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FIRST REPORT OF METALLO-β-LACTAMASES PRODUCING *Enterobacter* spp. STRAINS FROM VENEZUELA

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SUMMARY

Clinical strains of *Enterobacter* were isolated from Cumana's Central Hospital in Venezuela, and classified as *E. cloacae* (21), *E. aerogenes* (7), *E. intermedium* (1), *E. sakazakii* (1) and three unclassified. The strains showed high levels of resistance, especially to SXT (58.1%), CRO (48.8%), CAZ (46.6%), PIP (46.4%), CIP (45.2%) and ATM (43.3%). This is the first report for South America of bla_{VIM-2} in two *E. cloacae* and one *Enterobacter* sp., which also showed multiple mechanisms of resistance. Both *E. cloacae* showed bla_{TEM-1}, but only one showed $bla_{CTX-M-15}$ gene, while no bla_{SHV} was detected.

KEYWORDS: Carbapenemase; Metallobetalactamase; VIM; *Enterobacter;* Carbapenems.

INTRODUCTION

Species of the genus *Enterobacter* have been reported as an important source of intrahospital infections, especially those showing resistance to betalactams by the production of enzymes like extended spectrum beta-lactamases (ESBL) such as TEM, SHV, CTX, VEB, and carbapenemases such as VIM, KPC and GES[14]. This represents an important therapeutic challenge because of the few remaining treatment options, which gives rise to morbimortality and hospital expenses.

There are reports of *Enterobacter* strains producing metallo-β-lactamase (MBL) in different parts of the world, such as *E. cloacae* in Japan, Taiwan, Korea and Italy, as well as *E. aerogenes* in Japan and France[2]. However, no MBL-producing strains of *Enterobacter* have been reported anywhere in the Americas, except in Mexico[11] and Argentina[4].

METHODS

During August 2010 and March 2011, clinical strains of *Enterobacter* were isolated in the University Hospital Antonio Patricio de Alcala in Cumana, Venezuela. The use of the strains was approved by the patients or their relatives, according to the recommendations of the Bioethics and Biosecurity Committee of IIBCA, Universidad de Oriente, Cumana, Venezuela.

Isolated strains were inoculated in BHI broth, incubated for 12 hours at 37 ºC and later in MacConkey agar for 24 hours, in order to evaluate the morphological characteristics of the colonies to verify purity. For the classification of *Enterobacter* species tests for the fermentation of glucose and lactose in Kligler medium, use of citrate, arginine and malonate, use of MIO, LIA, and methyl red and Voges-Proskauer medium according to standard biochemical tests established for *Enterobacteriaceae*[8,16].

Antimicrobial susceptibility was assessed by Kirby-Bauer disk diffusion susceptibility test following the recommendations of the Clinical and Laboratory Standards Institute (CLSI). For tigecycline (TGC), we used the cutoff of the attached insert (Pfizer, INC).

Screening of extended spectrum beta-lactamases (ESBL), were carried out using the synergy effect between the antimicrobial disks CAZ, FEP, CRO, ATM and CTX surrounding AMC as well as the confirmatory test for ESBL was carried out using the combined disc test[7]. Additionally, in order to detect the presence of ESBL enzymes we used the modification suggested by SONG *et al.* (2007), in order to avoid the masking effect that a derepressed AmpC gene could produce[15]. For this, disks containing CAZ (30 µg) with and without clavulanic acid (10 µg) were added 3-aminophenyl boronic acid with a final amount of 400 µg.

The phenotypic detection of MBLs was carried out using IPM and MER disks on each side of a disk with ethylendiaminotetracetic acid-sodium mercaptoacetate (EDTA-SMA, 0.5 µmoles-3 µg) and the combined disc test (IMP, IMP-EDTA and MER, MER-EDTA)[6].

DNA extraction was carried out using the Wizard Genomic DNA kit (Promega) from the strains isolated after incubation in LB broth for 20 hours at 37 ºC. The bla_{VIM} gene was detected by PCR according

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to MENDEZ *et al.*[10]. Additionally, we detected the type 1 and 2 bla_{VIM} according to FIETT *et al.*[3]. The genes bla_{CTX-M} (EDELSTEIN *et al.* unpublished results, http://www.antibiotic.ru/en/pdfs/006-51.pdf), bla_{TEM} and bla_{SHV}[12] were also detected. Finally, in order to determine the clonality of the three *Enterobacter* strains showing a phenotype consistent with MBL by the IMP/MER/EDTA synergy test, we used ERIC-PCR[13].

RESULTS

The 33 strains of *Enterobacter* isolated were classified as *E. cloacae* (21), *E. aerogenes* (7), *E. intermedium* (1), *E. sakazakii* (1) and three were not possible to classify.

Ten of the strains were from infections acquired outside the hospital and most of the intrahospitalary strains were isolated in ICU (5), internal medicine areas A and B (4), soft surgery hall (4), pediatric area (3) and neonatology (3).

Antimicrobial susceptibility tests show high levels of resistance in most of the strains, with resistance to SXT (58.1%), CRO (48.8%), CAZ (46.6%), PIP (46.4%), CIP (45.2%) and ATM (43.3%) being the highest (Fig. 1). However, all the strains were sensitive to TGC. Results of the synergy test between CAZ/FEP/CRO/CTX/ATM with AMC in 16 of the strains were compatible with ESBL enzymes. We found that these strains showed the typical phenotypic effect for ESBL enzymes when using the combined disc test as a confirmatory. Additionally, three of the *Enterobacter* strains (two *E. cloacae* and one *Enterobacter* sp.) were resistant to cabapenems showing also synergy between IPM/MER and EDTA, typical of MBL enzymes (Table 1). ERIC-PCR patterns show no similarities among these strains. They showed resistance to multiple families of antibiotics (MDRs) and two of them also showed presence of ESBL by the synergy assay. These strains amplified for bla_{VIM-2} fragments (801 bp). Furthermore, both strains of *E. cloacae* amplified the typical fragment of the bla_{TEM} gene (1080 bp), but only one of them amplified the fragment of the bla_{CTX-M} gene (543 bp), while no bla_{SHV} gene was detected.

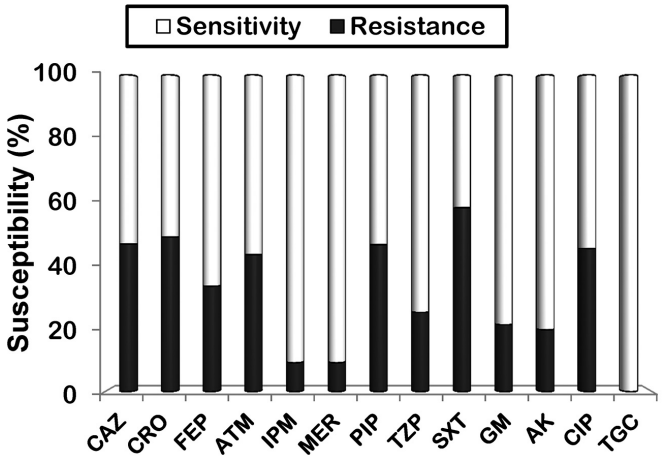

Fig. 1 - Antimicrobial susceptibility of the *Enterobacter* strains isolated in the Cumana hospital of Venezuela. CAZ: ceftazidime, CRO: ceftriaxone, FEP: cefepime, ATM: aztreonam, IPM: imipenem, MER: meropenem, PIP: piperacillin, TZP: piperacillin/tazobactam, SXT: trimethoprim-sulfamethoxazole, GM: gentamicin, AK: amikacin, CIP: ciprofloxacin and TGC: tigecycline.

Sequencing of the fragment of the bla_{VIM} gene, amplified using primers for type 2, produced sequences 100% homologous to bla_{VIM-2} reported in the GenBank in *P. aeruginosa* and other bacteria. Also, the sequences of the bla_{TEM} gene were 100% homologous to type 1 reported for many Enterobacteria. In addition, the fragment of the bla_{CTX-M} gene sequenced showed 100% homology with CTX-M-15 found in *E. coli* and other Enterobacteria.

DISCUSSION

According to our phenotypic tests, BLEA and ESBL-type of enzymes was very prevalent. On the other hand, we are not aware of previous reports of the presence of VIM-producing *Enterobacter* strain in any South American country. In Mexico, strains of *E. cloacae*

Table 1
Resistance pattern and epidemiological data of the three strains of *Enterobacter* showing bla_{VIM} type 2 MBLs

Species	Isolation date	Hospital area	Type of sample	Resistance pattern	Synergy tests	Detected genes
E. cloacae	August 2010	Soft Surgery	catheter	CAZ, FEP, IPM, MER, TZP, PIP, CRO, ATM, SXT, AK, GM	ESBL, MBL	bla_{VIM-2}, bla_{TEM-1}, $bla_{CTX-M-15}$
Enterobacter sp.	March 2011	ICU	bronchial secretion	CAZ, FEP, IPM, MER, TZP, PIP, CRO, ATM, SXT, CIP	AmpC, MBL	bla_{VIM-2}
E. cloacae	April 2011	Internal Medicine B	urine	CAZ, FEP, IPM, MER, TZP, PIP, CTX, ATM, SXT, CIP, AK	ESBL, MBL	bla_{VIM-2}, bla_{TEM-1}

ESBL: extended-spectrum betalactamase, MBL: metallo-β-lactamase, AmpC: de-repression of the chromosomal AmpC gene. ICU: intensive care unit. Acronyms of antibiotic as shown in the legend of Fig. 1.

have been shown to produce MBL[11]. These strains produced bla_{VIM-2} MBLs, the same type we found in this study. In Argentina, one strain of *E. cloacae* was reported containing bla_{IMP} gene, along with bla_{PER} and genes that confer resistance to aminoglycosides and quinolones[4]. This type of gene has been reported in Venezuela but only in strains of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*[5,9]. VIM-producing *P. aeruginosa* strains were previously found in Cumana hospital (ENSONY TOVAR & MARCOS DE DONATO, unpublished results). It seems very likely that the gene found in *P. aeruginosa* could have been transferred through mobile elements such as plasmid and/or integrons between these two species which are sharing the same environment, as previously reported[14], making possible the spread of this gene to many other bacteria species causing infection in this hospital environment.

The presence of multiple mechanisms of resistance in the bacteria isolated in the Cumana hospital causing intrahospital infections, especially in species of *Enterobacter,* which have natural resistance to several antibacterial drugs, suggests that more efficient preventive measures must be put in place in this hospital to avoid the survival and transmission of these strains. However, all the strains were susceptible to tigecycline, making it a suitable treatment for infections caused by MBL-producing enzymes in this hospital. This result agrees with numerous reports describing the use of tigecycline to treat infections caused by multidrug resistant bacteria, including those producing carbapenemases[14].

AUTHOR CONTRIBUTIONS

DM, LR and LC isolated the strains. HER, DM and MDD carried out the molecular analysis, BM, MG and NC helped in the bacteriological analysis. DM, HER and MDD wrote the manuscript and everyone reviewed the manuscript.

RESUMEN

Primer reporte de cepas de *Enterobacter* spp productoras de metalobetalactamasas de Venezuela

Cepas clínicas de Enterobacter fueron aisladas del Hospital central de Cumaná en Venezuela, y se clasificaron como *E. cloacae* (21), *E. aerogenes* (7), *E. intermedium* (1), *E. sakazakii* (1) y 3 sin clasificar. Las cepas mostraron altos niveles de resistencia, especialmente a SXT (58.1%), CRO (48.8%), CAZ (46.6%), PIP (46.4%), CIP (45.2%) and ATM (43.3%). Este es el primer reporte de América del Sur de bla_{VIM-2} en dos cepas de *E. cloacae* y una de *Enterobacter* sp., las cuales también mostraron múltiples mecanismos de resistencia. Ambas especies de *E. cloacae* mostraron genes bla_{TEM-1}, pero solo una mostro el gen $bla_{CTX-M-15}$, mientras que bla_{SHV} no fue detectado.

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