KPC and VIM producing Enterobacter cloacae strain from a hospital in northeastern Venezuela.

Dianny Martínez1, Daniel Marcano2, Hectorina Rodulfo3, Nurys Salgado2, Nirvia Cuaical2, Lucy Rodriguez1, Luisa Caña1, Belkis Medina1, Militza Guzman4 and Marcos De Donato3.

1Laboratorio de Bacteriología, Hospital Universitario “Antonio Patricio de Alcalá”. Cumaná, Venezuela.
2Instituto Nacional de Higiene Rafael Rangel, Ministerio del Poder Popular para la Salud. Caracas, Venezuela.
3Laboratorio de Genética Molecular, Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas, Universidad de Oriente. Cumaná, Venezuela.
4Laboratorio de Bacteriología Molecular, Departamento de Bioanálisis, Universidad de Oriente, Núcleo de Sucre. Cumaná, Venezuela.

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Abstract. An 83-year-old male patient is admitted to the central hospital in Cumaná, Venezuela with severe urinary infection, history of hospitalizations and prolonged antimicrobial treatments. A strain of Enterobacter cloacae was isolated showing resistance to multiple types of antibiotics (only sensitive to gentamicin), with phenotype of serine- and metallo-carbapenemases. Both, bla\textsubscript{VIM-2} and bla\textsubscript{KPC} genes were detected in the isolate. This is the first report of an Enterobacteriaceae species producing both KPC carbapenemase and VIM metallo carbapenemase in Venezuela. This finding has a great clinical and epidemiological impact in the region, because of the feasibility of transferring these genes, through mobile elements to other strains of Enterobacter and to other infection-causing species of bacteria.
Enterobacter cloacae producing KPC and VIM in a hospital in Venezuela

**Resumen.** En un paciente masculino de 83 años, que ingresó al Hospital de Cumaná, Venezuela, con diagnóstico de infección urinaria severa, antecedentes de hospitalización y diferentes tratamientos antimicrobianos durante largos periodos de tiempo, se aisló una cepa de Enterobacter cloacae, la cual evidenció resistencia a múltiples tipos de antibióticos (solo sensible a gentamicina) y con fenotipo de carbapenemasas de tipo serina y metalobetalactamasa. Los genes \( \text{bla}_{VIM-2} \) y \( \text{bla}_{KPC} \) fueron detectados en esta cepa. Este representa el primer reporte de una especie de Enterobacteriaceae productora simultánea de carbapenemasa KPC y metalobetalactamasa VIM en Venezuela. Esto tiene un gran impacto clínico y epidemiológico en la región por la posibilidad de transferencia de estos genes a otras cepas de Enterobacter u otras especies bacterianas causantes de infecciones, por medio de elementos móviles.

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**INTRODUCTION**

Resistance to a large number of broad-spectrum antimicrobials, including beta-lactams, is often reported in strains of Enterobacter and it is usually mediated by beta-lactamases. Considering that carbapenems constitute the most useful beta-lactams for the treatment of severe infections caused by resistant Enterobacteriales, it is of great concern the reports of serine-carbapenemase (KPC) or Metallo-beta-lactamases (MBLs) producing Enterobacter strains (1). In Venezuela, Enterobacter strains producing ESBLs, MBL and KPC have been reported (2-5), but the presence of multiple resistance genes in strains associated to infections represents an important finding with epidemiological impact for the treatment and control of nosocomial infections.

In April of 2012, an 83-year-old male patient was admitted to the Medicine Service of the University Hospital “Antonio Patricio de Alcalá” (HUAPA), in Cumaná, Venezuela, with the diagnostic impression of severe urinary infection. The patient had a history of previous hospitalizations in the Nephrology’s Service of this hospital because of his treatment of renal calculus, enlarged prostate, recurrent urinary and urethral infection. He had been using a urethral catheter for the last two years and was treated with ciprofloxacin. After his last admittance, a urine culture was indicated. The culture was carried out at the Clinical Bacteriological Laboratory of this hospital, and the antimicrobial susceptibility was done using the Kirby-Bauer method (6), according to the guidelines of CLSI (7) for Enterobacterials, using the following antimicrobials: piperacillin (PIP, 100 µg),
ticarcillin (TIC, 10 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), ampicillin/subactam (SAM, 10/10 µg), ceftazidime (CAZ, 30 µg), ceftotaxime (CTX, 30 µg), cefepime (FEP, 30 µg), aztreonam (ATM, 30 µg), ceftriaxone (GRO, 30 µg), ertapenem (ETP, 10 µg), imipenem (IPM, 10 µg), meropenem (MEM, 10 µg), netilmicin (NET, 30 µg), tobramycin (NN, 30 µg), amikacin (AK, 30 µg), gentamicin (GM, 10 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg) and ciprofloxacin (CIP, 5 µg).

The urine culture showed more than 10⁵ UFC/mL, isolating a strain of Enterobacter cloacae as the possible cause of the infection. This strain shows a multi-resistant profile to all the antimicrobials tested, except for GM.

The phenotypic detection of carbapenemases was carried out using the double disc synergy test (DDST), with discs of either IPM or MEM and ethylenediaminotetracetic acid-sodium mercaptoacetate (EDTA/SMA, 0.5 M/300 µg/mL) for MBLs and IPM with phenylboronic acid (300 µg) for serine-carbapenemases (8, 9). The polymerase chain reaction (PCR) of the bla⁷VIM, bla⁷VIM-2 and blaKPC genes was carried out using previously published primers (10-13), following the established protocols.

The DDST showed the presence of metallo-beta-lactamase and serine-carbapenemase in the isolated strain (Fig. 1). The presence of the blaVTM type 2 gene was demonstrated by PCR (Fig. 2), amplifying fragments of 381 and 801 bp for the general VIM and type 2, respectively. blaVTM was corroborated and the presence of blaKPC was demonstrated at the National Institute of Hygiene of Venezuela, when amplifying a second fragment of 593 bp and a fragment of 261 bp, respectively (Fig. 2C). Sequencing the fragment of 801 bp specific to blaVTM showed 100% homology to the published sequences in the GenBank for this type of the blaVTM gene.

Unfortunately, the patient died after 18 days of hospitalization and continuous antimicrobial treatment with different antibiotics.

**DISCUSSION**

The carbapenem-hydrolyzing enzyme KPC has disseminated widely among nosocomial pathogens, especially in Enterobacteriaceae. It has been found on transferable plasmids, and recovered from strains of E. cloacae, E. aerogenes and Enterobacter spp., as single isolates or in small outbreaks (14). The first detection of KPC-producing bacteria for Latin America, in this case in a strain of K. pneumoniae, was reported in 2005 (15) in Colombia. Later, KPC-producing bacterial strains have been reported in Puerto Rico and Trinidad.
and Tobago (16, 17). Recently, the KPC gene was detected in 61 E. coli, 333 K. pneumoniae, 99 P. aeruginosa, and 41 A. baumannii isolates in Puerto Rico (18), indicating the widespread dissemination of the KPC gene in clinically significant nosocomial isolates.

In Caracas, Venezuela, Marcano et al. (4) reported the presence of KPC in strains of K. pneumoniae and E. cloacae. In addition, a KPC-producing E. coli strain was isolated in a patient also from Caracas (19). The results in our study show the simultaneous presence of VIM and KPC type carbapenemases in E. cloacae from the general hospital of Cumaná, Venezuela. This is the first report of a strain of Enterobacteriaceae species carrying both KPC and VIM type bla genes in this hospital and in Venezuela.

On the other hand, only recently, two strains of E. cloacae and one of Enterobacter sp. were reported by our group to have blaVIM-2 genes in the general hospital of Cumaná (5). Before this report, no other studies have shown the presence of MBL-producing Enterobacter in any Latin American country, except for a report of strains of type 2 VIM-producing E. cloacae in México (20). This type of gene has been reported in Venezuela but only in strains of Pseudomonas aeruginosa (21, 22) and Klebsiella pneumoniae (23). VIM-producing P. aeruginosa strains were previously found in the general hospital of Cumaná (21), and it seems very likely that this gene could have been transferred from P. aeruginosa through mobile elements such as plasmid and/or integrons, as previously reported in other countries (24).

In recent years, Enterobacter spp. has been recognized as an increasingly important opportunistic pathogen particularly in debilitated and hospitalized patients. It is of epidemiologic relevance that the patient had received prior therapy with multiple antibiotics, showing the need for studies to determine the prevalence, sources and selection pressures responsible for the occurrence of this and other plasmid-encoded carbapenem-hydrolyzing betalactamases among clinically relevant species of Enterobacteriaceae. It is necessary that this hospital implements prevention plans to avoid the dissemination of carbapenemase-producing isolates and to restrict a tendency toward endemicity; the implementation of epidemiological control measures such as surveillance practices, strict contact isolation of patients harboring carbapenem-resistant members of the

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**Fig. 2.** Detection of blaVIM y blaKPC genes by PCR in the studied strain of Enterobacter cloacae (Ec) isolated in the general hospital of Cumaná, Venezuela. A: amplified fragment of 381 bp specific for general blaVIM. B: Amplification of a 801 bp fragment specific for blaVIM type 2. M: Molecular weight marker 100 pb (Axygen). C+: Positive control (ATCC 77297). C: Confirmation of the presence of the blaVIM and blaKPC, by the National Institute of Health of Venezuela showing a fragment of 261 bp and 893 bp, respectively. M: Molecular weight marker 50 pb (Invitrogen®). C+ VIM: Klebsiella pneumoniae (INH 77917) used as a positive control for the blaVIM gene, and C+KPC: K. pneumoniae (INH 636892) used as a positive control for the blaKPC gene.
Enterobacteriaceae and the restriction of the use of carbapenem only as the last resource (25).

The presence of both genes in this bacterial species is of great concern for its epidemiological implications, since these genes can be transferred to other strains of Enterobacter and other infection-causing bacteria, by plasmid or other mobile elements, as previously reported for Enterobacteriaceae in other countries (26).

REFERENCES


