REVIEW

Emerging carbapenemases in Gram-negative aerobes

P. Nordmann and L. Poirel

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique-Hôpitaux de Paris and Faculté de Médecine Paris-Sud, Université Paris XI, Paris, France

Carbapenemases may be defined as β-lactamases that significantly hydrolyze at least imipenem or/and meropenem. Carbapenemases involved in acquired resistance are of Ambler molecular classes A, B, and D. Class A, clavulanic acid-inhibited carbapenemases are rare. They are either chromosomally encoded (NMC-A, Sme-1 to Sme-3, IMI-1) in *Enterobacter cloacae* and *Serratia marcescens*, or plasmid encoded, such as KPC-1 in *Klebsiella pneumoniae* and GES-2 in *Pseudomonas aeruginosa*, the latter being a point-mutant of the clavulanic acid-inhibited extended-spectrum β-lactamase GES-1. The class B enzymes are the most clinically significant carbapenemases. They are metalloenzymes of the IMP or VIM series. They have been reported worldwide but mostly from South East Asia and Europe. Metalloenzymes, whose genes are plasmid and integron located, hydrolyze virtually all β-lactams except aztreonam. Finally, the class D carbapenemases are increasingly reported in *Acinetobacter baumannii* but compromise imipenem and meropenem susceptibility only marginally. The sources of the acquired carbapenemase genes remain unknown, as does the relative importance of the spread of epidemic strains as opposed to the spread of plasmid- or integron-borne genes. Because most of these carbapenemases confer only reduced susceptibility to carbapenems in Enterobacteriaceae, they may remain underestimated as a consequence of the lack of their detection.

Keywords β-Lactamase, carbapenems, antibiotic resistance, Gram-negative, aerobes

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INTRODUCTION

Reports of carbapenemases have been increasing over the last few years. This phenotypic grouping of enzymes is a heterogeneous mixture of β-lactamases belonging to molecular Ambler class A (penicillinases), class B (metalloenzymes) and class D (oxacillinases). These enzymes have the common property of hydrolyzing, at least partially, imipenem or meropenem together with other penicillin or cephalosporin antibiotics.

Metalloenzymes have been described as naturally occurring in several Gram-positive and Gram-negative bacterial species, such as *Bacillus cereus*, *Stenotrophomonas maltophilia*, *Flavobacterium* and *Chryseobacterium* species, *Aeromonas hydrophila*, *Legionella gormanii* and *Janthinobacterium lividum* [1–10]. The metalloenzymes are clavulanic acid resistant and susceptible to inhibition by divalent ion chelators such as EDTA. In *Chryseobacterium meningosepticum*, two unrelated class B carbapenemases are naturally expressed [8,9]. The naturally occurring class B enzymes are chromosomally encoded [10]. However, it has been found recently that the L-1 carbapenemase of *Stenotrophomonas maltophilia* might also be encoded by large plasmids (200 kb) [11]. None of these carbapenemases identified as naturally occurring mechanisms of resistance to carbapenems has been identified so far in acquired resistance.

Corresponding author and reprint requests: P. Nordmann, Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France
Tel: +33 1 45 21 20 19
Fax: +33 1 45 21 63 40
E-mail: nordmann.patrice@bct.ap-hop-paris.fr

THE AMBLER CLASS A CARBAPENEMASES

A few Ambler class A carbapenemases have been reported in rare enterobacterial isolates. They belong to the group 2f as defined by Bush et al. [12] and may form part of the so-called clavulanic acid-inhibited penicillinase group.

NMC-A was the first class A carbapenemase identified, from an *Enterobacter cloacae* clinical isolate NOR-1 in 1990 [13]. This strain was from a patient in a French hospital who had been treated with one intravenous bolus (500 mg) of imipenem [13]. *E. cloacae* NOR-1 had decreased susceptibility to imipenem, aztreonam and less so to meropenem, whereas it remained fully susceptible to extended-spectrum cephalosporins. NMC-A significantly hydrolyzes aminocarboxypenicillins, cephalothin, imipenem, and aztreonam (Table 1). Its activity is partially inhibited by clavulanic acid, tazobactam and sulbactam, as for most of the enzymes of the penicillinase group. NMC-A is distantly related to extended-spectrum penicillinases such as the derivatives of the TEM and SHV series. The NMC-A gene is chromosomally encoded. Expression of NMC-A was inducible [14]. Indeed, the $bla_{\text{NMC-A}}$ gene was preceded by a LysR-type regulatory gene similar to those found upstream of the naturally encoded cephalosporinase (AmpC-type) genes. The LysR-type regulator named NMC-R increases biosynthesis of the enzyme at a basal state and further increases its biosynthesis when β-lactam-mediated induction occurs [14]. We have shown recently that nucleotide substitutions in another gene, the *ampD* gene coding for an amidase (an enzyme involved in peptidoglycan biosynthesis), may co-regulate and lead to stable coexpression of the carbapenemase NMC-A together with overexpression of the naturally occurring AmpC-type enzyme of *Enterobacter cloacae* [15].

SME-1 has been identified from two *Serratia marcescens* isolates collected in London in 1982 prior to any carbapenem marketing [16]. These strains were resistant to amino-, carboxy- and ureidopenicillins, early-generation cephalosporins, imipenem and aztreonam. Although SME-1 shares only 68% amino acid identity with NMC-A [17], it had an overall similar hydrolysis profile. Its expression, like that of NMC-A, is also regulated by a LysR-type regulator but to a minor extent [18]. It is possible that *S. marcescens* isolates that have a bla_{SME} gene are *Serratia marcescens* subspecies that naturally encode this resistance determinant. Recently, SME-2 and SME-3 β-lactamases that are point-mutant derivatives of SME-1 have been identified from *S. marcescens* strains isolated in several geographic locations in the USA [19]. Genomic comparison of worldwide-encountered *S. marcescens* isolates that have SME-like enzymes suggests a global dissemination of a distinct *S. marcescens* subtype [20].

IMI-1 was the third type of chromosomally encoded carbapenemase of Ambler class A identified from two *E. cloacae* strains isolated in Southern California in 1984 [21]. IMI-1 shares 95% amino acid identity with NMC-A, has a very similar hydrolysis profile and is inducible, due to an upstream-located LysR-type regulatory gene.

More recently, a totally novel class A enzyme, KPC-1, has been reported from a *Klebsiella pneumoniae* isolate from the USA that was resistant to carbapenems, extended-spectrum cephalosporins and aztreonam (Table 1) [22]. Compared to the chromosome-encoded class A carbapenemases, KPC-1 activity is more inhibited by clavulanic acid and tazobactam, whereas its expression is not inducible. KPC-1 has weak amino acid identity with the most closely related enzymes SME-1 (45%), NMC-A (44%) and IMI-1 (43%). It hydrolyzes penicillins, first- and second-generation cephalosporins, aztreonam and carbapenems (meropenem as well as imipenem). Most of the amino acids putatively involved in the carbapenemase activity of chromosomally encoded class A carbapenemases (C69, S70, K73, S130, R164, E166, N170, D179, R220, K234 and C238 [22,23]) are found in its amino acid sequence, except for H105 and S237 instead of W105 and T237, respectively [22]. The discovery of $bla_{\text{KPC-1}}$ represents an additional threat in the evolving world of β-lactamase-mediated resistance in bacteria, since it was encoded by a 50-kb transferable plasmid of an enterobacterial species understood to be the source of well-known and worldwide extended-spectrum β-lactamases.

The most recently reported class A enzyme with carbapenemase activity is GES-2, which is a point-mutant derivative of the extended-spectrum β-lactamase GES-1 [24]. GES-2 was identified from a *Pseudomonas aeruginosa* isolate in South Africa [24]. GES-2, like GES-1, possesses cysteine residues in positions 69 and 238 that may form a disulfide bridge and that may explain the imipenem-binding properties [23,24]. GES-2 differs from GES-1 by a Gly-to-Asp substitution in Ambler position 170, located inside the omega loop. The omega loop (amino acid residues 162–179) is rather conserved in class A enzymes and may form the basis of their catalytic site. Kinetic analysis showed that GES-2 catalytic efficiency against imipenem was 100-fold higher than that of GES-1. However, GES-2 activity

Table 1 Main features of acquired carbapenemases

		Hydrolysis spectrum					Inhibited by			
Ambler classification	**Type of enzymes**	**Amino-penicillins**	**Ureido-penicillins**	**Extended-spectrum Cephalosporins**	**Aztreonam**	**Carbapenems**	**Chavulamic acid**	**EDTA**	**Organisms**	**Genetic location**
A	NmcA; Sme-1 to Sme-3; IMI-1	○	□	△	□	○	±	−	*E. cloacae; S. marcescens*	Chromosomal
A	KPC-1	○	○	□	○	○	+	−	*K. pneumoniae*	Plasmid
A	GES-2	○	○	○	○	□	+	−	*P. aeruginosa*	Plasmid; integron
B	IMP-1 to IMP-9	○	○	○	△	○	−	+	Enterobacteriaceae; Pseudomonaceae, *Alcaligenes* sp.; *Acinetobacter* sp.	Chromosomal; plasmid Integron
B	VIM-1 to VIM-3	○	○	○	△	○	−	+	*P. aeruginosa; P. putida; Acinetobacter baumannii*	Chromosomal; plasmid; integron
D	OXA-23 to OXA-27	○	□	○	△	□	±	−	*Acinetobacter baumannii*	Chromosomal; ± integron

(○) High level; (□) low level; (△) no hydrolysis.

against carbapenems remains 1000-fold less than that of SME-1 and NMC-A. GES-2 identification showed that class A extended-spectrum β-lactamases may become weak carbapenemases through a single amino acid substitution. Like bla_{KPC-1}, bla_{GES-2} was located on a large-size plasmid and on a class 1 integron.

Plasmid-mediated class A enzymes with carbapenemase activity may become clinically significant in species that naturally possess (e.g. *E. cloacae*, *P. aeruginosa*) or acquire additional mechanisms of carbapenem resistance such as low permeability and/or efflux.

THE AMBLER CLASS B METALLOENZYMES

IMP-1 was the first carbapenemase identified as a source of acquired resistance to carbapenems in a *S. marcescens* isolate in 1991 in Japan [25]. IMP-1, like most metallocarbapenemases, has a very broad substrate profile, including expanded-spectrum cephalosporins (cefotaxime, ceftazidime, cefepime) and carbapenems (imipenem, meropenem, panipenem). Only the monobactams are not hydrolyzed (Table 1). This metallo-β-lactamase is a binuclear zinc-dependent enzyme whose activity is susceptible to EDTA, whereas it is not inhibited by clavulanic acid, tazobactam, and sulbactam [26]. The same bla_{IMP-1} gene has also been characterized from several aerobic and aerobic/anaerobic Gram-negative species from patients hospitalized in Japan; Enterobacteriaceae, *P. aeruginosa*, *Brevium diminuta*, *Pseudomonas fluorescens*, *Burkholderia cepacia*, *Achromobacter* (*Alcaligenes*) *xylosoxydans*, *Pseudomonas putida* and *Acinetobacter baumannii* [26–29]. These reports suggested horizontal transfer of bla_{IMP-1}, particularly among *P. aeruginosa* and *S. marcescens* isolates, and/or repeated introduction into clinical settings from the environment. Senda et al. studied a total of 3700 *P. aeruginosa* isolates collected from 17 university hospitals in Japan from 1992 to 1994 [27]. They showed that 15 of 132 carbapenem-resistant *P. aeruginosa* isolates carried bla_{IMP}-like genes [27]. Acquisition of these genes is not always followed by expression of a high level of resistance to carbapenems [27,28]. The bla_{IMP} genes were often located on large-size plasmids transferable to Enterobacteriaceae. In a Japanese survey conducted in 1996–97, IMP-1-producing isolates accounted for 1.3% and 4.4% of *S. marcescens* and *P. aeruginosa* isolates, respectively [30]. The same authors also detected bla_{IMP-1} in *Escherichia coli* and *Citrobacter freundii* isolates.

Genetic analysis of the bla_{IMP-1} environment revealed typical features of integron-located genes, in particular cassette boundaries known as core sites and inverse core sites, defining gene cassettes. Integrons are genetic structures capable of integrating individual gene cassettes encoding antibiotic resistance genes. The gene cassettes are defined by a resistance gene preceded by a ribosomal binding site, and a recombination site, known as the 59-base element (59-be), located downstream of the integrated gene [31]. The bla_{IMP-1} gene cassette was found to be inserted into three different classes of integrons. Arakawa et al. studied another *S. marcescens* isolate from Japan producing the same enzyme [32]. They also found an integron-like element carrying the bla_{IMP} gene cassette. However, the integrase gene and the typical 59-be usually found downstream of bla_{IMP} were quite different. The putative integrase had only 61% amino acid identity with that of a typical class 1 integron. This was the first description of a class 3 integron in which bla_{IMP} was associated with an *aac(6′)-Ib* gene cassette encoding aminoglycoside resistance. In that case, the bla_{IMP}-containing integron was located on a large plasmid.

In another study, bla_{IMP-1} was located on a 36-kb plasmid and was part of a gene cassette inserted into a class 1 integron, In*31*, in *P. aeruginosa* [32,33]. In*31* belongs to a group of defective transposon derivatives that originated from Tn*402*-like ancestors such as In*0*, In*2* and In*5*. It comprises four additional gene cassettes (*aacA4*, *catB6*, *orfN*, *qacG*) (Figure 1) conferring resistance to aminoglycosides, chloramphenicol and quaternary ammonium compounds. IMP-1 is often found in Japanese isolates and it has recently been identified in isolates from other countries, especially from Europe. In 1999, Cornaglia et al. reported an IMP-1-producing *Acinetobacter baumannii* isolate from an Italian patient [34].

IMP-2 was found also from an *A. baumannii* isolate from Italy isolated in 1997 [35]. Bla_{IMP-2} was integron-borne but its 59-be was not related to that of bla_{IMP-1}, indicating a different origin of gene cassettes encoding these two β-lactamase variants. The bla_{IMP-2}-positive integron carried a DNA integrase gene typical of class 1 integrons and, in addition to the bla_{IMP-2} gene cassette, *aacA4* and *aadA1* gene cassettes expressing aminoglycoside

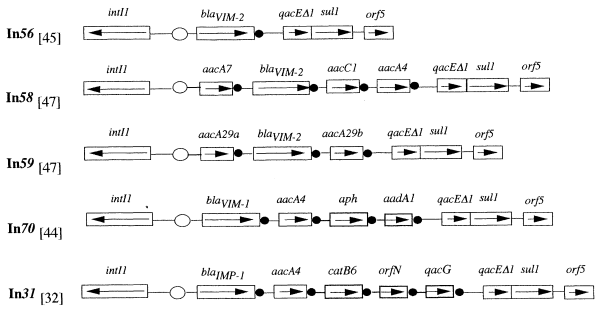

Figure 1 Compared structures of the class 1 integrons from *P. aeruginosa* that contain the gene cassettes encoding IMP-like and VIM-like carbapenemases. The *intI* integrase gene, which encodes an integrase, is part of the 5′-CS; the 3′-CS located downstream of the integrated gene cassette(s) includes the sulfonamide resistance gene *sul1*, the quaternary ammonium resistance determinant *qacEΔ1* and *orf5* of unknown function. Inserted gene cassettes are indicated by boxes, with an arrow indicating their transcriptional orientation. The 59-be recombination sites are represented by black circles and the *attI1* recombination site by white circles. References for characterized integrons are indicated as subscripts.

resistance. IMP-2 shares 85% amino acid identity with IMP-1, with none of the amino acid changes occurring among highly conserved residues of class B enzymes [35,36]. The kinetic parameters of IMP-2 are similar to those of IMP-1 for many β-lactam substrates, but differ particularly for ampicillin, carbenicillin, cephaloridin, and meropenem.

IMP-3 (previously named MET-1) was identified in a *Shigella flexneri* isolate from Japan [37]. This enzyme was encoded on a transferable plasmid by a class 1 integron-located gene. IMP-3 differs from IMP-1 by two amino acid changes. The Gly-to-Ser change at position 262 (class B BBL numbering [36]) in IMP-3 may restrict its substrate profile [37]. Indeed, IMP-3 does not significantly hydrolyze benzylpenicillin, ampicillin, ceftazidime, and imipenem. Therefore, it has been suggested that IMP-3 may be a progenitor of IMP-1 [38]. Identification of IMP-3 in *S. flexneri* signals the ongoing spread of metallocarbapenemases among community-acquired pathogens.

IMP-4 has been characterized from *Acinetobacter* isolates recovered from blood samples in Hong Kong between 1994 and 1998 [39]. These isolates were resistant to most β-lactams, including imipenem (MIC from 8 to 32 mg/L). IMP-4 has also been found in a *Citrobacter youngae* isolate obtained in 1998 in Hong Kong [40]. In that case, $bla_{\text{IMP-4}}$ was plasmid-borne and could be transferred by conjugation from *C. youngae* to *E. coli*. This report underlined the spread of these carbapenemase genes in enterobacterial isolates as a consequence of their plasmid location.

From 2000 to 2001, additional IMP variants were identified in several clinical strains of Gram-negative aerobes throughout the world (Table 2). They are often available only as accession numbers in the GenBank databases. $Bla_{\text{IMP-5}}$ has been found in an *A. baumannii* isolate from Portugal, $bla_{\text{IMP-6}}$ in a *S. marcescens* isolate from Japan [41], $bla_{\text{IMP-7}}$ in a *P. aeruginosa* isolate as a source of a nosocomial outbreak in Thailand, $bla_{\text{IMP-8}}$ in a *K. pneumoniae* isolate from Taiwan [42], and $bla_{\text{IMP-9}}$ in a *P. aeruginosa* isolate from China. In fact, IMP-like enzymes may now be divided into three major subgroups (Figure 2). The first subgroup includes IMP-1 and IMP-3 to −7, and the percentage of amino acid identity in this group ranges from 90% to 99%. The second subgroup includes IMP-2 and IMP-8, which differ by only two amino acid residues. The amino acid identity between these two subgroups ranges from 84% to 88%. $Bla_{\text{IMP-9}}$ reported from a *P. aeruginosa* isolate in Japan may be the unique representative of a third group, IMP-9 sharing 87% and 86% amino acid identity with IMP-1 and IMP-2, respectively. The genetic support of these novel bla_{IMP} genes is unknown.

VIM-1 was reported in Verona in Italy from a *P. aeruginosa* isolate in 1997 [43]. It was the first example of a representative of a novel family of acquired metalloenzymes. Although VIM-1 shares less than 30% amino acid identity with the IMP enzymes, it possesses the same broad-spectrum profile, including all β-lactams except aztreonam. Resistance to the monobactam aztreonam in the original VIM-1-producing *P. aeruginosa* isolate was probably due to additional mechanisms (efflux, cephalosporinase hyperproduction) [43]. As found for bla_{IMP} genes, $bla_{\text{VIM-1}}$ was also part of a gene cassette inserted into a class 1 integron. This integron carried an integrase gene typical of class 1 integrons and, in addition to a $bla_{\text{VIM-1}}$ gene cassette, an *aacA4* gene cassette encoding resistance to aminoglycosides (Figure 1). The $bla_{\text{VIM-1}}$-containing integron was probably chromosome located.

Very recently, the $bla_{\text{VIM-1}}$ gene was also found in an *Achromobacter xylosoxydans* isolate in the same Italian hospital [44]. This isolate exhibited broad-spectrum resistance to β-lactams, including

Table 2 IMP-like and VIM-like carbapenemases

Enzyme	Host	Origin	Integron	GenBank no.	Reference
IMP-1	*Serratia marcescens*	Japan	+	–	[25,32]
	Acinetobacter baumannii	Japan	?	–	[29]
	Pseudomonas aeruginosa	Japan	+	–	[27,28]
	Achromobacter xylosoxydans	Japan	?	–	[28]
	Pseudomonas putida	Japan, Taiwan	?	–	[28,51]
	Pseudomona stutzeri	Taiwan	?	–	[51]
	Klebsiella pneumoniae	Japan, Singapore	?	–	[28,59]
IMP-2	*Acinetobacter baumannii*	Italy	+	–	[35]
IMP-3	*Shigella flexneri*	Japan	+	–	[37,38]
IMP-4	*Acinetobacter baumannii*	Hong Kong	+	–	[39]
	Citrobacter youngae	China	+	–	[40]
IMP-5	*Acinetobacter baumannii*	Portugal	?	AAK27847	NP
IMP-6	*Serratia marcescens*	Japan	+	–	[41]
IMP-7	*Pseudomonas aeruginosa*	Canada	+	AF318077	NP
IMP-8	*Klebsiella pneumoniae*	Taiwan	?	–	[42]
IMP-9	*Pseudomonas aeruginosa*	China	?	AAK59385	NP
VIM-1	*Pseudomonas aeruginosa*	Italy	+	–	[43]
	Acinetobacter baumannii	Italy	+	–	NP
	Achromobacter xylosoxydans	Italy	?	–	[44]
VIM-2	*Pseudomonas aeruginosa*	France	+	–	[45,47]
		Greece	?	–	[50]
		Italy	+	–	[60]
		Spain	+	–	[61]
		Korea	?	AY029772	NP
	Acinetobacter baumannii	Korea	?	AF305559	NP
	Enterobacter cloacae	Korea	?	AF305559	NP
	Serratia marcescens	Korea	?	AY030343	NP
	Pseudomonas putida	Korea	?	AF291438	NP
	Acinetobacter genomosp. *3*	Italy	?	AF369871	NP
	Pseudomonas putida	Taiwan	?	–	[51]
	Pseudomonas stutzeri	Taiwan	?	–	[51]
VIM-3	*Pseudomonas aeruginosa*	Taiwan	?	–	[51]

?, unknown; NP, not published.

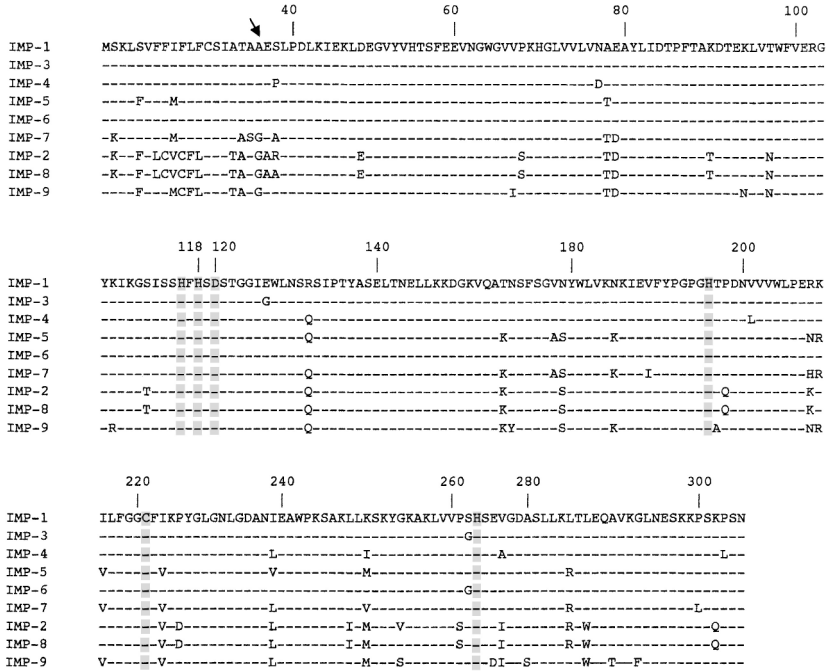

Figure 2 Comparison of the available amino acid sequences for IMP-like enzymes. The numbering is according to that reported recently for class B enzymes (BBL) [36]. Highlighted amino acids are those strictly conserved among class B β-lactamases. Dashes indicate identical amino acids. The arrow indicates the putative position of the leader peptide cleavage site.

carbapenems, and harbored a 30-kb non-conjugative plasmid carrying a class 1 integron. This integron (In*70*) contained four gene cassettes, three different aminoglycoside resistance genes being located downstream of the bla_{VIM-1} gene cassette (Figure 1). As observed for In*31* carrying the bla_{IMP-1} gene cassette, In*70* is flanked by inverted repeats, and a truncated *tni* module was detected in its 3′-part. Thus, In*70* can also be considered as a member of the group of class 1 integrons associated with defective transposon derivatives originating from Tn*402*-like elements.

Bla_{VIM-2} was identified first in southern France (Marseilles), not far from Italy, from a *P. aeruginosa* isolate in a blood culture of a neutropenic patient in 1996 [45]. This isolate was resistant to most β-lactams, including ceftazidime, cefepime and imipenem, whereas it remained susceptible to aztreonam. VIM-2 is closely related to VIM-1 (90% amino acid identity) and was encoded by a gene cassette that was the only resistance gene within the identified class 1 integron In*56* (Figure 1). Bla_{VIM-2} was located on a c. 45-kb plasmid. This plasmid was transferable by electroporation from *P. aeruginosa* to *P. aeruginosa*. Although hydrolysis profiles of VIM-1 and VIM-2 were similar for most β-lactam substrates, kinetic parameters for benzylpenicillin, ceftazidime and cefepime were different [46]. Thus, the structural differences between VIM-1 and VIM-2 may be functionally relevant [46]. Despite a consistent degree of sequence identity between bla_{VIM-1} and bla_{VIM-2}, the 59-bes associated with these genes differed in size and structure. This observation raises the question of a different origin of bla_{VIM-1} and bla_{VIM-2} gene cassettes as well as the corresponding carbapenemase genes. The G + C content of $bla_{VIM-1/VIM-2}$, averaging 56%, does not provide any clue to the putative origin of these genes, since it is known that the G + C content of naturally occurring β-lactamase genes is not always related to that of housekeeping genes of a given species (see, for example, bla_{SHV} and *K. pneumoniae* genes).

Since VIM-1- and VIM-2-positive *P. aeruginosa* isolates were isolated from the same southern part of Europe, a regional outbreak of *P. aeruginosa* producing related enzymes was not ruled out. However, two other *P. aeruginosa* isolates were subsequently found in the Paris area that harbored the same bla_{VIM-2} gene cassette [47]. In this latter case, the bla_{VIM-2} gene cassette formed part of different class 1 integrons, underlining its spread (Figure 1). The bla_{VIM-2}-positive integrons carried a variety of aminoglycoside resistance genes in addition to the sulfonamide resistance gene usually found in the 3′-CS.

A retrospective epidemiologic study in the hospital in Marseilles, France, where the first VIM-2-producing *P. aeruginosa* strain was isolated revealed that, from 1996 to 1998, 20 other VIM-2-positive *P. aeruginosa* isolates had been isolated, from patients hospitalized in different units. These isolates had indistinguishable genotypic patterns (personal data). Similarly, VIM-like-producing *P. aeruginosa* isolates were found to be the source of outbreaks in two university hospitals in Italy and in Greece during the same period [48–50]. In Greece, VIM-2 had been also identified, whereas VIM-1 was identified in Italy. Additionally, a *P. aeruginosa* strain that encodes a plasmid-mediated VIM-2 enzyme has been identified recently from Barcelona in Spain [61].

Very recently, VIM-2 and a novel variant of the VIM series, VIM-3, have been identified in *P. aeruginosa* isolates in Taiwan [51]. The VIM-3 amino acid sequence differs from that of VIM-2 by two amino acid changes. The bla_{VIM-3} gene was chromosomal [51]. Identication of a VIM-type enzyme outside Europe supported the idea of a possible worldwide spread of carbapenemases of the IMP as well as of the VIM series.

Detailed analysis of metalloenzyme-producing *P. aeruginosa* isolates revealed that they were involved in serious infections such as septicemia and pneumonia and that carbapenem-containing therapy may fail [45,48]. Expression of these β-lactamase genes may vary. Senda et al. reported, for example, for several *Pseudomonas* isolates involved in multifocal outbreaks, that the MIC values of imipenem ranged from 2 to >128 mg/L [27]. This variability of MIC values may explain the difficulty in detecting IMP- or VIM-positive isolates in a clinical laboratory, especially among Enterobacteriaceae [28]. Clinical microbiologists should be aware that Gram-negative isolates, including Enterobacteriaceae, *A. baumannii* and *Pseudomonas* species with borderline susceptibility to carbapenems, could be true carbapenemase producers. Difficulties in clinical detection, together with the plasmid, integron and sometimes transposon location of these carbapenemase genes, may enhance their spread. Thus, suspicious isolates should be tested for carbapenemase activity in reference laboratories. Detection of

metalloenzyme producers may also be based on a double disk synergy test using carbapenems and ion chelators [52,53].

THE OXACILLINASES WITH CARBAPENEMASE PROPERTY

Recently, five oxacillinases that confer some degree of resistance to carbapenems have been identified. These oxacillinases were from *A. baumannii* isolates from different parts of the world. OXA-23 (also named ARI-1 [54]) and OXA-27, from Scotland and Singapore, respectively [54,55], have 99% amino acid identity, whereas they share only 60% identity with a second group of oxacillinases with carbapenem-hydrolyzing properties consisting of OXA-24 (Spain), OXA-25 (Spain) and OXA-26 (Belgium), the latest enzymes differing only by a few amino acid substitutions [56]. An unsequenced oxacillinase with carbapenemase activity had been identified in France [57], and we have recently isolated a point-mutant derivative of OXA-26 with carbapenemase activity from an *A. baumannii* isolate from a patient transferred from Portugal (personal data) hospitalized in France.

The hydrolysis profiles of these oxacillinases are very similar. They hydrolyze imipenem and meropenem weakly, and do not hydrolyze extended-spectrum cephalosporins and aztreonam (Table 1). Their activity is inhibited by clavulanic acid, an uncommon property for oxacillinases, except for OXA-23, which is resistant to clavulanic acid. OXA-24 and OXA-27 hydrolyze benzylpenicillin and cephaloridine, while hydrolysis of oxacillin and cloxacillin is not detectable [55]. The amino acid sequence analysis of these oxacillinases may indicate that a Y-to-F substitution (position 154) in the YGN typical motif of oxacillinases may explain part of the carbapenem hydrolysis activity of these enzymes [54–56].

A careful comparison of MICs of β-lactams for oxacillinase-producing clinical strains and those for *E. coli* recombinant strains once the oxacillinase genes are cloned and expressed indicates that these oxacillinases have weak carbapenemase activity. These enzymes may contribute to carbapenem resistance if other carbapenem resistance mechanisms are present, such as impermeability or efflux. There has been a report of a nosocomial outbreak involving such oxacillinases [58].

While most (if not all) oxacillinase genes form part of gene cassettes in class 1 integrons, the genetic support of these oxacillinase genes remains unclear. While $bla_{\text{OXA-23}}$ was not found in a typical integron, an imperfect inverted repeat sequence similar to known 59-bes and two GTTA sequence recombination sites in the 5′- and 3′-ends of the gene were identified in its 3′-downstream-located region, thus suggesting that $bla_{\text{OXA-23}}$ was a form of gene cassette [54]. While $bla_{\text{OXA-24}}$ was likely to be chromosomally located, the genetic support of the other oxacillinase/carbapenemase genes is unknown. The fact that these oxacillinases have been reported so far only from *A. baumannii* isolates remains intriguing.

CONCLUSION

This review emphasizes that acquired carbapenemases are increasingly reported worldwide among nosocomial and community-acquired Gram-negative aerobes. Enzymes that may be clinically worrying are of the IMP and VIM series in *P. aeruginosa* and Enterobacteriaceae *and* of the oxacillinase type in *Acinetobacter baumannii*. These enzymes confer resistance to carbapenems, once expressed from Gram-negative species that possess either a naturally occurring (*P. aeruginosa*, *Acinetobacter* sp.) or an acquired (Enterobacteriaceae) low level of outer membrane permeability/efflux. The only exception would be the recently reported plasmid-encoded class A enzyme KPC-1 from a *K. pneumoniae* isolate that confers high-level resistance to carbapenems by itself.

Thus, clinical detection of carbapenemase producers remains difficult based on a simple phenotypic analysis of antibiotic susceptibility testing. With the exception of the rare class A enzymes with carbapenemase activity, detection of these enzymes by a synergy test based on clavulanate- and imipenem-containing disks remains elusive.

A PCR-based technique may be used for detection of known metalloenzymes. Detection of acquired class B enzymes may be based on the degenerated primers IMP-A (5′-gaa ggy gtt tat gtt cat ac-3′) and IMP-B (5′-gta mgt ttc aag agt gat gc-3′) for bla_{IMP}-like genes, and on primers VIM-B (5′-atg gtg ttt ggt cgc ata tc-3′) and VIM-F (5′-tgg gcc att cag cca gat c-3′) for the bla_{VIM}-like genes.

One of the most interesting aspects of further studies will be to estimate precisely the prevalence

of carbapenemases among clinically relevant Gram-negative isolates and to analyze the selection power of β-lactam and non-β-lactam antibiotics, since the carbapenemase genes are often physically linked to at least aminoglycoside resistance genes in integron structures.

The origin of these carbapenemase genes remains unknown. It is very likely that Enterobacteriaceae do not represent the natural reservoir of these enzymes. Determination of the reservoirs, which may be environmental, may help to prevent dissemination of carbapenemase genes and may provide insights into the molecular mechanism of integron and gene cassette formation.

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