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VIM-19, a Metallo-β-Lactamase with Increased Carbapenemase Activity from *Escherichia coli* and *Klebsiella pneumoniae*▽

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Two carbapenem-resistant isolates, one *Escherichia coli* isolate and one *Klebsiella pneumoniae* isolate, recovered from an Algerian patient expressed a novel VIM-type metallo-β-lactamase (MBL). The identified bla_{VIM-19} gene was located on a ca. 160-kb plasmid and located inside a class 1 integron in both isolates. VIM-19 differed from VIM-1 by the Asn215Lys and Ser228Arg substitutions, increasing its hydrolytic activity toward carbapenems. Site-directed mutagenesis experiments showed that both substitutions were necessary for the increased carbapenemase activity of VIM-19. This study indicates that MBLs with enhanced activity toward carbapenems may be obtained as a result of very few amino acid substitutions.

Acquired metallo-β-lactamases (MBLs) are emerging resistance determinants in clinically relevant Gram-negative species (5, 32). These enzymes confer broad-spectrum β-lactam resistance, including resistance to carbapenems (28, 32). In addition, their potential for rapid and wide dissemination make them of great concern (3, 32). Nine types of acquired MBLs have been reported so far, the IMP, VIM, SPM, GIM (25, 32), SIM (14), KHM (29), AIM (D. Yong, T. R. Walsh, J. Bell, B. Ritchie, R. Pratt, and M. A. Toleman, presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 25 to 28 October 2008), NDM (34), and DIM types (L. Poirel, J. M. Rodriguez-Martinez, N. Al Naiemi, Y. Debets-Ossenkopp, and P. Nordmann, presented at the 19th European Congress of Clinical Microbiology and Infectious Diseases, Helsinki, Finland, 16 to 19 May 2009). These MBLs are usually encoded by genes on plasmids and associated with mobile genetic elements, mostly class 1 integrons and IS*CR* elements (25, 28, 30, 32). They have been identified in *Pseudomonas* spp. and more rarely in members of the family *Enterobacteriaceae* and *Acinetobacter* spp. Those enzymes corresponding to the so-called VIM type have been classified into three clusters according to their amino acid sequences (subgroups VIM-1, VIM-2, and VIM-7) (www.lahey.org/Studies). Those enzymes share similar hydrolytic properties, even if some slight hydrolytic differences have been noticed (6). We report here the identification and characterization of a new VIM variant, belonging to the VIM-1 cluster which exhibits an increased carbapenem-hydrolyzing activity compared with VIM-1.

MATERIALS AND METHODS

Bacterial strains and plasmids. Identification of *Escherichia coli* DIH-1 and *Klebsiella pneumoniae* DIH-2 was performed by using the API 20E system (bioMérieux, Marcy l'Etoile, France). *Pseudomonas aeruginosa* V4 (4) and *Acinetobacter* sp. 154 (7) were used as bla_{VIM-1} and bla_{VIM-4} positive controls, respectively. *E. coli* TOP10 was used as the host strain for cloning, and *E. coli* J53 (resistant to azide) was used as the host for conjugation assays.

Antimicrobial agents and MIC determinations. The antimicrobial agents and their sources have been described elsewhere (21). Susceptibility testing was performed by disk diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France), as previously described. The MICs were determined by Etest (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar plates at 37°C (21). Results of susceptibility testing were recorded according to the CLSI guidelines (2). MBL detection tests were performed using an Etest strip (AB Biodisk).

Cloning experiments, PCR, and DNA sequencing. Whole-cell DNAs were extracted as previously described (1). PCR screening for MBL-encoding genes bla_{VIM} and bla_{IMP} and for extended-spectrum β-lactamases (ESBLs) encoding genes bla_{TEM}, bla_{SHV}, and bla_{CTX-M} were performed as described previously (26). PCR combinations performed as described previously (26) were performed in order to identify the class 1 integron 5′ and 3′ extremities, including the search for Tn*402*-related transposon structures described previously (22). In order to express the different bla_{VIM} genes in an identical background, cloning of the bla_{VIM-1}, bla_{VIM-4}, and bla_{VIM-19} genes was performed in *E. coli* TOP10 as described previously (23), using the ZeroBlunt TOPO PCR cloning kit (Invitrogen, Cergy-Pontoise, France) followed by selection on plates containing 50 μg/ml of amoxicillin and 30 μg/ml of kanamycin. The PCR amplicon encompassing the entire sequence of the bla_{VIM} genes used for cloning was obtained with primers VIM-CasA (5′-TATGCCGCACCCACCCCTATG-3′) and VIM-CasB (5′-ATGCTACTCGGCGACTGAGC-3′). Those amplicons did not include the original promoter region of the bla_{VIM} genes in order to express those genes under the control of the same promoter provided by plasmid pCR-BluntII-TOPO. The corresponding recombinant strains were used for MIC determinations.

Both strands of the cloned DNA inserts of recombinant plasmids were sequenced by using an Applied Biosystems sequencer (ABI 377). The nucleotide and deduced protein sequences were analyzed with software available over the Internet from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/).

Site-directed mutagenesis. The VIM-19 β-lactamase identified contained a single substitution (Asn215Lys) compared to the sequence of VIM-4 and two substitutions (Asn215Lys and Ser228Arg) compared to the sequence of VIM-1. Therefore, we aimed to evaluate the role of the single Asn215Lys substitution in increased carbapenemase activity. For this purpose, a site-directed mutagenesis protocol was used as described by the manufacturer (QuikChange site-directed mutagenesis kit; Stratagene). Recombinant plasmid pVIM-1 was used as the template in PCR amplification with primers VIM-1-N215K-F (5′-GTCCCGTCAGCGAAAGTGCTATACGG-3′) and VIM-1-N215K-R (5′-CCGTATAGCACTTTCGCTGACGGGAC-3′). It gave rise to recombinant plasmid pVIM-1-N215K, which was subsequently transformed into *E. coli* TOP10. Sequence analysis of the inserts confirmed the presence of the expected mutation, which led to the N215K replacement in the mature β-lactamase VIM-1-N215K.

IEF analysis. The β-lactamase extract from a culture of *E. coli* TOP10 harboring recombinant plasmid pVIM-19 was subjected to analytical isoelectric focusing (IEF) analysis as described previously (15). The focused β-lactamases were detected by overlaying the gel with 1 mM nitrocefin (Calbiochem, La Jolla, CA).

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TABLE 1. MICs of β-lactams for *E. coli* and *K. pneumoniae* isolates and strains[a]

β-Lactam(s)[b]	MIC (μg/ml) of β-lactam for:								
	E. coli DIH-1 (VIM-19)	*K. pneumoniae* DIH-2 (VIM-19)	*E. coli* TOP10 (pVIM-19)	*E. coli* TOP10 (pVIM-4)	*E. coli* TOP10 (pVIM-1)	*E. coli* TOP10 (pVIM1-N215K)	*E. coli* J53(pR3) (VIM-19)	*E. coli* TOP10	*E. coli* J53
Amoxicillin	>512	>512	>512	>512	>512	>512	>512	4	4
Amoxicillin + CLA	>512	>512	>512	>512	>512	>512	>512	4	4
Ticarcillin	>512	>512	>512	>512	>512	>512	>512	4	4
Ticarcillin + CLA	>512	>512	>512	>512	>512	>512	>512	4	4
Piperacillin	>512	>512	256	256	256	256	256	1	1
Piperacillin + TZB	>512	>512	256	256	256	256	256	1	1
Cefuroxime	>512	>512	>512	>512	>512	>512	>512	2	4
Ceftazidime	>512	128	32	32	512	512	32	0.06	0.06
Cefotaxime	>512	>512	64	64	32	64	64	0.12	0.12
Cefepime	16	16	2	1	16	16	2	0.06	0.06
Cefpirome	32	64	2	2	32	32	2	0.06	0.12
Cefoxitin	>512	>512	256	256	256	256	256	4	4
Aztreonam	4	2	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Imipenem	8	8	8	4	1	1	4	0.06	0.06
Meropenem	4	4	1	0.5	0.25	0.25	0.5	0.016	0.016
Ertapenem	16	16	2	0.75	0.125	0.125	1.5	0.006	0.006

[a] The *E. coli* and *K. pneumoniae* isolates and strains follow: *E. coli* DIH-1 and *K. pneumoniae* DIH-2 clinical isolates; *E. coli* TOP10 strains harboring recombinant plasmid pVIM-19, pVIM-4, pVIM-1-N215K, or pVIM-1 expressing β-lactamase VIM-19, VIM-4, VIM-1-N215K, or VIM-1, respectively; *E. coli* J53 transconjugant containing the natural plasmid expressing VIM-19 from *E. coli* DIH-1; and *E. coli* TOP10 and *E. coli* J53 reference strains.

[b] CLA, clavulanic acid at a fixed concentration of 4 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml.

β-Lactamase purification. Cultures of *E. coli* TOP10 harboring recombinant plasmid pVIM-19 were grown overnight at 37°C in 4 liters of Trypticase soy broth containing amoxicillin (100 μg/ml) and kanamycin (30 μg/ml). β-Lactamase VIM-19 was purified by ion-exchange chromatography. Briefly, the bacterial suspension was pelleted, resuspended in 50 ml of 100 mM sodium phosphate buffer (pH 7.0) plus 50 μM $ZnCl_2$ and 1 mM $MgCl_2$, sonicated, cleared by ultracentrifugation, and treated with DNase. The extract was then dialyzed against 50 mM Bis-Tris buffer (pH 6.5) plus 50 μM $ZnCl_2$ and 1 mM $MgCl_2$ and loaded onto a preequilibrated Q-Sepharose column. The β-lactamase-containing fractions were eluted with a linear NaCl gradient (0 to 1 M). The same procedure was repeated using a 30 mM cacodylate buffer (pH 6.5) plus 50 μM $ZnCl_2$ and 1 mM $MgCl_2$. Finally, fractions containing the highest β-lactamase activities were pooled and subsequently dialyzed overnight against 50 mM HEPES buffer (pH 7.5) including 50 μM $ZnCl_2$ and 1 mM $MgCl_2$. The β-lactamase activity was determined qualitatively using nitrocefin hydrolysis (Oxoid, Dardilly, France). The protein content was measured using the Bio-Rad DC protein assay. The purification factor was measured by comparing the activities of the VIM-19 crude extract and purified enzyme using 100 μM imipenem as the substrate.

Kinetic studies. Kinetic measurements (k_{cat} and K_m) of purified β-lactamase VIM-19 were performed spectrophotometrically as described previously (24).

Plasmid content and conjugation assays. Plasmid DNAs of *E. coli* DIH-1 and *K. pneumoniae* DIH-2 were extracted by using the Kieser method (16). *E. coli* NCTC50192 harboring four plasmids of 154, 66, 48, and 7 kb was used as the size marker for plasmids. Plasmid DNAs were analyzed by agarose gel electrophoresis as described previously (16). Direct transfer of the β-lactam resistance markers into *E. coli* J53 was attempted by liquid mating-out assays at 37°C. Selection was performed on agar plates supplemented with amoxicillin (50 μg/ml) and azide (100 μg/ml).

Nucleotide sequence accession number. The nucleotide sequence reported in this work has been deposited in the GenBank nucleotide database under accession no. FJ822963.

RESULTS

Characteristics of *E. coli* and *K. pneumoniae* isolates. This study was initiated by the isolation of carbapenem-resistant *E. coli* and *K. pneumoniae* isolates in January 2008 in our hospital. They had been recovered from rectal swabs obtained at the hospital entrance (systematic screening for multidrug-resistant bacteria) from a 30-year-old patient transferred from Algiers, Algeria, where he had been hospitalized 10 days after injuries caused by a terrorist attack in December 2007.

Isolates *E. coli* DIH-1 and *K. pneumoniae* DIH-2 were resistant to most β-lactams, including imipenem and ertapenem (Table 1). These isolates were also resistant to all aminoglycosides and to chloramphenicol, tetracycline, trimethoprim, and sulfonamides; they were susceptible only to fluoroquinolones and colistin (data not shown). MBL detection tests were positive for both isolates, and PCR screening for MBL-encoding genes identified a novel bla_{VIM} type in both isolates that was defined as bla_{VIM-19} (http://www.lahey.org/Studies/). Synergy tests performed with clavulanic acid and cefepime showed the production of an ESBL only in *K. pneumoniae* DIH-2. PCR for ESBL genes followed by sequencing identified the $bla_{CTX-M-3}$ gene in *K. pneumoniae* DIH-2.

Genetic support of bla_{VIM-19}. Analysis of the genetic environment of bla_{VIM-19} in isolates DIH-1 and DIH-2 showed that it was part of a gene cassette located at the first position of a class 1 integron. However, PCR combinations failed to identify the 3′ extremity (either 3′ CS or Tn*402*-like backbone) of the class 1 integron and consequently possible additional gene cassettes. Conjugation experiments produced *E. coli* transconjugants exhibiting an MBL phenotype using both *E. coli* DIH-1 and *K. pneumoniae* DIH-2 as donors, containing a single 160-kb plasmid (named pR3) harboring the bla_{VIM-19} gene. Both transconjugants additionally expressed resistance to kanamycin, tobramycin, gentamicin, chloramphenicol, trimethoprim, tetracycline, and sulfonamides. This result suggested strongly that the bla_{VIM-19}-positive plasmids identified in *E. coli* DIH-1 and *K. pneumoniae* DIH-2 were identical. In addition, an *E. coli* transconjugant expressing VIM-19 in addition to an ESBL (evidenced through a slight synergy between clavulanate and aztreonam) was obtained only with *K. pneumoniae* DIH-2 as the donor strain, in which a 70-kb plasmid was identified that harbored the $bla_{CTX-M-3}$ gene. No transconjugant expressing the ESBL CTX-M-3 only was obtained, suggesting that the

FIG. 1. Comparison of the amino acid sequences of VIM-19, VIM-1, VIM-4, VIM-1 (N215K), and VIM-2. Dashes indicate conserved residues. The differences found between VIM-1 and VIM-19 are boxed. Residues in boldface type belong to the His and Cys active sites of MBLs. The numbering is according to the updated BBL scheme (10). The vertical arrow indicates the signal peptide cleavage site.

plasmid harboring $bla_{CTX-M-3}$ might be mobilizable by that carrying the bla_{VIM-19} gene.

Characterization of the VIM-19 MBL. β-Lactamase VIM-19 differed from VIM-4 by a single amino acid substitution (Asn215Lys) and from VIM-1 by two substitutions (Asn215Lys and Ser228Arg) (Fig. 1). As expected, expression of the bla_{VIM-1}, bla_{VIM-4}, and bla_{VIM-19} genes in *E. coli* TOP10 conferred resistance or reduced susceptibility to all β-lactams except to aztreonam (Table 1). However, the MICs of imipenem, meropenem, and ertapenem were higher for VIM-19 than those for VIM-1 and VIM-4 once cloned in *E. coli*, suggesting the involvement of residues Lys215 and Arg228 in higher carbapenemase activity. In contrast, the MICs of ceftazidime and cefepime for *E. coli* carrying pVIM-19 [*E. coli* (pVIM-4)] were lower than those obtained for *E. coli* (pVIM-1), but similar to those for *E. coli*(pVIM-4) (Table 1). Those results strengthened the role of the Ser228 residue in higher hydrolysis of expanded-spectrum cephalosporins. The resistance pattern of the *E. coli* recombinant strain expressing the Asn215Lys-mutated VIM-1 was similar to the pattern of a strain expressing VIM-1, thus ruling out the possibility that Lys215 alone could play a role in the higher carbapenemase activity of VIM-19 (Table 1).

IEF analysis identified a pI of 5.2 for β-lactamase VIM-19 that was purified to near homogeneity (>95%) as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis (data not shown), and the purification factor was estimated to be 40-fold. β-Lactamase VIM-19 hydrolyzed all tested β-lactams except aztreonam. Kinetic data showed that VIM-19 hydrolyzed imipenem and meropenem at higher levels than VIM-1 did (6- and 7-fold, respectively), although cefepime was less hydrolyzed (Table 2). VIM-19 hydrolyzed imipenem more efficiently than it hydrolyzed meropenem, as observed for VIM-1 (Table 2). Those data are in accordance with the resistance phenotype observed for the *E. coli* recombinant strains. Higher catalytic efficiencies were also

TABLE 2. Kinetic parameters of VIM-19 and VIM-1 enzymes

β-Lactam	Kinetic parameter of enzyme						k_{cat}/K_m ($\mu M^{-1}s^{-1}$) ratio for VIM-19/VIM-1
	VIM-19[a]			VIM-1[b]			
	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\mu M^{-1}s^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\mu M^{-1}s^{-1}$)	
Benzylpenicillin	300	1,300	5	850	30	0.05	100
Piperacillin	250	860	4	3,500	1,860	0.5	8
Cefoxitin	60	30	0.5	130	25	0.2	2.5
Cefotaxime	30	900	30	250	170	0.7	30
Ceftazidime	>1,000	>20	0.02	800	60	0.1	
Cefepime	350	100	0.5	145	550	4	0.12
Aztreonam	ND[c]	ND	ND	>1,000	<0.01	<0.0001	
Imipenem	40	250	6	1.5	2	1	6
Meropenem	15	25	2	50	15	0.3	7
Ertapenem	200	15	0.1				

[a] Data are the means of three independent experiments. Standard deviations were within 15% of the means.
[b] VIM-1 values were reported by Franceschini et al. (8).
[c] ND, no detectable hydrolysis (<0.01 s^{-1}) for a maximum amount of 5 μg of purified enzyme and up to 200 nmol of substrate.

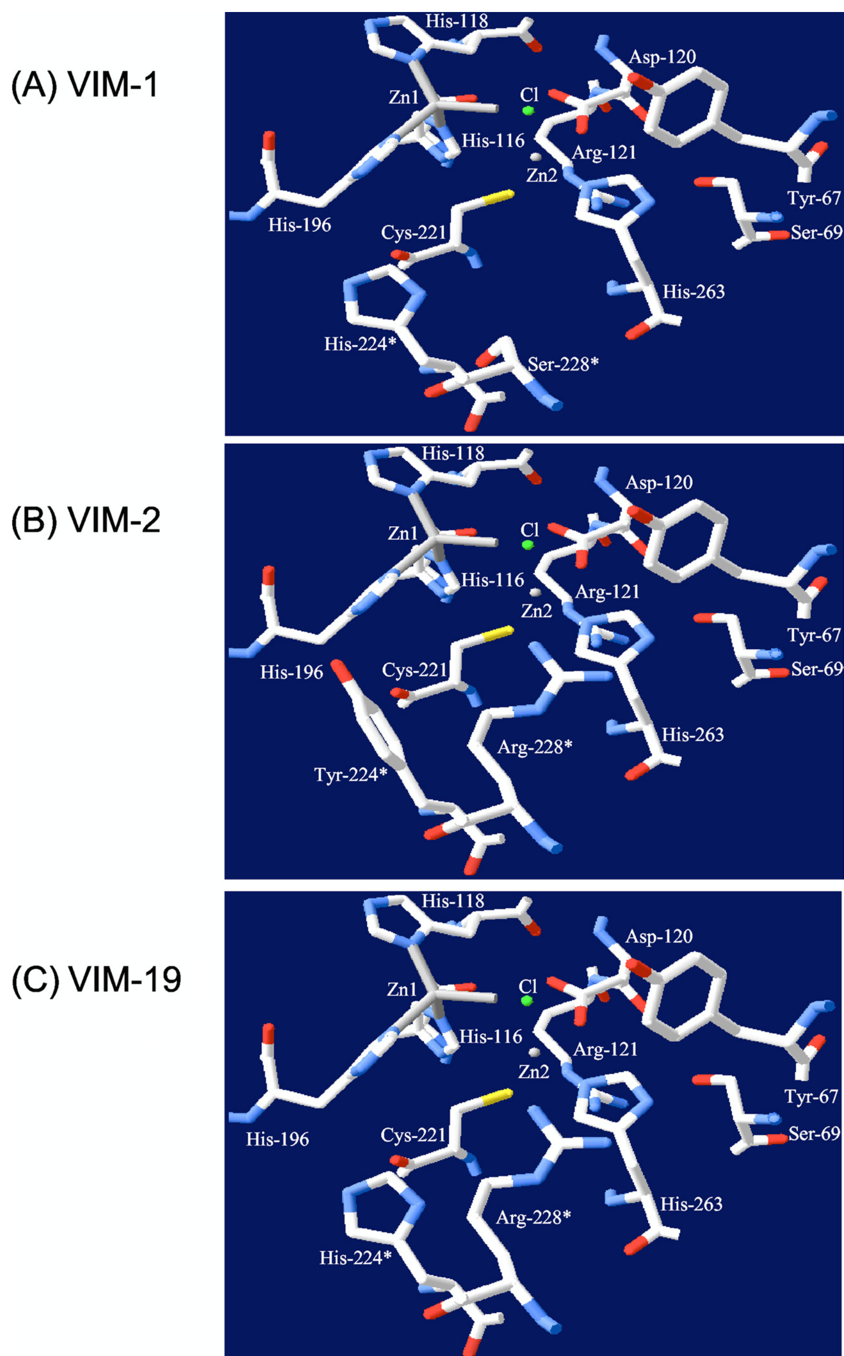

FIG. 2. Comparative lateral view of the active site (His and Cys sites) of VIM-1 (A), VIM-2 (B) (Protein Data Bank entry 1ko3) (11), and VIM-19 (C). The asterisks indicate the positions of the two residues which differ between VIM-1 (His224 and Ser228), VIM-2 (Tyr224 and Arg228), and VIM-19 (His224 and Arg228). The representation has been determined using the software Swiss-Pdb Viewer available at www.expasy.org/spdbv/ (12, 19, 20). The colors of the atoms are standard for the program and are as follows: white, carbon; red, oxygen; blue, nitrogen; yellow, sulfur; gray, zinc ion; green sphere, chloride ion. Residues are numbered according to the BBL standard numbering scheme (9).

observed for VIM-19 compared to VIM-1 with benzylpenicillin, piperacillin, and cefotaxime (100-, 8-, and 30-fold, respectively).

DISCUSSION

We identified here a novel MBL, VIM-19, that had a higher ability to hydrolyze carbapenems associated with a lower ability to hydrolyze several expanded-spectrum cephalosporins. Differences in the hydrolysis parameters of β-lactams had been already noticed for some distantly related VIM enzymes, especially between VIM-1 and VIM-2, but interestingly here the peculiar properties of VIM-19 are based on only two substitutions.

The Lys215 residue in VIM-19 is unique compared to all

VIM-type amino acid sequences and is located outside the active site. We showed here that this specific substitution alone was not responsible for the higher carbapenemase activity observed with VIM-19, even if a slight increased carbapenemase activity was noticed. However, the association of amino acids Lys215 together with Arg228 significantly increased the activity of VIM-19 against carbapenems compared to VIM-1 and VIM-4. That observation might be paralleled with that made for VIM-11 by Marchiaro et al. (18), indicating that amino acid substitutions located outside the active site may modulate the hydrolytic properties of the enzyme.

The Arg228 residue is the second residue leading to a higher carbapenemase activity of VIM-19. It has been shown that this same residue, which is present in the VIM-2 sequence, defined a precise positively charged space for substrate binding by forming with its side chain a kind of "wall" that encloses one side of the active site (11). The presence at position 228 of a Ser residue instead of an Arg residue may eliminate this physical restraint, consequently opening that area (11). This might explain the lower affinity for imipenem (higher K_m values) of VIM-19 and VIM-2 compared to VIM-1, but the paradoxical better affinity of VIM-19 for meropenem compared to VIM-1 remains unexplained (Table 2 and Fig. 1). Note that our results also showed that the Arg228 residue in VIM-19 is responsible for a lower hydrolytic activity toward ceftazidime and cefepime.

The VIM-1 and VIM-2 enzymes have been shown to possess two distinct three-dimensional (3D) structures, and their primary structures diverge significantly with 25 different amino acids (11). Those two enzymes exhibit differences in their respective His and Cys active sites (His224 and Ser228 for VIM-1 and Tyr224 and Arg228 for VIM-2). The corresponding residues in VIM-19 consist of His224 and Arg228, therefore being an hybrid active site between VIM-1 and VIM-2, which is also shared with VIM-4 and VIM-7 (27, 31). Considering previous kinetic studies comparing VIM-2 and VIM-1 (6, 8, 11, 24), it has been shown that the affinities of both enzymes for penicillins are different, but also that their affinities for carbapenems may differ, with VIM-1 hydrolyzing imipenem, cefepime, and cefpirome better than VIM-2, and conversely VIM-2 hydrolyzing meropenem more efficiently than VIM-1 (32). The data obtained for VIM-19 compared to VIM-1 are in accordance with those of previous observations, considering that the active site of VIM-19 resembles the active site of VIM-2 more than that of VIM-1 (Fig. 2).

This study identified MBL producers for the first time in Algeria, thus indicating a likely dissemination in North Africa, whereas VIM-2 and VIM-4 have been reported in Tunisia (13, 17). Its plasmid and integron location may be the source of its spread as exemplified by its identification in two different enterobacterial species. The identification of VIM-19 constitutes the first example of an evolution of a VIM-type enzyme toward increased hydrolytic activity, which results from just a single amino acid substitution. A similar observation had been made among the IMP-type MBL family, with the IMP-6 variant differing from IMP-1 by a Ser196Gly substitution (thus another location inside the protein structure) and consequently possessing the ability to hydrolyze meropenem more efficiently (33).

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REFERENCES

1. **Bellais, S., D. Aubert, T. Naas, and P. Nordmann.** 2000. Molecular and biochemical heterogeneity of class B carbapenem-hydrolyzing β-lactamases in *Chryseobacterium meningosepticum*. Antimicrob. Agents Chemother. **44:** 1878–1886.
2. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial susceptibility testing. 18th informational supplement. Clinical and Laboratory Standards Institute, Wayne, Pa.
3. **Cornaglia, G., M. Akova, G. Amicosante, R. Canton, R. Cauda, J.-D. Docquier, M. Edelstein, J.-M. Frère, M. Fuzi, M. Galleni, H. Giamarellou, M. Gniadkowski, R. Koncan, B. Libisch, F. Luzzaro, V. Miriagou, F. Navarro, P. Nordmann, L. Pagani, L. Peixe, L. Poirel, M. Souli, E. Tacconelli, A. Vatopoulos, and G. M. Rossolini.** 2007. Metallo-β-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. Int. J. Antimicrob. Agents **29:**380–388.
4. **Corvec, S., L. Poirel, J.-W. Decousser, P.-Y. Allouch, H. Drugeon, and P. Nordmann.** 2006. Emergence of carbapenem-hydrolysing metallo-β-lactamase VIM-1 in *Pseudomonas aeruginosa* isolates in France. Clin. Microbiol. Infect. **12:**941–942.
5. **Crowder, M. W., J. Spencer, and A. J. Vila.** 2006. Metallo-β-lactamases: novel weaponry for antibiotic resistance in bacteria. Acc. Chem. Res. **39:** 721–728.
6. **Docquier, J.-D., J. Lamotte-Brasseur, M. Galleni, G. Amicosante, J.-M. Frère, and G. M. Rossolini.** 2003. On functional and structural heterogeneity of VIM-type metallo-β-lactamases. J. Antimicrob. Chemother. **51:**257–266.
7. **Figueiredo, S., L. Poirel, A. Papa, V. Koulourida, and P. Nordmann.** 2008. First identification of VIM-4 metallo-beta-lactamase in *Acinetobacter* spp. Clin. Microbiol. Infect. **14:**289–290.
8. **Franceschini, N., B. Caravelli, J.-D. Docquier, M. Galleni, J.-M. Frère, G. Amicosante, and G. M. Rossolini.** 2000. Purification and biochemical characterization of the VIM-1 metallo-β-lactamase. Antimicrob. Agents Chemother. **44:**3003–3007.
9. **Galleni, M., J. Lamotte-Brasseur, G. M. Rossolini, J. Spencer, O. Dideberg, and J.-M. Frère.** 2001. Standard numbering scheme for class B β-lactamases. Antimicrob. Agents Chemother. **45:**660–663.
10. **Garau, G., I. García-Sáez, C. Bebrone, C. Anne, P. Mercuri, M. Galleni, J.-M. Frère, and O. Dideberg.** 2004. Update of the standard numbering scheme for class B β-lactamases. Antimicrob. Agents Chemother. **48:**2347–2349.
11. **Garcia-Saez, I., J.-D. Docquier, G. M. Rossolini, and O. Dideberg.** 2008. The three-dimensional structure of VIM-2, a Zn-β-lactamase from *Pseudomonas aeruginosa* in its reduced and oxidised form. J. Mol. Biol. **375:**604–611.
12. **Guex, N., and M. C. Peitsch.** 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis **18:**2714–2723.
13. **Ktari, S., G. Arlet, B. Mnif, V. Gautier, F. Mahjoubi, J. M. Ben, M. Bouaziz, and A. Hammami.** 2006. Emergence of multidrug-resistant *Klebsiella pneumoniae* isolates producing VIM-4 metallo-β-lactamase, CTX-M-15 extended-spectrum β-lactamase, and CMY-4 AmpC β-lactamase in a Tunisian university hospital. Antimicrob. Agents Chemother. **50:**4198–4201.
14. **Lee, K., J. H. Yum, D. Yong, H. M. Lee, H. D. Kim, J.-D. Docquier, G. M. Rossolini, and Y. Chong.** 2005. Novel acquired metallo-β-lactamase gene, bla_{SIM-1}, in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. Antimicrob. Agents Chemother. **49:**4485–4491.
15. **Mammeri, H., L. Poirel, and P. Nordmann.** 2003. In vivo selection of a chromosomally encoded β-lactamase variant conferring ceftazidime resistance in *Klebsiella oxytoca*. Antimicrob. Agents Chemother. **47:**3739–3742.
16. **Mammeri, H., M. Van de Loo, L. Poirel, L. Martinez-Martinez, and P. Nordmann.** 2005. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrob. Agents Chemother. **49:**71–76.
17. **Mansour, W., L. Poirel, D. Bettaieb, O. Bouallegue, N. Boujaafar, and P. Nordmann.** 2009. Metallo-β-lactamase-producing *Pseudomonas aeruginosa* isolates in Tunisia. Diagn. Microbiol. Infect. Dis. **64:**458–461.
18. **Marchiaro, P., P. E. Tomatis, M. A. Mussi, F. Pasteran, A. M. Viale, A. S. Limansky, and A. J. Vila.** 2008. Biochemical characterization of metallo-β-lactamase VIM-11 from a *Pseudomonas aeruginosa* clinical strain. Antimicrob. Agents Chemother. **52:**2250–2252.
19. **Peitsch, M. C.** 1996. ProMod and Swiss-Model: Internet-based tools for

automated comparative protein modelling. Biochem. Soc. Trans. **24:**274–279.

20. **Peitsch, M. C., T. N. Wells, D. R. Stampf, and J. L. Sussman.** 1995. The Swiss-3DImage collection and PDB-Browser on the World-Wide Web. Trends Biochem. Sci. **20:**82–84.
21. **Philippon, L. N., T. Naas, A. T. Bouthors, V. Barakett, and P. Nordmann.** 1997. OXA-18, a class D clavulanic acid-inhibited extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **41:** 2188–2195.
22. **Pitout, J. D., G. Revathi, B. L. Chow, B. Kabera, S. Kariuki, P. Nordmann, and L. Poirel.** 2008. Metallo-β-lactamase-producing *Pseudomonas aeruginosa* isolated from a large tertiary centre in Kenya. Clin. Microbiol. Infect. **14:**755–759.
23. **Poirel, L., A. Carrër, J. D. Pitout, and P. Nordmann.** 2009. Integron mobilization unit as a source of mobility of antibiotic resistance genes. Antimicrob. Agents Chemother. **53:**2492–2498.
24. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J.-D. Cavallo, and P. Nordmann.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.
25. **Poirel, L., J. D. Pitout, and P. Nordmann.** 2007. Carbapenemases: molecular diversity and clinical consequences. Future Microbiol. **2:**501–512.
26. **Poirel, L., G. F. Weldhagen, T. Naas, C. De Champs, M. G. Dove, and P. Nordmann.** 2001. GES-2, a class A β-lactamase from *Pseudomonas aeruginosa* with increased hydrolysis of imipenem. Antimicrob. Agents Chemother. **45:**2598–2603.
27. **Pournaras, S., A. Tsakris, M. Maniati, L. S. Tzouvelekis, and A. N. Maniatis.** 2002. Novel variant (bla_{VIM-4}) of the metallo-β-lactamase gene bla_{VIM-1} in a clinical strain of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **46:**4026–4028.
28. **Rossolini, G. M., E. Mantengoli, J. D. Docquier, R. A. Musmanno, and G. Coratza.** 2007. Epidemiology of infections caused by multiresistant gram-negatives: ESBLs, MBLs, panresistant strains. New Microbiol. **30:**332–339.
29. **Sekiguchi, J., K. Morita, T. Kitao, N. Watanabe, M. Okazaki, T. Miyoshi-Akiyama, M. Kanamori, and T. Kirikae.** 2008. KHM-1, a novel plasmid-mediated metallo-β-lactamase from a *Citrobacter freundii* clinical isolate. Antimicrob. Agents Chemother. **52:**4194–4197.
30. **Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. IS*CR* elements: novel gene-capturing systems of the 21st century? Microbiol. Mol. Biol. Rev. **70:** 296–316.
31. **Toleman, M. A., K. Rolston, R. N. Jones, and T. R. Walsh.** 2004. bla_{VIM-7}, an evolutionarily distinct metallo-β-lactamase gene in a *Pseudomonas aeruginosa* isolate from the United States. Antimicrob. Agents Chemother. **48:** 329–332.
32. **Walsh, T. R., M. A. Toleman, L. Poirel, and P. Nordmann.** 2005. Metallo-β-lactamases: the quiet before the storm? Clin. Microbiol. Rev. **18:**306–325.
33. **Yano, H., A. Kuga, R. Okamoto, H. Kitasato, T. Kobayashi, and M. Inoue.** 2001. Plasmid-encoded metallo-β-lactamase (IMP-6) conferring resistance to carbapenems, especially meropenem. Antimicrob. Agents Chemother. **45:**1343–1348.
34. **Yong, D., M. A. Toleman, C. G. Giske, H. S. Cho, K. Sundman, K. Lee, and T. R. Walsh.** 21 September 2009. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob. Agents Chemother. doi:10.1128/AAC.00774-09.