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Investigação dos genes *bla*$_{KPC}$ e *bla*$_{NDM}$ em Enterobactérias recebidas em um Laboratório de Saúde Pública

Investigation of blaKPC and blaNDM genes in Enterobacteriaceae received in a Public Health Laboratory

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RESUMO

Introdução: A produção de carbapenemases dos tipos KPC e NDM é um importante mecanismo enzimático de resistência aos carbapenêmicos em bactérias da família Enterobacteriaceae. Estas enzimas degradam os antibióticos beta-lactâmicos e são codificadas pelos genes *bla*$_{KPC}$ e *bla*$_{NDM}$, que podem estar localizados em elementos genéticos móveis como plasmídeos e transposons. **Objetivos:** Avaliar a taxa de positividade de *bla*$_{KPC}$ e *bla*$_{NDM}$ em enterobactérias resistentes aos carbapenêmicos recebidos no Intituto Adofo Lutz (IAL) de São José do Rio Preto e pesquisar dados epidemiológicos dos pacientes cujos isolados foram recuperados. **Métodos:** No período de junho de 2015 a abril de 2019 foram recebidos isolados bacterianos resistentes aos carbapenêmicos da região de São José do Rio Preto. No laboratório de bacteriologia e biologia molecular foram realizadas a extração de DNA e a PCR em tempo real para investigação dos genes *bla*$_{KPC}$ e *bla*$_{NDM}$. Em seguida, foi feito o levantamento dos dados epidemiológicos, tais como, o município de origem, idade e gênero dos pacientes cujos isolados bacterianos foram recuperados. **Resultados:** A amostragem total do estudo foi de 934 isolados de enterobactérias provenientes de diferentes hospitais localizados em cinco municípios da região. Destes; 93,4% foram positivos para bla$_{KPC}$, sendo 96,3% em isolados do gênero *Klebsiella* sp. e 1,85% dos isolados do gênero *Enterobacter* sp. e da espécie *Escherichia coli* , respectivamente; 52,5% dos isolados foram obtidos de mulheres e 84,4% de pacientes idosos. O gene *bla*$_{NDM}$ foi detectado apenas em três isolados, sendo dois deles provenientes de culturas de vigilância. **Conclusão:** Os resultados gerados evidenciaram que enterobactérias produtoras de KPC estão disseminadas em todas unidades de saúde dos cinco municípios estudados, sugerindo que os isolados de *Klebsiella sp.* carreadores de *bla*$_{KPC}$ possam ser endêmicos nestas instituições. Pudemos também notar o importante papel das culturas de vigilância na prevenção da disseminação de genes de resistência, como observado para *bla*$_{NDM}$ neste estudo.

Palavras-Chave: Infecções Bacterianas; Carbapenêmicos; Farmacorresistência Bacteriana.

ABSTRACT

Introduction: *KPC and NDM carbapenemases production is an important enzymatic mechanism of resistance to carbapenens in bacteria belonging to the Enterobacteriaceae family. These enzymes degrade virtually all beta-lactam antibiotics and are encoded by the bla*$_{KPC}$ *and bla*$_{NDM}$ *genes, which can be in mobile genetic elements such as plasmids and transposons.* ***Objectives:*** *This study evaluated the positivity rate of the presence of bla*$_{KPC}$ *and bla*$_{NDM}$ *genes in carbapenem-resistant enterobacteria received at the Instituto Adolfo Lutz (IAL) of São José do Rio Preto, Brazil and determined the epidemiological data related to the patients whose isolates were recovered.* ***Methods:*** *From June 2015 to April 2019, bacterial isolates were obtained from different hospitals located in five municipalities in São José do Rio Preto region. In the bacteriology and molecular biology laboratory, DNA extraction and real-time PCR were performed to investigate the bla*$_{KPC}$ *and bla*$_{NDM}$ *genes. Afterwards, epidemiological data were surveyed such as the municipality of origin, age, and gender of the patients whose bacterial isolates were recovered.* ***Results:*** *A total of 934 enterobacteria isolates were recovered from the different hospitals. Of these; 93.4% were positive for bla*$_{KPC}$*, with 96.3%, 1.85%, and 1.85% of the isolates belonged to the Klebsiella genus, Enterobacter genus, and Escherichia coli species, respectively. Also, 52.5% and 84.4% of the isolates were obtained from women and elderly patients, respectively. The bla*$_{NDM}$ *gene was detected only in three isolates, two of which originated from surveillance cultures.* ***Conclusion:*** *Therefore, KPC-producing enterobacteria are widespread in all health units of the five municipalities that were studied, suggesting that the blaKPC-carrying Klebsiella sp. isolates may be endemic in these institutions. Additionally, there is a significant role of surveillance cultures in preventing the spread of resistance genes, as observed for bla*$_{NDM}$ *in this study.*

Keywords: *Bacterial Infections; Carbapenems; Drug Resistance, Bacterial.*

Contribuição dos autores: LZM: coleta de dados, tabulação de dados e redação do manuscrito. ECA: execução dos testes laboratoriais. DFM: execução dos testes laboratoriais. APL: tabulação dos dados. FMTB: delineamento do estudo, execução dos testes laboratoriais, discussão dos achados e revisão do manuscrito. MMCNS: delineamento do estudo e revisão do manuscrito. MPS: orientação do projeto, delineamento do estudo, discussão dos achados e elaboração do manuscrito.

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INTRODUCTION

Healthcare-associated infections are defined as infections that manifest themselves during hospitalization or after discharge. These infections represent a serious global public health issue, as they lead to longer hospitalization, higher healthcare costs, and increased morbidity and mortality rates[1-2]. Members of the Enterobacteriaceae family, mainly the species *Klebsiella pneumoniae*, *Enterobacter cloacae*, and Escherichia coli, are well-known on account of their high capacity to acquire resistant mechanisms as well as their frequent association with severe opportunistic infections in hospitalized patients, mainly in immunosuppressed individuals with underlying diseases such as wounds, intra-abdominal infections, urinary tract infections and sepsis[3-4].

Carbapenems are a class of antimicrobial agents reserved for infections caused by multidrug-resistant microorganisms. However, resistance to carbapenems has increased. It is becoming a serious public health threat, representing a major limitation in establishing effective antimicrobial therapy[5]. This type of antimicrobial resistance is spreading at an alarming rate, resulting in major outbreaks and treatment failure of community-acquired and nosocomial infections caused by the clinically relevant carbapenem producing *Enterobacteriaceae*[6].

Two particularly important carbapenemases in Enterobacteriaceae are encoded by the genes bla_{KPC} (*Klebsiella pneumoniae* carbapenemase) and bla_{NDM} (New Delhi Metallo-β-lactamase-1). The first report of the bla_{KPC} gene occurred in the United States , in 1996, in a K. pneumoniae isolate, while in Brazil, the first report was in Recife, in 2009[7].

The bla_{NDM} gene was first reported , in 2009, in New Delhi (India), from *K. pneumoniae*[8]; this gene is common in the Enterobacteriaceae family and has been detected in several species such as *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter cloacae*[9]. In Brazil, the first cases of NDM-1 infection and colonization were confirmed at the Conceição Hospital in Porto Alegre, Brazil, where five patients were infected or colonized between September 2012 and April 2013[10].

The objectives of this study were to evaluate the positivity rate of bla_{KPC} and bla_{NDM} genes in all carbapenem resistant bacterial clinical isolates received at the IAL of São José do Rio Preto from June 2015 to April 2019. Afterwards, we determined the species, city of origin, age, and gender of the patients who were infected with the carbapenem resistant bacterial clinical isolates.

METHODS

Selection of Isolates

Resistance to at least one carbapenem antibiotic was the criterion for receiving the studied isolates at the Adolfo Lutz Institute between June 2015 and April 2019. These isolates were obtained from five hospitals from the largest city; São José do Rio Preto (around 460,000 inhabitants) and from hospitals of nearby cities such as Catanduva, Votuporanga, Fernandópolis, and Jales, with an estimated population of 122,000, 95,000, 69,000, and 49,000 inhabitants, respectively. The bacterial isolates were recovered from positive cultures of several clinical specimens, such as blood, urine, tracheal aspirate, bronchoalveolar lavage, biopsy tissue fragments, and catheters. We received the bacterial isolates already identified from the origin hospital, and to check the species identification, we carried out the biochemical tests in our institute. This study was approved by the Research Ethics Committee (number 26144619.4.0000.0059).

DNA Extraction

DNA extraction was performed using a boiling protocol in which colonies of a recent pure culture (up to 24 h) grown on PROBAC MacConkey agar, TSA, or blood plates were selected and resuspended in 500 µL sterile water in a 1.5 mL tube, vortexed, and then incubated at 95–99˚C for 10 min. This mixture was heat shocked on ice for three to five minutes and then centrifuged at 16.1 rcf for three minutes. After centrifugation, the supernatant was collected and packed in PCR reactions for later use.

Detection of bla_{KPC} and bla_{NDM} genes by real time PCR

The investigation of bla_{KPC} and bla_{NDM} genes was performed by the multiplex real-time PCR method using the primers and protocol from the Centers of Disease Control (USA)[11]. The cycling conditions for qPCR were initial enzymatic activation at 95°C for three minutes, followed by 40 cycles of 95°C for 3 seconds, annealing, and then extension at 60°C for 3 seconds.

RESULTS

A total of 934 carbapenem-resistant enterobacteria isolates were received from health services or hospitals located across five cities in the Northwest of São Paulo state. Most of these isolates (n = 887, 89.32%) belonged to the *Klebsiella genus*, with *K. pneumoniae* being the most frequent species (n = 857, 91.55%), followed by Enterobacter sp. (n = 32, 3.42%) and *Escherichia coli* (n = 17, 1.82%).

KPC Results

Of the 934 patients considered cases; 93.4% of the isolates (873/934) were positive for bla_{KPC}, with 96.3% (841/873), 1.85% (16/873), and 1.85% (16/873) of the isolates detected in *Klebsiella* sp., *Enterobacter* sp. and *Escherichia coli*, respectively. It was observed that the *K. pneumoniae* specie presented the highest positive rate, corresponding to 93.2% (814/873).

Table 1 (below) reports the characteristics of patients with bla_{KPC} carriers. The highest proportion of the isolates came from patients admitted in hospitals located at São José do Rio Preto and Catanduva (the two most populous cities), followed by Votuporanga, Jales, and Fernandópolis. As expected, the positivity rate of bla_{KPC} was higher in isolates from the most populous cities, except for Jales, which presented a larger number of bla_{KPC} isolate carriers when compared to Fernandópolis.

Table 1. Characteristics of the bla_{KPC} positive isolates and their epidemiological data of the patients.

Variables		Number (N)	Percentage (%)
Species/Genus	*K. pneumoniae*	814	93.2
	Klebsiella sp.	27	3.1
	Enterobacter sp.	16	1.8
	Escherichia coli	16	1.8
City	São José do Rio Preto	782	89.6
	Catanduva	35	4.0
	Votuporanga	30	3.4
	Fernandópolis	2	0.2
	Jales	24	2.7
Sex	Male	415	47.5
	Female	458	52.5
Age Group (years)	0–1	9	1.0
	2–18	3	0.3
	19–44	58	6.6
	45–59	102	11.7
	60–90	663	76.0
	>90	38	4.4
Clinical Specimen	Urine	317	36.30
	Tracheal aspirate	181	20.73
	Secretions	117	13.40
	Surveillance swab	78	8.93
	Blood	66	7.56
	Biopsy	87	9.97
	Fluids	10	1.15
	Bronchoalveolar lavage	8	0.90
	Other	9	1.03

Many of the isolates were from females (52.5%); while 80.3% were from individuals older than 60 years. The most frequent clinical specimens were urine (n = 317, 36.3%), followed by tracheal aspirate (n = 181, 20.73%), secretions (n=117, 13.4%) and others (Table 1).

NDM Results

The *bla*$_{NDM}$ gene was detected twice in *Klebsiella pneumoniae* isolates and once in an Escherichia coli isolate. The bla$_{NDM}$-positive strains were all derived from São José do Rio Preto (Table 2).

Table 2. Patient characteristics of the blaNDM positive isolates in São José do Rio Preto-SP city.

Patient	Species	Age (years)	Sex	Year	Clinical Specimen
1	*E. coli*	70	Male	2016	Surveillance Swab
2	*K. pneumoniae*	60	Male	2017	Surveillance Swab
3	*K. pneumoniae*	69	Female	2018	Biopsy

DISCUSSION

In this study, the *bla*$_{KPC}$ gene was widespread in all the studied cities. They are the main cause of carbapenem resistance in the majority of the Enterobacteriaceae isolates. These results have corroborated with the data published from several other Brazilian institutions[12-15].

The *K. pneumoniae* species corresponded to the majority of the *bla*$_{KPC}$ gene carriers, followed by *Escherichia coli* and *Enterobacter cloacae*, as reported in other studies worldwide[16,17]. Similar results were found in a study carried out in Belo Horizonte, in which most of the infections were caused by KPC carrying Klebsiella pneumoniae[2].

The high prevalence of the bla$_{KPC}$ gene is a major concern owing to the KPC enzyme, which has the capacity to inactivate carbapenems and other extended spectrum beta-lactams, such as cephalosporins; thus, causing a significant decline in treatment options for the patients, leading to prolonged hospitalizations and higher mortality rates[18]. In addition, this gene has a high potential for dissemination via mobile genetic elements, and high mortality rates have been associated with infections caused by KPC-producing isolates[19]. These data showed the need for the adoption of better control measures, such as isolation of the infected patient, adequate hand washing, and disinfection of the environment to avoid the dissemination of bla$_{KPC}$.

To the best of our knowledge, this is the first study that has collected data on antimicrobial carbapenem resistance profiles and positivity rates of bla$_{KPC}$ and bla$_{NDM}$ genes in clinical isolates from the cities of Catanduva, Votuporanga, Jales, and Fernandópolis. In addition, it is the first report on bla$_{KPC}$ in hospitals located in these cities, showing important and relevant epidemiological data for this region.

This study found high rates of the bla$_{KPC}$ gene in elderly people, showing that this population needs additional care due to their vulnerability. A study conducted in a Brazilian hospital[20] showed that old age and the use of mechanical ventilations were potent risk factors in the dissemination of KPC. Other studies have also defined age-dependence along with mechanical breathing, heart disease, impaired functional status, cancer, and admission to intensive care units (ICUs) as risk factors for mortality due to infections by *K. pneumoniae*-KPC[21, 22].

Throughout the study period, the *bla*$_{NDM}$ gene was detected only three times in three different years (2016, 2017 and 2018) in isolates from two different institutions. In this case, it is interesting to observe the importance of surveillance cultures, as two of the *bla*$_{NDM}$ genes were recovered from surveillance swabs from the same hospital, suggesting that gene dissemination could be contained. Therefore, we have demonstrated that early detection and notification to surveillance authorities are very important for a rapid response to prevent gene dissemination.

CONCLUSION

In conclusion, KPC bacteria producers are prevalent in our regional hospitals, mainly in isolates of *Klebsiella* sp. in the five studied cities and that the elderly people were the most affected. Therefore, control measures, especially simple strategies such as ensuring proper hand hygiene by health professionals, which are useful in fighting the spread of these pathogens carrying *bla*$_{KPC}$ resistance genes. In addition, we highlight the importance of surveillance *bla*$_{KPC}$- positive cultures in preventing the spread of resistance genes, similar to *bla*$_{NDM}$ in our study.

REFERENCES

1. World Health Organization. Prevention of hospital-acquired infections: a practical guide [monografia na Internet]. 2. ed. Geneva: WHO; 2002. [acesso em 2019 Ago 22]. Disponível em: http://apps.who.int/iris/bitstream/handle/10665/67350/WHO_CDS_CSR_EPH_2002.12.pdf?sequence=1&isAllowed=y
2. Alvim ALS, Couto BRGM, Gazzinelli A. Epidemiological profile of healthcare-associated infections caused by Carbapenemase-producing Enterobacteriaceae. Rev Esc Enferm USP. 2019;53:e03474. http://dx.doi.org/10.1590/S1980-220X2018001903474
3. Abramowicz L, Gerard M, Martiny D, Delforge M, De Wit S, Konopnicki D. Infections due to carbapenemase-producing bacteria, clinical burden, and impact of screening strategies on outcome. Med Mal Infect. 2020;50(8):658-64. doi: 10.1016/j.medmal.2019.12.011
4. Zhou H, Zhang K, Chen W, Chen J, Zheng J, Liu C, et al. Epidemiological characteristics of carbapenem-resistant Enterobacteriaceae collected from 17 hospitals in Nanjing district of China. Antimicrob Resist Infect Control. 2020;9(5):2-10. https://doi.org/10.1186/s13756-019-0674-4
5. 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
6. Elshamy AA, Aboshanab KM. A review on bacterial resistance to carbapenems: epidemiology, detection and treatment options. Future Sci OA. [Internet]. 2020;6(3): FSO438. doi: 10.2144/fsoa-2019-0098
7. Monteiro J, Santos AF, Asensi MD, Peirano G, Gales AC. First report of KPC-2-producing Klebsiella pneumoniae strains in Brazil. Antimicrob Agents Chemother. 2009;53(1):333- 4. https://doi.org/10.1128/AAC.00736-08
8. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother. 2009;53(12):5046-54. doi: 10.1128/AAC.00774-09
9. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int. 2014;2014:1-12. doi: 10.1155/2014/249856
10. Rozales FP, Ribeiro VB, Magagnin CM, Pagano M, Lutz L, Falci DR, et al. Emergence of NDM-1-producing Enterobacteriaceae in Porto Alegre, Brazil. Int J Infect Dis. 2014;25:79-81. DOI:https://doi.org/10.1016/j.ijid.2014.01.005
11. Centers for Disease Control and Prevention homepage na Internet]. 2011 [acesso em 2019 Set 6]. Multiplex Real-Time PCR detection of K. pneumoniae carbapenemase (KPC) and New Delhi metallo-?-lactamase (NDM-1). [aproximadamente 2 telas]. Disponível em: https://www.cdc.gov/hai/settings/lab/kpc-ndm1-lab-protocol.html
12. Nicolás MF, Ramos PIP, Carvalho FM, Camargo DRA, Alves CFM, Morais GL, et al. Comparative genomic analysis of a clinical Isolate of Klebsiella quasipneumoniae subsp. similipneumoniae, a KPC-2 and OKP-B-6 Beta-Lactamases Producer Harboring Two Drug-Resistance Plasmids from Southeast Brazil. Front Microbiol. 2018;9:220. doi: 10.3389/fmicb.2018.00220
13. Rosa JF, Rizek C, Marchi AP, Guimaraes T, Miranda L, Carrilho C, et al. Clonality, outer-membrane proteins profile and efflux pump in KPC- producing Enterobacter sp. in Brazil. BMC Microbiol. 2017;17(1):69. doi: 10.1186/s12866-017-0970-1
14. Silva KE, Cayô R, Carvalhaes CG, Sacchi FPC, Rodrigues-Costa F, Ramos da Silva AC, et al. Coproduction of KPC-2 and IMP-10 in Carbapenem-Resistant Serratia marcescens Isolates from an Outbreak in a Brazilian Teaching Hospital. J Clin Microbiol. 2015;53(7):2324-8. doi: 10.1128/JCM.00727-15
15. Biberg CA, Rodrigues ACS, Carmo SF, Chaves CEV, Gales AC, Chang MR. KPC-2-producing Klebsiella pneumoniae in a hospital in the Midwest region of Brazil. Braz J Microbiol. 2015;46(2):501-4. http://dx.doi.org/10.1590/S1517-838246246220140174
16. Logan LK, Weinstein RA. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis. 201;215(Supl1):S28-36. doi: 10.1093/infdis/jiw282

17. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, et al. Epidemiology of Carbapenem-Resistant Enterobacteriaceae Infections: report from the China CRE Network. Antimicrob Agents Chemother. 2018;62(2):e01882-17. doi: 10.1128/AAC.01882-17
18. Manageiro V, Romão R, Moura IB, Sampaio DA, Vieira L, Ferreira E, et al. Molecular epidemiology and risk factors of Carbapenemase-Producing Enterobacteriaceae isolates in Portuguese Hospitals: results from European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE). Front Microbiol. 2018; 9:2834. DOI: 10.3389/fmicb.2018.02834
19. Wang Z, Qin R-R, Huang L, Sun L-Y. Risk Factors for Carbapenem-resistant Klebsiella pneumoniae infection and mortality of Klebsiella pneumoniae infection. Chin Med J. 2018;131(1):56–62. https://doi.org/10.4103/0366-6999.221267
20. Tuon FF, Rocha JL, Toledo P, Arend LN, Dias CH, Leite TM, et al. Risk factors for KPC-producing Klebsiella pneumoniae bacteremia. Braz J Infect Dis. 2012;16(5):416-9. https://doi.org/10.1016/j.bjid.2012.08.006
21. Lee C-R, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of Carbapenemase-Producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol. 2016;7:895. https:doi.org/10.3389/fmicb.2016.00895
22. Zhu W-M, Yuan Z, Zhou H-Y. Risk factors for carbapenem-resistant Klebsiella pneumoniae infection relative to two types of control patients: a systematic review and meta-analysis. Antimicrob Resist Infect Control. 2020;9(1):23. doi: 10.1186/s13756-020-0686-