JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

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

Liliana Gomez-Gamboa[1], Armindo Perozo-Mena[2], Jose Bermudez-Gonzalez[3], Christty Villavicencio[1], Jessica Villasmil[4], Messaria M Ginestre[2], Jesvy Velasquez[1]

[1] *School of Medicine, Medical Faculty, University of Zulia, Maracaibo, Venezuela*
[2] *School of Bioanalysis, Medical Faculty, University of Zulia, Maracaibo, Venezuela*
[3] *Bios Venezuela, CA Maracaibo, Venezuela*
[4] *School of Bioanalysis, Medical Faculty, University of Zulia, Maracaibo, Venezuela*

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-Baüer 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 *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-2}$, *bla*$_{OXA-3}$, *bla*$_{OXA-15}$ and *bla*$_{OXA-21}$ were determined.

Results: The bacterial species identified were *Klebsiella pneumoniae* complex (181), *Pseudomonas aeruginosa* (51), and *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 *K. pneumoniae* isolates (77.9%). VIM-type gene was identified in *P. aeruginosa* (86.27%) and *K. pneumoniae* isolates (3.87%). There was not detection of IMP-type and OXA-type genes.

Conclusions: We found a predominance of *K. pneumoniae* KPC producers and a high rate of VIM-producing *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.

J Infect Dev Ctries 2021; 15(1):163-167. doi:10.3855/jidc.13567

(Received 28 July 2020 – Accepted 04 September 2020)

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 (*Klebsiella pneumoniae* carbapenemase)-producing *K. pneumoniae* and *Enterobacter cloacae* were first described between 2009 and 2010 [4] and *K. pneumoniae* harboring *bla*$_{VIM}$ (Verona Integron-encoded Metallo-β-lactamase)-type were reported in 2008 [5]. Currently KPC-2 and NDM-1 (New Delhi metallo-β-lactamase) are the genes which have been predominantly identified in *K. pneumoniae* [6]. VIM-2 was identified for first time in 2002 in isolates of *Pseudomonas aeruginosa* [7] and IMP (Imipenemase)-producing *P. aeruginosa* was described in 2013 [8]. The most frequent carbapenemases in *Acinetobacter* spp. are carbapenem-hydrolyzing class D β-lactamases (CHDLs) and secondly, metalloenzymes such as VIM, IMP and NDM. OXA (Oxacillinase)-23 is the most widely disseminated class D-carbapenemase in *A. baumannii* isolates from Latin American countries [9], and Fritsche et al. reported the first OXA-23-producing *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 isolates 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 χ^2 and Fisher's exact tests. p values ≤0.05 were considered to be statistically significant.

The resistance to carbapenems was performed by Kirby-Baüer 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 *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-2}$, *bla*$_{OXA-3}$, *bla*$_{OXA-15}$ and *bla*$_{OXA-21}$ 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 1. Phenotypic and Genotypic detection of Carbapenemase-producing *K. pneumoniae* complex, *P. aeruginosa* and *A. baumannii-calcoaceticus* complex isolates.

Isolate	Methods used				Genes detected			
	MHT	PB	EDTA	BCT	*bla*$_{IMP}$	*bla*$_{VIM}$	*bla*$_{KPC}$	*bla*$_{OXA-2}$
K. pneumoniae	161 (88.95)	164 (90.61)	15 (8.29)	161 (88.95)	0	7 (3.87)	141 (77.9)	0
P. aeruginosa	24 (47.06)	NA	39 (76.47)	NA	0	44 (86.27)	0	0
A. baumannii-calcoaceticus	48 (40.34)	NA	NA	75 (63.03)	0	0	0	0

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 bla_{KPC} has only been identified in *K. pneumoniae* complex in the present study.

Class B β-lactamases, or metallo-β-lactamases (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 bla_{VIM} 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 bla_{IMP} gene was not detected in carbapenemase-producing bacteria in this study.

Acquired class D carbapenem-hydrolyzing β-lactamases 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-lactamases, enzymes that are currently regarded as non carbapenemases, have catalytic efficiencies against carbapenems similar to those of well-recognized Carbapenem-hydrolyzing class D β-lactamases (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 $bla_{\text{OXA-23}}$ gene (87.5%) and the $bla_{\text{NDM-1}}$ gene (37.5%) were detected in Venezuela and the coexistence of $bla_{\text{OXA-23}}$/$bla_{\text{NDM-1}}$ 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 *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 *K. pneumoniae* complex. Likewise, MHT showed low sensitivity (50%) and specificity of 71% for detection of *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 *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 *Enterobacterales*, *P. aeruginosa* and *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 *K. pneumoniae* serine carbapenemase producers. To phenotypic detection of carbapenemase-producing *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 *K. pneumoniae* complex carbapenemase producers and sensitivity of 82% and 57% of specificity for the detection of carbapenemase-producing *P. aeruginosa.*

OXA-type carbapenemases common to carbapenem-resistant *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 *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 *A. baumannii,* with a sensitivity of 95% and a specificity of 100% for detecting carbapenemases commonly associated with *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 *K. pneumoniae* isolates in Dr. Adolfo Pons´s Hospital in Maracaibo, Venezuela, followed by VIM-producing *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.

Acknowledgements

This study was supported by grants from Humanistic and Technological Scientific Development Council (CONDES) VAC-CC-0251-17 from The University of Zulia (LUZ-Venezuela).

References

1. An J, Lai K, Ma Y, Guo L (2018) Emergence of multiple carbapenemase-producing organisms in single patients : an increasing threat to treatment of infection. J Antimicrob Chemother 73: 544-546.
2. Logan LK, Weinstein RA (2017) The epidemiology of carbapenem-resistant *Enterobacteriaceae*: The impact and evolution of a global menace. J Infect Dis 215 Suppl 1: 1-9.
3. Maya JJ, Ruiz SJ, Blanco VM, Gotuzzo E, Guzman-Blanco M, Labarca J, Salles M, Quinn JP, Villegas MV (2013) Current status of carbapenemases in Latin America. Expert Rev Anti Infect Ther 11: 657-667.
4. Marcano D, De Jesus A, Hernandez L, Torres L (2011) Frequency of enzymes associated with decreased sensitivity to beta-lalactamics in enterobacteria isolates, Caracas, Venezuela. Rev Panam Salud Publica 30: 529-534 [Article in Spanish].
5. Marcano D, Pasteran F, Rapoport M, Faccone D, Ugarte C, Salgado N, Payares D, Spadola E, López Y, Maggi G, Galas M, Sánchez D (2008) First isolation of a VIM-producing *Klebsiella pneumoniae* from a seven-year-old child in Venezuela. J Infect Dev Ctries 2: 241-244. doi: 10.3855/jidc.270.
6. Gómez-Gamboa L, Barrios-Camacho H, Durán-Bedolla J, Sánchez-Pérez A, Reyna-Flores F, Perozo-Mena A, Zabala I, Rodríguez-Medina N, Martínez-Barnetche J, Téllez-Sosa J, Valdovinos-Torres H, Garza-Ramos U (2019) Molecular and genetic characterization of carbapenemase-producing bacteria in Venezuela. J Chemother 31: 349-353.
7. Mendes RE, Castanheira M, Garcia P, Guzman M, Toleman MA, Walsh TR (2004) First isolation of *bla*$_{VIM-2}$ in Latin America: report from the SENTRY Antimicrobial Surveillance Program. Antimicrob Agent Chemother 48: 1433-1434.
8. Perozo-Mena A, Castellano-González M, Chávez K, Ling-Toledo E, Arraiz N (2013) Assessment of phenotypic methods for metallobetalactamase detection in clinical isolates of *Pseudomonas aeruginosa*. Kasmera 41: 115-126.
9. Rodríguez CH, Nastro M, Famiglietti A (2018) Carbapenemases in *Acinetobacter baumannii*. Review of their dissemination in Latin America. Rev Argent Microbiol 50: 327-333.
10. Fritsche T, Sader H, Toleman M, Walsh T, Jones R (2005) Emerging metallo-β-lactamase-mediated resistances: a summary report from the worldwide SENTRY antimicrobial surveillance program. Clin Infect Dis 41 Suppl 4: 276-278.
11. Kassim A, Omuse G, Premji Z, Revathi G (2016) Comparison of Clinical Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing guidelines for the interpretation of antibiotic susceptibility at a University teaching hospital in Nairobi, Kenya: a cross-sectional. Ann Clin Microbiol Antimicrob 15 (21):1-7.
12. Sánchez-Bautista A, Coy J, García-Shimizu P, Rodríguez JC (2018) From CLSI to EUCAST guidelines in the interpretation of antimicrobial susceptibility: What is the effect in our setting? Enferm Infecc Microbiol Clin 36 (4): 229-232.
13. Escandón-Vargas K, Reyes S, Gutiérrez S, Villegas MV (2017) The epidemiology of carbapenemases in Latin America and the Caribbean. Expert Rev Anti Infect Ther 15: 277-297.
14. Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA (2018) Carbapenemase-Producing Organisms: A Global Scourge. Clin Infect Dis 66: 1290-1297.
15. Tamma PD, Simner PJ (2018) Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. J Clin Microbiol 56: 1-13.
16. Clinical and Laboratory standard institute (CLSI) (2016) Performance standards for antimicrobial susceptibility testing, 26th informational supplement. CLSI document M100-S26 (ISBN 1-56238-924-6).
17. Woodford N, Tierno PM, Young K, Tysall L, Palepou MI (2004) Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A β-lactamase, KPC-3 , in a New York medical center. Antimicrob Agents Chemother 48: 4793-4799.
18. Briñas L, Zarazaga M, Sáenz Y, Ruiz-Larrea F, Torres C (2002) β-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob Agents Chemother 46: 3156-3163.
19. Mathers A (2016) Mobilization of carbapenemase-mediated resistance in *Enterobacteriaceae.* Microbiol Spectr 4: 201-212.
20. Perozo-Mena A, Castellanos-Gonzalez M, Ling E, Gomez L, Ginestre M, Rincon G (2016) Presence of KPC-type carbapenemase in clinical isolates of *Klebsiella pneumoniae* from intensive care unit patients. Kasmera 44: 44-52 [Article in Spanish].
21. Martínez D, Marcano D, Rodulfo H, Salgado N, Cuaical N, Rodriguez L (2015) KPC and VIM producing *Enterobacter cloacae* strain from a hospital in northeastern Venezuela. Invest Clin 56: 182-187.
22. Antunes NT, Lamoureaux TL, Toth M, Stewart NK, Frase H, Vakulenko SB (2014) Class D beta-lactamases: Are they all carbapenemases ?. Antimicrob Agent Chemother 58: 2119-2125.
23. Pires J, Novais Â, Peixe L (2013) Blue-Carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. J Clin Microbiol 51: 4281-4283.
24. Han JH, Goldstein EJC, Wise J, Bilker WB, Tolomeo P, Lautenbach E (2017) Epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in a network of long-term acute care hospitals. Clin Infect Dis 64: 839-844.
25. Rolain JM, Cornaglia G (2014) Carbapenemases in *Enterobacteriaceae*: The magnitude of a worldwide concern. Clin Microbiol Infect 20: 819-820.
26. Thomson G, Turner D, Brasso W, Kircher S, Guillet T, Thomson K (2017) High-stringency evaluation of the automated BD Phoenix CPO detect and Rapidec Carba NP tests for detection and classification of carbapenemases. J Clin Microbiol 55: 3437-3443.

Corresponding author

Liliana Gomez Gamboa, MD, PhD
Microbiology Chair, School of Medicine, Medical Faculty,
University of Zulia, Av. 20, next to Castillo Plaza Hospital,
Paradise Sector.
4004, Maracaibo, Venezuela.
Tel: 58 412 2382372
Email: lilianagomezgamboa@gmail.com

Conflict of interests: No conflict of interests is declared.