes do not appear to be ubiquitous parts of the genetic background of the species. The G+C contents of the bla_{OXA-51} family genes vary between 39 and 40%, which is within the range of the G+C contents of *A. baumannii* genes.[61] As the bla_{OXA-51} family genes seem to be absent from some *A. baumannii* strains this may imply that the genes are not omnipresent in the species, but present rather in subpopulations of *A. baumannii*.

Apart from bla_{OXA-51} family genes, the G+C contents of the other bla_{OXA} genes in *A. baumannii* vary between 34 and 38% implying that the latter genes have evolved in other species. Some of these genes have subsequently been embedded in the *A. baumannii* chromosome. When investigated, the chromosomal OXA-type carbapenemase genes have not been found to be associated with integrons or insertion sequences.[39,41]

Experiments using imipenem for induction of OXA-60 in *R. pickettii* induced an elevated biosynthesis of the enzyme;[50] whereas the induction of OXA-50 in *P. aeruginosa* and OXA-69 in *A. baumannii* failed when challenged with either imipenem or cefoxitin.[43,52]

The investigation by Kong *et al*.[62] implies why the induction of the OXA-50/PoxB carbapenemase production in *P. aeruginosa* failed. The synthesis of the OXA-50/PoxB carbapenemase seems to be suppressed by AmpR,[62] the LysR transcriptional activator of the ubiquitous AmpC β-lactamase.[63] In the presence of a β-lactam antibiotic the expression of the OXA-50/PoxB enzyme is more reduced than in the absence of the drug.[62] The AmpR also regulates expression of other genes that are not in the least associated with β-lactam resistance.[62]

The *ampC* genes in *A. baumannii* and *S. oneidensis* are not linked to an upstream-located *ampR* gene. In some *A. baumannii* strains, the *ampC* gene seems to be preceded by a transposase gene. The possibility that the production of OXAs of *A. baumannii* and *S. oneidensis* may be regulated by other LysRs cannot be entirely excluded. Alternatively, induction may be executed by non-β-lactam compounds like the induction of other β-lactamases.[64,65]

In *S. oneidensis* and *S. algae*, the bla_{OXA-54},[46] bla_{OXA-55}[47] and $bla_{OXA-SHE}$ genes (AY066004) are linked to downstream located and divergently transcribed genes encoding putative transcriptional regulators of the LysR-type. However, induction experiments indicating involvement of these transcriptional activators in expression of the bla_{OXA} genes are still lacking. Challenge with cefoxitin and imipenem for induction of the bla_{OXA-54} gene expression have been performed, but induction failed.[46]

It cannot completely be ruled out that the gene products of the *lysR* genes located downstream from the bla_{OXA}-type genes in *S. oneidensis* and *S. algae* may act in a similar manner as AmpR in *P. aeruginosa*. In *A. baumannii* and *R. pickettii*, no regulator gene has been found immediately downstream from the bla_{OXA} gene.

Origin of OXA-type carbapenemases

Environmental microbiota may carry chromosomal genes conferring resistance to antibiotics as a protection against antibiotic-producing soil micro-organisms. Imipenem is an N-formimidoyl derivative of thienamycin, which is a natural product of the soil organism *Streptomyces cattleya*.[66] Possession of carbapenem-hydrolysing enzymes would thus be beneficial for soil bacteria. As a result of this natural selective pressure, the organisms may originally have evolved enzymes with carbapenemase activities or they may have acquired these genes from other bacteria through plasmids acting as vehicles. The genes may either have remained located on plasmids or subsequently become inserted into the chromosome by recombination, co-integration or transposition.

Barlow and Hall[67] have estimated that bla_{OXA} genes have been on plasmids for millions of years, and the ancestors of the mobile OXA-type carbapenemases may thus have disappeared long ago. A more recent mobilization of chromosomal genes to plasmids and interspecies transfer and integration into the chromosome have been described for the *ampC* genes.[68–70]

The first identified isolate expressing an OXA-type carbapenemase was the OXA-23-producing *A. baumannii* from Scotland.[29] The isolate was recovered in 1985 before or at the time when imipenem was approved for general use. Likewise, class A β-lactamases with carbapenem-hydrolysing properties were recorded prior to imipenem being licensed for clinical use. Those were IMI-1 and SME-1 which were characterized from isolates collected in 1982 in UK and in 1984 in USA, respectively.[71,72]

These findings strongly suggest that the clinical use of imipenem is not responsible for the evolution of the class A and D carbapenemases; the enzymes were probably present in bacteria long before this.

Biochemical properties of OXA-type carbapenemases

Mature OXA-type carbapenemases contain between 243 and 260 amino acid residues with experimentally determined molecular masses that vary from 23 to 35.5 kDa. The OXA-69 enzyme is the first OXA-type carbapenemase that has been shown to form a dimer,[52] similar to many class D oxacillinases. The isoelectric points (pIs) of the enzymes vary between 5.1 and 9.0 (Table 1).

The active-site serine β-lactamases operate by a simple three-step model involving acylation and deacylation, which is schematically represented by

$$S + E \underset{k_{+1}}{\overset{k_{-1}}{\leftrightarrows}} ES \underset{k_{+2}}{\overset{k_{-2}}{\leftrightarrows}} E\text{–}S^* \overset{k_{+3}}{\rightarrow} E + P \qquad (1)$$

In the first step, the β-lactamase (E) and substrate (S) interact into a Henri–Michaëlis complex (ES) which may either dissociate or form a transient acyl-enzyme intermediate (E–S*). The intermediate is formed as a result of a nucleophilic attack on the carbonyl carbon atom of the β-lactam ring by the γ-O of Ser[70], and the intermediate is subsequently rapidly deacylated releasing a ring-opened product (P) and the intact enzyme.

The three-step acylation–deacylation mechanism is identical to the serine-active β-lactamases, but the catalytic pathways of class D β-lactamases differ from those operating in class A and from AmpC β-lactamases.[73]

In Eqn. 1, k_{+1}, k_{-1}, k_{+2}, k_{-2} and k_{+3} are microscopic rate constants. Both acylation, represented by k_{+2}, and deacylation (k_{+3}) are usually rapid steps resulting in high turnover numbers [$k_{cat} = (k_{+2}\cdot k_{+3})/(k_{+2}+k_{+3})$] and catalytic efficiencies [$k_{cat}/K_m = (k_{+1}\cdot k_{+2})/(k_{+1}+k_{+2})$].[74] The acylation rate (formation of the acyl-enzyme intermediate) is represented by k_{cat}/K_m whereas the turnover number, k_{cat}, corresponds to the deacylation rate.[74]

The steady-state kinetic constants, k_{cat} and K_m, have been determined for a number of purified OXA-type carbapenemases

Table 2. Steady-state kinetic parameters, k_{cat} (s^{-1}) and k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$), for hydrolysis of various β-lactam antibiotics by OXA-type carbapenemases

Enzyme	PEN	AMP	PIP	TIC	CEF	LOR	OXA	CTX	CAZ	IPM	MEM	Ref.
OXA-40												
k_{cat}	5	5	1	1	3	5	2	–	20	0.1	–	41
k_{cat}/K_m	220	20	50	20	50	5	0.03	–	10	15	–	
OXA-48												
k_{cat}	245	340	75	45	3	2	25	10	4	2	0.1	42
k_{cat}/K_m	6100	65	185	820	150	75	830	58	1	145	0.5	
OXA-50												
k_{cat}	110	<3	<2	<0.1	0.2	<2	<0.2	–	–	0.1	<0.02	43
k_{cat}/K_m	140	<3	<2	<0.1	<1	<2	<0.2	–	–	5	<0.02	
OXA-54												
k_{cat}	120	540	20	30	3	1	35	15	NC	1	0.1	46
k_{cat}/K_m	2000	125	85	125	15	20	500	10	NC	250	1	
OXA-55												
k_{cat}	4	8	3	1	0.6	10	5	–	2	0.1	0.05	47
k_{cat}/K_m	160	15	30	50	10	15	10	–	0.5	5	0.1	
OXA-58												
k_{cat}	5.5	1	2.5	1	0.1	–	1.5	–	–	0.1	<0.01	48
k_{cat}/K_m	110	8	50	4	1	–	2	–	–	13.5	<0.15	
OXA-60												
k_{cat}	420	60[a]	>300	200	–	>0.5	>130	–	2	0.5	–	50
k_{cat}/K_m	11350	190	<150	520	–	<0.5	<65	–	2	260	–	
OXA-69												
k_{cat}	0.2	0.06	0.2	0.3	0.004	0.05	0.2	–	–	0.1	0.06	52
k_{cat}/K_m	0.28	0.25	0.11	0.05	0.02	0.02	0.06	–	–	0.03	0.01	
OXA-type[b]												
k_{cat}	120	90[a]	–	16	99	114	192	3.6	<0.4	0.6	1.2	53
k_{cat}/K_m	24000	6400	–	1950	880	2000	2500	20[c]	<10[c]	1500[c]	960[c]	

PEN, benzylpenicillin; AMP, ampicillin; PIP, piperacillin; TIC, ticarcillin; CEF, cefalotin; LOR, cefaloridine; OXA, oxacillin; CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem.
–, Not determined.
NC, not calculated because of too low initial rate of hydrolysis.
[a]Amoxicillin used as substrate.
[b]The sequence of the OXA-type carbapenemase is unknown.
[c]K_m determined as K_i by substrate competition.

(Table 2). Very low values of both k_{cat} and K_m can result in a high k_{cat}/K_m value (apparent high catalytic efficiency), but in conjunction k_{cat} and k_{cat}/K_m reflect the genuine catalytic efficiency of a β-lactamase against a β-lactam antibiotic.[74]

Even with an extremely low value of k_{cat} for hydrolysis of a β-lactam, the agent may still be hydrolysed efficiently *in vivo*, because the organism may produce exceptionally large periplasmic concentrations (up to 1 mM) of the β-lactamase.[75] As the maximal velocity attainable for the β-lactam degradation (V_{max}) is equal to the product of k_{cat} and the enzyme concentration $[E]_0$ ($V_{max} = k_{cat} \cdot [E]_0$),[75] the low k_{cat} value may be counterbalanced by a high $[E]_0$. If a specific porin deficiency besides restricts the penetration of the antibiotic into the bacteria, a large fraction of the entering β-lactam can be degraded which may result in resistance to that antibiotic.

Phenotypically, many class D β-lactamases belong to the Bush subgroup 2d β-lactamases which are defined as enzymes hydrolysing oxacillin at a rate >50% of that for benzylpenicillin.[27] However, most of the OXA-type carbapenemases hydrolyse oxacillin at a much lower rate. Only OXA-25, OXA-26, OXA-55[40,47] and the enzymes described by Hornstein *et al.*[53] and Afzal-Shah *et al.*[54] hydrolyse oxacillin at rates >50% than that for benzylpenicillin and thus comply fully with the definition of the subgroup 2d β-lactamases.

The substrate specificities of the OXA-type carbapenemases are diverse, but generally the enzymes hydrolyse penicillins (benzylpenicillin, ampicillin, piperacillin and ticarcillin) and the narrow-spectrum cephalosporins, cefalotin and cefaloridine efficiently, while the extended-spectrum β-lactams, ceftazidime, cefotaxime and aztreonam, are not or only very poorly hydrolysed (Table 2).

Most of the OXA-type carbapenemases have low hydrolytic activities against imipenem and especially against meropenem. Except for OXA-69[52] and the pI 6.9 enzyme from *A. baumannii*,[54] all OXA-type carbapenemases show higher affinity (lowest K_m) for imipenem than for any other tested β-lactam antibiotic. Most of the OXA-type carbapenemases exert their hydrolysis of imipenem by a combination of a low turnover number and high affinity for the drug. Meropenem is consistently hydrolysed at a much lower rate than imipenem, but not by the OXA-type enzyme characterized by Hornstein and co-workers.[53]

OXA-48, OXA-54 and the unsequenced OXA-type carbapenemase described by Hornstein *et al.*[53] however, hydrolyse,

Table 3. *In vitro* susceptibilities (MIC in mg/L) of *E. coli* transconjugants or transformants expressing OXA-type carbapenemases (references in parentheses)

Enzyme	OXA-23 (34)	OXA-24 (39)	OXA-40 (41)	OXA-48 (42)	OXA-54 (46)	OXA-55 (47)	OXA-58 (48)	OXA-60 (50)	OXA- 69 (52)
Ampicillin or amoxicillin[a]	>1024	128	>512	>512	>512	128	>512	256	16
+Clavulanate[b]	>1024	64	>512	>512	>512	128	128	256	8
Ticarcillin	>1024	256	>512	>512	>512	>512	>512	256	8
+Clavulanate	>1024		>512	>512	>512	256	256	256	8
Piperacillin	32		256	128	32	4	8	2	4
+Tazobactam[c]	4		128	128	32	4	8	2	4
Cefalotin	16		8	64	4	4	8	2	4
Cefuroxime		4	8	8	4	4	4	4	4
Cefoxitin	4	4		8	4	1		4	
Ceftazidime	0.25	0.25	0.5	0.12	0.12	0.12	0.12	0.5	0.06
Cefotaxime	0.06	0.06	0.12	0.25	0.12	0.06	0.12	<0.06	0.06
Cefepime		0.12	0.25	0.06	0.06	0.06	0.12	<0.06	0.06
Aztreonam	0.12	0.12	0.12	0.06	0.06	0.06	0.12	0.12	0.06
Imipenem	0.5	1	2	2	1	0.25	0.5	0.5	0.06
Meropenem	0.5	0.125	0.5	0.25	0.12	0.06	0.5	<0.06	0.06

[a]Ampicillin differs from amoxicillin only by a hydroxy group in the 6′-substituent. Ampicillin is an α-carboxyphenylpenicillin while amoxicillin is an α-carboxyhydroxyphenylpenicillin.
[b]Clavulanate was used at a fixed concentration of 2 mg/L.
[c]Tazobactam was used at a fixed concentration of 4 mg/L.

imipenem at levels significantly higher than those of the other OXA-type carbapenemases (Table 2). The kinetic parameters of these three enzymes for imipenem are similar to those of the zinc-dependent carbapenemases, L1e, VIM-1 and JOHN-1.[76–78]

Generally, class D β-lactamases are inhibited less efficiently by clavulanate than the majority of the other group 2 β-lactamases to which the class D enzymes belong. In keeping with this inhibition profile, all OXA-type carbapenemases are inhibited more efficiently by tazobactam than by clavulanate.

A classical feature of most class D β-lactamases is the inhibition by NaCl, which is attributed to the presence of a Tyr residue in the Tyr[144]-Gly-Asn motif.[41] OXA β-lactamases having a Phe-Gly-Asn element instead (OXA-23, OXA-25, OXA-26, OXA-27, OXA-40, OXA-49 and OXA-72) are not or only weakly inhibited by NaCl. Surprisingly, OXA-24 holding the triad Phe-Gly-Asn is inhibited by NaCl.[39]

Susceptibility patterns

Table 3 illustrates the *in vitro* susceptibilities of *Escherichia coli* transconjugants or transformants that carry recombinant plasmids encoding an OXA-type carbapenemase.

In the *E. coli* derivatives, OXA-type carbapenemases confer high-level resistance to aminopenicillins (ampicillin or amoxicillin) and carboxypenicillins (ticarcillin). The susceptibilities to ureidopenicillins (piperacillin) are diverse as only OXA-40 and OXA-48 provide resistance to the drugs. The susceptibilities of the *E. coli* derivatives to cephalosporins, including narrow-spectrum cephalosporins (cefalotin and cefuroxime), the oxyimino-cephalosporins (ceftazidime and cefotaxime) and the 7-α-methoxy-cephalosporins (cefoxitin), oxacephems (moxalactam) and monobactams (aztreonam) are more or less unchanged compared with the reference strains. Only OXA-48 provides resistance to cefalotin.

The effects of the inhibitors, clavulanate and tazobactam, on the susceptibility of the *E. coli* transconjugants or transformants are also compiled in Table 3. These *E. coli* strains are not susceptible to the action of the inhibitors, as the penicillin resistance is unchanged after addition of an inhibitor, with the exception of OXA-23 that shows an 8-fold reduction in MIC of piperacillin in the presence of tazobactam.

The OXA-48-producing *K. pneumoniae* isolate shows a high-level resistance to carbapenems (MIC = 64 mg/L).[42] This may be the result of a combined action of a high production of OXA-48, owing to the association of the bla_{OXA-48} gene with IS*1999*[59] and the lack of a 36 kDa porin.[42]

The low level of resistance (MIC ≤ 8 mg/L) to imipenem and meropenem conferred by the naturally produced OXA-type carbapenemases in *P. aeruginosa*,[43] *A. baumannii*[45] and *R. pickettii*[50] may depend on a combination of low activity of the enzymes against carbapenems and the amount of enzyme produced.

The nosocomial isolates of *A. baumannii* producing carbapenemases, apart from OXA-51, show high-level resistance to imipenem and meropenem (MIC >> 8 mg/L),[29,33,35,38–41,48,49,53] but the OXA-type carbapenemases in the *E. coli* derivatives provide only reduced susceptibility to the carbapenems (MIC ≤ 2 mg/L) (Table 3). This may indicate that additional resistance factors in the nosocomial isolates co-operate to enhance the resistance to carbapenems.

Alterations in the access of antibiotics to the organisms may contribute to a high level of resistance to the carbapenems. In an *A. baumannii* isolate carrying OXA-24, the reduced expression of two outer membrane porins (OMPs) may restrict the membrane permeability and add to the high MICs to imipenem and meropenem (128 and 256 mg/L, respectively).[79] The high level of carbapenem resistance (MICs ≥ 32 mg/L) of the *A. baumannii* isolates producing OXA-23 and OXA-58 may be caused by elevated expression of both the OXA-type carbapenemase and an efflux

pump.[80] The linkage of the bla_{OXA-23} and bla_{OXA-58} genes to the insertion sequences IS*AbA1* and IS*AbA3*, respectively, may lead to an increased production of the enzymes probably because the ISs may supply the genes with strong promoter sequences.[80]

In *A. baumannii*, a combined action of the AmpC β-lactamase with weak carbapenemase activity and reduced expression of OMPs may also result in resistance to carbapenems.[81] Other mechanisms involve diminished production of PBPs, reduced affinity of PBPs for carbapenems[82,83] or loss of OMPs.[21,84–86]

Most of the nosocomial isolates of *A. baumannii* producing OXA-type carbapenemases exhibit high MICs to oxyimino-cephalosporins and to the 7-α-methoxy-cephalosporin cefoxitin,[39,40,53,54] which may be caused by hyperproduction of the ubiquitous AmpC β-lactamase.[87] Other β-lactamases have also been recorded in OXA-type carbapenemase-producing isolates, and those are TEM-1-like enzymes,[38,39,42,54] SHV-2a,[42] OXA-22[50] and OXA-47[42] together with some unidentified β-lactamases.[40,54] These enzymes possess no detectable activity against carbapenems, but they may contribute to the overall β-lactam resistance profile of the isolates.

Many of the *A. baumannii* clinical strains were also resistant to several conventional non-β-lactam antibiotics, such as aminoglycosides, fluoroquinolones, sulphonamides, chloramphenicol and tetracycline.

Structure–function relationships

The crystal structures of the prototypic class D β-lactamases, OXA-1, OXA-2 (Protein Data Bank, http://www.rcsb.org/pdb, Entry 1K38) and OXA-10 have been determined.[73,88] Despite large evolutionary distances between the three enzymes (identities between 23.3 and 33.7%), the overall three-dimensional structures are very similar.[88] Thus, it may be hypothesized that the structures of the OXA-type carbapenemases may be very similar to the structures of the class D oxacillinases.

The crystal structure of the OXA-13 oxacillinase complexed with meropenem has been reported.[89] Binding of meropenem to the apoenzyme leads to displacement of residues and loops, especially the catalytically essential Ser^{118}. The OXA-13:meropenem complex is not hydrolysed because the distance between the catalytic water molecule and the acyl carbonyl of meropenem is too great.[89]

At present, it is not known which amino acid residues play an essential role for the carbapenemase activity in the OXA-type carbapenemases as comprehensive investigations involving site-directed mutagenesis have not been published. Multiple sequence alignment of the class D β-lactamases has revealed that a residue common to all OXA-type carbapenemases was also present in the same position in at least some of the class D oxacillinases that do not show carbapenemase activity and vice versa. Consequently, it appears that no specific residue(s) is solely responsible for the carbapenemase activity of the OXA-type carbapenemases. Rather, it is more likely that specific residues in enzymes may interact in a way to cause a specific local conformation of the active site leading to a proper positioning of the catalytic water molecule and consequently to hydrolysis of the carbapenem:OXA complex.

Conclusion

This review underlines that the OXA-type carbapenemases are increasingly reported worldwide. The vast majority of OXA-type carbapenemases has so far been found in non-enterobacterial species, especially *A. baumannii*. The OXA-type carbapenemases, OXA-50, OXA-51 and OXA-60, seem to be naturally occurring enzymes in the species *P. aeruginosa*, *A. baumannii* and *R. pickettii*, respectively.

Most of the OXA-type carbapenemases confer only reduced susceptibility to the carbapenems, but unless secondary resistance mechanisms, such as altered permeability, reduced affinity of PBPs for carbapenems or increased efflux, are involved the clinical detection of organisms producing these enzymes remains difficult.

It has been experimentally shown that an organism producing an enzyme with very low activity against a substrate may evolve enzymes that are much more efficient when the organism is exposed to the substrate.[90] This aspect has clearly been illustrated by the SHV β-lactamases which have evolved from broad-spectrum β-lactamases to extended-spectrum, inhibitor-resistant or carbapenem-hydrolysing enzymes. AmpC β-lactamases with weak carbapenemase activity have been described in both *A. baumannii* and *P. aeruginosa*. Thus, it may be anticipated that the clinical use of carbapenems may lead to the emergence of *P. aeruginosa* and *A. baumannii* producing more efficient mutant enzymes. This prospect is worrisome as *A. baumannii* and *P. aeruginosa* account for many severe nosocomial infections.

The chromosomal location of many of the OXA-type carbapenemase encoding genes has certainly contributed to the slow spread of these genes. By inappropriate use of carbapenems the plasmid-encoded genes will likely spread, owing to the increased selective pressure.

The ancestors of most of the mobile carbapenem-hydrolysing OXA β-lactamases are unknown.

Transparency declarations

None to declare.

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