Detection of carbapenemase-producing bacteria in a public healthcare center from Venezuela

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Abstract

Introduction: The dramatic increase in the prevalence and clinical impact of infections caused by Carbapenemase-Producing Bacteria in the nosocomial setting in Latin America represents an emerging challenge to public health. The present study detected carbapenemase-producing Gram-negative bacteria in patients from a Hospital from Venezuela, by phenotypic and genotypic methods.

Methodology: The bacterial identification was carried out using conventional methods. The resistance to carbapenems was performed by Kirby-Bauer disk diffusion method, according to CLSI recommendations. The modified Hodge Test, double-disk with phenylboronic acid, double-disk with EDTA and Blue Carba Test were performed to detect phenotypic carbapenemase producers. The carbapenemase-encoding genes \( \text{bla}_{KPC} \), \( \text{bla}_{VIM} \), \( \text{bla}_{IMP} \), \( \text{bla}_{OXA-2} \), \( \text{bla}_{OXA-3} \), \( \text{bla}_{OXA-15} \) and \( \text{bla}_{OXA-21} \) were determined.

Results: The bacterial species identified were \textit{Klebsiella pneumoniae} complex (181), \textit{Pseudomonas aeruginosa} (51), and \textit{Acinetobacter baumannii-calcoaceticus} complex (119). KPC-type was detected in 40.17% of isolates and VIM-type in 14.53%. KPC-type gene was only identified in \textit{K. pneumoniae} isolates (77.9%). VIM-type gene was identified in \textit{P. aeruginosa} (86.27%) and \textit{K. pneumoniae} isolates (3.87%). There was not detection of IMP-type and OXA-type genes.

Conclusions: We found a predominance of \textit{K. pneumoniae} KPC producers and a high rate of VIM-producing \textit{P. aeruginosa}. The epidemiology of CPB in Venezuela is rapidly evolving, and enhanced surveillance and reporting are needed across the healthcare continuum.

Key words: carbapenemase-producing bacteria; KPC; VIM.


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Introduction

The spread of Carbapenemase-Producing Bacteria (CPBs), a major public health threat, continues to increase on a global level and is associated with significant morbidity and mortality [1,2]. Carbapenemases belonging to all of the three classic classes (A, B and D) have been identified all over the Latin American region [3].

In Venezuela, sporadic cases of CPB have been reported; KPC (\textit{Klebsiella pneumoniae} carbapenemase)-producing \textit{K. pneumoniae} and \textit{Enterobacter cloacae} were first described between 2009 and 2010 [4] and \textit{K. pneumoniae} harboring \( \text{bla}_{VIM} \) (Verona Integron-encoded Metallo-\( \beta \)-lactamase)-type were reported in 2008 [5]. Currently KPC-2 and NDM-1 (New Delhi metallo-\( \beta \)-lactamase) are the genes which have been predominantly identified in \textit{K. pneumoniae} [6]. VIM-2 was identified for first time in 2002 in isolates of \textit{Pseudomonas aeruginosa} [7] and IMP (Imipenemase)-producing \textit{P. aeruginosa} was described in 2013 [8]. The most frequent carbapenemases in \textit{Acinetobacter} spp. are carbapenem-hydrolyzing class D \( \beta \)-lactamases (CHDLs) and secondly, metalloenzymes such as VIM, IMP and NDM. OXA (Oxacillinase)-23 is the most widely disseminated class D-carbapenemase in \textit{A. baumannii} isolates from Latin American countries [9], and Fritsche et al. reported the first OXA-23-producing \textit{Acinetobacter} spp. strain isolated in Venezuela in 2002 [10].

The Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines are the most popular breakpoint guidelines used in antimicrobial susceptibility testing worldwide. Several studies show acceptable level of agreement between EUCAST and CLSI zone diameter breakpoints to
carbapenems for Gram-negative bacilli and laboratories with similar antibiotic susceptibility patterns may choose to adopt either guideline without fear of significantly altering reported antibiotic susceptibility [11,12].

The increased frequency of reports on carbapenemases in Latin America and the Caribbean suggests they have successfully spread and become endemic in some countries [13]. The detection of carbapenemase-mediated carbapenem resistance is essential for patient management, infection control, and public health efforts [14]. Currently, characterization of the underlying mechanism of carbapenem resistance is not undertaken by most clinical microbiology laboratories for therapeutic decision-making. However, understanding if an organism is carbapenemase producing and, if so, the class of carbapenemase (s) produced has treatment implications [15]. Therefore, the aim of this study was to detect KPC, VIM and OXA carbapenemases in carbapenem-resistant Gram-negative bacteria in a Hospital from Venezuela, by phenotypic and genotypic methods.

Methodology

The study was performed with three hundred fifty one (n = 351) carbapenem-resistant or-intermediate clinical isolates recovered during 2016 and 2017 (24-month period) in a 192-bed tertiary-care teaching hospital (Dr. Adolfo Pons Hospital) in Maracaibo, Venezuela. The bacterial identification was carried out using conventional methods. A total of one hundred eighty one (n = 181) isolates of K. pneumoniae complex, fifty one (n = 51) P. aeruginosa and one hundred nineteen (n = 119) A. baumannii-calcoaceticus complex were included in the study. Most of the strains were isolated from men (53.08%), among adult patients (20.6%) hospitalized in Intensive Care Units (ICUs) (58.64%). The majority of bacterial species (39.9%) were isolated from VAP (Ventilation Associated Pneumonia), 20.63% from UTI (Urinary Tract Infection) and, 17.42% from bacteremia.

The association with the carbapenemase-producing isolate and the gender and age group of the patients, hospital settings and type of sample was calculated using χ² and Fisher’s exact tests. p values ≤0.05 were considered to be statistically significant.

The resistance to carbapenems was performed by Kirby-Bauer disk diffusion method, according to CLSI recommendations [16]. The modified Hodge Test (MHT), double-disk with phenylboronic acid (PBA), double-disk with EDTA and Blue Carba Test (BCT) were performed to detect phenotypic carbapenemase producers. The isolation, identification and phenotypic detection of carbapenemase-producing bacteria were conducted at Dr. Adolfo Pons’s Bacteriological Laboratory, Maracaibo, Venezuela. The carbapenemase-encoding genes blaKPC, blaVIM, blaIMP, blaOXA-2, blaOXA-3, blaOXA-15 and blaOXA-21 were determined [17,18]. The genotypic detection was conducted at Bacteriological Reference Laboratory, University’s Hospital, Maracaibo, Venezuela.

Results

The results of phenotypic detection of carbapenemase-producing bacteria were presented in Table 1. To phenotypic detection of carbapenemase-producing K. pneumoniae complex, the MHT showed sensitivity of 99%, 45% of specificity, PPV 86.3%, PNV 90%, RV+ 1.8% and RV- 0.02. The double-disk with phenyl boronic acid showed sensitivity of 99%, 38% of specificity, PPV 84.8%, PNV 88.2%, RV+ 1.6% and RV- 0.03 and the BCT showed sensitivity 99%, specificity 50%, PPV 87.6%, PNV 100%, RV+ 1.7% and RV- 0.03.

On the other hand, to phenotypic detection of metallo-β-lactamase in P. aeruginosa, the MHT showed 50% of sensitivity, specificity of 71%, PPV 91.7%, PNV 18.5%, RV+ 1.7%, RV- 0.7 and the double disk with EDTA showed a sensitivity of 82%, specificity 57%, PPV 92.3%, PNV 33.3%, RV+ 1.9% and RV- 0.32.

To phenotypic detection of carbapenemase-producing A. baumannii-calcoaceticus complex, the

<table>
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<tr>
<th>Isolate</th>
<th>Methods used</th>
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<tr>
<td></td>
<td>MHT</td>
<td>PB</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>161 (88.95)</td>
<td>164</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>24 (47.06)</td>
<td>NA</td>
</tr>
<tr>
<td>A. baumannii-calcoaceticus</td>
<td>48 (40.34)</td>
<td>NA</td>
</tr>
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Values are presented as n (%). MHT: modified Hodge test; PB: phenylboronic double disk; EDTA double disk; BCT: Blue Carba test; NA: not available.
MHT showed 40.34 % (48/119) of positive results, 63.03 % (75/119) of isolates were positive to the BCT method and 31.93 % (38/119) of the isolates were positive in both methods. The carbapenemases were identified as follows: KPC-type in 141/351 of isolates (40.17%) and VIM-type in 51/351 of isolates (14.53%). KPC-type gene was only identified in K. pneumoniae complex (77.9%). Forty four VIM-type genes were identified in P. aeruginosa isolates (86.27%) and seven in K. pneumoniae complex isolates (3.87%). There was not detection of IMP-type and OXA-type genes (Table 1).

There was not statistically significant association between the carbapenemase-producing isolate and the gender and age group of the patients, hospital settings and type of sample.

**Discussion**

The epidemiology of carbapenem resistance in Enterobacterales around the globe has been dominated by the dissemination of three distinct Ambler classes of β-lactamases: NDM, KPC, and OXA-48-like [19].

KPC enzymes are the most prevalent class A carbapenemase worldwide [13] and has been extensively reported in K. pneumoniae and other Enterobacterales [14]. KPC-producing K. pneumoniae is widespread in the United States, but is also endemic in some European countries such as Greece and Italy [14] and were also described in Enterobacterales in the Caribbean, South America, China, and Israel [19].

The first Latin American report of a KPC-producing K. pneumoniae took place in Medellin, Colombia in 2005 and soon afterwards, was also reported all around Latin American countries. In Venezuela, KPC-producing K. pneumoniae and E. cloacae isolates were first described between 2009 and 2010 [4]. We found a clear predominance (77.9%) of K. pneumoniae complex KPC producers, as observed in previous studies describing isolates in Venezuela [6,20].

KPC enzymes have also been identified in other Gram-negative pathogens including P. aeruginosa [14]. The identification of KPC in P. aeruginosa was reported for the first time globally in Colombia in 2007 and KPC-producing A. baumannii-calcoaceticus clinical isolates were described first in Puerto Rico in 2009 [13]; nevertheless, the gene blaKPC has only been identified in K. pneumoniae complex in the present study.

Class B β-lactamas, or metallo-β-lactamas (MBLs), are commonly identified in Enterobacterales and P. aeruginosa. Among the MBLs, NDM, VIM, and IMP enzymes are the most frequently identified worldwide. IMP-producing Gram-negative bacteria are mainly detected in China, Japan, and Australia, mostly in A. baumannii. VIM producers are most often found in Italy and Greece (Enterobacterales) and in Russia (P. aeruginosa) [14].

In Latin America, VIM-2 was identified for first time in 2002 in Chile and Venezuela in isolates of Pseudomonas fluorescens and P. aeruginosa, respectively [7,10], and it has also been reported in P. aeruginosa isolates in Brazil, Argentina, Mexico, Uruguay, Peru and Costa Rica [13]. Overall, in Latin America and the Caribbean, rates of VIM-producing organisms are 2-19% among carbapenem-resistant P. aeruginosa isolates [13], but in the present study, the rate of VIM-producing P. aeruginosa isolates was much more high (86.27%).

The blaVIM gene was also identified in K. pneumoniae complex (3.87%) in this study. In contrast to Europe, few reports of VIM-producing Enterobacterales exist in Latin America and the Caribbean [13]. In Venezuela, VIM-2 was first reported in 2008 among isolates of K. pneumoniae [5] and more recently in Enterobacter spp. [21].

Overall, studies comprising carbapenem-resistant P. aeruginosa isolates in Latin America and the Caribbean have reported rates of IMP production of 1-16% [13]. However, the blaIMP gene was not detected in carbapenemase-producing bacteria in this study.

Acquired class D carbapenem-hydrolyzing β-lactamas are commonly reported in A. baumannii (mainly OXA-23, OXA-24/40, and OXA-58-like enzymes), but not in P. aeruginosa. OXA-48 and derivatives (eg, OXA-181 and OXA-232) have been detected in Enterobacterales, hydrolyze narrow-spectrum β-lactams and weakly hydrolyze carbapenems, but spare broad-spectrum cephalosporins (ceftazidime, cefepime) [14]. Otherwise, OXA-2 and OXA-10 β-lactamas, enzymes that are currently regarded as non carbapenemases, have catalytic efficiencies against carbapenems similar to those of well-recognized Carbapenem-hydrolyzing class D β-lactamas (CHDLs) and are capable of conferring resistance to these last-resort antibiotics when expressed in A. baumannii [22]. However, OXA-2-producing A. baumannii isolate was not detected in this study.

Apart from the OXA-51-related family typically present in A. baumannii, six subgroups of acquired CHDLs have been identified in Latin America and the Caribbean, namely OXA-23-like, OXA-40/24-like, OXA-58-like, OXA-48-like, OXA-143-like, and OXA-
235-like enzymes [13]. In Venezuela, the first OXA-23-producing Acinetobacter spp. strain was detected in 2002 [10]. More recently, A. baumannii harboring the \textit{bla}\textit{OXA-23} gene (87.5%) and the \textit{bla}\textit{NDM-1} gene (37.5%) were detected in Venezuela and the coexistence of \textit{bla}\textit{OXA-23/bla}\textit{NDM-1} genes was identified in two of the isolates [6].

On the other hand, the MHT is probably the most well-known approach for carbapenemase detection. This assay demonstrates acceptable sensitivity for most carbapenemases, particularly KPC enzymes, but low sensitivity for MBLs [15]. For U.S. collections of \textit{Enterobacterales}, where KPC producers comprise greater than 95% of carbapenemases [23,24], the sensitivity of the MHT has been reported to be between 93% and 98% [25], like the sensitivity observed in the present study (99%). However, the MHT showed low specificity (45%) in this study for the detection of carbapenemase-producing \textit{K. pneumoniae} complex. Likewise, MHT showed low sensitivity (50%) and specificity of 71% for detection of \textit{P. aeruginosa} carbapenemase producers. As isolates producing extended-spectrum β-lactamases (ESBLs) or AmpC cephalosporinases in conjunction with porin mutations often yield false-positive MHT results, the MHT has limited specificity, reported at approximately 91% [10,15]. During the past years, the rates of infections by ESBL-producing isolates have greatly increased. As observed in a previous study in Venezuela, 63.64% of \textit{K. pneumoniae} isolates were ESBLs producers [6].

The Blue-Carba is a biochemical modified test (BCT) that was validated for the detection of carbapenemase-producing strains directly from bacterial cultures and it detected all \textit{Enterobacterales}, \textit{P. aeruginosa} and \textit{A. baumannii} carbapenemase producers with 100% sensitivity and 100% specificity [23]. In the present study, BCT showed the highest sensitivity and specificity for detection of \textit{K. pneumoniae} serine carbapenemase producers. To phenotypic detection of carbapenemase-producing \textit{A. baumannii-calcoaceticus} complex, the BCT showed 63.03% of positive isolates and 31.93% of the isolates were positive in both, BCT and MHT.

Targeted phenotypic carbapenemase assays compare carbapenem activity with and without the presence of inhibitors (e.g., PBA for KPC and EDTA for MBL). Targeted carbapenemase tests offer straightforward, affordable, and accurate options for the detection of KPC, MBL, and OXA-48-like carbapenemase producers. One high stringency evaluation of the assay reported a sensitivity of 97% and specificity of 69% to detect carbapenemase producers. Furthermore, it classified carbapenemases appropriately for 85% of class A, 72% of class B, and 89% of class D carbapenemases [26]. Similar results were obtained in the present investigation; the double-disk with PBA showed sensitivity of 99% and 38% of specificity for the detection of \textit{K. pneumoniae} complex carbapenemase producers and sensitivity of 82% and 57% of specificity for the detection of carbapenem-producing \textit{P. aeruginosa}.

OXA-type carbapenemases common to carbapenem-resistant \textit{A. baumannii} strains are known to be inefficient at hydrolyzing the β-lactam ring of carbapenem antibiotics. The elevated carbapenem MICs observed in carbapenem-resistant \textit{A. baumannii} strains are largely attributable to additional manifestations of resistance, such as reduced porin expression or upregulation of efflux pumps. The CarbAcineto NP test was designed to overcome some of the impediments associated with detecting carbapenemases produced by \textit{A. baumannii}, with a sensitivity of 95% and a specificity of 100% for detecting carbapenemases commonly associated with \textit{A. baumannii} [15].

The spread of CPB remains a significant clinical and public health concern. Consequently, reliable detection of carbapenemase production is an essential first step in combating this problem [15]. The epidemiology of CPB in Venezuela is rapidly evolving, and enhanced surveillance and reporting are needed across the healthcare continuum. Data on regional prevalence rates of carbapenemase-mediated carbapenem resistance are critical for development of coordinated approaches to CPB control [24].

Infections caused by carbapenemase producers are associated with high rates of morbidity and mortality, limited therapeutic options for the treatment, prolonged hospital stays, and overall increased healthcare costs. Therefore, infection prevention remains one of the critical approaches for preventing the spread of such organisms [6,25].

**Conclusions**

In summary, KPC was the most common carbapenemase detected in \textit{K. pneumoniae} isolates in Dr. Adolfo Pons’s Hospital in Maracaibo, Venezuela, followed by VIM-producing \textit{P. aeruginosa}. Finally, considering the increasing identification of carbapenemase-producing bacteria in this hospital, systematic carriage screening at hospital admission, additional surveillance studies, and early detection of such isolates are required to limit their further spread.
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References

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