ABCS HEALTH SCIENCES

REVIEW ARTICLE

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Occurrence of *bla*$_{KPC}$ gene in clinical isolates of *Pseudomonas aeruginosa* from Brazil

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Pseudomonas aeruginosa is one of the main microorganisms causing healthcare-related infections. The rise of carbapenem-resistant *P. aeruginosa* (CRPA) strains has become a serious public health problem. Dissemination of the enzyme *Klebsiella pneumoniae* carbapenemase (KPC) encoded by the *bla*$_{KPC}$ gene cause the inactivation of β-lactam antibiotics being one of the mechanisms involved in this resistance. Given the above, the objective of this review was to evaluate the occurrence of the *bla*$_{KPC}$ gene in clinical isolates of *P. aeruginosa* in Brazil. For this, the online databases used were: Lilacs, SciELO and PubMed. The search for articles included articles published from 2012 to 2020, using the following keywords: *bla*$_{KPC}$ (KPC), *Pseudomonas aeruginosa,* and Brazil (in Portuguese and English). Initially, 30 publications eligible for inclusion in this review were identified. After the first analysis, two articles were excluded due to duplication. Subsequently, titles and abstracts were evaluated, 15 articles were excluded because they did not fit the theme, and 13 articles that met the inclusion criteria were read in full. In these studies, the presence of the *bla*$_{KPC}$ gene was investigated in 566 clinical isolates of *P. aeruginosa* in Brazil, with 86 (15.2%) positive samples found. Pernambuco was the state with the highest number of articles and positive samples, respectively, 38.5% (5/13), and 65.1% (56/86). This study reinforces the need to investigate the occurrence of this gene in all regions of the country in CRPA, aiming to understand how its dissemination occurs and to promote prevention and therapeutic strategies.

Keywords: *Pseudomonas aeruginosa*; carbapenem-resistant Enterobacteriaceae; Brazil; hospital infections.

How to cite this article: Lima et al. Occurrence of *bla*$_{KPC}$ gene in clinical isolates of *Pseudomonas aeruginosa* from Brazil. ABCS Health Sci. 2022;47:e022306 https://doi.org/10.7322/abcshs.2020198.1646

Received: Oct 29, 2020
Revised: Apr 26, 2021
Approved: May 20, 2021

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Declaration of interests: nothing to declare

INTRODUCTION

Pseudomonas aeruginosa is included in the group of six pathogens known as ESKAPE, which is formed by *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter* spp., *P. aeruginosa,* and *Enterobacter* spp. *P. aeruginosa* is one of the main causes of healthcare-related infections (HAIs) in the world, in addition, has a high capacity to become resistant to antimicrobials, which places this bacterium on the list of priority microorganisms for the development of infection control and prevention strategies of the world health organization[1]. The main colonization sites for these bacilli are the upper respiratory and urinary tracts, including the kidneys. Due to the previous colonization of these sites, *P. aeruginosa* is commonly reported in surgical, wound, urinary tract infections (UTI), pneumonia, dermatitis, and systemic respiratory infections in patients with cystic fibrosis and bacteremia[2]. Several factors of virulence are involved

in the pathogenesis of *P. aeruginosa* infection, which is divided into three phases: bacterial adhesion and colonization, local invasion, and systemic spread of the infection. However, this infection can be stopped at any of these steps[3].

This microorganism is related to several types of HAIs, such as pneumonia associated with mechanical ventilation, central venous catheter infection, urinary tract infections, infections in transplant patients, and in addition to being frequently reported as an important etiological agent in patients with cystic fibrosis[4]. This pathogen is prevalent in hospital environments, due to its ability to survive on biotic and abiotic surfaces, such as medical and hospital equipment, being transmitted from patient to patient[5].

This microorganism has a high ability to become resistant to antimicrobials and there have been increasing cases of infections with the worldwide occurrence of strains of *P. aeruginosa* resistant to carbapenems (CRPA), mainly in patients admitted to intensive care units (ICUs) with comorbidities. These factors led the World Health Organization (WHO) to consider *P. aeruginosa* as one of the priority pathogens for research and development of new drugs[6]. Among the risk factors for infection or colonization with CRPA described, we can highlight the previous exposure to carbapenems, the number of days of treatment with carbapenems, and co-infection with *Escherichia coli*[7]. Cases of pneumonia and bloodstream infections are among the main infections by *P. aeruginosa*, with cases of pneumonia most associated with the appearance of CRPA[8].

The emergence of strains of CRPA is related to different mechanisms, among which stand out the occurrence of mutations that affect the permeability of these microorganisms to carbapenems, in addition to the overexpression of efflux systems to multiple drugs that can confer resistance to a wide variety of antimicrobials[9]. In addition to these mechanisms, the production of β-lactamases enzymes, including metallo-β-lactamases, should be highlighted, with SPM-1 standing out in Brazil, whose occurrence is endemic, almost all cases outside Brazil being related to hospitalizations in the country, except the study reporting the presence of *P. aeruginosa* isolates positive for bla_{SPM-1} recovered from burn injuries of patients treated at a hospital in Iran[10]. There is no reference to the travel history of these patients.

Other important enzymes are carbapenemases, especially *Klebsiella pneumoniae* carbapenemase (KPC), which has broad hydrolytic activity against cephalosporins, monobactams, and carbapenems[11]. The KPC enzyme has high global dissemination, being widely detected in members of the *Enterobacteriales* order, being less frequent among non-fermenting Gram-negative bacilli (BGNNF), although there are several reports of the occurrence of this enzyme in *P. aeruginosa* in the world. The description of first report of this enzyme in this microorganism occurred in Colombia in 2007[12]. So far, its occurrence in *P. aeruginosa* has been reported in the following countries: in the Americas: Brazil[13], Argentina[14], Mexico[15], Puerto Rico[16], Trinidad and Tobago[17], United States of America (USA)[18] and Canada[19]. In Europe: Germany[20] and Spain[21]; in Asia: China[22], India[23], and Iran[24].

To date, 73 variants of the bla_{KPC} gene responsible for encoding the KPC enzyme have been described, with KPC-2 being the most frequently reported. Moreover, the spread of this gene is mainly related to the transposon Tn4401, which is transported by plasmids of approximately 100 kb[25]. In Brazil, all detected cases of this enzyme in *P. aeruginosa* are related to the KPC-2 enzyme. It is not yet fully elucidated how this gene reached BGNNF, but it is suggested that this phenomenon occurred through a genetic transfer with *Enterobacteriales*[25].

The major concern related to the production of the KPC enzyme in strains of *P. aeruginosa* is due to the ability that this microorganism has to acquire resistance mechanisms, in addition to having intrinsic resistance to some antimicrobials, allowing the reduction of therapeutic options for treatment of infections by this bacterium[26].

There are few studies evaluating combinations of drugs against strains of CRPA, including the use of carbapenems, which makes it difficult to determine which are the best therapeutic options for infections by this microorganism[27]. According to Fritzenwanker et al.[28] for the treatment of CRPA infections that produce KPC or GES enzymes sensitive to ceftazidime/avibactam (CAZ-AVI), antimicrobial therapy should be the combination of CAZ-AVI with colistin (polymyxin E), while for strains resistant to CAZ-AVI, the combination of colistin and meropenem should be used. According to Karaikos et al.[29] in a review study, the most effective drugs for the treatment of *P. aeruginosa* infections in Europe were colistin and CAZ-AVI respectively. In addition, it has also been shown that studies have failed to pinpoint the best therapy for the treatment of CRPA infections, demonstrating that there is a small advantage of colistin monotherapy compared to combined colistin therapy with another anti-*Pseudomonas*. Another important point demonstrated is that the implantation of ceftolozano-tazobactam as the therapy of choice for the treatment of CRPA is an excellent option and can be associated with colistin, fosfomycin, and plazomycin[29].

Given the above, the objective of this review was to evaluate the occurrence of the bla_{KPC} gene in clinical isolates of *P. aeruginosa* in Brazil and evaluate the spread of this gene in the country.

METHODS

The systematic literature review was carried out between February and September 2020 using the online databases: Lilacs, Scientific Electronic Library Online (SciELO) and US National Library of Medicine, and the National Institutes of Health (PubMed). The following descriptors were used, in Portuguese

and English: bla_{KPC}, KPC, *Pseudomonas aeruginosa,* and Brazil. The "AND" intercessor was used.

For the research of the articles, the following inclusion criteria were used: articles that related to the theme of detection of the bla_{KPC} gene in *P. aeruginosa* in Brazil, articles that had been published in 2012, the year of the first report of the bla_{KPC} gene in this microorganism in Brazil and articles published only in English, Portuguese or Spanish. Through this search procedure, 30 potentially eligible publications were initially identified for inclusion in this review.

RESULTS

In total, 30 articles were found resulting from the search, according to the descriptors used, in the databases searched. After the initial analysis, which evaluated the possible duplication of articles in the different databases, two articles were excluded. Subsequently, titles and abstracts were evaluated, and 15 articles were excluded because they did not fit the theme. After this stage, 13 studies met the inclusion criteria and moved on to the next stage where the articles were read in full, as can be seen in the flowchart, Figure 1. Table 1 presents the main results found in these studies[30-40].

After reading the 13 articles in full, it was seen that the studies investigated the presence of the bla_{KPC} gene in 566 clinical isolates of *P. aeruginosa* in Brazil, with 86 (15.2%) positive samples found. Pernambuco was the state with the highest number of articles and positive samples, respectively, 38.5% (5/13), 65.1% (56/86) Figure 2.

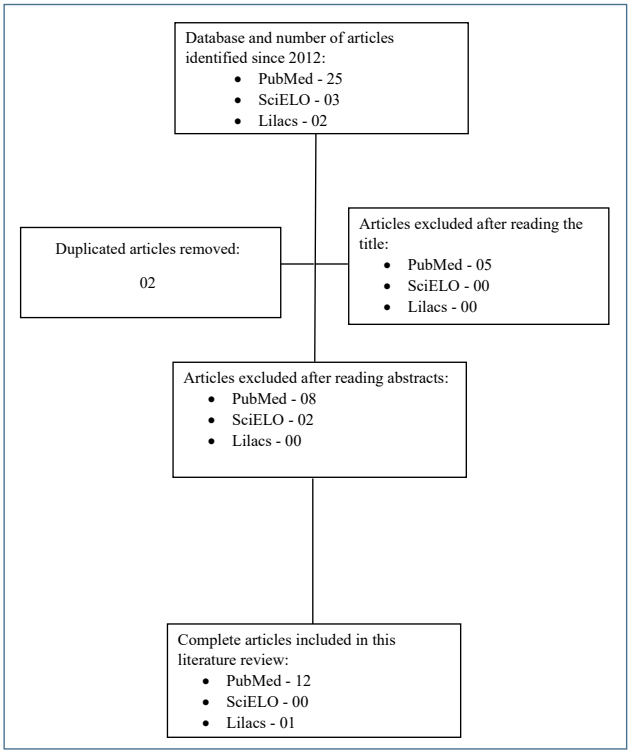

Figure 1: Flowchart of article selection for inclusion in the review.

DISCUSSION

Nordmann & Poirel[41] evaluated a review study related to epidemiology and the diagnosis of carbapenem resistance in Gram-negative bacteria and observed the prevalence of 64.6% of strains of CRPA in Latin America, with the most prevalent samples coming from tracheal secretion cultures and UTIs.

The screening of *P. aeruginosa* isolates producing the KPC enzyme occurs mainly through resistance to carbapenems (imipenem and meropenem). Some phenotypic tests have been used in the detection of this enzyme and have good sensitivity and specificity, especially the Carba-NP tests and the modified Hodge test, with the first one showing better results in the detection of the KPC enzyme[42].

Santos et al.[11] evaluated the epidemiological and antimicrobial resistance trends in clinical isolates of *P. aeruginosa* from Rio de Janeiro over the years 1995-2015, detecting a significant increase in resistance to carbapenems, which varied from 4.5% to 94.1%. Meanwhile, Lima et al.[30] evaluated the occurrence of high production of carbapenemase enzymes in a study carried out in Recife, Pernambuco, carried out with 65 isolates of *P. aeruginosa*, coming from two public hospitals, describing a frequency of 63.1 % (41⁄65) carrying the bla_{KPC} gene, in samples from patients with various infections resulting from 36% tracheal secretion, 17% urine and 11% cerebrospinal fluid.

Most of the studies that comprised this review were limited to detecting the occurrence of the bla_{KPC} gene[13,30,31,35,36,40]. Although it is important to detect the occurrence of the gene, it is essential to understand how its dissemination occurs, the type of clone involved, as well as the type of vector plasmid. The gene encoding the KPC enzyme is generally described in the transposon Tn4401, which has a variable length, according to the genes it incorporates. The dissemination of the bla_{KPC} gene has been successful mainly in isolates of *K. pneumoniae* belonging to clone ST258. In P. aeruginosa, little data is described on this dissemination[43]. The plasmids described in the Brazilian studies are mentioned in Table 1.

Cavalcanti et al.[39] described the dissemination of the bla_{KPC} gene in two isolates belonging to clone ST235 and one isolate from clone ST244. Similar data were reported by Petroli et al.[34] who reported the occurrence of the bla_{KPC} gene in an isolate of clone ST235. Interestingly, this strain was isolated from UTIs in 2008, long before the publication of the first report of this gene in *P. aeruginosa* in Brazil, which occurred in 2012. The clone ST235 was first detected in isolates in Colombia and is related to strains with a high index of resistance to antimicrobials. Differing from these findings, Santos et al.[11] reported the dissemination of the bla_{KPC} gene in three isolates belonging to clone ST1560 and two isolates belonging to clone ST1944.

Carrara-Marroni et al.[38] described the location of the bla_{KPC} gene in four CRPA isolates, the gene is located on the chromosome of three

Table 1: Summary of articles selected for review

Author	Year of publication	State	No. of samples (*P. aeruginosa*)	Positive samples for bla_{KPC}	ST	Gene location
Lima et al.[30]	2020	Pernambuco	65	41	NA	NA
Santos et al.[11]	2019	Rio de Janeiro	88	5	ST1560 (3);ST1944 (2)	NA
Galetti et al.[31]	2019	Minas Gerais	1	1	ST381	Plasmid pBH6
Scavuzzi et al.[32]	2019	Pernambuco	14	2	NA	NA
Santos et al.[33]	2018	Minas Gerais	1	1	ST2584	Plasmid IncQ1 group
Petroli et al.[34]	2018	Londrina	1	1	ST235	Plasmid
Chaves et al.[35]	2017	São Paulo	29	7	They analyzed the ST of only three isolates, and all belonged to the ST277	NA
Jacomé et al.[36]	2016	Pernambuco	58	8	NA	NA
Galetti et al.[37]	2016	Minas Gerais	1	1	ST244	Plasmid pBH6
Carrara-Marroni et al.[38]	2015	Londrina	170	4	NA	NA
Cavalcanti et al.[39]	2015	Pernambuco	9	3	ST235(2); ST244(1)	Suggest that it is located on IncU-type plasmids
Rizek et al.[40]	2014	São Paulo	127	10	NA	NA
Jacomé et al.[13]	2012	Pernambuco	2	2	NA	NA

NA: Not available

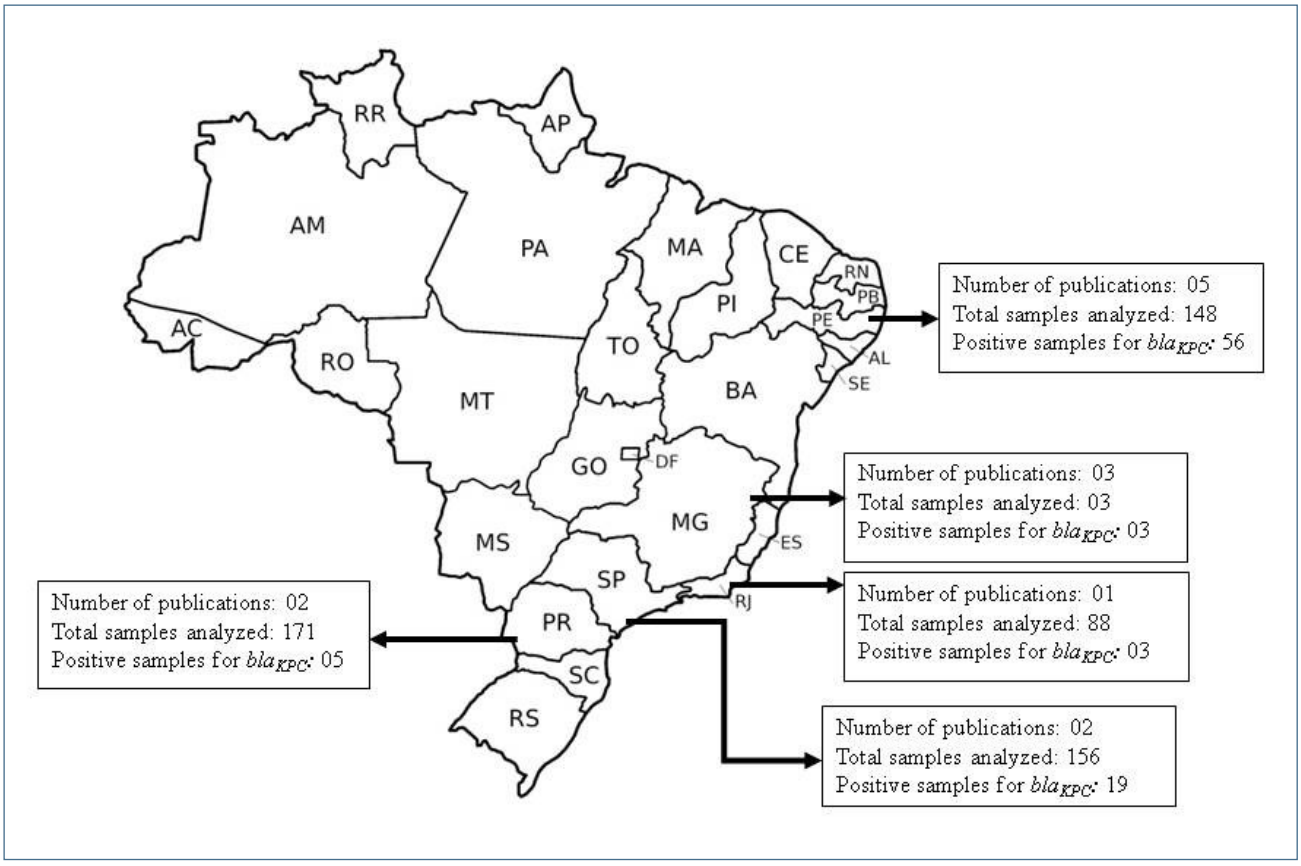

Figure 2: Data on the number of publications on the occurrence of the bla_{KPC} gene in clinical isolates of *P. aeruginosa*, the total samples analyzed, and the number of positive samples.

isolates and the plasmids in the fourth isolate. However, they did not characterize the type of plasmid in which the gene was inserted. Galetti et al.[37] characterized a small new plasmid carrying the bla_{KPC} gene, called pBH6, in a CRPA strain belonging to clone ST244.

Santos et al.[33] analyzed the genome of a CRPA belonging to a different clone ST2584, which had recently been described. In addition, they detected that the bla_{KPC} gene was inserted into an IncQ1-like plasmid. In another study, Galetti et al.[31] characterized a new plasmid carrying the bla_{KPC} gene, where they described the presence of the previously described pBH6 plasmid associated with several bacteriophage genes in a CRPA strain belonging to the ST381 clone.

The increase in the number of HAIs cases caused by strains of CRPA in Brazil and worldwide is a serious public health problem, and it is necessary to evaluate the resistance mechanism involved in the development of this resistance, in addition to the need for the rational use of antimicrobials to the shorter hospital stay of patients and consequently the lower occurrence of development of CRPA strains.

Conclusion

Few studies have been dedicated to assessing the occurrence of the bla_{KPC} gene among CRPA isolates in Brazil, and most studies that carry out this detection stop their analysis there, without conducting further research to understand the genetic mechanisms involved in its dissemination. Given the data on the dissemination of the bla_{KPC} gene in Brazil and the impact caused by CRPA infections carrying this gene, it is clear the need to investigate the occurrence of these genes in this microorganism in all regions of the country, as well as to evaluate the environment gene in which it is inserted to understand its dynamics of dissemination and thus interrupt its chain of transmission. In addition, it is essential to adopt measures to prevent HAIs, and the rational use of antimicrobials to reduce the time between patients, especially in ICUs to prevent the occurrence and spread of strains of CRPA in the hospital environment.

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