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KPC and VIM producing *Enterobacter cloacae* strain from a hospital in northeastern 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, $bla_{\text{VIM-2}}$ and bla_{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.

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Enterobacter cloacae **productor de KPC y VIM en un hospital del noreste de Venezuela.**

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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 $bla_{\text{VIM-2}}$ y 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 betalactams, is often reported in strains of *Enterobacter* and it is usually mediated by betalactamases. Considering that carbapenems constitute the most useful betalactams 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.

CASE DESCRIPTION

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/sulbactam (SAM, 10/10 μg), ceftazidime (CAZ, 30 μg), cefotaxime (CTX, 30 μg), cefepime (FEP, 30 μg), aztreonam (ATM, 30 μg), ceftriaxone (CRO, 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 10^5 UFC/mL, isolating a strain of *Enterobacter cloacae* as the possible cause of the infection. This strain shows a multiresistant 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 ethylendiaminotetracetic acid-sodium mercaptoacetate (EDTA/SMA, 0.5 M/300μg/mL) for MBLs and IPM with phenylboronic acid (300 μg) for serine-carbapenemases (8, 9). The polymerase chain reaction (PCR) of the bla_{VIM}, bla_{VIM-2} and bla_{KPC} 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 bla_{VIM} type 2 gene was demonstrated by PCR (Fig. 2), amplifying fragments of 381 and 801 bp for the general VIM and type 2, respectively. bla_{VIM} was corroborated and the presence of bla_{KPC} was demonstrated at the National Institute of Hygiene of Venezuela, when amplifying a second fragment of 893 bp and a fragment of 261 bp, respectively (Fig. 2C). Sequencing the fragment of 801 bp specific to bla_{VIM-2} showed 100% homology to the published sequences in the GenBank for this type of the bla_{VIM} gene.

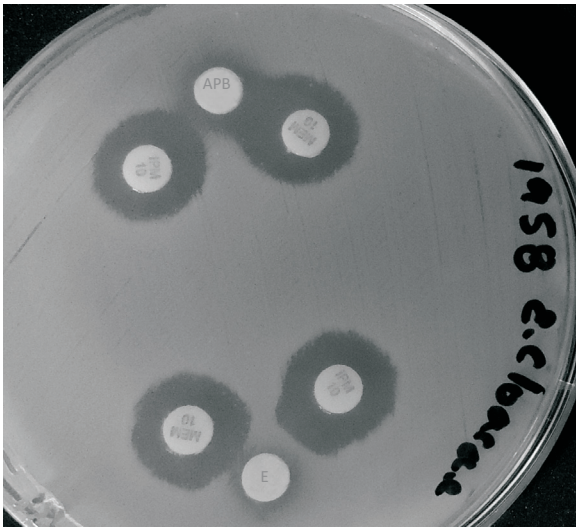

Fig. 1. Phenotype of metallo-beta-lactamase and serin-carbapenemase using the double disc synergy test (DDST) in a strain of *Enterobacter cloacae* isolated in the general hospital of Cumaná, Venezuela. IPM: imipenem, MER: meropenem, E: ethylendiaminotetracetate 750 μg /sodium mercaptoacetate 2 mg, APB: Phenylboronic acid (300 μg).

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

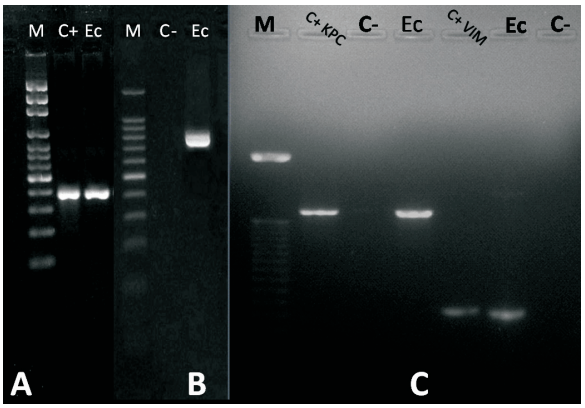

Fig. 2. Detection of bla_{VIM} y bla_{KPC} genes by PCR in the studied strain of *Enterobacter cloacae* (Ec) isolated in the general hospital of Cumaná, Venezuela. A: amplified fragment of 381 bp specific for general bla_{VIM}. B: Amplification of a 801 bp fragment specific for bla_{VIM} type 2. M: Molecular weight marker 100 pb (Axygen). C+: Positive control (ATCC 77297). C: Confirmation of the presence of the bla_{VIM} and bla_{KPC} 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 bla_{VIM} gene, and C+KPC: *K. pneumoniae* (INH 636892) used as a positive control for the bla_{KPC} gene.

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. pneumonie* 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 bla_{VIM-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

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).

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