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Class A carbapenemases

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Carbapenems, such as imipenem and meropenem, are most often used to treat infections caused by enterobacteria that produce extended-spectrum β-lactamases, and the emergence of enzymes capable of inactivating carbapenems would therefore limit the options for treatment. Carbapenem resistance in Enterobacteriaceae is rare, but class A β-lactamases with activity against the carbapenems are becoming more prevalent within this bacterial family.

The class A carbapenemases can phylogenetically be segregated into six different groups of which four groups are formed by members of the GES, KPC, SME, IMI/NMC-A enzymes, while SHV-38 and SFC-1 each separately constitute a group.

The genes encoding the class A carbapenemases are either plasmid-borne or located on the chromosome of the host. The bla_{GES} genes reside as gene cassettes on mainly class I integrons, whereas the bla_{KPC} genes and a single bla_{IMI-2} gene are flanked by transposable elements on plasmids.

Class A carbapenemases hydrolyse penicillins, classical cephalosporins, monobactam, and imipenem and meropenem, and the enzymes are divided into four phenotypically different groups, namely group 2br, 2be, 2e and 2f, according to the Bush–Jacoby–Medeiros classification system. Class A carbapenemases are inhibited by clavulanate and tazobactam like other class A β-lactamases.

Keywords: class A β-lactamases, carbapenem resistance, Enterobacteriaceae, *Pseudomonas aeruginosa*

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β-Lactamases are bacterial enzymes that protect the organisms against the lethal actions of β-lactam antibiotics, such as penicillins, cephalosporins and carbapenems, by breaking down the indispensable β-lactam bond in the antibiotics rendering the drugs antimicrobially inert.[1] The β-lactamases are considered as the primary cause of bacterial resistance to these drugs.[2]

Based on amino acid sequence identities, β-lactamases can be divided into four disparate molecular classes, labelled A, B, C and D.[3–5] Class A, C and D β-lactamases use a catalytically active serine residue for inactivation of the β-lactam drug,[6] whereas the molecular class B enzymes are metalloenzymes that require zinc as a metal cofactor for their catalytic activities.

Carbapenems, such as imipenem and meropenem, diffuse easily into bacteria[7,8] and have a very broad spectrum of activity as the drugs are active against many Gram-negative, Gram-positive and anaerobic bacteria.[9,10] The targets of carbapenems are the penicillin-binding proteins (PBPs).[8,11]

MIC breakpoints for imipenem and meropenem resistance in members of the Enterobacteriaceae and in non-fastidious Gram-negative pathogens, such as *Pseudomonas aeruginosa*, have been established by the CLSI (formerly NCCLS) as $\geq$16 mg/L while the CLSI susceptibility breakpoints were defined as $\leq$4 mg/L.

Enterobacteriaceae and *P. aeruginosa* have a plethora of mechanisms to elude the action of carbapenems. Carbapenem resistance in Enterobacteriaceae can be mediated by metallo-β-lactamases, but enterobacteria carrying genes encoding these enzymes are not always resistant to the carbapenems.[12] Carbapenem resistance in Enterobacteriaceae can also be conferred by class A carbapenemases (this review) or on rare occasions by OXA-type carbapenemases.[13] Other resistance mechanisms are attributed to altered affinity of PBPs for carbapenems,[14,15] increased efflux of the β-lactam antibiotics,[16,17] decreased permeability of the outer membrane,[15–19] or to a combination of reduced permeability and high-level production of a β-lactamase,[20–26] typically a class C β-lactamase.

The carbapenems are recommended as first-line therapy for severe infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs),[27] and the emergence of carbapenem-resistant enterobacteria is therefore worrisome as the antimicrobial armamentarium consequently is restricted.

In *P. aeruginosa*, class B enzymes account for most of the resistance to carbapenems.[12] However, loss of the outer membrane protein, OprD, overexpression of drug efflux pumps and combinations thereof with hyperproduction of the chromosomal class C β-lactamase also play important roles in the resistance to

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carbapenems.[28–31] Resistance to imipenem seems, however, not to be mediated solely by efflux pumps.[32]

Another microorganism of major concern is *Acinetobacter baumannii* which displays a high incidence of resistance to carbapenems owing to the expression of OXA-type carbapenemases.[13,33]

Class A β-lactamases

Class A β-lactamases appear to be the most diverse and widely distributed class of the β-lactamases as the encoding genes have been found in several bacterial phyla, such as Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes and Proteobacteria, including α-, β- and γ-proteobacteria. The genes do not reside in all genera of these phyla.

A multiple sequence alignment of the class A β-lactamases shows 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 consensus numbering for class A β-lactamases[34]). The second motif, the invariable Ser_{130}-Asp-Asn, is equivalent to the variable Ser-X-Val/Ile element in class D β-lactamases and Tyr-Ala/Ser-Asn in class C β-lactamases, while the Lys_{234}-Thr/Ser-Gly triad is common to the vast majority of serine-active β-lactamases. Another conserved residue in class A β-lactamases is Glu_{166} which acts as a general base in the acylation step.[35]

Many class A β-lactamases are very efficient in hydrolysing benzylpenicillin and ampicillin, and consequently, the enzymes have been characterized primarily as penicillinases. According to their functional properties, β-lactamases are divided into different groups.[36] Class A β-lactamases are assigned to the functional group 2 which is segregated into several subgroups. The members of Bush group 2 generally hydrolyse penicillins and classical cephalosporins efficiently, and most of the enzymes are effectively inhibited by clavulanic acid.[36]

Clusters of class A carbapenemases

A phylogenetic analysis of class A carbapenemases together with other class A β-lactamases from bacteria belonging to different phyla shows that the carbapenemases form six distantly related branches (Figure 1), and the clusters share amino acid sequence identities ranging from 32% to 70%. The high divergence among the groups of class A carbapenemases suggests that the clusters may have derived from different ancestors.

The GES enzymes

In 1998, a *Klebsiella pneumoniae* isolate was recovered in a French hospital from an infant that previously had been hospitalized in French Guiana.[37] The β-lactamase characterized from this clinical strain was accordingly named GES-1 (Guiana extended-spectrum), and at present, the GES family enzymes comprise nine different members (Table 1) of which only four, GES-1, -2, -4 and -5, show a measurable enzymatic activity against carbapenems. However, the carbapenemase activity of GES-1 is so low that it seems reasonable to consider the enzyme as an ESBL rather than a carbapenemase. In this review, we have adopted the nomenclature of the GES enzymes accordingly to the Lahey Clinic web site (http://www.lahey.org/Studies). The enzymes of the GES family differ from each other by one to four amino acid substitutions. At present, the phylogenetically closest related β-lactamase to the GES family enzymes is BEL-1 from *P. aeruginosa*[38] with identities ranging from 50% to 51%.

The GES enzymes have mainly been found in *P. aeruginosa*, but the enzymes have also been observed in members of Enterobacteriaceae (Table 1). Isolates producing GES enzymes with carbapenemase activity have been collected predominantly in Europe, South Africa and the Far East (Table 1).

In March–July 2000, an outbreak of clonally related GES-2-producing *P. aeruginosa* isolates occurred in Republic of South Africa[46] while an outbreak involving an endemic strain of *K. pneumoniae* expressing GES-5 took place in Korea in 2004.[60]

Apart from the bla_{GES-7} gene in an *Escherichia coli* isolate described by Galani *et al.*,[54] the bla_{GES} genes have all been found as gene cassettes on integrons. The *E. coli* isolate which harbours the chromosomally located bla_{GES-7} gene is so far the only bla_{GES} gene that has not been identified on an integron.[54] Closer investigations on the genetic environment of the bla_{GES-7} gene have not been published.

From some clinical isolates, the resistance phenotype associated with a GES enzyme could be transferred by conjugation to recipients[45–47,49,50,52,55,56,59,60,62] while others were not successful under the experimental conditions used.[37,43,48–50] The conjugal transfer may indicate that the bla_{GES}-carrying integrons are present on transferable plasmids. In one instance, the resistance phenotypes were transferred to a recipient by electroporation but not by conjugation[37] which may signify that the bla_{GES-1} integron is located on a non-transferable plasmid. Other isolates appear to have the bla_{GES}-carrying integrons inserted into the chromosome of the host.[40,54,57,58]

The gene cassettes coding for the GES enzymes were examined by an *in silico* analysis. The results show that the cassettes characterized in the isolates collected in Europe and South Africa differ only in the structural genes for the GES enzymes. These cassettes are 1020 bp in length, as opposed to the 929 bp long cassettes in the four South American isolates.[37,42] The reduced length is caused by identical truncations of the 59 base elements (bes) suggesting that the four cassettes may have a common origin. No data have been published on the bla_{GES-1} gene cassette in the *P. aeruginosa* isolate from Argentina[43] and consequently it is unknown how long the gene cassette in fact is.

Except for differences in the bla_{GES-3} and bla_{GES-4} genes, the two GES-encoding cassettes described in *K. pneumoniae* isolates from Japan[47,48] are identical, but marginally different from the other analogous cassettes.

Owing to the differences between the 59 bes of the GES encoding cassettes, it is conceivable that the bla_{GES} genes have been mobilized from their ancestor on at least two separate occasions in different geographic locations.

The KPC enzymes

The second group encompasses the KPC enzymes (acronym of *K. pneumoniae* carbapenemase). The four variants that up to now have been described all possess carbapenemase activity and they differ from each other by one to three amino acid

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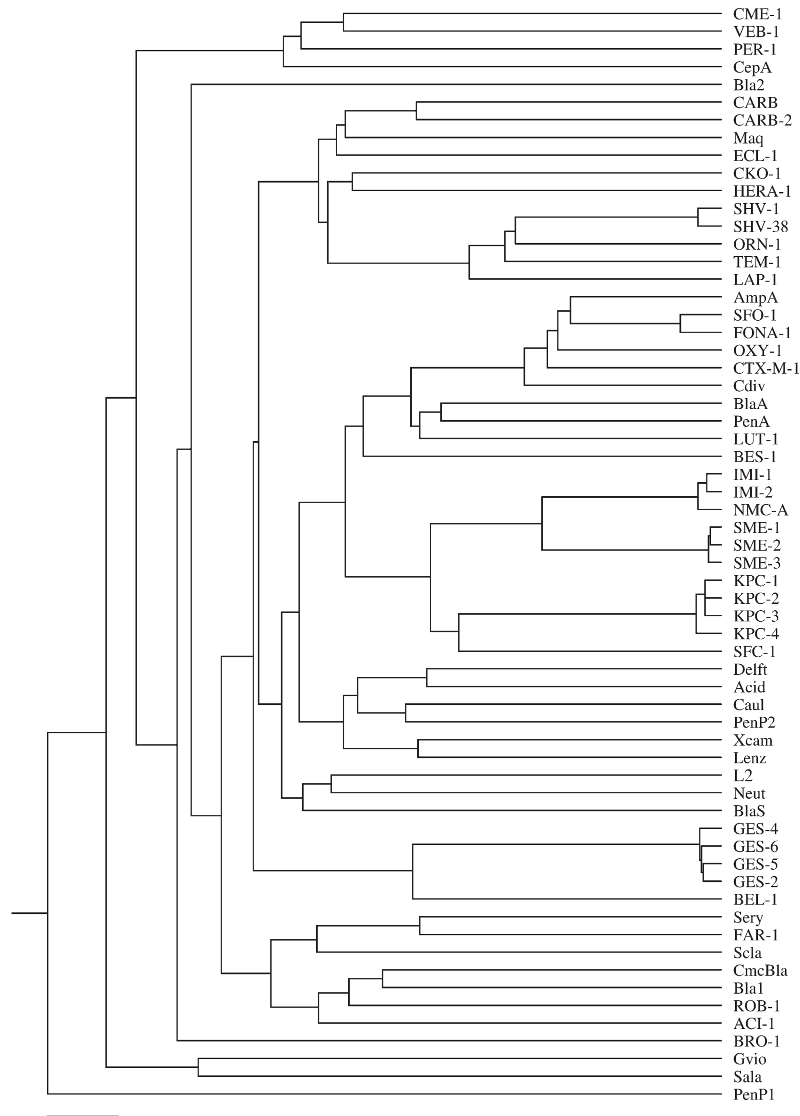

Figure 1. Rooted dendrogram obtained for 62 representative precursor class A β-lactamases including the class A carbapenemases. The tree was constructed by the use of the www-based services, Clustal W algorithm (http://www.genebee.msu.su/clustal/basic.html) and Phylodendron—Phylogenetic tree printer (http://iubio.bio.indiana.edu/treeapp/treeprint-sample2.html). The enzymes referred by both a GenBank accession number and a locus tag are putative but contain the conserved motifs common to the class A β-lactamases. The members of the different groups of class A carbapenemases are GES-2, *P. aeruginosa* (AF347074); GES-4, *K. pneumoniae* (AB116723); GES-5, *Escherichia coli* (AY494717); GES-6, *K. pneumoniae* (AY494718); IMI-1, *Enterobacter cloacae* (U50278); IMI-2, *E. cloacae* (AY780889); KPC-1, *K. pneumoniae* (AF297554); KPC-2, *K. pneumoniae* (AY034847); KPC-3, *K. pneumoniae* (AF395881); KPC-4, *Enterobacter* sp. (AY700571); NMC-A, *E. cloacae* (Z21956); SFC-1, *Serratia fonticola* (AY354402); SHV-38, *K. penumoniae* (AY079099); SME-1, *Serratia marcescens* (U60295); SME-2, *S. marcescens* (AF275256); SME-3, *S. marcescens* (AY504237).

Included enzymes are ACI-1, *Acidaminococcus fermentans* (GenBank Acc. No. AJ007350); Acid, *Acidovorax* sp. JS42 (AASD01000003, AjsDRAFT_3009); AmpA, *Leminorella grimontii* (AM422900); BEL-1, *P. aeruginosa* (DQ089809); BES-1, *S. marcescens* (AF234999); BlaA, *Yersinia enterocolitica* (X57074); BlaS, *Mycobacterium smegmatis* (AY332268); Bla1, *Bacillus cereus* (X06599); Bla2, *Francisella tularensis* (NC_008245, YP_666764.1); BRO-1, *Moraxella cattarhalis* (Z54180); CARB, *Vibrio fischeri* (AY438037); CARB-2, *E. coli* (DQ157752); Caul, *Caulobacter* sp. K31 (AATH01000006, CaulDRAFT_1231); Cdiv, *Citrobacter diversus* (X62610); CepA, *Bacteroides fragilis* (L134772); CKO-1, *Citrobacter koseri* (AJ609504); CME-1, *Elizabethkingia meningoseptica* (AJ006275); CmcBla, *Amycolatopsis lactamdurans* (Z13971); CTX-M-1, *E. coli* (X92506); Delft, *Delftia acidovorans* (AAVD01000001, DaciDRAFT_5465); ECL-1, *E. coli* (EF104648); FAR-1, *Nocardia farcinica* (AF024601); FONA-1, *Serratia fonticola* (AJ251239); Gvio, *Gloeobacter violaceus* (BA000045, gl10595); HERA-1, *Escherichia hermannii* (AF311385); L2, *Bradyrhizobium japonicum* (NC_004463, bl10941); LAP-1, *E. cloacae* (EF026092); Lenz, *Lysobacter enzymogenes* (M97392); LUT-1, *Pseudomonas luteola* (AY695112); Maq, *Marinobacter aquaeolei* (NZ_AALG01000023, MaquDRAFT_0322); Neut, *Nitrosomonas eutropha* (NC_008344, Neut_1060); ORN-1, *Raoultella ornithinolyticus* (AY307386); OXY-1, *Klebsiella oxytoca* (AJ871864); PenA, *Burkholderia pseudomallei* (AY032868); PER-1, *P. aeruginosa* (Z21957); PenP1, *Prochlorococcus marinus* (NC_005042, Pro1145); PenP2, *Rhodopseudomonas palustris* (NC_005296, RPA0362); ROB-1, *Mannheimia haemolytica* (X52872); Sala, *Sphingopyxis alaskensis* (NC_008048, Sala_0174); Scla, *Streptomyces clavuligerus* (Z54190); Sery, *Saccharopolyspora erythraea* (AM420293, SACE_1374); SFO-1, *E. cloacae* (AB003148); SHV-1, *K. pneumoniae* (X98098); TEM-1, *P. aeruginosa* (X54607); VEB-1, *E. coli* (AF205943); Xcam, *Xanthomonas campestris* (NC_007508, XCV3293).

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Table 1. Summary of GES-producing bacteria, their geographical distribution and year of isolation

Enzyme	Location[a]	Host	Country	Year	Accession no.	Reference(s)
GES-1[b]	P	*K. pneumoniae*	France	1998	AF156486	37
GES-1[c]	P	*K. pneumoniae*	Portugal	1999	AY219651	39
GES-1	C	*P. aeruginosa*	France	1999	AF355189	40
GES-1	?	*P. aeruginosa*	Brazil	?	—	41
GES-1	?	*P. aeruginosa*	Brazil	2001	DQ236170	42
GES-1	?	*P. aeruginosa*	Argentina	2002	—	43
GES-1	?	*S. marcescens*[d]	Holland	2002–3	—	44
GES-1-type[e]	?	*P. aeruginosa*	PR China	?	DQ333893	—
GES-2	P	*P. aeruginosa*	RSA	2000	AF326355	45
GES-2	P	*P. aeruginosa*	RSA	2000	AF347074	46
GES-3	P	*K. pneumoniae*	Japan	2002	AB113580	47
GES-4	P	*K. pneumoniae*	Japan	2002	AB116723	48
GES-5[f]	P	*E. coli*	Greece	?	AY494717	49
GES-5[f,g]	?	*K. pneumoniae*	Korea	2003	—	50
GES-5	?	*P. aeruginosa*	Brazil	2001	DQ236171	42
GES-5	?	*P. aeruginosa*	RSA	?	EF190326	—
GES-5-type[e,h]	?	*P. aeruginosa*	PR China	2004	AY953375	51
GES-6[f]	P	*K. pneumoniae*	Greece	?	AY494718	49
GES-7[f,i]	P	*E. cloacae*[j]	Greece	1999	AF208529	52
GES-7[f,i]	C	*E. coli*	Greece	2001	—	53,54
GES-7[f,i]	P	*E. coli*	Greece	2002	AY260546	55
GES-7[f,i]	P	*K. pneumoniae*	Greece	1998–2000	—	56
GES-8[f,k]	C[l]	*P. aeruginosa*	Greece	1998	AF329699	57
GES-9	C[l]	*P. aeruginosa*	France	2004	AY920928	58

The GES enzymes without measurable carbapenemase activity (GES-3, GES-7, GES-8 and GES-9) have also been included in the table.
[a]Genetic location: P, plasmid; C, chromosome.
[b]The *K. pneumoniae* isolate was recovered in France, but the patient was transferred from French Guiana.[51]
[c]An endemic strain of *K. pneumoniae* producing GES-1 caused an outbreak in Lisbon, Portugal, between 1999 and 2001.[59]
[d]Outbreak of GES-1-producing *S. marcescens* in Groningen, Holland, between 2002 and 2003.[44]
[e]Partial sequence.
[f]Numbering according to the Lahey Clinic web site (http://www.lahey.org/Studies).
[g] GES-5-producing *K. pneumoniae* caused an outbreak in Korea in 2004.[60] Another GES-5-producing *K. pneumoniae* isolate from Korea collected in 2004 has been described.[61]
[h]Partial GES-5-type enzymes have been identified in two *P. aeruginosa* isolates collected from another parts of P.R. China (DQ660416 and DQ660417).
[i]Formerly known as IBC-1.
[j]An outbreak of GES-7-producing *E. cloacae* isolates of different genotypes occurred in a Greek hospital in 1998–2000.[62]
[k]Formerly known as IBC-2.
[l]Probably chromosomal.

substitutions. The nearest phylogenetically related enzyme to the KPCs is another class A carbapenemase, SFC-1, from *Serratia fonticola* (Figure 1) with an identity of roughly 61%.

Despite the fact that KPC refers to the species in which the KPC-1 enzyme was originally identified, members of this group have also been found in several other enterobacteria and in *P. aeruginosa* (Table 2). The vast majority of these KPC producers has been collected in the Northeastern parts of USA (Table 2).

Carbapenem-resistant klebsiellae were not reported in a US nationwide surveillance study from 1998 to 2001.[81] Ensuing nationwide or citywide (New York City) survey studies of *K. pneumoniae* collected from different hospitals showed that the incidence of bla_{KPC}-positive isolates has risen gradually.[74,82–86] Most of the bla_{KPC}-carrying isolates in each individual study belonged to the same ribotype.[82,85]

A screening investigation during September–October 2004 of *E. coli*, *Enterobacter* spp. and *K. pneumoniae* collected from four hospitals in Brooklyn, New York City, showed that none of the *E. coli* or *Enterobacter* spp. isolates carried a bla_{KPC} gene, whereas 24% of the *K. pneumoniae* isolates possessed a bla_{KPC-2} or bla_{KPC-3} gene.[84] The majority (88%) of the bla_{KPC}-carrying isolates belonged to the same ribotype.[84] To date, KPC-producing enterobacteria in USA have been reported from North Carolina, New Jersey, Maryland, Pennsylvania, Massachusetts, Delaware, Arkansas, Ohio, Virginia, New York and New York City.

An outbreak of carbapenem-resistant *K. pneumoniae* happened in 2000–01 in a Manhattan hospital of New York.[77] Most of the isolates were found to be genetically related and produced KPC-3.

Two outbreaks involving imipenem-resistant *K. pneumoniae* occurred in continuation of each other (2003–04) at two hospitals in Brooklyn, New York City.[83] All the isolates carried the KPC-2 carbapenemase and the majority of the isolates belonged to the same ribotype.[83] In 2004, a major outbreak of a clonal KPC-2-producing *K. pneumoniae* occurred in a medical centre

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Table 2. Summary of KPC carbapenemase-producing bacteria, their geographical distribution and year of isolation

Enzyme	Location[a]	Host	Country	State[b]	Year	Accession no.	Reference(s)
KPC-1	P	*K. pneumoniae*	USA	NC	?	AF297554	63
KPC-2	P	*C. freundii*	Colombia	—	2006	—	64
KPC-2[c]	?	*C. freundii*	USA	NY*	2000–05	—	65
KPC-2	?	*E. aerogenes*	USA	NY*	2003	—	66
KPC-2[d]	?	*E. cloacae*	USA	NY*	2001	—	66
KPC-2	?	*E. hormaechei*	USA	NY		—	67
KPC-2	P	*Enterobacter* sp.	USA	MA	2001	—	68
KPC-2[e]	P	*E. coli*	Israel	—	2005	—	69
KPC-2[f]	?	*E. coli*	USA	OH	2004	—	65
KPC-2[g]	P	*K. oxytoca*	USA	NY	1998	AY210886	70
KPC-2	P	*K. pneumoniae*	China	—	2004	DQ897687	71
KPC-2[h]	P	*K. pneumoniae*	Colombia	—	2005	DQ523564	72
KPC-2[i]	P	*K. pneumoniae*	France	—	2005	—	73
KPC-2[j]	P	*K. pneumoniae*	USA	MD	1998	AY034847	74
KPC-2	C,P	*P. aeruginosa*	Colombia	—	2006	—	64
KPC-2	P	*S. enterica*[k]	USA	MD	1998	AF481906	75
KPC-2	P	*S. marcescens*	China	—	2006	DQ899729	76
KPC-3[l]	?	*C. freundii*	USA	OH	2001	—	65
KPC-3	?	*E. coli*	USA	NY			67
KPC-3[m]	?	*E. cloacae*	USA	NY*	2000	AY522950	65,66
KPC-3	?	*E. gregoviae*	USA	NY*	2002	—	65
KPC-3[n]	P	*K. pneumoniae*	USA	NY	2000–01	AF395881	77
KPC-3	?	*S. marcescens*	USA	NY*	2000	—	65
KPC-4	?	*Enterobacter* sp.	Scotland	—	?	AY700571	—

[a]Genetic location: P, plasmid; C, chromosome.
[b]State: AR, Arkansas; MA, Massachusetts; MD, Maryland; NC, North Carolina; NY, New York; NY*, New York City; OH, Ohio; VA, Virginia.
[c]A KPC-2-producing isolate of *C. freundii* was recovered in 2005 in Mineola, NY.[65] A *C. freundii* isolate producing a KPC-like β-lactamase was collected in Delaware.[65]
[d]Among *E. cloacae* isolates collected between 2000 and 2002, a KPC-2-producing *E. cloacae* was recovered in Boston, MA.[78]
[e]Four genetically unrelated isolates of *E. coli* were recovered from four different patients without any obvious epidemiological links and none of the patients had recently travelled to the east coast of USA.[69]
[f]KPC-2 was also found in *E. coli* isolates collected in 2005 in New York City.[65,79]
[g]KPC-2 was also found in a *K. oxytoca* isolate collected in 2003 in AR. *K. oxytoca* and *E. cloacae* isolates producing KPC-like enzymes were also found in VA between 2000 and 2004.[65,67]
[h]Another isolate of *K. pneumoniae* carrying the bla_{KPC-2} gene was also identified in Colombia, 2005.[72]
[i]Prior to hospitalization in France, the patient had been treated in a hospital in New York, USA.[73]
[j]Among *K. pneumoniae* isolates collected between 2000 and 2002, a KPC-2-producing *K. pneumoniae* was recovered in New York.[78] In 2005, a KPC-2-producing *K. pneumoniae* was isolated in a rural community of Pennsylvania.[80] KPC-2 was also found in *K. pneumoniae* isolates collected in New York City in 2000–05 and in Mineola, NY in 2004 and in 2005.[65]
[k]*Salmonella enterica* serotype Cubana.
[l]KPC-3 was also found in *C. freundii* isolates collected in 2002 and 2005 in New York City.[65]
[m]KPC-3 was also found in other *E. cloacae* isolates collected in 2000, 2001, 2003 and 2004 in New York City.[65,66]
[n]KPC-3 was also found in *K. pneumoniae* isolates collected in 2002 in New York City.[65]

in New York City and this clone has subsequently become endemic in the center.[65] The KPC-2-producing *K. pneumoniae* clone was later found in a medical centre in Mineola, NY,[65] which is indicative of clonal expansion. Clonal spread between two hospitals has also been reported for KPC-2-producing *E. coli* found in New York City and Cleveland, OH, USA.[65]

These facts imply that the KPC determinants have the potential to disseminate rapidly among human pathogens. The emergence of carbapenem resistance in enterobacteria is worrisome because the carbapenem resistance often may be associated with resistance to many β-lactam and non-β-lactam antibiotics, and this will eventually limit effective therapeutic regimens.

The prevalence of KPC-producing isolates may be clinically unrecognized because several factors can complicate their detection. In the absence of other mechanisms, the KPC carbapenemases may not confer resistance to carbapenems but only reduced susceptibility[74,84] and some phenotypic tests may suggest KPC producers are ESBL producers.[74] The presence of carbapenem resistance in *K. pneumoniae* is sometimes overlooked when automated susceptibility testing systems using low inocula are employed.[83,84,87] These difficulties consequently imply that the incidence of KPC-producing *K. pneumoniae* isolates may be underestimated and an inaccurate identification of carbapenem-resistant *K. pneumoniae* may also have fatal consequences.

The observations of bla_{KPC} genes in isolates from Israel and Columbia[69,72] are a matter of concern as the infected patients from whom the isolates were recovered had no records of recent

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travel to the eastern parts of USA. These findings indicate that bla_{KPC} genes from a common but unknown ancestor may have been mobilized in these areas or that bla_{KPC}-carrying microorganisms may have been transferred passively from the United States by other vectors. There is no published data on travels outside of P.R. China for the patients from whom the KPC-2-producing isolates were collected.[71,76]

The genes encoding the KPC carbapenemases all appear to be plasmid-borne, apart from the bla_{KPC-2} in *P. aeruginosa* (Table 2). None of the genes has, however, been found in a gene cassette, owing to the lack of the characteristic core site of the 59 be. Instead, the bla_{KPC} genes are associated with mobile elements. Identical sequences coding for a putative IS*1*-like ATP-binding protein have been found upstream from the bla_{KPC-2} genes in *K. pneumoniae*[72] and *Salmonella enterica*.[75] Apart from a deletion of six nucleotides resulting in a loss of two amino acids, a partial but otherwise identical sequence of the gene is also found upstream from the bla_{KPC-2} gene in *Klebsiella oxytoca*.[70]

Downstream from the bla_{KPC-1} and bla_{KPC-2} genes in *K. pneumoniae* and *S. enterica*,[63,72,75] the 3′-end of a *tnp* gene was identified. Owing to lack of sequence data, it is not known whether any of the other bla_{KPC} genes have the same genetic environment.

The genetic contexts of the bla_{KPC} genes were studied by *in silico* analyses and the investigations showed that the non-coding region downstream from the bla_{KPC} genes separate into two types. One type holding 249 bp is identified exclusively in the *K. pneumoniae* isolates, whereas the other type with 248 bp and a slightly different nucleotide sequence is found only in *K. oxytoca* and *S. enterica* producing KPC-1 and KPC-2, respectively.

The SME enzymes

The SME enzymes have been found exclusively in *Serratia marcescens* (*S. marcescens* enzyme) (Table 3) and the three variants differ by one to two amino acid substitutions. Geographically, SME-producing isolates have been collected in UK[88] and infrequently and sporadically across USA (MA, IL, TX, OH, WA and CA).[65,67,89,90]

The bla_{SME} genes are seemingly not ubiquitous in *S. marcescens*, but the genes are more likely present in only a subpopulation of the species. The genes are presumed to be chromosomal and non-mobile, but no data have, however, been presented to document this assumption.

Among the *S. marcescens* isolates producing an SME enzyme, the bla_{SME-1} gene is so far the only one that is found associated with a gene encoding a regulator of the LysR type.[93] Owing to the lack of sequence data, it is not known whether any of the other bla_{SME} genes is linked to a LysR regulator gene. The upstream sequence (123 bp long) from the bla_{SME-3} gene[92] is 100% identical to the equivalent sequence from the bla_{SME-1} gene which suggests that the bla_{SME-3} gene may have an upstream-located LysR encoding gene. The genetic environments of the bla_{SME} genes have not been investigated and consequently it remains unknown whether the genes are parts of mobile elements.

Only *S. marcescens* strain S6 has been studied for induction of the SME-1 enzyme.[93] Induction with imipenem resulted only in a marginal increase of activity against imipenem[93] suggesting that SME-1 may be produced at a high basal level. This presumed high basal level of expression of the SME-1 enzyme may be mediated by the SmeR regulator operating as an activator of the bla_{SME-1} gene transcription in the absence of a β-lactam inducer, but in the presence of an inducer, it acts more strongly.[93] The inducible expression of the other SME enzymes in the remaining *S. marcescens* isolates has not been investigated.

The IMI/NMC-A enzymes

The fourth group encompasses the IMI/NMC-A enzymes (imipenemase/non-metallocarbapenemase-A) which form two subgroups, IMI and NMC-A, respectively. The NMC-A enzyme deviates by eight amino acid substitutions from the two IMI variants which differ by two substitutions from each other. The

Table 3. Summary of IMI/NMC-A and SME carbapenemase-producing bacteria, their geographical distribution and year of isolation

Enzyme	Location[a]	Host	Country	Year	Accession no.	Reference
SME-1	C	*S. marcescens*[b]	UK	1982	U60295	91
SME-2	?	*S. marcescens*	USA	1992	AF275256	90
SME-3	C	*S. marcescens*	USA	2003	AY584237	92
IMI-1	C	*E. cloacae*	USA	1984	U50278	94
IMI-2	P	*E. asburiae*[c]	USA	1999–2001	DQ173429	96
IMI-2	P	*E. cloacae*	China	2001	AY780889	95
NMC-A	C	*E. cloacae*	France	1990	Z21956	97
NMC-A	C	*E. cloacae*	Argentina	2000	AJ536087	98
NMC-A	C	*E. cloacae*	USA[d]	?	—	99

[a]Genetic location: P, plasmid; C, chromosome.
[b]Another isolate of *S. marcescens* also producing SME-1 was recovered from another patient hospitalized at the same hospital in 1982.[88]
[c]Environmental isolates.
[d]*E. cloacae* isolates producing NMC-A have been recovered in Seattle, WA,[99] and in New York.[67]

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enzymes have been found sporadically (Table 3), both in clinical isolates[94,95,97–99] and in environmental isolates from rivers in USA.[96]

The clinical isolates carrying the chromosomal bla_{NMC-A} gene all have an adjacent *nmc-R* gene.[98–100] The NmcR regulator affects the transcription of its linked *bla* gene in the same manner as the SmeR regulator[100] and AmpD has been found to be involved in the regulation of the Nmc-A carbapenemase.[101]

The bla_{IMI-1} gene and its linked *imi-R* gene coding for a LysR transcriptional regulator are embedded in the chromosome of the host,[94] whereas the bla_{IMI-2} genes and their *imi-R* genes are plasmid-borne.[95,96] The adjacent regions flanking the bla_{IMI-R}–bla_{IMI-1} complex have not been analysed, but the complex may possibly be a part of a composite transposon.

The plasmid-borne *imi-R*–*imi-2* gene complexes in *Enterobacter cloacae* and *Enterobacter asburiae* appear to be flanked by transposable elements.[95,96] The bla_{IMI-R}–bla_{IMI-2} region in *E. asburiae* is surrounded upstream by an IS*2*-like fragment and downstream by a transposase gene similar to the *tnpA* gene of Tn*2501*.[96] The bla_{IMI-R}–bla_{IMI-2} unit in *E. cloacae* is flanked on both sides by structures related to the *tnpA* gene of Tn*903* and further upstream by an IS*2*-like element.[95] Originally, these transposable elements may have been involved in mobilization of the bla_{IMI} genes to the chromosome and to the plasmids, but whether they still are mobile is not known. If the transposable elements became inactive some time after the mobilization, it may explain the rare occurrence of the enzymes in these species.

Challenging of the IMI-producing isolates with imipenem results in substantial increases of activity against imipenem,[94,96] whereas the IMI-2-producing *E. cloacae* showed a less strong induction.[95] A high basal level of expression of IMI-2 because of mutational derepression may explain why the production of this enzyme is only marginally induced.[95]

The SFC-1 and SHV-38 enzymes

Each of the last two groups contains a single enzyme, namely SFC-1 and SHV-38, from isolates of *S. fonticola* and *K. pneumoniae*, respectively. The *S. fonticola* strain was an environmental isolate from Portugal,[102] whereas the *K. pneumoniae* isolate was recovered in France,[103] and both enzymes are chromosomally encoded. The SFC-1 enzyme is not ubiquitous in *S. fonticola* because it is found only in this environmental isolate and not in other strains of *S. fonticola*.[102] The SHV-38 enzymes differ by a single substitution from the broad-spectrum β-lactamase, SHV-1.

Origin of class A carbapenemases

The soil organism, *Streptomyces cattleya*, is a natural producer of the carbapenem compound, thienamycin,[104,105] and imipenem is an *N*-formimidoyl derivative of thienamycin. Environmental microbiota carrying genes encoding for carbapenem-hydrolysing enzymes would thus be better endowed to survive in this environment.

The first identified isolate expressing a class A carbapenemase was the SME-1-producing *S. marcescens* from London.[88] The isolate was recovered in 1982 before imipenem was approved for general use. Similarly, the *E. cloacae* isolate producing IMI-1 was collected in 1984 in USA.[94] Likewise, a class D carbapenemase, OXA-23, was identified in an *A. baumannii* isolate which was recovered in 1985 in UK prior to launch of imipenem for clinical use.[106]

The imipenem-resistant phenotype in these instances preceded the clinical use of imipenem because the drug was first allowed for clinical use in 1985 in USA.[1] These findings strongly suggest that the clinical use of imipenem has not been responsible for the evolution of the class A and D carbapenemases. The enzymes were probably present in bacteria long before, but the ancestors may have faded into the mists of history.

From the dendrogram in Figure 1, it appears that the IMI/NMC-A, SME, KPC and SFC-1 enzymes share a common origin which is quite distinct from the ancestors of the GES enzymes and SHV-38. The latter enzyme is the only class A carbapenemase with an identified ancestor.[107] The intrinsic carbapenemase activity of all members of the IMI/NMC-A, SME, KPC and SFC-1 clusters suggests that the ancestral enzyme(s) may also have held the ability to hydrolyse carbapenems. It is also noticeable that the only clusters of class A carbapenemase genes associated with a LysR regulator gene are those most identical to each other, i.e. the bla_{IMI}, bla_{NMC-A} and bla_{SME} genes, which appear to have descended from a common ancestor (Figure 1). The encoding genes are, however, far from ubiquitous in the species that carry them.

The cluster including the GES-type carbapenemases also encompasses ESBLs and a cephamycinase. The ability to hydrolyse carbapenems is evidently not an innate property of all GES enzymes, but instead some GES enzymes may have evolved the carbapenemase activity by amino acid substitutions. Thus, it cannot be excluded that the ancestral enzyme(s) of the GES cluster enzymes may have lacked the ability to hydrolyse carbapenems.

Biochemical properties of class A carbapenemases

Mature class A carbapenemases are monomeric enzymes that contain between 265 and 269 amino acid residues with experimentally determined molecular masses that range from 25 to 32 kDa. The isoelectric points of the enzymes vary from 5.8 to 9.7.

Like other active-site serine β-lactamases, class A carbapenemases also proceed via a simple three-step mechanism involving acylation and deacylation.[6]

Class A carbapenemases can phenotypically be divided into different subgroups of the group 2 β-lactamases accordingly to the Bush–Jacoby–Medeiros classification scheme.[36] As GES-1, GES-2 and SHV-38 hydrolyse the extended-spectrum cephalosporins, cefotaxime or ceftazidime, at a rate $\geq$10% than that for benzylpenicillin, the enzymes are assigned to subgroup 2be which encompasses the ESBLs. The SME, IMI and NMC-A enzymes are all assigned to the Bush subgroup 2f β-lactamases, which are enzymes hydrolysing imipenem at a rate $\geq$200% than for benzylpenicillin.[36]

The kinetic data in Table 4 indicate that GES-4 and the KPC enzymes are better cephalosporinases than penicillinases, whereas the hydrolysis rates for extended-spectrum cephalosporins are lower than for penicillins. The KPC enzymes were originally assigned to the Bush subgroup 2f.[63,70] The enzymes of

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Table 4. The steady-state kinetic parameters, k_{cat} (s^{-1}), K_m (μM) and k_{cat}/K_m (mM^{-1} s^{-1}), for *in vitro* hydrolysis of various β-lactam antibiotics by purified class A carbapenemases

Enzyme	PEN	AMP	LOR	CEF	CTX	CAZ	FOX	IPM	MEM	ATZ	Reference
GES-1											
k_{cat}	2.8	13[a]	53	179	68	380	0.9	0.003	NA	NC	37
K_m	40	200	2000	3400	4600	2000	30	45	NA	NC	
k_{cat}/K_m	70	65	26	52	15	188	33	0.07	NA	NC	
GES-2											
k_{cat}	0.4	0.7[a]	0.5	0.3	2.2	ND	NC	0.004	NC	NC	45
K_m	4	25.8	7.7	3	890	>3000	NC	0.45	NC	NC	
k_{cat}/K_m	96	26	65	112	2.5	ND	NC	9	NC	NC	
GES-4											
k_{cat}	130	19	490	NA	17	2.5	85	0.38	NA	NC	48
K_m	160	62	2200	NA	700	1500	810	4.7	NA	NC	
k_{cat}/K_m	810	310	230	NA	24	1.7	110	81	NA	NC	
GES-5											
k_{cat}	317	NA	190	50	2.9	0.3	9.6	1.2	NA	NA	61
K_m	370	NA	506	577	341	394	650	4.2	NA	NA	
k_{cat}/K_m	860	NA	380	87	8.5	0.8	15	286	NA	NA	
KPC-1											
k_{cat}	32	110	340	75	14	0.1	0.3	12	3	20	63
K_m	23	130	560	53	160	94	120	81	12	310	
k_{cat}/K_m	1400	850	610	1400	880	1.1	2.5	150	250	65	
KPC-2											
k_{cat}	51	210	530	69	22	≤0.12	0.31	15	4	30	70
K_m	27	230	500	82	220	ND	180	51	15	360	
k_{cat}/K_m	1900	910	1050	840	100	ND	1.7	295	265	85	
KPC-3											
k_{cat}	NA	77	364	153	52	3	0.05[b]	45	6	NA	108
K_m	NA	65	261	44	95	88	970[c]	23	4	NA	
k_{cat}/K_m	NA	1200	1400	3500	550	34	0.05	1950	1500	NA	
SME-1											
k_{cat}	19.3	181	980	NA	<0.98	0.07	<0.15	104	8.9	108	90
K_m	16.7	488	770	NA	NC	NC	NC	202	13.4	259	
k_{cat}/K_m	1150	370	1250	NA	NC	NC	NC	515	660	420	
SME-2											
k_{cat}	21.3	204	1081	NA	<1.0	<0.09	<0.17	136	7.3	140	90
K_m	17.7	609	859	NA	NC	NC	NC	313	9.6	277	
k_{cat}/K_m	1200	335	1250	NA	NC	NC	NC	435	760	505	
SME-3											
k_{cat}	6.27	539	1370	NA	1.48	0.14	0.30	322	3.22	148	92
K_m	2.2	420	401	NA	140	169	620	154	5.8	769	
k_{cat}/K_m	2850	1280	3410	NA	11	0.8	0.5	2090	960	190	
IMI-1											
k_{cat}	36	190	2000	120	3.4	0.0068	0.3	89	10	51	94
K_m	64	780	1070	130	190	270	45	170	26	26	
k_{cat}/K_m	560	240	1900	920	18	0.024	6.7	520	380	380	
NMC-A											
k_{cat}	260	816[a]	NA	2820	286	4.7	5.0	1040	12	707	109
K_m	28	90	NA	185	956	90[c]	93[c]	92	4.35	125	
k_{cat}/K_m	9300	9060	NA	15 200	300	52	62	11 300	2750	5600	

Continued

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Table 4. *continued*

Enzyme	PEN	AMP	LOR	CEF	CTX	CAZ	FOX	IPM	MEM	ATZ	Reference
SHV-38											
k_{cat}	100	1800[a]	40	5	1	110	NA	0.01	NA	3	103
K_m	13	35	150	100	800	3800	NA	24	NA	5500	
k_{cat}/K_m	7700	51 000	270	50	1	30	NA	0.5	NA	0.5	

PEN, benzylpenicillin; AMP, ampicillin; LOR, cephaloridine; CEF, cephalothin; CTX, cefotaxime; CAZ, ceftazidime; FOX, cefoxitin; IPM, imipenem; MEM, meropenem; ATZ, aztreonam; NA, not available; NC, not calculated because of too low initial rate of hydrolysis; ND, not determinable because of very high K_m values.
[a]Amoxicillin used as substrate.
[b]The k_{cat} value was determined by a high concentration of the enzyme hydrolysing a low concentration of the substrate.
[c]K_m determined as K_i by substrate competition.

subgroup 2f have relative rates of hydrolysis of imipenem that are ≥200% than of benzylpenicillin,[36] whereas the analogous relative rates of the subgroup 2e enzymes are ≪50% than of cephaloridine.[36] The relative rates of hydrolysis of imipenem of GES-4 and the KPC enzymes are ≪50% than of cefaloridine and ≪200% than of benzylpenicillin. As the hydrolysis rates of other β-lactams also correspond to those of subgroup 2e enzymes, it seems reasonable to categorize the GES-4 and KPC enzymes as subgroup 2e enzymes rather than subgroup 2f enzymes. This classification is also corroborated by the fact that the enzymes do not have high affinities for aztreonam (Table 4), and they are inhibited by clavulanate, although only weakly in comparison with the subgroup 2f enzymes. Table 4 also demonstrates that the relative rate of hydrolysis of imipenem of GES-5 is ≪50% than of cefaloridine and ≪200% than of benzylpenicillin, and besides, GES-5 seems to hydrolyse penicillin at a higher relative rate than classical and extended-spectrum cephalosporins. Thus, it is seems justified to classify GES-5 as a Bush subgroup 2br enzyme which includes broad-spectrum penicillinases inhibited by clavulanate.[36]

As seen with OXA-type and zinc-dependent carbapenemases, the class A carbapenemases hydrolyse meropenem at a much lower rate than imipenem whereas their affinity for meropenem consistently is much higher (lower K_m) than for imipenem (Table 4).

A multiple sequence alignment of the GES enzymes shows that enzymes displaying carbapenemase activity (GES-2, -4, -5 and -6) hold either a Ser or an Asn residue (GES-2) at position 170 (Ambler numbering),[34] whereas the remaining GES enzymes all have a Gly residue. The residue at position 170 is located in the Ω-loop which forms the bottom of the active site.[48]

The GES-6 enzyme confers reduced susceptibility to carbapenems in an *E. coli* transconjugant,[49] but the *in vitro* kinetic parameters of GES-6 have not been determined. A disc diffusion bioassay showed, however, that GES-6 also has detectable carbapenemase activity like GES-5.[49] Because GES-6 holds the same Gly-170 to Ser substitution as GES-5 it is conceivable that GES-6 may also possess measurable enzymatic activities against carbapenems.

The GES-4 enzyme which differs from GES-3 only by a Gly-170 to Ser substitution degrades not only carbapenems but also the cephamycin, cefoxitin (Table 4). This property is in contrast to the GES-3 enzyme which hydrolyses neither carbapenems nor cefoxitin to measurable extents.[48] GES-5 holds the same Gly-170 to Ser substitution as GES-4 and also displays cephamycinase activity (Table 4). The GES-6 enzyme has detectable carbapenemase activity,[49] but the MIC of the GES-6-producing *E. coli* transformant to cefoxitin has not been determined.

The GES family enzymes possess an unusual phenotypic plasticity, like the TEM and SHV enzymes, because the GES enzymes have activities against carbapenems, extended-spectrum β-lactams, cephamycins or monobactam.

The difference between the broad-spectrum penicillinase, SHV-1 and SHV-38 is an Ala-146 to Val substitution (Ambler numbering)[34] owing to a single transition. Among the available 78 different SHV enzymes in the GenBank database, only SHV-38 possesses Val in this position, all other SHV β-lactamases have an Ala residue and none of these enzymes has carbapenemase activity. SHV-38 does not hydrolyse meropenem in measurable amounts.[103]

Structure–function relationship

Class A β-lactamases are folded into two globular domains, one entirely α-helical and the second with five β-strands surrounded by α-helices.[110–112] The catalytic site with the active-site serine residue, Ser_{70}, is located at the interface between the two domains. The B3 strand (residues 230–238) forms one wall of the active-site cavity while the bottom of this cavity consists of the so-called Ω-loop encompassing residues 160–180.[110] Other key residues involved in the formation of the active site are the canonical triads, S_{130}DN and K_{234}TG, together with the invariant Glu_{166} that acts as a general base.

Despite only moderate amino acid sequence identities among the clusters of class A carbapenemases, the enzymes may share similar protein folds enabling them to hydrolyse carbapenems. Apart from SHV-38, all class A carbapenemases seem to contain a unique disulphide bridge between Cys_{69} and Cys_{238}, and the bond appears to stabilize the protein fold.[113] The S–S bond seems to be critical for the biological function of SME-1 as mutant enzymes without the bond do not confer resistance to β-lactams.[113,114] It is, however, not likely that the disulphide bond between these two residues in any class A β-lactamase is a prerequisite for hydrolysis of carbapenems as an engineered S–S bond in the ESBL, Toho-1, results in a continually unmeasurable hydrolysis of imipenem.[115] Therefore, it seems reasonable to assume that other structural features are required for hydrolysis of carbapenems. This is also consistent with the findings by

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Majiduddin and Palzkill[116] that multiple residues may affect the conformation of the active-site cavity.

The crystal structures of SME-1, NMC-A and the SHV-38-related SHV-1 have been determined and the enzymes share an overall structural organization with other class A β-lactamases.[113,117,118]

In contrast to other class A β-lactamases, including the NMC-A enzyme, SME-1 lacks the catalytic water molecule that is generally positioned in the substrate-binding site between Ser_{70} and Glu_{166}.[113] This water molecule typically serves as the deacylating agent of the acylated enzyme, but instead, another water molecule situated in the oxyanion hole of SME-1 is hypothesized to serve as the catalytic water molecule.[113]

In SME-1, the His_{105} residue is positioned on one side of the active-site cleft where the residue is involved in binding of the antimicrobial compound.[113] This residue is replaced by Tyr in SME-3 resulting in moderate changes of some *in vitro* kinetic parameters (Table 4), most noticeable for imipenem.

The Ser_{237} residue in SME-1 contributes significantly to catalytic activity against imipenem[119] because the side chain of Ser_{237} is stabilized by formation of hydrogen bonds between the side chain and Arg_{220}.[113] Replacement of Ser_{237} with Ala whose side chain is not capable of forming hydrogen bonds results in a 5-fold reduction of k_{cat} for imipenem.[119]

The crystal structure of the NMC-A enzyme described by Swarén *et al.*[118] shows that the distance between the Ser_{70} and Glu_{166} residues is longer than in SME-1 which allows for accommodation of the typical catalytic water molecule.[113] Compared with other class A β-lactamases devoid of carbapenemase activity, shifts in positions of Asn_{132} in the conserved SDN motif and residues at positions 237–240 adjacent to the KTG triad in NMC-A have occurred.[118]

Among the GES enzymes, the Gly-170 to Ser substitution appears to be critical for carbapenemase activity, but it is unknown how this substitution affects the catalytic mechanism of the enzymes. It is possible that the side chain of the substituted Ser residue forms new hydrogen bonds in the active site which eventually may lead to carbapenem hydrolysis. However, this has to await the elucidation of the crystal structure of a GES carbapenemase which currently is underway.[61]

The SHV-38 enzyme differs from the broad-spectrum penicillinase, SHV-1, by an Ala-146 to Val substitution and in SHV-1 this residue is exposed to the solvent.[117] Owing to the larger size and especially the more hydrophobic nature of the side chain of Val, this substitution may induce subtle structural perturbations in SHV-38 which may favour the insertion and hydrolysis of carbapenems in the active site of SHV-38. It is known from the IMP metallo-β-lactamase that a substitution remotely located from the active-site has an improving effect on the catalytic efficiency towards imipenem.[120]

Apart from the disulphide bridge, no major structural differences have been recorded between class A carbapenemases and non-carbapenemases. Therefore, it is possible that interactions between specific residues lining the active-site pocket bring about the spatial and mechanistic basis for carbapenem hydrolysis.

Conclusions

The number of different class A β-lactamases with carbapenemase activity is still limited and the occurrence of most class A carbapenemases is at present sporadic. The most widely dispersed enzymes, the GES and KPC enzymes, are encoded by genes located on transferable elements facilitating their dissemination which is also reflected in the geographical distribution of the GES and KPC enzymes. The KPC carbapenemases have established themselves especially in *K. pneumoniae* recovered from the Northeastern parts of USA, but the enzymes have also been recorded outside USA. In the light of the rapid spread of the CTX-M enzymes and the plasmid-borne AmpC β-lactamases, it is conceivable that a similar scenario may occur for the GES and KPC carbapenemases. Carbapenems are often used to combat severe infections caused by multiresistant pathogens because the drugs resist hydrolysis by most β-lactamases, including ESBLs and derepressed chromosomal AmpC β-lactamases. A wider dissemination of the GES and KPC carbapenemases would therefore pose therapeutic problems as the options for treatment may be restricted, possibly with fatal consequences.

Transparency declarations

None to declare.

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