

BRIEF REPORT

MOLECULAR CHARACTERIZATION OF CARBAPENEMASES IN PERU DURING 2019

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ABSTRACT

Resistance to carbapenems is a public health problem. This study presents the identification of carbapenemase enzymes in Enterobacteriaceae, *Pseudomonas spp.* and *Acinetobacter spp.* present in strains from 30 institutions that provide health services in Peru as part of the quality control process in diagnoses. Phenotypic confirmation and enzymatic identification were performed using the Blue CARBA test and the synergy test with phenylboronic acid and ethylenediaminetetraacetic acid/sodium mercaptoacetic acid discs. 185 strains with carbapenemases were identified: 78 in Enterobacteriaceae, 61 in *P. aeruginosa* and 46 in *Acinetobacter spp.* The types of carbapenemases identified were: *blaKPC*, *blaNDM*, *blaIMP*, *blaVIM*, *blaOXA-23*, *blaOXA-24*, *blaOXA-51* and the *blaVIM/IMP* co-production. It is important to strengthen the promotion of the rational use of antimicrobials and epidemiological surveillance in the country's hospitals.

Keywords: Drug resistance; Carbapenemases; Acinetobacter; Enterobacteriaceae; Pseudomonas; Peru (source: MeSH NLM).

INTRODUCTION

Infections caused by bacteria that are multiresistant to antibiotics are a global public health problem due to the great impact they have on morbidity and mortality; they caused nearly 250,000 deaths in 2017⁽¹⁾. Because of this, the World Health Organization (WHO) published a list of priority bacteria to be monitored due to their greater risk to human health and the hospital environment, including species such as *Acinetobacter baumannii*, *Pseudomonas spp.* and the carbapenem-resistant Enterobacteriaceae family⁽²⁾.

These species and especially gram-negative carbapenemase enzyme-forming bacteria⁽³⁾ have been reported since the 1990s⁽⁴⁾ and are currently categorized into enzyme families of different classes (A, B, C and D). Some of these classes, such as the metallo- β -lactamases (MBLs) initially represented a threat in few geographic areas, but later expanded to areas of Europe, Asia and America, and now is considered an epidemic that generates resistance to multiple drugs⁽⁵⁾.

In Latin America and the Caribbean, classes A, B and D described by Ambler⁽⁶⁾ were identified; the KPC type (isolated for the first time in 2001 in the United States) predominates among the mobile genetic elements and integrons of these 3 classes⁽⁷⁾. This suggests that specific strains have successfully spread, becoming endemic in some countries such as Brazil, Colombia, Argentina and Mexico⁽⁸⁾. Therefore, the aim of this study is to describe the current status of circulating carbapenemases in Peru in order to strengthen epidemiological surveillance of healthcare-associated infections (HAIs) and multidrug-resistant bacteria,

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as well as to strengthen the rational use of antimicrobials program (RUAP) in public and private hospitals.

THE STUDY

Descriptive observational cross-sectional study conducted on strains from 30 health care institutions (HCI) from 12 regions of Peru from January to December 2019. The population consisted of 331 strains sent for diagnostic confirmation to the National Referral Laboratory for Intrahospital Infections (LRNIH) of the Instituto Nacional de Salud (INS).

For the identification of the strains, we used selective culture media such as MacConkey agar and biochemical media (Citrato, TSI, LIA, MIO, Urea) ⁽⁹⁾, of the Becton and Dickinson brand. Antimicrobial sensitivity was determined by the Kirby Bauer method and the minimum inhibitory concentration (MIC) by the Epsilon Test method (E-Test, Liofilchem) following the interpretation guidelines for halos proposed by the CLSI (Clinical and Laboratory Standards Institute) ⁽¹⁰⁾. We used the Blue CARBA test for phenotypic confirmation of carbapenemase production ⁽¹¹⁾, and for identification, we used the synergy test with phenylboronic acid discs (APB, Liofilchem) and ethylenediaminetetraacetic acid/sodium mercaptoacetic acid (EDTA/SMA, Bioanalyse). Internal quality controls for sensitivity discs and culture media were performed with strains from the Antimicrobial Service of the National Referral Laboratory for Antimicrobial Resistance INEI-ANLIS Dr. Carlos G. Malbrán in Argentina.

Bacterial DNA extraction was obtained from previously identified carbapenem-resistant strains using the supernatant for polymerase chain reaction (PCR) amplification using specific primers designed for *blaKPC*, *blaNDM*, *blaIMP*, *blaVIM*, *blaOXA-23*, *blaOXA-24*, *blaOXA-51*, *blaOXA-58*, *blaOXA-48*, and *blaOXA-143* ⁽¹²⁾. Finally, the products were analyzed on a 1.5% agarose gel in an ultraviolet (UV) transilluminator (Figure 1) using positive and negative controls obtained from the INEI-ANLIS Latin American Quality Control Program strain collection.

Regarding ethical considerations, we maintained strict confidentiality of the data from the institutions from which the strains were obtained, as we followed the regulations of the Helsinki Declaration. This research was carried out within the framework of the quality control process for

KEY MESSAGES

Motivation for the study: In Peru, resistance to carbapenems is a public health problem, there is an increasing dissemination of multidrug-resistant bacteria encoded mostly by plasmids that can pass from one bacterial genus to another; therefore, it is necessary to know the types of genes circulating in the country.

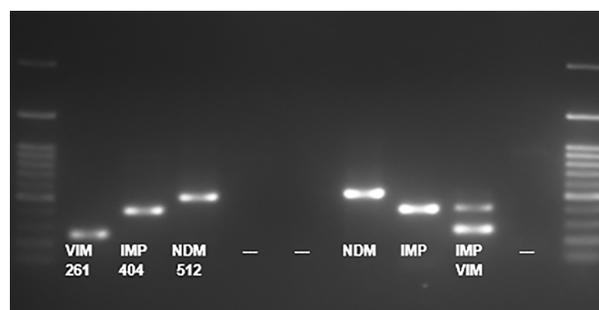
Main findings: We identified 185 strains with class A, B and D carbapenemase enzymes from 30 health care institutions in Peru during 2019. Their prevalence was 59.7%, class B was the most frequent, and the most frequently detected genes were *blaNDM*, *blaIMP*, *blaOXA24*-like, *blaKPC* and *blaOXA23*-like.

Implications: Information on the health impact of carbapenem resistance should help strengthen epidemiological surveillance programs.

diagnostic confirmation of samples sent to the INS from HCIs nationwide. Finally, the approval of an Ethics Committee was not required due to the characteristics previously described.

FINDINGS

A total of 331 strains were obtained from 12 regions of the country, 70% (n=21) from MINSAs HCIs and 23% (n=7) from EsSalud. Most HCIs were located in Lima and Callao (56.7%;



N°	Code	Species	PCR
M	Marcador	Ladder 100 pb GeneDirex	--
1	OPS 228	<i>Providencia rettgeri</i> (VIM)	VIM
2	OPS 222	<i>Acinetobacter ursingii</i> (IMP)	IMP
3	OPS 234	<i>Proteus mirabilis</i> (NDM)	NDM
4	ATCC 25922	<i>Escherichia coli</i> (negative control)	Neg
5	Mix PCR	System control	Neg
6	IIH-054-20	<i>Klebsiella pneumoniae</i>	NDM
7	IIH-061-20	<i>Pseudomonas aeruginosa</i>	IMP
8	IIH-062-20	<i>Pseudomonas aeruginosa</i>	IMP/VIM
9	IIH-067-20	<i>Escherichia coli</i>	Neg
M	Marker	Ladder 100 pb GeneDirex	--

Figure 1. Polymerase chain reaction products observed in 1.5% agarose gel under ultraviolet light

n=17). Of these strains, 21 were initially excluded because they were not viable or were contaminated, resulting in a final sample of 310 viable isolates. Of these, 87.4% (n=271) were gram-negative bacteria. The prevalence of carbapenemases was 59.7% (n=185), of which 42.2% (n=78) corresponded to Enterobacteriaceae; 32.9% (n=61) to *P. aeruginosa* and 24.9% (n=46) to *Acinetobacter spp.* Additionally, 27.7% (n=86) were strains sensitive to carbapenems.

Regarding the geographical distribution of carbapenemases, Figure 2 shows distribution by region and, in the case of Lima, by district based on the location of the HCI. A greater number of carbapenemase isolates were found in MINSA (18.4%) and EsSalud (81.6%) institutions, and in the regions of Cusco (12.4%), La Libertad (6.4%), Callao (6.4%) and Lambayeque (4.9%).

Carbapenemases from class A were reported in 12.4% (n=23) of the cases, with the *blaKPC* type found in *Klebsiella pneumoniae* and *Escherichia coli*. According to the type of carbapenemases, class A was reported in 12.4% (n=23), with the *blaKPC* type found in *Klebsiella pneumoniae* and *Escherichia coli*. Class B was reported in 62.7% (n=116) of the cases, with *blaNDM* type (n=57) found in *K. pneumoniae*, *E. coli*, *Providencia rettgeri*; and in *P. aeruginosa*, *blaIMP* type (n=32) and *blaVIM* type (n=13) as well as *blaIMP/VIM* type coproduction (n=14) were found. Class D was reported in 24.9% (n=46) with only *blaOXA23*-like and *blaOXA24*-like types found in *Acinetobacter spp.* The *blaOXA51*-like gene weakly hydrolyzes penicillins and carbapenems and is used as a species marker for *A. baumannii*, and was not reported in this series (Table 1).

Antimicrobial resistance was found in 50% (n=39) of Enterobacteriaceae, mainly against fosfomicin (therapeutic option for carbapenem-resistant strains); regarding *Pseudomonas spp.*, resistance to ceftolozane/tazobactam was reported in all cases and in *Acinetobacter spp.* resistance to minocycline was 38.4% (n=17).

DISCUSSION

In 1988 the first carbapenemase was reported in Japan, and five years later it was reported for the first time in a Latin American country (Argentina), initiating a chain of reports up to the present time^(8,13). In 2013 in Peru the first case of *blaKPC-2* carbapenemases in a *K. pneumoniae* strain derived from a blood culture at the Hospital Nacional Arzobispo Loayza was reported⁽¹⁴⁾. Since that first report, cases have increased, even showing the presence of resistance genes⁽¹⁵⁾.

Regarding class A carbapenemases, the *blaKPC* type identified in this study is the third most frequently reported (12.4%) and is present in 23 strains of Enterobacteriaceae (22 in *K. pneumoniae* and 1 in *E. coli*) in four regions of the country (Arequipa, Lima, Lambayeque and Callao). This gene was first reported in 2005 in Colombia⁽⁸⁾ and was the first *blaKPC* reported in Peru (Lima)⁽¹⁴⁻¹⁶⁾. Its increased and distributed presence in HCIs in highly populated regions is an important finding, since its presence in blood is associated with high lethality rates in hospitalized patients receiving effective antibiotic treatment⁽¹⁷⁾.

Class B was the most frequent type of carbapenemase reported in this study (62.7%), 53 *blaNDM* gene strains were identified (44 in *K. pneumoniae*; 9 in *E. coli*), which were found in six regions of the country (Ancash, Cusco, Lambayeque, Loreto, Lima and Callao). The first worldwide report of these strains was in Sweden in 2008 in a patient from India⁽⁸⁾ and later in 2011 the first case in Latin America was identified in Guatemala^(8,18). In Peru, this strain was first reported in 2013 at the Edgardo Rebagliati Martins National Hospital and subsequently spread throughout the national territory^(15,18). The *blaVIM* gene was found in 7.0% (n=13) of the total carbapenemases analyzed in this study and present in *P. aeruginosa* strains from three HCIs in Lima and Loreto.

Regarding the *blaIMP* gene in our study, we found it in 17.3% of the total samples, being detected in *P. aeruginosa* strains from ten HCIs in six regions (Apurímac, Ayacucho, Cusco, Loreto, Lima and Callao). The previously mentioned genes are among the most widespread in the world. In Peru, they were first reported in 2013⁽¹⁹⁾. These strains use a plasmid diffusion mechanism that facilitates replication in accelerated proportions and with low energy consumption, which allows the transfer of genes, such as those related to anti-microbial resistance, favoring their dissemination in hospitals and larger regions.

This study reports for the first time the co-production of *blaIMP/VIM* carbapenemases in *P. aeruginosa* strains, found in 7.6% (n=14) of the total samples from five HCIs in Lima, Loreto and Callao. Its existence can be explained by plasmid transmission since both are encoded by class 1 integrons that have been described since 2006 in Poland⁽²⁰⁾. Their importance for public health lies in their simple transmission mechanism and the consequent multiplying and disseminating effect related to antibacterial resistance.

Class D carbapenemases were the second most frequent (24.9%), with 27 strains with the *blaOXA24*-like gene in

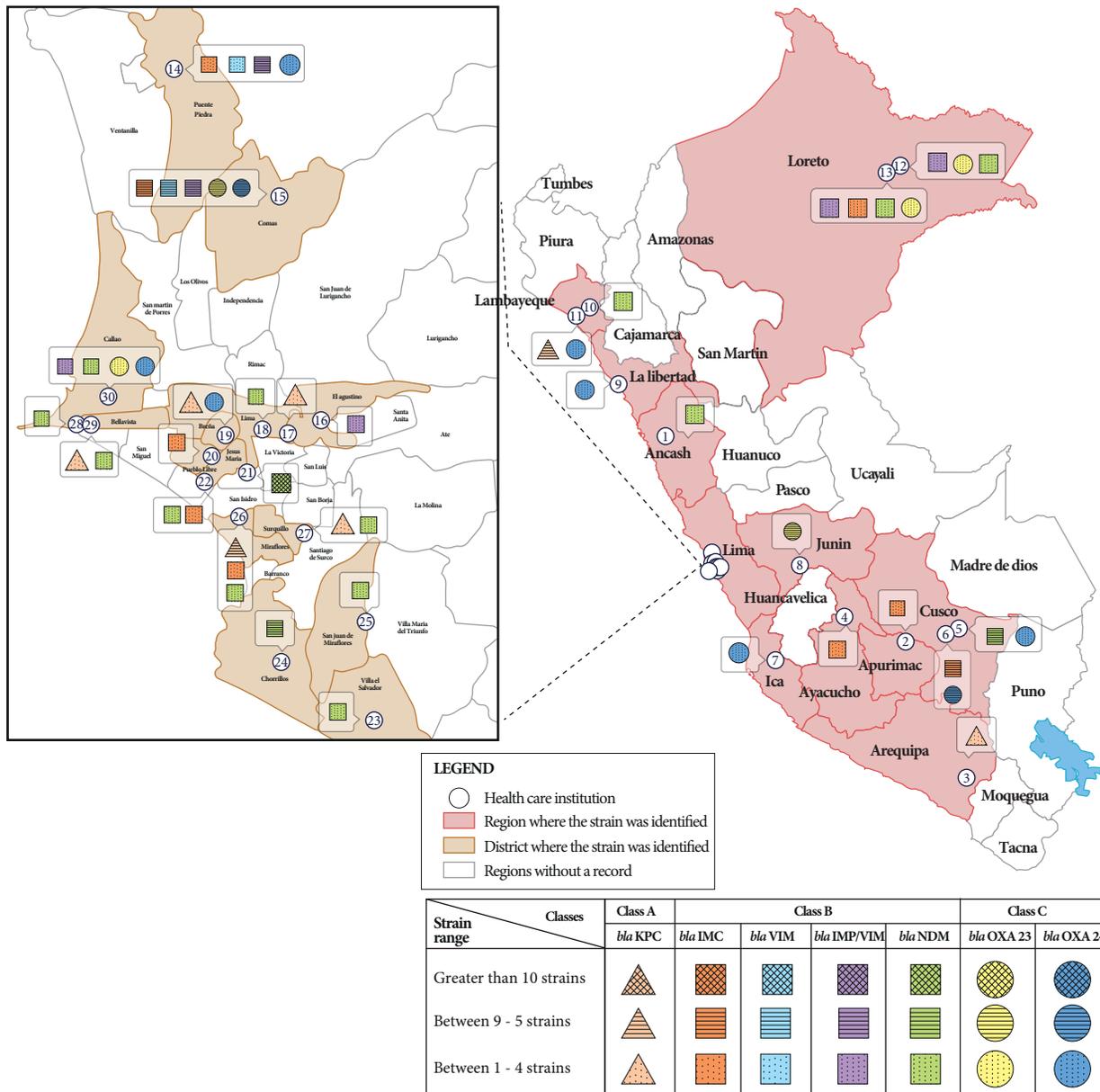


Figure 2. Distribution of Class A, B and D Carbapenemases in Peru during 2019, by geographic area.

1: Víctor Ramos Guardia Hospital (MINSa), 2: Abancay Regional Hospital (MINSa), 3: Alberto Seguin Escobedo Hospital (ESSALUD), 4: Ayacucho Regional Hospital (MINSa), 5: Cusco Regional Hospital (MINSa), 6: Antonio Lorena Hospital (MINSa), 7: Ica Regional Hospital (MINSa), 8: Daniel Alcides Carrión Regional Hospital of Junín (MINSa), 9: Trujillo Teaching Regional Hospital (MINSa), 10: Luis Heyen Inchaustegui Hospital (ESSALUD), 11: Lambayeque Regional Hospital (MINSa), 12: Iquitos Hospital III (ESSA- LUD), 13: Loreto Hospital Regional (MINSa), 14: Carlos Lanfranco La Hoz Hospital (MINSa), 15: Sergio E. Bernales Hospital (MINSa), 16: Hipólito Unanue Hospital (MINSa), 17: Dos de Mayo Hospital (MINSa), 18: Grau Emergency Hospital (ESSALUD), 19: Instituto Nacional del Niño (MINSa), 20: Santa Rosa Hospital (MINSa), 21: Edgardo Rebagliati Martins Hospital (ESSALUD), 22: Central Military Hospital, 23: H. Emergency Hospital Villa el Salvador (MINSa), 24: Instituto Nacional de Rehabilitación (MINSa), 25: María Auxiliadora Hospital (MINSa), 26: FAP Central Hospital, 27: Instituto Nacional de Enfermedades Neoplásicas (MINSa), 28: Daniel Alcides Carrión Hospital (MINSa), 29: Alberto Sabogal Sologuren Hospital (ESSALUD), 30: A. Leonardo Barton Hospital (ESSALUD).

Acinetobacter spp. from nine HCIs from Cusco, Ica, La Libertad, Lambayeque, Lima and Callao; and 19 with the *bla*OXA23-like gene in five HCIs from Junín, Loreto, Lima and Callao. No strains with *bla*OXA 48-like, *bla*OXA58-like and *bla*OXA143-like genes were found despite the fact

that specific primers were created for them. In the scientific literature, the *bla*OXA-24 gene was identified for the first time in 1997 in Spain and *bla*OXA-23 for the first time in 1997 in Scotland⁽⁸⁾. In Peru these genes were isolated for the first time in 2014 at HCIs in Lima⁽¹⁹⁾, and possess an amplified

Table 1. Types of carbapenemase according to strain in health care institutions in Peru in 2019.

Carbapenemase Type	n (185)	%
Class A (n=23; 12.4 %)		
KPC (<i>Klebsiella pneumoniae</i>)	22	11.9
KPC (<i>Escherichia coli</i>)	1	0.5
Class B (n=116; 62.7%)		
NDM (<i>Klebsiella pneumoniae</i>)	44	23.8
NDM (<i>Escherichia coli</i>)	9	4.9
NDM (<i>Providencia rettgeri</i>)	2	1.1
NDM (<i>Pseudomonas aeruginosa</i>)	2	1.1
VIM (<i>Pseudomonas aeruginosa</i>)	13	7.0
IMP (<i>Pseudomonas aeruginosa</i>)	32	17.3
IMP/VIM (<i>Pseudomonas aeruginosa</i>)	14	7.6
class D (n=46; 24.9%)		
OXA-23-like (<i>Acinetobacter spp.</i>)	19	10.3
OXA-24-like (<i>Acinetobacter spp.</i>)	27	14.6
OXA-48-like	0	0.0
OXA-51-like	0	0.0
OXA-58-like	0	0.0
OXA-143-like	0	0.0

KPC: *Klebsiella pneumoniae* producer of carbapenemase, NDM: New Delhi metallo-beta-lactamase, IMP: imipenem-hydrolyzing metallo-beta-lactamase, VIM: Verona integron-encoded metallo-beta-lactamase.

spectrum of activity on cephalosporins and carbapenems. An example of this is *Acinetobacter baumannii*, which has acquired antibiotic resistance mechanisms through the production of oxacillinase, in addition to resistance against carbapenems, aminoglycosides, quinolones and polymyxins, which complicates treatment, not only considering the broad spectrum of resistance but also the limitations in diagnosis

REFERENCES

- World Health Organization. Antibacterial agents in clinical development. An analysis of the antibacterial clinical development pipeline, including tuberculosis. Geneva: WHO; 2018 (Accessed on September 22, 2020). Available at: https://www.who.int/medicines/areas/rational_use/antibacterial_agents_clinical_development/en/.
- World Health Organization. Global priority list of antibiotic resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: WHO; 2017. (Accessed on September 22, 2020). Available at: <http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/>.
- Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA *et al.* Carbapenemase-Producing Organisms: A Global Scourge. *Clin Infect Dis*. 2018;66(8):1290-7. doi:10.1093/cid/cix893.
- Jaurin B, Grundstrom T. AmpC cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of β -lactamases of the penicillinase type. *Proc Natl Acad Sci USA*. 78(8):4897-901. doi:10.1073/pnas.78.8.4897.
- Bush K. Past and Present Perspectives on β -Lactamases. *Antimicrob Agents Chemother*. 2018;62(10):e01076-18. doi:10.1128/AAC.01076-18.
- Ambler RP. The structure of β -lactamases. *Phil Trans R Soc Lond B*. 1980;289:321-31. doi:10.1098/rstb.1980.0049.
- Vera-Leiva A, Barria-Loaiza C, Carrasco-Anabalón S, Lima C, Aguayo-Reyes A, Domínguez M, *et al.* KPC: *Klebsiella pneumoniae* carbapenemase, principal carbapenemase en enterobacterias. *Rev Chil Infectol*. 2017;34(5):476-484. doi:10.4067/S0716-10182017000500476.
- Escandón-Vargas K, Reyes S, Gutiérrez S, Villegas MV. The epidemiology of carbapenemases in Latin America and the Caribbean. *Expert Rev Anti Infect Ther*. 2017;15(3):277-297. doi:10.1080/14787210.2017.1268918.

9. Sacaquispe R, Ventura G. Manual de procedimientos bacteriológicos de Infecciones Intrahospitalarias. Lima: Instituto Nacional de Salud; 2001 (Accessed on September 22, 2020). Available at: http://ftp2.minsa.gob.pe/descargas/OGCI/proyectosterminados/Proyecto_vigia/Doc12.pdf.
10. Performance Standards for Antimicrobial Susceptibility Testing. Clinical Laboratory Stand; 2020 (Accessed on September 22, 2020). Available at: <https://clsi.org/standards/products/microbiology/documents/m100/>
11. Laboratorio Nacional de Referencia en Antimicrobianos, INEI-ANLIS "Dr. Carlos G. Malbrán." Blue Carba- Detección rápida de carbapenemasas directo de placas de cultivo. Protocolo del Servicio ANTIMI- CROBIANOS; 2014 (Accessed on September 22, 2020). Available at: <http://antimicrobianos.com.ar/ATB/wp-content/uploads/2014/10/BLUE-CARBA-.pdf>.
12. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis.* 2011;70(1):119-23. doi:10.1016/j.diagmicrobio.2010.12.002.
13. Rada AM, Hernández-Gómez C, Restrepo E, Villegas MV. Distribución y caracterización molecular de betalactamasas en bacterias Gram negativas en Colombia, 2001-2016. *Biomédica.* 2019;39:199-20. doi:10.7705/biomedica.v39i3.4351.
14. Velásquez J, Hernández R, Pamo O. Klebsiella pneumoniae resistente a los carbapenemes. Primer caso de carbapenemasa tipo KPC en Perú. *Rev Soc Peru Med Interna.* 2013;26(4):5.
15. Sacaquispe-Contreras R, Bailón-Calderón H. Identificación de genes de resistencia a carbapenémicos en enterobacterias de hospitales de Perú, 2013-2017. *Rev Peru Med Exp Salud Pública.* 2018;35(2):259-64. doi:10.17843/rpmesp.2018.352.3829.
16. Krapp F, Amaro C, Ocampo K, Astocondor L, Hinostroza N, Riveros M, et al. Comprehensive characterization of the emerging carbapenem-resistant klebsiella pneumoniae clinical isolates from a public Hospital in Lima, Peru. *Open Forum Infect Dis.* 2018;5(1). doi:10.1093/ofid/ofy210.1022.
17. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber, MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. *Lancet Infect Dis.* 2013;13(9):785-796. doi:10.1016/S1473-3099(13)70190-7.
18. Organización Panamericana de la Salud. Alerta epidemiológica: primer hallazgo de carbapenemasas de tipo New Delhi metalobetalactamasas (NDM) en Latinoamérica. Washington, D. C: OPS; 2011 (Accessed on September 22, 2020). Available at: <https://www.sdpt.net/ALERTAEPIDEMIOLOGICO1.htm>
19. Levy-Blitchein S, Roca I, Plasencia-Rebata S, Vicente-Taboada V, Velásquez-Pomar J, Muñoz L, et al. Emergence and spread of carbapenem-resistant Acinetobacter baumannii international clones II and III in Lima, Peru. *Emerg Microbes Infect.* 2018;7(1):1-9. doi:10.1038/s41426-018-0127-9.
20. Lee K, Lee WG, Uh Y, Ha GY, Cho J, Chong Y. VIM- and IMP-Type Metallo- β -lactamase- Producing Pseudomonas spp. and Acinetobacter spp. in Korean Hospitals. *Emerg Infect Dis.* 2003;9(7):4. doi:10.3201/eid0907.030012.