

Case Report

First isolation of a VIM-producing *Klebsiella pneumoniae* from a seven-year-old child in Venezuela

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Abstract

Background: VIM-type metallo-beta-lactamases (MBLs) exhibit hydrolytic activity against most beta-lactam antibiotics, including carbapenems. So far, VIM-type-producing *Klebsiella pneumoniae* isolates had not been reported in Latin America.

Methodology: In July 2005, a carbapenem-resistant *Klebsiella pneumoniae* was isolated from a urine sample collected from a 7-year-old girl hospitalized at the Hospital de Niños "J. M. de los Ríos" in Caracas, Venezuela. This strain was identified using conventional biochemical tests. The susceptibility analysis was conducted by disk diffusion, and MICs for Imipenem and Meropenem were performed by agar dilution. For the phenotypic detection of MBL we used the Imipenem-EDTA/SMA double-disk diffusion method. The hydrolytic activity against carbapenems was determined by the Masuda microbiological method. Purified protein was subjected to isoelectric focusing (IEF). Detection of antimicrobial resistance genes was performed by PCR amplification with specific VIM primers.

Results: The strain showed resistance to most beta-lactam antibiotics, quinolones and aminoglycosides, but remained susceptible to Aztreonam and Cefepime. The use of phenotypic and microbiological methods detected the presence of a metallo-beta-lactamase. By IEF we visualized three bands at pI 5.4, 7.6 and 7.9, corresponding to reduced-spectrum beta-lactamases, and a band at pI 5.8 that corresponded to the metallo-beta-lactamase. PCR screening of *bla* genes revealed the presence of *bla*_{VIM}, with an amplicon of 261 bp. **Conclusions:** This is the first report of a MBL-mediated carbapenem-resistant *Klebsiella pneumoniae* in Latin America, which constitutes a public health concern in our region since their transference to other microorganisms with multiple antibiotic resistance mechanisms will increase the antimicrobial resistance problem.

Key Words: *Klebsiella pneumoniae*, VIM, carbapenems, Venezuela.

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Introduction

The VIM-type metallo-beta-lactamases (MBLs) exhibit a wide spectrum of hydrolytic activity against beta-lactam antibiotics, including carbapenems, and their respective *bla* genes are located in the variable region of class 1 integrons [1,2]. These enzymes, that belong to the B class of Ambler's molecular classification and group 3 of Bush, Jacoby and Medeiros's functional classification, have the following characteristics: they're active against carbapenems; they do not hydrolyse monobactams; they are inhibited by metallic ions' chelant agents such as EDTA; they are not inhibited by clavulanic acid, sulbactam or tazobactam; and they present one or two zinc ions in their active site, which bond to water molecules

that intervene in the hydrolytic inactivation of the beta-lactam ring [3-7]. The most frequent MBLs in America are the VIM type, and they have 12 allelic variants, with the VIM-2 variant the most disseminated worldwide [3]. The first South American VIM was reported in 2002 in Venezuela in *Pseudomonas aeruginosa*, being found in two hospitals in Caracas and in one in Ciudad Bolívar [3, 8].

Until now, most VIM-producing *K. pneumoniae* are susceptible to Aztreonam (a poor substrate for the VIM MBL) and Gentamicin [9]. So far this type of isolate had not been reported in Venezuela, but now we report a case of a patient with a complicated urinary infection caused by VIM-type-producing *Klebsiella pneumoniae*.

Case Report

In July 2005, after a Ceftazidime week-long treatment, one carbapenem-resistant strain of *Klebsiella pneumoniae* (185362) was isolated at the Hospital de Niños “J.M de los Ríos” in Caracas, Venezuela from a urine sample collected by probe from a 7-year-old female patient with a history of a left urethral implant, neurogenic bladder, urethrocystoplasty and repeated urinary infections since April 2005. The strain was submitted to the Instituto Nacional de Higiene “Rafael Rangel” (Caracas, Venezuela) for confirmation of its unusual resistance to Imipenem and Cefoxitin) and later referred to the Instituto Nacional de Enfermedades Infecciosas ANLIS “Dr. Carlos Malbran” (Buenos Aires, Argentina) for additional testing.

The strain was identified using conventional biochemical tests (Difco Laboratories, Detroit, Michigan USA), evaluating glucose and lactose fermentation (Kligler agar), glucose metabolism pathway (MRVP broth), malonate metabolism (Malonate broth), citrate metabolism (Citrate agar), lysine, arginine and ornithine decarboxylation (Moeller decarboxylase broth), urea hydrolysis (Christensen agar), arabinose, rhamnose and inositol fermentation (Andrade broth), fenilalanine metabolism (Fenilalanine agar), indole production and motility (MIO agar).

The susceptibility analysis was conducted by disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [10] using Mueller Hinton agar (Difco Laboratories) and Ampicillin, Amoxicillin/Clavulanic Acid, Cefoxitin, Cefotaxime, Ceftazidime, Cefpodoxime, Imipenem, Meropenem, Ciprofloxacin, Gentamicin, Amikacin, Aztreonam disks (Oxoid Ltd). The antimicrobial agents used for susceptibility testing were obtained from standard laboratory powders. MIC for Imipenem (Merck Sharp & Dohme), Meropenem (Astra Zeneca) and Aztreonam (Bristol Mayers) was performed by agar dilution according to CLSI guidelines [11].

For the phenotypic detection of MBL the Imipenem-EDTA/SMA double-disk diffusion method was applied [12], using EDTA/SMA (Sigma Chemicals) disks 0.4 mM produced in our laboratory. The hydrolytic activity against carbapenems was determined by the Masuda microbiological method [13].

Purified protein was subjected to isoelectric focusing (IEF) as described previously [14]. β -lactamases from *Klebsiella pneumoniae* 185362 were visualized by the iodometric method described by Labia & Barthélémy [15]. Crude preparations from bacteria possessing β -lactamases of known isoelectric points (pI) were used as standards.

Detection of antimicrobial resistance genes was performed by PCR amplification using primers VIM-F (5'-AGTGGTGAGTATCCGACAG-3') and VIM-R (5'-ATGAAAGTGCCTGGAGAC-3') [16] and the GeneAmp PCR reagent kit (Applied Biosystems). DNA templates were prepared by lysing two colonies of the *K. pneumoniae* strain in 100 μ L of boiling water, and 5 μ L was used for the PCR. Amplification was performed in a DNA thermal cycler GeneAmp PCR system 2400 (Applied Biosystems) in a final volume of 50 μ L containing 50 pmol of each primer (Invitrogen), 25 μ M of each dNTP, 1.5 mM MgCl₂ and 1 U of Taq polymerase. PCR products were visualized by electrophoresis on 0.8% agarose gels in TBE buffer (pH 7.0) and stained with 1% ethidium bromide.

The strain showed resistance to Ampicillin, Amoxicillin/Clavulanic Acid, Cefoxitin, Cefotaxime, Ceftazidime, Cefpodoxime, Imipenem, Meropenem, Ciprofloxacin, Gentamicin and Amikacin, remaining susceptible to Aztreonam. The use of the Imipenem-EDTA/SMA double-disk diffusion method and the Masuda method for Imipenem and Meropenem suggested the presence of a metallo- β -lactamase. The Imipenem and Meropenem MIC were conducted by agar dilution according to CLSI guidelines, resulting in 16 μ g/ml (Resistant) for Imipenem, 8 μ g/ml (Intermediate) for Meropenem and 0.25 μ g/ml (Susceptible). By CLSI MIC determination, Imipenem restored its activity after the addition of EDTA 0.4 mM (from 16 μ g/ml to 0.12 μ g/ml) as well as Meropenem (from 8 μ g/ml to 0.25 μ g/ml), which confirmed the inhibition of the Imipenem/Meropenem resistance enzyme by EDTA and thus the presence of a metallo- β -lactamase.

By isoelectric focusing three bands were identified at pI 5.4, 7.6 and 7.9, corresponding to β -lactamases without carbapenemase activity, and there was also a band at pI 5.8 with carbapenemase activity that corresponded to the

MBL. PCR screening of *bla* genes revealed the presence of *bla*_{VIM}, with an amplicon of 261 bp.

Discussion

Panresistant *K. pneumoniae* have been found in the intensive care units of various Athens hospitals since 2001, coinciding with the emergence of MBL-positive enterobacteria [9]. This is the first report of a MBL-mediated carbapenem-resistant *Klebsiella pneumoniae* in Venezuela, which constitutes a public health concern in our country and requires efficient detection and intervention to preserve antibiotic options. The presence of the resistance enzymes described above in bacteria in our hospitals is of great relevance, since their transfer from *P. aeruginosa* to other microorganisms that also have multiple antibiotic resistance mechanisms (i.e. Enterobacteriaceae) will increase the antimicrobial resistance problem, and this phenomenon is already occurring in some countries with the subsequent rise in health care costs and mortality rates. Tato *et al.* [17,18] describe an ongoing outbreak of infection and colonization with MBL-producing gram-negative bacteria that is resistant to most widely used antibiotics. They report a situation that is perilously close to a state of endemicity, whereby untreatable organisms are entrenched in their institution, having a mortality rate of almost 50% among patients who were infected with these organisms [17, 18]. This report, and others like it [18-20], provide an important signal that we need to rapidly attune our surveillance, clinical microbiology, and infection control programs to the coming threat of increasing antibiotic resistance in gram-negative bacilli. It is very important that MBL screening is routinely made to gram-negative bacilli in clinical laboratories, because failure to control outbreaks involving carbapenemase-producing organisms will eventually lead to a situation where all possible empirical treatment options are useless.

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