RBFHSS
Revista Brasileira de Farmácia Hospitalar e Serviços de Saúde

Original Paper

Open Access

Profile of Carbapenemase Enzyme Encoding Genes in bacterial strains from a Teaching Hospital in Juiz de Fora-MG

Bárbara Oliveira REIS[1], Cristiane Marcos-Soares-Dias FERREIRA[1]

[1]Hospital Universitário da Universidade Federal de Juiz de Fora, Rua Catulo Breviglieri, s/n - Santa Catarina, Juiz de Fora - MG

Corresponding author: Reis BO, barbarareisjf@gmail.com

Submitted: 28-05-2022 Resubmitted: 18-08-2022 Accepted: 26-08-2022

Peer review: blind reviewers

Abstract

Objectives: To describe genes that generate resistance to Carbapenems in bacteria isolated from a teaching hospital, observing epidemiological and genetic aspects related to their resistance. **Methods:** Cross-sectional study of 37 bacterial strains obtained from September 2017 to October 2018, in a teaching hospital in Juiz de Fora - MG, previously classified as multidrug-resistant by the criteria of the Clinical and Laboratory Standards Institute (CLSI). Such strains were submitted to the search for resistance genes- bla_{KPC}, bla_{NDM} and bla_{OXA-48} - through Polymerase Chain Reaction. The species, microbiological material, resistance gene, sex and age of the individuals in the samples were identified. **Results:** There was a balance in the distribution of sex between the samples and the mean age of the individuals was 63.86 years. The species *Klebsiella oxytoca* was identified in 1 sample (2.7%), *Enterobacter cloacae* in 1 sample (2.7%) and *Klebsiella pneumoniae* in 35 samples (94.6%). The blaKPC gene was detected in 81.1% of the samples, while bla_{NDM} was not detected in the strains of this study. Even though bla_{OXA-48} was not tested in all samples, it was positive in 17% and, in addition, 8% of individuals had no resistance genes detected in their bacterial strains. **Conclusion:** Bacteria with genes encoding resistance to carbapenems and, mainly, bla_{KPC} strains, circulate widely, leading to reduced therapeutic options and increased risk of serious infections. Therefore, the prudent choice of antimicrobials combined with prevention strategies and hospital surveillance should be prioritized.

Keywords: Carbapenems; MDR genes; Bacterial Pharmacoresistance; Carbapenem-Resistant Enterobacteriaceae; beta-Lactamases.

Perfil de Genes Codificadores de Enzimas Carbapenemases em cepas bacterianas de um Hospital de Ensino de Juiz de Fora-MG

Resumo

Objetivos: Descrever genes geradores de resistência a Carbapenêmicos em bactérias isoladas de um hospital escola, observando aspectos epidemiológicos e genéticos relacionados à resistência das mesmas. **Métodos:** Estudo transversal de 37 cepas bacterianas obtidas de setembro de 2017 a outubro de 2018, em um hospital de ensino de Juiz de Fora- MG, previamente classificadas como multirresistentes pelos critérios do Clinical and Laboratory Standards Institute (CLSI). Tais cepas foram submetidas à pesquisa de genes de resistência- bla_{KPC}, bla_{NDM} e bla_{OXA-48} - através de Reação em Cadeia de Polimerase. Identificaram-se a espécie, o material microbiológico, o gene de resistência, o sexo e a idade dos indivíduos referentes às amostras. **Resultados:** Houve equilíbrio na distribuição do sexo e a idade média dos indivíduos foi 63,86 anos. A espécie *Klebsiella oxytoca* foi identificada em 1 amostra (2,7%), *Enterobacter cloacae* em 1 amostra (2,7%) e *Klebsiella pneumoniae* em 35 amostras (94,6%). O gene bla_{KPC} foi detectado em 81,1% dos pacientes, enquanto bla_{NDM} não foi detectado nas cepas deste estudo. Mesmo não tendo sido realizada a pesquisa de bla_{OXA-48} em todos os materiais, ele foi positivo em 17% e, além disso, 8% dos indivíduos não tiveram genes de resistência detectados em suas cepas bacterianas. **Conclusão:** As bactérias com genes codificadores de resistência aos Carbapenêmicos e, principalmente, de cepas $bla_{KPC,}$ circulam amplamente, gerando redução das opções terapêuticas e aumento do risco de infecções graves. Portanto, a escolha prudente de antimicrobianos aliada a estratégias de prevenção e vigilância hospitalar devem ser priorizadas.

Palavras-chaves: Carbapenêmicos; Genes MDR; Farmacorresistência Bacteriana; Enterobacteriáceas Resistentes a Carbapenêmicos; beta-Lactamases.

Introduction

Antimicrobial resistance is currently one of the most relevant public health problems at the global level, as it increases morbidity and mortality in patients with Healthcare-Associated Infections by more than 50%, due to the lower availability of viable therapeutic options[1,2]. It is associated with error or absence of the diagnosis of pathologies, a situation in which physicians end up prescribing unnecessarily to avoid complications. In this context, there is also use of falsified medications or inappropriate in terms of dosage, administration route and coverage spectrum, limited professional training for efficient choice of antimicrobials and for carrying out hygiene measures, unsatisfactory sanitary control, and globalization with intense exchange of people and elements[3].

Although having a multifactorial cause, the main responsible for dissemination of the resistance genes are people themselves, whether due to imprudence or to lack of dexterity. In addition, despite all the publications, campaigns and information disseminated, irrational use of antimicrobials continues to increase, generating both financial costs and thousands of lives lost[3].

Antibiotics of the Carbapenem class have characteristically one of the broadest and most important antimicrobial spectra, due to their action against Gram-negative bacteria that produce chromosomal cephalosporinase enzymes and extended-spectrum beta-lactamases, being considered the last option for the treatment of serious infections by Gram-negative bacteria, even in monotherapy. Their spectrum also includes Gram-positive cocci, fermenting and non-fermenting Gram-negative bacilli and Gram-positive and Gram-negative anaerobials, including *Bacteroides fragilis.* And in this context, the emergence of carbapenem hydrolyzing beta-lactamases (Carbapenemases) threatens the enormous clinical usefulness of this class of antibiotics and creates the challenge of "extreme drug resistance" in Gram-negative microorganisms, or superbacteria[4,5].

During the studies conducted by Ambler, beta-lactamases were classified according to their amino-acid homology. While Classes A, C and D share a serine residue at their active site, Class B enzymes require the presence of some metal, usually zinc, for their activity [and are therefore referred to as metallo-beta-lactamases (MBL)]. Bush, Jacoby and Medeiros expanded and subdivided classification of beta-lactamases into Groups 1 to 4 according to enzyme substrate and inhibition profile by beta-lactamases inhibitors. NDM carbapenemases are MBL and therefore belong to Group 3a according to Bush, Jacoby and Medeiros. OXA carbapenemases belong to Class D according to Amber and to the 2df functional group[5]. The *Klebsiella Pneumoniae Carbapenemase* (KPC) group is clinically the most important in Class A, as it confers resistance to most beta-lactams. The genes that encode the KPC enzyme can be integrated into the bacterial chromosome although, most of the times, they are located in mobile elements, such as plasmids or transposons, which are transferable between different bacterial strains, species and genera. Thus, clinical outbursts are frequently complex, encompassing varied degrees of genetic dissemination mediated by clones, plasmids or transposons[4,6].

Given their relevance, several KPC variants have already been identified and some of them hydrolyze beta-lactams at variable rates, which can contribute to different susceptibility profiles in KPC-producing bacteria when tested *in vitro*. In Latin America, the KPC-type enzyme-producing bacterium was reported for the first time in 2006, with isolation of *K. pneumoniae* strains containing the bla_{KPC} gene in Colombia. It is noted that the KPC-type enzyme encoding gene can be transmitted from *Klebsiella sp*. to other genera, including *E. coli, P. aeruginosa, Citrobacter, Salmonella, Serratia* and *Enterobacter spp*[4,6,7].

It is noted that KPC is present in bacteria that colonize or infect patients with severe health conditions, being one of the most feared Healthcare-Associated Infections. Acquisition of KPC-producing bacteria is associated with prolonged hospitalization time, permanence in intensive care units, use of invasive devices, immunosuppression and multiple antibiotic treatments. For this reason, contact precautions cannot be overlooked, as they prevent the transmission of microorganisms that carry genes of this resistance mechanism, disseminated by direct or indirect contact with the patients or their environment[7,8].

In 2013, there was a rapidly controlled KPC outbreak in a ward at the NHS Trust, a Teaching Hospital from Leeds, United Kingdom. The infection prevention and control strategy that was immediately implemented targeted five main areas: hand hygiene, cohort and private room, triage, environmental cleaning, and education. In studying such outbreak, the authors reported that there was considerable potential for the rapid spread of Carbapenemase-encoding genes between *Klebsiella sp.* and *Enterobacteriaceae*, even with the implementation of extensive infection control interventions[9].

The objective of this study was to describe the presence of carbapenem-resistance enzyme encoding genes in bacteria isolated from materials obtained from patients admitted to a teaching hospital, evaluating epidemiological and genetic aspects related to resistance to carbapenems present in these microorganisms.

Methods

The study was cross-sectional, where data from all 37 bacterial strains obtained from September 22nd, 2017 to October 3rd, 2018 (1-year period) were analyzed in a teaching hospital from Juiz de Fora-MG, and then sent to investigate resistance-enzyme encoding genes in a regional laboratory, as they were classified as multiantimicrobial-resistant through an automated DNA fingerprint method. The in-hospital classification of resistance was achieved because they presented high Minimum Inhibitory Concentrations according to the Clinical and Laboratory Standards Institute (CLSI) criteria for the following: Amikacin, Ampicillin, Ampicillin+Sulbactam, Cefepime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, Meropenem, Piperacillin-Tazobactam and Sulfamethoxazole-Trimetropim.

The PCR was performed between 2017 and 2018 at the Octávio de Magalhães Institute/Central Public Health Laboratory of MG (*Laboratório Central de Saúde Pública de MG*, LACEN-MG), belonging to the Ezequiel Dias Foundation (*Fundação Ezequiel Dias*, FUNED). FUNED received from the hospital of origin the strain already isolated that had the result of the sensitivity test or of other phenotypic tests showing resistance to carbapenems. Identification of the microorganism was confirmed at FUNED. In 2017/2018, identification was performed by means of the manual (biochemical tests) and/or automated (Vitek 2 - Biomerieux) methods. After identification, the resistance genes were investigated by means of conventional PCR, with DNA extraction by thermal lysis of the bacterial suspension. Conventional PCR was performed using the in-house method (without resorting

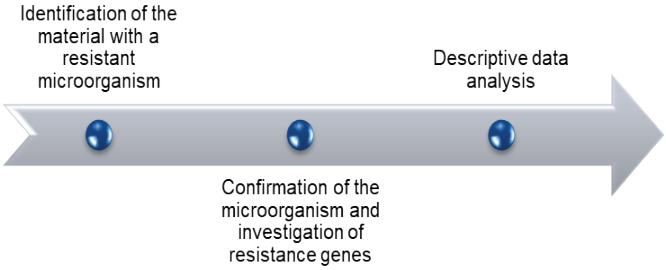
to commercial closed kits), with isolated purchase of primers, enzymes, master mix, etc. Detection was performed by means of agarose gel electrophoresis, with ethidium bromide revelation. When implemented at FUNED, this PCR followed the protocol of the Fiocruz-RJ National Reference Laboratory. Initially, a duplex reaction for KPC and NDM was standardized for enterobacteria. Investigation of the OXA-48 gene was later included. Currently, the analysis is already different since, for KPC/OXA-18/NDM, research it is done by real-time PCR (Triplex) and identification of bacteria is by mass spectrometry (MALDI-TOF).

The bla_{KPC} and bla_{NDM} genes were investigated in all 37 strains and bla_{OXA-48} in 24 of them, depending on availability of resources. It is noted that other important genes encoding serine-carbapenemases (such as bla_{GES} and bla_{OXA-23}) and metallo-carbapenemases (such as bla_{VIM} and bla_{IMP}) were not investigated, as they are not part of the standard analysis list of the reference laboratory to which the local samples are sent, and could contribute to even more relevant results.

In the analysis of each of the collected materials, the species and microbiological material obtained were identified, as well as the type of carbapenemase decoding resistance gene, in addition to the gender and age of the individuals whose material was collected. All the information was stored and tabulated in *Microsoft Excel* 2007 spreadsheets.

The program used for the statistical analysis was the *Statistical Package for the Social Sciences* (SPSS), version 27.0., and the significance level adopted for all the tests was 5%. Age distribution was verified with the Shapiro-Wilk test and was presented as mean and standard deviation. Balance in gender distribution was checked with Pearson's Chi-square test. The materials analyzed, the microorganisms found, the genes observed and the genes investigated were presented as absolute and relative frequencies (Figure 1).

Figure 1. Research phases



The research sought to meet the recommendations set forth in Resolution 466/2012 of the National Health Council, being submitted to the appreciation of the Research Ethics Committee of the University Hospital belonging to the Federal University of Juiz de Fora, with CAAE: 19716919.2.0000.5133.

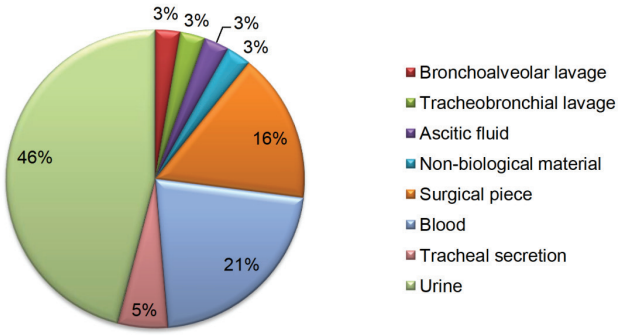
Results

The study included 37 collection procedures: 23 in women, 13 in men and only 1 in a boy, resulting in a mean age of 63.86 (± 21.23) years old. All samples came from patients undergoing treatment for healthcare-associated infections, characterized by diagnosis 48 hours after hospital admission. Only one collection procedure was performed and a single bacterium was isolated for each patient.

Age distribution was verified by means of the Shapiro-Wilk test and normality was not observed, with p-value = 0.033. The gender analysis in the contingency table showed that there was no expected absolute frequency below 5, allowing use of Pearson's Chi-square test to verify whether there was balance in this distribution. The p-value = 0.139 found in the test evidenced balance in gender distribution in this study.

The *Klebsiella oxytoca* species was identified in 1 sample (2.7%) of ascitic fluid, *Enterobacter cloacae* in 1 sample (2.7%) of tracheobronchial lavage and *Klebsiella pneumoniae* in: 17 urine samples, 8 blood samples, 6 surgical pieces, 2 samples of tracheal secretions, 1 sample of bronchoalveolar lavage and 1 sample of non-biological material, being identified in a total of 35 samples (94.6%) (Graph 1).

Graph 1. Frequency of the materials analyzed



Adults and older adults were the most affected individuals in the hospital of this study, whereas only one child was detected with a multidrug-resistant bacterium. There was no general predilection for gender but there was predominance of urinary tract infection, frequently diagnosed in the female gender: 14 out of 17 urine collections. The surgical piece and tracheal secretion samples were equally divided (50%) between the genders. In blood, 6 out of 8 (75%) individuals were male. The lavages, ascitic fluid and non-biological material corresponded to female patients.

The bla_{KPC} gene was detected in 81.1% (30/37 samples), while the bla_{NDM} gene was not detected in the strains of this study (0%). Even though it was not possible to investigate bla_{OXA-48} in 13 materials, it was positive in 4 strains among the 24 samples in which it was analyzed (17%). Finally, no resistance gene was detected in 3 samples from all 37 individuals (8%) (Table 1).

Discussion

The results elucidate the presence of genes that encode resistance enzymes which imply difficulty for the therapeutic management of patients with infections associated with multidrug-resistant microorganisms. Such Carbapanemase-enzyme decoding genes generate a direct impact on mortality and on the epidemiological control of health centers, and knowledge about the profile of the infections is essential to define initial therapeutic strategies[1,9].

Recently, the KPC gene was detected in ST131 *E. coli*, a multidrug-resistant and internationally epidemic clone. Knowing that *Escherichia sp.* is a community pathogen, the species has become a potential vehicle for KPC spread in community settings[8-10].

RBFHSS
Revista Brasileira de Farmácia Hospitalar e Serviços de Saúde

Table 1. Multi-drug resistant strains in a Teaching Hospital from Juiz de Fora-MG

Date	Gender	Age	Material	Microorganism	bla_{KPC}	bla_{NDM}	bla_{OXA-48}
09/22/2017	F	70	Surgical piece	Klebsiella pneumoniae	0	0	1
09/29/2017	F	74	Urine	Klebsiella pneumoniae	1	0	Not investigated
10/05/2017	F	72	Bronchoalveolar lavage	Klebsiella pneumoniae	1	0	Not investigated
10/11/2017	F	62	Non-biological material	Klebsiella pneumoniae	1	0	Not investigated
10/19/2017	F	69	Urine	Klebsiella pneumoniae	1	0	Not investigated
10/24/2017	F	95	Urine	Klebsiella pneumoniae	1	0	Not investigated
11/11/2017	M	2	Blood	Klebsiella pneumoniae	1	0	Not investigated
11/17/2017	F	69	Blood	Klebsiella pneumoniae	1	0	Not investigated
11/21/2017	M	62	Surgical piece	Klebsiella pneumoniae	0	0	1
11/24/2017	M	76	Surgical piece	Klebsiella pneumoniae	0	0	1
12/08/2017	M	98	Blood	Klebsiella pneumoniae	0	0	Not investigated
01/08/2018	M	78	Blood	Klebsiella pneumoniae	1	0	Not investigated
01/16/2018	M	74	Surgical piece	Klebsiella pneumoniae	1	0	Not investigated
01/22/2018	F	44	Blood	Klebsiella pneumoniae	1	0	Not investigated
01/25/2018	F	72	Urine	Klebsiella pneumoniae	0	0	Not investigated
01/28/2018	F	85	Urine	Klebsiella pneumoniae	1	0	Not investigated
02/01/2018	F	83	Urine	Klebsiella pneumoniae	1	0	0
02/22/2018	F	20	Urine	Klebsiella pneumoniae	1	0	0
02/22/2018	F	67	Urine	Klebsiella pneumoniae	1	0	0
02/23/2018	M	52	Blood	Klebsiella pneumoniae	0	0	1
03/07/2018	F	32	Urine	Klebsiella pneumoniae	0	0	0
06/03/2018	F	65	Surgical piece	Klebsiella pneumoniae	1	0	0
06/11/2018	M	90	Blood	Klebsiella pneumoniae	1	0	0
06/12/2018	F	61	Urine	Klebsiella pneumoniae	1	0	0
06/11/2018	M	90	Blood	Klebsiella pneumoniae	1	0	0
06/12/2018	F	41	Ascitic fluid	Klebsiella oxytoca	1	0	0
07/12/2018	F	59	Urine	Klebsiella pneumoniae	1	0	0
07/13/2018	F	23	Tracheobronchial lavage	Enterobacter cloacae	1	0	0
07/16/2018	F	89	Urine	Klebsiella pneumoniae	1	0	0
07/17/2018	F	48	Urine	Klebsiella pneumoniae	1	0	0
09/05/2018	M	63	Urine	Klebsiella pneumoniae	1	0	0
09/05/2018	M	67	Tracheal secretion	Klebsiella pneumoniae	1	0	0
09/13/2018	M	65	Urine	Klebsiella pneumoniae	1	0	0
09/18/2018	M	75	Urine	Klebsiella pneumoniae	1	0	0
09/24/2018	F	70	Surgical piece	Klebsiella pneumoniae	1	0	0
09/27/2018	M	60	Urine	Klebsiella pneumoniae	1	0	0
10/03/2018	F	41	Tracheal secretion	Klebsiella pneumoniae	1	0	0

According to the Brazilian Bulletin on Patient Safety and Quality in Health Services, of the 22,499 notifications of microorganisms causing Primary Bloodstream Infections in ICUs for adults in 2015, the most frequent ones were as follows, with variations according to region: *Klebsiella Pneumoniae* (16.9% n=3,805), followed by Coagulase-Negative *Staphylococcus* (16.5% n=3,703), *Staphylococcus aureus* (13.2% n=2,734), *Acinetobacter sp.* (12.2% n=2,734) and *Pseudomonas aeruginosa* (10.0% n=2,242)[11].

Two international institutions, the *Centers for Disease Control and Prevention* and the *Center for Disease Prevention and Control*, highlight the following as one of the key strategies to address the problem of microbial resistance: creation of programs aimed at improving and assessing appropriate use of antimicrobials by promoting optimal selection of the ideal antimicrobial regimen[12-13].

In Brazil and to such end, the National Guideline for the Development of a Program to Manage Use of Antimicrobials in Health Services was created in 2017[12-13]. Both in the Program actions and in this study, it was observed that enteric Gram-negative bacteria, especially *Klebsiella pneumoniae*, continue to be the protagonists of multidrug resistance, mainly due to the presence of the bla_{KPC} gene[14,15].

The bla_{NDM} gene was detected in Brazil for the first time in 2013, in Porto Alegre, having presented lower prevalence when compared to bla_{KPC} and bla_{OXA-48}[16]. In this study herein presented there was no genetic identification of NDM strains, similarly to another large research study conducted in 2016 at an ICU from Rio de Janeiro[17]. In addition to that, in the 24 samples in which the bla_{OXA-48} gene was analyzed, it was found with a comparatively lower frequency (17%) in relation to the prevalence of bla_{KPC} (81%), which was only detected in 3 surgical pieces and 1 blood sample. This result is similar to several Brazilian studies that reported concomitance of bla_{KPC} with the bla_{OXA} group in *K. pneumoniae*[17-18]. Of the 3 materials in which it was not possible to identify the genes, 2 did not have the bla_{OXA-48} gene researched and could be related to other types of genes in addition to the 3 under investigation.

In a recent study[19], an analysis was performed with notified samples from 43 patients with healthcare-associated infection by KPC obtained from hospitals in the city of Rio de Janeiro, between January and June 2021. The mean age was 71 years old and there was 58% prevalence of the female gender. The main foci were as follows: urinary (65.1%), pulmonary (16.2%), hematogenous (11.6%) and bone (2.3%). Frequency for the bla_{KPC} gene was 65.1%, while it was 23.2% for bla_{NDM}, although no bla_{OXA-48} or MCR-1 genes were found.

Another study, with a larger sample size, evaluated the phenotypic and genotypic profile of carbapenem-resistant *Klebsiella pneumoniae* through the isolation of 170 samples from colonized and infected patients assisted in a high-complexity hospital from the city of São Paulo, between 2009 and 2014. All the isolates were submitted to real-time PCR for detection of the bla_{CTX-M}, bla_{SHV}, bla_{TEM}, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{GES}, bla_{VIM} and bla_{IMP} genes, and presented, without exception, high levels of resistance to all the antimicrobials tested, as well as 100% presence of the bla_{KPC} gene[20].

A systematic literature review from 2021 carried out in the SciELO, Medline, LILACS and PubMed databases located 95 articles, of which 58 were included to identify the incidence of *Klebsiella pneumoniae* with KPC resistance factor in adults at hospitals from the Midwest, Southeast and South Brazilian regions, in addition to the antimicrobial resistance profile between 2006 and 2016. The South region obtained the highest prevalence and the Ertapenem antimicrobial showed almost 100% resistance in all the states[21].

As a limitation of the study herein reported, it is to be noted that only 3 genes were investigated, in addition to the fact that research of the bla_{OXA-48} gene was only carried out in 24 of the 37 bacterial strains, as well as the impossibility of generalization, given that the data are only from a single local hospital. The study design does not allow making causality assertions, although it corroborates similar findings in previous studies conducted in other loci and presents a warning. Thus, it would be suitable to conduct multicenter studies with larger sample sizes in the future. The profile of resistance genes is carefully described and the predominance of *Klebsiella Pneumoniae* in the environment of a public teaching hospital is noted. In view of the multidrug resistance context, a warning is issued about the need for professional training for adequate choice of antimicrobials with coverage spectrum for this microorganism, in addition to hygiene and health surveillance measures. These measures culminate in preserving the therapeutic possibilities and in reducing mortality in those who evolved with severe HAIs[22,23].

Conclusion

The data obtained are a proof that bacteria which contain carbapenem-resistance genes circulate widely in teaching hospitals, with predominance of bla_{KPC} strains, and that they are a public health problems[1,2], as they increase morbidity and mortality in the patients as a result of the reduction in the available therapeutic options and the increased risk of serious infections. Therefore, prudent choice of antimicrobials according to the coverage spectrum required, prevention strategies and hospital surveillance should be prioritized to avoid the spread of bacteria carrying resistance genes, with early detection of infected or colonized patients[22,23].

Funding sources

The research did not receive funding for its conduction.

Collaborators

Reis BO assisted in conception, design, data analysis and interpretation, and in writing of the article and relevant critical review of the intellectual content.

Ferreira CMSD assisted in conception and design.

Conflict of interest statement

The authors declare that there are no conflicts of interest in relation to this article.

References

1. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med. 2012. 18(5):263-72. DOI: 10.1016/j.molmed.2012.03.003.
2. Macedo MLAP, Cartaxo RS, Alemida TCC, *et al*. Mecanismos de resistência e detecção das beta-lactamases. UNOPAR Cient., Ciênc. Biol. Saúde Londrina. 2005. 7(1): 59-63. DOI: https://doi.org/10.17921/2447-8938.2005v7n1p%25p.
3. Del Fiol FS, Lopes LC, Toledo MI, *et al*. Perfil de prescrições e uso de antibióticos em infecções comunitárias. Revista da Sociedade Brasileira de Medicina Tropical. 2010. 43(1):68-72. DOI: http://www.dx.doi.org/10.5935/2238-3182.20170069.
4. WHO, World Health Organization. Global Action Plan on Antimicrobial Resistance. 2015. Available in: http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf.
5. Andrade LN, Darini ALC. Bacilos gram-negativos produtores de beta-lactamases: que bla bla bla é esse?. Journal Of Infection Control, 2017. 1(6): 16-25. Available in: https://jic-abih.com.br/index.php/jic/article/view/173.
6. Munoz-Price LS, Poirel EU, Bonomo RA, *et al.* Epidemiologia clínica da expansão global das carbapenemases de Klebsiella pneumoniae. Lancet Infect Dis. 2013; 13: 785-96. DOI: https://doi.org/10.1016/S1473-3099(13)70190-7.
7. El Fertas-aissani R, Messai Y, Alouache S, *et al*. Virulência e padrões de susceptibilidade a antibióticos de cepas de Klebsiella pneumoniae isoladas de diferentes espécimes clínicos. Pathol Biol. 2013; 61: 209–216. DOI: https://doi.org/10.1016/j.patbio.2012.10.004.
8. De Belder D, Lucero C, Rapoport M, *et al*. Genetic diversity of KPC-producing Escherichia coli, Klebsiella oxytoca, Serratia marcescens, and Citrobacter freundii isolates from Argentina. Microbial Drug Resistance, 2018. 24(7), 958-965. DOI: https://doi.org/10.1089/mdr.2017.0213.
9. Martin J, Phan HT, Findlay J, *et al*. Covert dissemination of carbapenemase-producing Klebsiella pneumoniae (KPC) in a successfully controlled outbreak: long-and short-read whole-genome sequencing demonstrate multiple genetic modes of transmission. Journal of Antimicrobial Chemotherapy, 2017. 72(11), 3025-3034. DOI: https://doi.org/10.1093/jac/dkx264.
10. Pitout JD. Extraintestinal pathogenic Escherichia coli: an update on antimicrobial resistance, laboratory diagnosis and

treatment. Expert Rev. Anti. Infect. Ther. 2012 10:1165–1176. DOI: https://doi.org/10.1586/eri.12.110.

11. Brasil, ANVISA. Boletim de Segurança do Paciente e Qualidade em Serviços de Saúde nº 14: Avaliação dos indicadores nacionais das Infecções Relacionadas à Assistência à Saúde (IRAS) e Resistência microbiana do ano de 2015. 2016. Available in: https://www20.anvisa.gov.br/segurancadopaciente/index.php/publicacoes/item/boletim-seguranca-do-paciente-e-qualidade-em-servicos-de-saude-n-16-avaliacao-dos-indicadores-nacionais-das-infeccoes-relacionadas-a-assistencia-a-saude-iras-e-resistencia-microbiana-do-ano-de-2016.
12. CDC, Centers for Disease Control and Prevention. Antimicrobial Use and Resistance (AUR) Module. 2017. Available in: https://www.cdc.gov/nhsn/pdfs/pscmanual/11pscaurcurrent.pdf.
13. ECDC, European Centre for Disease prevention and Control. Healthcare-associated infections. 2016. Available in: https://ecdc.europa.eu/en/healthcare-associated-infections.
14. Brasil, ANVISA. Programa Nacional de Prevenção e Controle de Infecções Relacionadas à Assistência à Saúde (2016-2020). 2016. Available in: https://www20.anvisa.gov.br/segurancadopaciente/index.php/publicacoes/item/pnpci-ras-2016-2020.
15. Moreira VC, Freire D. Klebsiella pneumoniae e sua resistência a antibióticos. Available in: http://www.cpgls.pucgoias.edu.br/6mostra/artigos/SAUDE/VANESSA%20CARVALHO%20MOREIRA.pdf.
16. Campos JC. Estudo genotípico e fenotípico de bacilos Gram-negativos produtores de carbapenemase do tipo New Delhi metalo-β-lactamase. São Paulo: Faculdade de Ciências Farmacêuticas. 2017. DOI: 10.11606/T.9.2017.tde-18102017-152216.
17. Flores C, Maria CPA, Kayo B, *et al.* Detection of antimicrobial resistance genes in beta-lactamaseand carbapenemase-producing Klebsiella pneumoniae by patient surveillance cultures at an intensive care unit in Rio de Janeiro, Brazil. J. Bras. Patol. Med. Lab. 2016. 52(5):284-292. DOI: https://doi.org/10.5935/1676-2444.20160049.
18. Fehlberg LC, Carvalho AM, Campana EH, *et al*. Emergence of Klebsiella pneumoniae-producing KPC-2 carbapenemase in Paraíba. Northeastern Brazil. Braz. J. Infect. Dis. 2012. 16: 577–580. DOI: http://dx.doi.org/10.1016/j.bjid.2012.07.001.
19. Ledesma LA, Moura CAB, Oliveira SS, *et al.* Klebsiella pneumoniae produtoras de carbapenemases (KPC) no Rio de Janeiro: frequência dos genes blakpc, blandm, blaoxa-48, mcr-1 e análise da concentração inibitória mínima (CIM) de polimixina b pelo teste de microdiluição em caldo nas amostras. The Brazilian Journal of Infectious Diseases. 2022. 26 (S1): 102253. DOI: https://doi.org/10.1016/j.bjid.2021.102253.
20. Fonseca JM. Caracterização microbiológica de Klebsiella pneumoniae produtora de enzima KPC isoladas em pacientes de um hospital de alta complexidade durante o período de cinco anos (2009–2014). 2019. Available in: https://repositorio.unifesp.br/handle/11600/59221.
21. Marçal TVG, Costa LF, Nicoletti DR, *et al*. Incidência de KPC (Klebsiella Pneumoniae Carbapenemase) em adultos internados em hospitais nas regiões do Brasil de 2006 a 2016: revisão bibliográfica. SaudColetiv (Barueri). 2021, 11(62):5174-91. Available in: http://www.revistas.mpmcomunicacao.com.br/index.php/saudecoletiva/article/view/1339.
22. Acar JF. Consequences of bacterial resistance to antibiotics in medical practice. Clin Infect Dis. 1997. 24 (S1):17-18. DOI: https://doi.org/10.1093/clinids/24.Supplement_1.S17.
23. Julian D, Dorothy D. Origins and Evolution of Antibiotic Resistance. Microbiol Mol Biol Rev. 2010; 74(3): 417–433. DOI: https://doi.org/10.1128/MMBR.00016-10.