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Novel VIM Metallo-β-Lactamase Variant, VIM-24, from a *Klebsiella pneumoniae* Isolate from Colombia▽

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We report the emergence of a novel VIM variant (VIM-24) in a *Klebsiella pneumoniae* isolate in Colombia. The isolate displays MICs for carbapenems below the resistance breakpoints, posing a real challenge for its detection. The bla_{VIM-24} gene was located within a class 1 integron carried on a large plasmid. Further studies are needed to clarify its epidemiological and clinical impact.

The emergence of acquired metallo-β-lactamases (MBLs) among Gram-negative strains is a matter of significant concern worldwide, due to their successful dissemination capacity, increasing diversity, and variability of resistance phenotypes (5, 19, 24). Detection of MBL-producing strains is challenging (5), especially in *Enterobacteriaceae*, as some strains display MICs below the resistance breakpoint established by the Clinical and Laboratory Standards Institute (CLSI) (5, 22). In addition, discrepancies in susceptibility testing of carbapenems with MBL producers have been reported (9).

To the best of our knowledge, in Latin America, VIM-producing *Enterobacteriaceae* have been limited to Mexico (18) and Venezuela (15), and no IMP enzymes have been reported within this family. In Colombia, the first report of a VIM was in a carbapenem-resistant *Pseudomonas aeruginosa* strain harboring VIM-8 found in a single city (6), followed by several disseminated carbapenem-resistant *P. aeruginosa* strains producing VIM-2 (23). We now describe a novel VIM in a *Klebsiella pneumoniae* isolate, which confers a minimal increase in the MICs to selected β-lactams and exhibits variable MICs to imipenem when tested by different methods, posing a real challenge for its detection.

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K. pneumoniae 5639 was isolated from a blood culture of a 4-month-old Colombian female hospitalized in the intensive care unit (ICU) of a hospital located in Barranquilla, Colombia. The patient had an *Escherichia coli* intra-abdominal infection secondary to intestinal obstruction, requiring treatment with piperacillin-tazobactam followed by cefepime plus clindamycin. After a complicated clinical course, the abscess was surgically drained, and she was successfully treated with meropenem, which was given when blood cultures reported a *K. pneumoniae* (isolate 5639) susceptible (MIC of ≤1 μg/ml) to this antibiotic.

Isolate 5639 was sent to the International Center for Medical Research and Training (CIDEIM) as part of a carbapenemase surveillance study, where identification was confirmed by using the Vitek 2 test (bioMérieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was performed by the broth microdilution (BMD) method (Sensititre panels; TREK Diagnostic Systems, Westlake, OH) and Vitek 2, and the results were interpreted according to the CLSI guidelines (3, 4). Tigecycline breakpoints for *Enterobacteriaceae* were defined based on the U.S. Food and Drug Administration guidelines (susceptible, MIC of ≤2 μg/ml), and polymyxin B breakpoints were based on the CLSI cutoff for *Acinetobacter* spp. (resistant, MIC of ≥4 μg/ml), as previously recommended (7).

The strain was tested in triplicate with a standardized inoculum, as discrepancies in cefepime MICs between the referral hospital and CIDEIM, as well as decreased susceptibility to imipenem (MIC of 2 μg/ml), were observed. Results from susceptibility testing for selected antibiotics are summarized in Table 1. Imipenem was additionally tested by Etest, obtaining variable MICs of 1 to 2 μg/ml. Further testing was performed, including characterization of β-lactamases by isoelectric focusing (IEF) as described by Matthew et al. (16), and three-dimensional (3D) testing as previously reported (21). IEF revealed the presence of a single β-lactamase with a pI of 7.9, and a positive 3D test indicated the presence of a carbapenemase. Screening by PCR of β-lactamases bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{KPC}, bla_{IMP}, and bla_{VIM} revealed the presence of a bla_{VIM} gene, while the other resistance determinants were not detected, consistent with the IEF results. Even though there is a debate about the possible universality of SHV β-lactamase in *K. pneumoniae* (1, 10), our results suggested the absence of the SHV β-lactamase gene in the *K. pneumoniae* 5639 isolate.

Sequencing revealed a novel bla_{VIM} gene, designated bla_{VIM-24} (nucleotide sequence accession no. HM855205). This novel gene differed from that coding for VIM-2, the closest related enzyme, and the previously reported variants by a sub-

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TABLE 1. MICs of selected antibiotics for *K. pneumoniae* strain 5639 and *E. coli* strain J53 and its transconjugants

Strain or TC	Method	MIC or MIC range (μg/ml) of[a]:											
		ETP	IPM	MEM	FEP	CAZ	CTX	TZP	SAM	AMK	TGC	PMB	CIP
K. pneumoniae 5639	BMD	0.25	0.25–2.0	≥0.12	≤1	4	4	16/4	32/16	32	0.5–1	0.5–1	2
	Vitek 2	≤0.5	≤1.0	≤0.25	≤1	4	ND[b]	ND	≥32	≥64	1	ND	1
E. coli J53	BMD	≤0.12	≤0.12	≤0.12	≤1	≤2	≤1	≤8/4	ND	≤8	≤0.12	2	≤0.5
	Vitek 2	≤0.5	≤1.0	≤0.25	≤1	≤1	ND	ND	4	≤1	≤0.5	ND	≤0.25
E. coli J53 VIM-24 TC[c]	BMD	0.25–0.5	0.5	≤0.12	2–4	16–32	8	16/4–32/4	ND	≤8	≤0.12	1–2	≤0.5
	Vitek 2	≤0.5	2–4	≤0.25	≤1	8–16	ND	ND	≥32	8–16	≤0.5	ND	0.5

[a] ETP, ertapenem; IPM, imipenem; MEM, meropenem; FEP, cefepime; CAZ, ceftazidime; CTX, cefotaxime; TZP, piperacillin-tazobactam; SAM, ampicillin-sulbactam; AMK, amikacin; TGC, tigecycline; PMB, polymixin B; and CIP, ciprofloxaxin.
[b] ND, not determined.
[c] Shown are the MIC ranges for four transconjugants.

stitution at position 614 from G to T, changing the amino acid from Arg to Leu (Arg205→Leu). Detection and mapping of the class 1 integron were carried out by using specific primers for the 5′ and 3′ conserved segments (13, 17), as well as primers targeting the VIM gene cassette. The analysis revealed that bla_{VIM-24} was the first gene cassette of a class 1 integron, followed by *aacA7*, *catB3*, and *arr-3* gene cassettes. The promoter region analysis indicated that these gene cassettes were preceded by a strong P1 promoter and the inactive form of P2.

S1 nuclease coupled with pulsed-field gel electrophoresis (PFGE) was used for the detection and size estimation of plasmids (2). This methodology revealed the presence of five plasmids ranging in size between 46 and 172 kb (Fig. 1A). The bla_{VIM}-specific probe hybridized with the plasmid band of approximately 172 kb (Fig. 1B), indicating that the gene was carried on a large plasmid, as previously reported (11, 20). The extrachromosomal location of bla_{VIM-24} was confirmed by digesting *K. pneumoniae* 5639 DNA with I-Ceu-I endonuclease (14), followed by PFGE (Fig. 1C) and hybridization with probes for 16S rRNA genes (Fig. 1D) and bla_{VIM} (Fig. 1E). When comparing 16S rRNA gene and bla_{VIM} hybridization results, the bla_{VIM} gene was localized in a position different from that of the chromosomal fragments. Mating experiments using *Escherichia coli* J53 as the recipient strain and amikacin as the selection marker (8 μg/ml) confirmed that bla_{VIM-24} was encoded in a conjugative plasmid. Transconjugants (TC) were PCR positive for the bla_{VIM} gene and display a MIC for imipenem of between 2 and 4 μg/ml when tested by Vitek 2 and 0.5 μg/ml when tested by BMD (Table 1).

The production of metallo-β-lactamases (MBL) is a growing health problem worldwide (5, 22) that poses challenges for the treatment of infections due to Gram-negative bacteria. The increase in diversity, as evidenced by the growing number of new variants of the already known MBLs, as well as the recent detection of the New Delhi MBL (12), is particularly worrisome. We now report the emergence of a VIM-producing *K. pneumoniae* strain in Colombia, which caused the first documented case of an infection with MBL-producing *Enterobacteriaceae* in the country. Surveillance for similar strains is warranted considering the known association of bla_{VIM} with mobile genetic elements and the fact that *Klebsiella pneumoniae* carbapenemase (KPC) is already disseminated in Colombia, which could lead to the emergence of *K. pneumoniae* isolates coproducing KPC and VIM, as has been reported in Greece (8).

Although VIM-producing *Enterobacteriaceae* isolates were initially recognized by their *in vitro* resistance to carbapenems (22), this strain displays MICs for carbapenems below the current resistant breakpoints (ertapenem, ≥1 μg/ml; imipenem, ≥4 μg/ml; and meropenem, ≥4 μg/ml) (4). This represents a real challenge for its detection and hinders the esti-

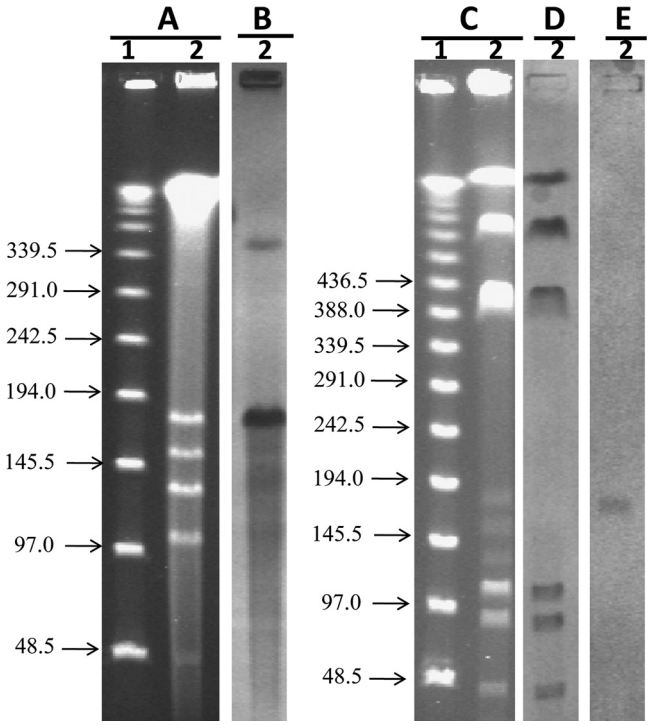

FIG. 1. Localization of the bla_{VIM-24} gene in *K. pneumoniae* isolate 5639. Shown are the results of S1 nuclease and I-Ceu-I digestions, pulsed-field gel electrophoresis, and hybridizations. (A and B) S1 restriction and PFGE of total DNA (A) and hybridization with a probe specific to bla_{VIM} (B). (C to E) I-Ceu-I fragment restriction pattern of total DNA after PFGE (C) and hybridization with a probe specific to 16S rRNA genes (D) and bla_{VIM} (E). Lane 1, lambda ladder (molecular sizes in kilobases are shown to the left); lane 2, *K. pneumoniae* isolate 5639.

mation of its real incidence. Because MICs may be unreliable in detecting MBLs, special tests (such as the 3D test) could be helpful in detecting the resistance mechanisms involved. It seems prudent that infection control procedures should apply to all proven MBL-producing isolates regardless of their actual level of susceptibility.

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