Complex Clonal and Plasmid Epidemiology in the First Outbreak of Enterobacteriaceae Infection Involving VIM-1 Metallo-β-Lactamase in Spain: Toward Endemicity?

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(See the editorial commentary by Paterson and Doi on pages 1179–81)

Background. We report the emergence and spread of metallo-β-lactamases (MBLs) among enterobacterial isolates at Ramón y Cajal University Hospital (Madrid, Spain).

Methods and results. During the period from March 2005 through September 2006, 25 patients (52% of whom were in the intensive care unit) were infected and/or colonized with single or different MBL-producing Enterobacteriaceae isolates (Klebsiella pneumoniae, 14 patients; Enterobacter cloacae, 12 patients; Escherichia coli, 1 patient; and/or Klebsiella oxytoca, 1 patient). Clonal analysis (XbaI pulsed-field gel electrophoresis) revealed that all K. pneumoniae isolates belonged to the same clone, but 6 patterns were found among the E. cloacae isolates. Carbenpens were affected to different degrees (minimum inhibitory concentration, =1 to >8 μg/mL), as were aminoglycosides and ciprofloxacin. The blaVIM-1 MBL gene was present in all isolates; in addition, the blaSHV-12 extended-spectrum β-lactamase gene was detected in K. pneumoniae and E. coli isolates. The blaVIM-1 gene was detected within a 4.0-kb class 1 integron (blaVIM-1–aacA4–dfrII–aadA1–catB2) in K. pneumoniae and E. coli and in a 2.5-kb class 1 integron (blaVIM-1–aacA4–aadA1) in E. cloacae and K. oxytoca isolates. The blaVIM-1 gene was transferable (filter-mating) in 14 of 14 K. pneumoniae isolates, 4 of 11 E. cloacae isolates, and 1 of 1 E. coli isolate. A 60-kb plasmid belonging to the IncI1 group was detected in the epidemic VIM-1-K. pneumoniae clone. Plasmids of 300- or 435-kb belonging to IncH12 group were found among E. cloacae isolates.

Conclusions. K. pneumoniae-MBL monoclonal epidemics coexisted with E. cloacae-MBL multiclonal epidemics in our hospital. The spread of the blaVIM-1 gene among Enterobacteriaceae was driven by clonal spread associated with intergeneric plasmid transfer with different class I integron platforms. Such complex epidemiology might anticipate endemicity and should be considered for the design of containment epidemiology strategies.

Carbapenems play an important role in the treatment of nosocomial infections caused by multidrug-resistant gram-negative bacteria. They are very stable to hydrolysis by most β-lactamases, including extended-spectrum β-lactamases (ESBLs) [1]. However, the emergence of carbapenemases, such as acquired metallo-β-lactamases (MBLs), and other β-lactamases affecting carbapenems is becoming a therapeutic challenge [2]. MBLs have a broad substrate profile that includes carbapenems. These enzymes confer high-level resistance to most other β-lactams, with the exception of aztreonam, and they are not inhibited by class A β-lactamase inhibitors [3, 4].

MBLs have been categorized in 2 major groups: IMP- and VIM-type enzymes. However, other groups, such as SPM, GIM, and SIM, have also been reported [4]. Genes encoding these MBLs can be plasmid-mediated or chromosomally mediated and are usually located within integrons [3]. Although IMP and VIM derivate have been described worldwide, IMP-type MBLs are predominant in Southeast Asia, whereas VIM-type
MBLs are more common in Europe [4]. The first VIM-type enzyme, VIM-1, was detected in a Pseudomonas aeruginosa isolate recovered in 1997 in Verona, Italy [5]. VIM enzymes have since been found mainly in nonfermenting gram-negative bacteria but are increasingly being detected in members of the Enterobacteriaceae family [6]. Most VIM-producing Enterobacteriaceae isolates that have been reported are sporadic and clonally unrelated, although clonal epidemics have been also described, and some countries, such as Greece, are close to an endemic situation [7–10].

Acquired MBL-producing enterobacterial isolates are still very uncommon in Spain. Until now, and to the best of our knowledge, only 2 enterobacterial isolates have been reported: an Escherichia coli isolate responsible for a urinary tract infection and a fecal Klebsiella pneumoniae isolate [11]. Our study describes the first outbreak of infection due to multidrug-resistant Enterobacteriaceae carrying blaVIM, in Spain. The outbreak involved 4 different species (K. pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, and E. coli), different plasmids, and different blaVIM. Genetic environments. These results show complex epidemiology of this emerging problem in Europe.

**METHODS**

**Patients and bacterial isolates.** From March 2005 through September 2006, a total of 25 patients were infected and/or colonized with 28 MBL-producing Enterobacteriaceae isolates. The isolates were recovered at Ramon y Cajal University Hospital, a 1200-bed teaching hospital in Madrid, Spain. Twenty-seven isolates (only 1 isolate per species and patient) were recovered from hospitalized patients; the remaining isolate was recovered from an outpatient who had been hospitalized 2 months earlier. Of these 28 isolates, 27 were available for further study. The patients who shared a room with an infected or colonized patient were routinely tested for the presence of MBL-producing isolates in rectal swabs, which were cultured on selective MacConkey agar medium that contained 1 µg of ceftazidime per milliliter. Phenotypic analysis and MBL characterization were also performed on the isolates that grew on these plates. An epidemic K. pneumoniae isolate carrying blaVIM, and blaSHV, that had caused outbreaks of infection in France and Greece was included for clonal comparison [7, 9, 12].

Preliminary species identification and antimicrobial susceptibility testing were performed using the Wider automated system (Francisco Soria Melguizo). MICs of imipenem, meropenem, ertapenem, and aztreonam were also determined by Etest (AB Biodisk). Susceptibility to non-β-lactam antibiotics was assessed by the disk diffusion test, and isolates that were found to have intermediate susceptibility were considered to be resistant. Results were interpreted according to the Clinical and Laboratory Standards Institute guidelines [13]. The presence of MBL and ESBL production was screened by the double-disk synergy test using 10 µL of EDTA 0.5 M versus imipenem and ceftazidime (15 mm apart) or amoxicillin-clavulanic acid versus ceftazidime, cefepime, and aztreonam (25 mm apart), respectively [14, 15].

**Clonal relatedness.** Chromosomal DNA was digested with XbaI and subjected to pulsed-field gel electrophoresis. Clonal relatedness was established by comparison of band patterns [16]. The electrophoresis conditions used were 24 h at 6 V/cm at 14°C, with initial and final pulse times of 10 and 40 s, respectively, with use of a CHEF-DRIII system (Bio-Rad).

**Characterization of β-lactamases.** Preliminary identification was performed by isoelectrofocusing, as described elsewhere [17]. Crude sonic extract from E. coli harboring TEM-1 (pI, 5.4), TEM-4 (pI, 5.9), TEM-3 (pI, 6.3), SHV-2 (pI, 7.6), CTX-M-9 (pI, 8.1), and SHV-12 (pI, 8.2) were used as controls. Molecular characterization of β-lactamases was performed by PCR amplification of blaTEM-1, blaSHV, blaOXA-1, and blaIMP genes using primers and conditions described elsewhere, as well as additional sequencing of the PCR products obtained [3, 17–19].

**Analysis of the genetic environment of MBL genes.** Association of blaVIM with class 1 integrons was established by amplification of the variable region of class 1 integrons. Similarity among integrons was determined by comparison of restriction fragment–length polymorphism patterns corresponding to PCR products digested with AluI. One integron of each restriction fragment–length polymorphism type was sequenced using specific primers: VIM-F (5′-TGG GCC ATT CAG CCA GAT C-3′) [3], VIM-R (5′-ATG GGT TTT GGT CGC ATA TC-3′) [3], VIM-FI (5′-GAT CTT TGT GAA TGG CCC A-3′; present report), 5′CS (5′-GCG ATC AAA CGA GCA AG-3′) [20], 3′CS (5′-AAC CAG ACT TGA CCT GAT-3′) [20], aacA4-F (5′-AGC CAC CCA GTG ATG C-3′), aadA1-F (5′-GCT GGC CGT ACA TTT GTA CG-3′), aadA1-FI (5′-CGT GGC CGT ACA TTT GTA CG-3′), and aadA1-F2 (5′-GCG GAC ATC ACC AAG GTA-3′; present report).

**Transfer of resistance and plasmid content.** Conjugal transfer of blaVIM-1 was screened by filter mating at a 1:10 donor–recipient ratio using E. coli strain BM21 (which is nalidixic acid and rifampin resistant, lactose fermentation positive, and plasmid free) as recipient [21]. Transconjugants were selected on Luria-Bertani agar plates containing ceftazidime (4 µg/mL) and rifampin (300 µg/mL) and were incubated at 37°C for 24 h. Presence of blaVIM in the transconjugants was confirmed by PCR. Plasmid size was determined in E. coli transconjugants (or wild-type strains, in the absence of transfer) by the technique described by Barton et al. [22]. Plasmids were classified according to their incompatibility group using the PCR replicon-typing scheme described by Carattoli et al. [23]. Correspondence of the replicons amplified in the transconjugants with plasmids containing blaVIM was validated by hybridization.
of S1-digested genomic DNA with both bla_{VIM-1} and the incompatibility group replicon probe amplified in each case. Transfer and hybridization were performed using standard procedures [24]. Labeling and detection were performed using ECL kits (Amersham Life Sciences) in accordance with the manufacturer’s instructions.

RESULTS

Patients and bacterial isolates. A total of 25 patients were found to be colonized or infected with MBL-producing isolates during the study period. Ten patients were infected with K. pneumoniae, and 7 were infected with E. cloacae. Two patients were infected with both K. pneumoniae and E. cloacae, and 1 patient was infected with K. pneumoniae and colonized with E. coli. In addition, 3 patients were colonized with E. cloacae, 1 was colonized with K. pneumoniae, and 1 was colonized with K. oxytoca.

Thirteen patients (52%) were located in intensive care units, 7 were located in medical wards (28%), and 4 were located in surgical wards (16%); the remaining patient (4%) was an outpatient who had been hospitalized 2 months earlier in a general surgery ward (table 1). The isolates were recovered mainly from blood (12 of 25 patients) and respiratory secretions (8 of 25 patients) and were less frequently recovered from urine, wound, catheter, and rectal swab specimens. In some cases, isolates were recovered from >1 sample from the same patient (table 1). The overall mortality rate among patients infected or colonized with an MBL-harboring organism was very high (45%), and 67% of this mortality rate was attributable to the presence of the resistant organisms [25].

Antimicrobial susceptibility and detection of MBL and ESBL. Isolates were characterized by reduced and variable susceptibility to carbapenems. Most isolates had imipenem and meropenem MICs within the range of susceptibility, as stated by the Clinical and Laboratory Standards Institute guidelines (i.e., an MIC of $\leq 1$ to $>8 \mu g/mL$) (table 2). Ertaopenem displayed higher MICs. According to Clinical and Laboratory Standards Institute criteria, all MBL-producing isolates had resistance to all penicillin inhibitor combinations and broad-spectrum cephalosporins tested, except for cefepime. As determined on the basis of European Committee on Susceptibility Testing breakpoints (http://www.eucast.org), all isolates were also considered to be resistant to cefepime. All K. pneumoniae and E. coli isolates were resistant to aztreonam, whereas E. cloacae isolates showed variable susceptibility to this compound. Resistance to non-β-lactam antibiotics also varied, although all K. pneumoniae isolates were resistant to gentamicin, tobramycin, and amikacin and demonstrated reduced susceptibility to ciprofloxacin.

The MBL screening test (EDTA double-disk synergy test) yielded positive results for all isolates, whereas the double-disk synergy test to detect ESBLs yielded a positive result only with aztreonam for E. coli and K. pneumoniae isolates.

Clonal relatedness. All MBL-producing K. pneumoniae isolates demonstrated identical XbaI PFGE patterns (KPMBL-A). However, high clonal diversity was observed among E. cloacae isolates (12 isolates corresponding to 6 PFGE types, which were designated ECLMBL 1–6) (figure 1). Moreover, PFGE revealed different restriction patterns between KPMBL-A and the epidemic K. pneumoniae isolate reported in France [9, 12].

Characterization of β-lactamases. Isoelectrofocusing revealed a β-lactamase band with pl value of ~5.2 in all isolates. Both E. coli and K. pneumoniae isolates showed 2 more bands, with pl values of 5.4 and 8.2, and an additional band (pl >8.5) was also seen in E. cloacae isolates; this is consistent with the chromosomal AmpC cephalosporinase. PCR analysis and ampicillin sequencing revealed the presence of the MBL VIM-1 in all 28 MBL-producing isolates and the β-lactamase TEM-1 plus the ESBL SHV-12 in the E. coli and all K. pneumoniae isolates.

Analysis of the genetic environment of bla_{VIM,gene}. Two class 1 integrons containing the bla_{VIM-1} gene were identified and arbitrarily designated types A and B. Type A integron, which contained the gene cassette array bla_{VIM-1}-aacA4-dfrH1-aadA1-catB2 (4.0 kb in size), was identified in K. pneumoniae and E. coli isolates. Type B consisted of bla_{VIM-1}-aadA4-aadA1 genes (2 kb in size) and was recovered from E. cloacae and K. oxytoca isolates.

Plasmid characterization. Resistance to β-lactams was transferable by conjugation from all MBL-producing K. pneumoniae isolates, 4 of 11 E. cloacae isolates, and the sole E. coli isolate, but not from the K. oxytoca isolate. A plasmid band of ~60 kb was detected in all transconjugants obtained from K. pneumoniae and E. coli isolates. It is of note that this E. coli isolate was recovered from a patient who had been colonized with K. pneumoniae 3 months earlier. Plasmid bands of 300 and 435 kb were detected in 3 and 1 transconjugants, respectively, obtained from different E. cloacae isolates. Amplification and further sequencing of regions corresponding to basic replicons revealed that the 60-kb plasmid belonged to IncHI1 group and the 300- and 435-kb plasmids belonged to IncH12 group. Hybridization of S1-digested genomic DNA with bla_{VIM-1} and each replicon probe confirmed these results (figure 2). Plasmid bands of 300 kb were also observed in K. oxytoca isolates and in 6 E. cloacae isolates that did not transfer the resistance phenotype, and a single plasmid band of 235 kb was detected in 1 E. cloacae isolate.

DISCUSSION

Spread of acquired MBLs among Enterobacteriaceae is increasing worldwide [6]. In recent years, several outbreaks caused by IMP- and VIM-producing enterobacterial isolates have been described [4, 7–9, 26, 27]. VIM-1–producing Enterobacteriaceae are now recognized as important public health threats worldwide.
Table 1. Epidemiological data of metallo-\(\beta\)-lactamase–producing enterobacterial isolates.

<table>
<thead>
<tr>
<th>Species, PFGE group</th>
<th>No. of patients</th>
<th>Ward(s) (no. of patients)</th>
<th>Source(s) (no. of patients)</th>
<th>Integron size, kb</th>
<th>Integron type</th>
<th>Plasmid size, kba</th>
<th>Plasmid Inc group</th>
<th>Conjugation frequency</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em>: KPMBL-A</td>
<td>14</td>
<td>Cardiology (2), oncology (1), urology (1), surgical cardiology (2), cardiovascular ICU (5), surgical neurology ICU (2), general surgery ICU (1)</td>
<td>Rectal swabs (8), blood (6), respiratory secretions (6), urine (4), wound (2), catheter (4)</td>
<td>4.0</td>
<td>A</td>
<td>60, 40, 90, 200</td>
<td>IncI1</td>
<td>(10^{-1}) to (10^{-4})</td>
<td>Sm, (Sp), (Gm), Net, Tb, Km, Ak, Su, Tp, (Te, Cm)</td>
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<tr>
<td><em>Escherichia coli</em>: ECMBL-1</td>
<td>1</td>
<td>Urology (1)</td>
<td>Rectal swab (1)</td>
<td>4.0</td>
<td>A</td>
<td>60</td>
<td>IncI1</td>
<td>(10^{-4})</td>
<td>(Sm, Sp), Km, (Su, Tb, Te, Cm)</td>
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<tr>
<td><em>Klebsiella oxytoca</em>: KOMBL-1</td>
<td>1</td>
<td>Pneumology (1)</td>
<td>Respiratory secretions (1)</td>
<td>2.5</td>
<td>B</td>
<td>300</td>
<td>ND</td>
<td>NT</td>
<td>Sm, Sp, Km, Su, Te, Cm</td>
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<td><em>Enterobacter cloacae</em></td>
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<tr>
<td>ECLMBL-0(^b)</td>
<td>1</td>
<td>Surgical neurology ICU (1)</td>
<td>Urine (1), respiratory secretions (1)</td>
<td>...</td>
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<tr>
<td>ECLMBL-1</td>
<td>2</td>
<td>Pediatric cardiology (1), Pediatric cardiology ICU (1)</td>
<td>Blood (2), catheter (1)</td>
<td>2.5</td>
<td>B</td>
<td>300</td>
<td>ND(^c)</td>
<td>NT</td>
<td>Sm, Sp, (Km), Su, (Te, Cm)</td>
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<tr>
<td>ECLMBL-2</td>
<td>2</td>
<td>Surgical neurology ICU (1), outpatient (1)</td>
<td>Urine (2)</td>
<td>2.5</td>
<td>B</td>
<td>235, 290, 300</td>
<td>ND</td>
<td>NT</td>
<td>Sm, (Sp, Tb, Km), Su, Te, Cm</td>
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<tr>
<td>ECLMBL-3</td>
<td>2</td>
<td>Cardiovascular ICU (1), internal medicine (1)</td>
<td>Blood (2)</td>
<td>2.5</td>
<td>B</td>
<td>95, 300, 435</td>
<td>IncH12</td>
<td>(10^{-4}) to (10^{-7})</td>
<td>Sm, (Sp), (Km), Su, (Te, Cm)</td>
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<tr>
<td>ECLMBL-4</td>
<td>1</td>
<td>Gastroenterology (1)</td>
<td>Blood (1)</td>
<td>2.5</td>
<td>B</td>
<td>300</td>
<td>IncH12</td>
<td>(10^{-7})</td>
<td>Sm, Sp, Km, Tb, Su, Te, Cm</td>
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<tr>
<td>ECLMBL-5</td>
<td>1</td>
<td>Hematology (1)</td>
<td>Blood (1)</td>
<td>2.5</td>
<td>B</td>
<td>150, 300</td>
<td>IncH12</td>
<td>(10^{-4})</td>
<td>Sm, Sp, Km, Tb, Su, Te, Cm</td>
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<tr>
<td>ECLMBL-6</td>
<td>3</td>
<td>Medical ICU (1), cardiovascular ICU (2)</td>
<td>Catheter (1), rectal swabs (3)</td>
<td>2.5</td>
<td>B</td>
<td>95, 300</td>
<td>ND</td>
<td>NT</td>
<td>Sm, Sp, Km, (Tb, Su, Cm)</td>
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</table>

**NOTE.** Plasmid sizes and resistance to a given antibiotic of the transconjugants are presented in boldface; parentheses indicate that not all studied isolates showed a resistance phenotype. Nalidixic acid and ciprofloxacin were not tested in the transconjugants. Ak, amikacin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; ICU, intensive care unit; ND, not done; Net, netilmicin; NT, no transfer; Sm, streptomycin; Sp, spectinomycin; Su, sulphonamide; Tb, tetracycline; Te, trimethoprim.

\(^a\) Each number represents the plasmid size expressed in kb of different plasmids.

\(^b\) This isolate was not available for typing and further studies.

\(^c\) Plasmid incompatibility group was studied only in the transconjugants.
aceae were first identified in an *E. coli* isolate recovered from a urine specimen of a patient hospitalized in 2001 in Greece [28]. This enzyme has also been detected in *E. cloacae, K. pneumoniae*, and *Proteus mirabilis* isolates in Greece [7, 8, 10, 27, 29, 30] and has also been reported in *K. pneumoniae* isolates from France and in *K. pneumoniae* and *E. coli* isolates from Spain [9, 11, 12]. However, only VIM–1–producing *E. coli, P. mirabilis*, and mainly *K. pneumoniae* isolates were involved in hospital outbreaks of infection in France in 2001. More recently, an outbreak caused by an epidemic *K. pneumoniae* strain, which was closely related to one of the epidemic isolates from Greece, was detected in France [9]. The present study reports the first clonal outbreak of infection caused by multidrug-resistant Enterobacteriaceae carrying *bla*<sub>VIM-1</sub> in Spain; also, to our knowledge, this is the first hospital outbreak of VIM-producing Enterobacteriaceae infection involving multiple genera, plasmids, and integrons platforms. Our study reveals a complex epidemiology of VIM–1 involving both dissemination of epidemic isolates and spread of different plasmids among 4 different enterobacterial species (*K. pneumoniae, K. oxytoca, E. cloacae*, and *E. coli*) in our hospital, and this may anticipate endemicity.

During an 8-month period, *K. pneumoniae* strains were isolated from 14 hospitalized patients in 7 different wards and intensive care units. Clonally related *E. cloacae* isolates were also obtained from different hospital locations and persisted over time. These findings confirm the risk of spread of these organisms with the transfer of patients to different wards and the persistence of these clones through time, as occurs with other multidrug-resistant gram-negative bacilli [31].

Implementation of epidemiological control measures and use of surveillance cultures have helped to prevent persistence of epidemics over time and to restrict a tendency toward endemicity [9]. In our outbreak, control measures consisted of identifying MBL carriers with serial surveillance cultures among patients hospitalized in the areas where MBL-carrying patients were located, implementing contact isolation, recommending reduction in carbapenem use, and reinforcing the application of barrier precautions and hand washing. During our outbreak, an additional study (from January through April 2006) of intestinal colonization with MBL-producing isolates, including 114 hospitalized and 455 ambulatory patients who were treated at our hospital, was performed [32]. Only 4 patients (0.7%) had fecal carriage MBL identified, and all 4 were hospitalized in areas where patients infected with MBL-producing isolates had been detected.

In the time since we obtained the results included in this manuscript, we have detected 9 new patients who were infected or colonized with enterobacterial isolates expressing an MBL phenotype. Two other patients infected with VIM–1–producing *P. aeruginosa* have also been detected, with a different integron (unpublished results). We are prospectively observing the evolution of VIM–1 spread. Nevertheless, our current situation, 2 years since it first arose, is far from that in some institutions in Greece, where several institutions have these types of isolates [7, 8].

Table 2. Susceptibility profile of metallo-β-lactamase–producing enterobacterial isolates.

<table>
<thead>
<tr>
<th>Method, antibiotic</th>
<th>Klebsiella pneumoniae, KPMBL-A (n = 14)</th>
<th>ECLMBL-1 (n = 2)</th>
<th>ECLMBL-2 (n = 2)</th>
<th>ECLMBL-3 (n = 1)</th>
<th>ECLMBL-4 (n = 1)</th>
<th>ECLMBL-5 (n = 3)</th>
<th>ECLMBL-6 (n = 1)</th>
<th>Klebsiella oxytoca, KOMBL-1 (n = 1)</th>
<th>Escherichia coli, ECLMBL-1 (n = 1)</th>
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<td>Microdilution</td>
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<td>Amoxicillin–clavulanic acid</td>
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<td>Piperacillin–tazobactam</td>
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<td>Ceftazidime</td>
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<td>Cefotaxime</td>
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<td>Cefepime</td>
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<td>Meropenem</td>
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<td>Amikacin</td>
<td>&gt;16 &lt;4 to 8 &lt;4 &lt;4 &lt;4 &lt;4 &lt;4 &lt;4</td>
<td>&lt;4 &lt;4 &lt;4 &lt;4 &lt;4 &lt;4 &lt;4 &lt;4</td>
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<tr>
<td>Ciprofloxacin</td>
<td>1 to &gt;4 &lt;0.12 &gt;4 1–2 1 &gt;0.12 1–2 &gt;4</td>
<td>1–2 1 &gt;0.12 1–2 &gt;4</td>
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<tr>
<td>Etest (AB Biodiski)</td>
<td>Imipenem 0.38 to &gt;32 3–4 1.5–3 1–1.5 1 0.5 4 to &gt;32 0.19 16</td>
<td>0.38 to &gt;32 3–4 1.5–3 1–1.5 1 0.5 4 to &gt;32 0.19 16</td>
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<tr>
<td>Meropenem</td>
<td>0.064–1 0.38–2 0.38–0.75 0.25–1 0.25 0.25 0.25–1 0.25 0.25 0.25–1 0.25 0.25–1 0.25 0.25–1 0.25 0.25–1</td>
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<tr>
<td>Ertapenem</td>
<td>2 to &gt;32 &gt;32 &gt;32 &gt;32 12 16 &gt;32 32 12 to &gt;32 0.50 8</td>
<td>2 to &gt;32 &gt;32 &gt;32 &gt;32 12 16 &gt;32 32 12 to &gt;32 0.50 8</td>
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<tr>
<td>Aztreonam</td>
<td>48 to 256 0.75 to &gt;256 6 to &gt;256 0.38–2 0.06 0.75 0.75–64 1 &gt;256</td>
<td>48 to 256 0.75 to &gt;256 6 to &gt;256 0.38–2 0.06 0.75 0.75–64 1 &gt;256</td>
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**NOTE.** Data are for 1 isolate per patient.
E. coli belonged to the incompatibility group I, which seems to be currently involved in the dissemination of different ESBL genes in the community [33, 34]. The conjugative IncHI2 plasmids had variable size (300–435 kb), which may be associated with recombinatorial events, as has also been observed for other IncHI2 plasmids carrying bla_{VIM}[18]. Other European studies have also detected enterobacterial conjugative plasmids containing bla_{VIM} genes, but these genes were associated with the broad host range plasmid group IncN. The diversity of conjugative plasmids and the frequent location of integrons containing bla_{VIM} genes on transposable platforms might enhance the spread and persistence of these genes among different enterobacterial species in different areas and confirm the high risk of intraspecies and interspecies horizontal spread of MBL genes [18, 35, 36].

As has been reported elsewhere with regard to MBL-producing Enterobacteriaceae, the MICs of carbapenem are usually below the susceptible breakpoints, as defined by the Clinical and Laboratory Standards Institute [13]. The finding that MBL-producing Enterobacteriaceae strains are susceptible to carbapenems may have important clinical consequences, because infections caused by these organisms may not respond favorably to carbapenem treatment [2]. Differences in the carbapenem resistance levels among isolates of the same PFGE type were also observed in other studies [7, 8, 37] and may be due to variable expression of the MBL gene as a consequence of different promoters or differences in the gene copy number [38]. Nevertheless, the most probable reason for the variation in the MICs of carbapenem may be the existence of additional resistance mechanisms, such as porin deficiency and/or impaired permeability, and, eventually, the presence of an ESBL [7, 8, 37]. Most of our isolates were resistant to aztreonam, although...
this antibiotic is not hydrolyzed by MBLs [3]. In *K. pneumoniae* and *E. coli* isolates, the aztreonam resistance can be explained by the production of ESBLs in *E. cloacae* isolates, it was probably associated with the presence of derepressed AmpC.

Reduced and variable susceptibility to carbapenems and the possible coexistence of other β-lactamases make the phenotypic detection of MBL-producing Enterobacteriaceae difficult in routine assays. Adequate screening methods should be applied when an increase in the MIC of carbapenem is observed, independent of susceptibility to aztreonam [2]. In our experience, the double-disk synergy test allowed correct MBL detection in all isolates—even in isolates with carbapenem MICs within the susceptible range.

In conclusion, wide and rapid dissemination of multidrug-resistant plasmids containing * bla* _VIM-1_—carrying integrons among different Enterobacteriaceae, as was seen in our study, is of great clinical concern. The broad hydrolysis profile of the VIM-1 enzyme and the association of * bla* _VIM-1_ with genes encoding other antibiotic resistance determinants (e.g., aminoglycoside resistance) severely limit the therapeutic options for the treatment of infection with VIM-1-producing organisms. It seems necessary to adopt measures for the prevention of their dissemination and the spread of genetic elements harboring the MBL-encoding genes. In the absence of such measures, MBL-producing Enterobacteriaceae may significantly contribute to the emergence of nosocomial infections that antibiotics cannot treat.

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Potential conflicts of interest.

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