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Proceeding Paper

Dissemination of Resistance to Carbapenems Worldwide, Due to the Acquisition of *bla*KPC Genes in Clinical Isolates of *Pseudomonas aeruginosa*: A Systematic Review †

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Abstract: The spread of *bla*KPC-harboring *Pseudomonas aeruginosa* is considered a serious public health problem. A systematic review in PubMed and EMBASE was performed to determine the possible mechanisms of dissemination of *bla*KPC among isolates of *P. aeruginosa* resistant to carbapenems, and to consolidate the epidemiological information of these strains circulating worldwide. We found 494 *bla*KPC-*P. aeruginosa* isolates from 12 countries, from these 46% harbored *bla*KPC in a plasmid structure. Also, different NTEKPC and Tn*4401* elements surrounding b*la*KPC have been reported. In addition, the different unrelated reported sequence types reveal an alarming dynamic of increase in carbapenem-resistant *P. aeruginosa* clones.

Keywords: *Pseudomonas aeruginosa*; carbapenems; KPC; mobile genetic elements; antibiotic resistance; systematic review

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1. Introduction

Infections caused by *Pseudomonas aeruginosa* resistant to antibiotics have become a serious public health problem, representing a risk factor for hospitalized patients [1,2]. The prevalence of carbapenem-resistant *P. aeruginosa* (CRPA) has increased rapidly, threatening the efficacy of these antibiotics, and limiting the effective therapeutic options [2,3]. At present, multiple mechanisms of carbapenem resistance have been described for *P. aeruginosa*, including the production of carbapenemase enzymes, the repression of the OprD porin and the overexpression of efflux pumps [3–5]. Additionally, a wide variety of enzimes like GES, IMP, VIM, NDM and KPC have been reported for this bacterium [1,6].

KPC have a great clinical impact due to their high hydrolyzing efficiency of carbapenems [3], and their ability to spread using mobile genetic elements (MGE) such as plasmids and transposons [5,7]. The reports of *P. aeruginosa* carrying *bla*KPC are increasing [6,8]; however, the genetic mechanisms that have favored the dissemination of KPC in this species are still unclear. In this study, a systematic review was done to determine the possible mechanisms of dissemination of the *bla*KPC gene, among isolates of CRPA and to consolidate the information of the epidemiological knowledge of these strains circulating worldwide.

2. Methods

A systematic review was conducted following the published guidelines for the development of systematic reviews [9]. The protocol was registered in the Prospective International Registry of Systematic Reviews (PROSPERO) of the National Institute of Health Research (Registration code: CRD42022320686).

2.1. Search Strategy

Online searches were performed through the PubMed and EMBASE database for articles published up to end of September 2021, without language or geographic location restriction. A combination of keywords and controlled vocabulary (MeSH/Emtree terms) was formulated into the search, including terms related to the pathogen, carbapenem antibiotics, the resistance gene *bla*$_{KPC}$ and some dissemination platforms or MGE that could be associated with the spread of these strains worldwide.

2.2. Inclusion and Exclusion Criteria

Full texts retrieved items were screened to determine their eligibility according to the predefined selection criteria. We included studies reporting in a descriptive manner, isolates of *P. aeruginosa* with the following characteristics: (i) isolates obtained from human patients who have been treated in the hospital setting, (ii) isolates not susceptible to at least one type of carbapenem antibiotic, and (iii) *bla*$_{KPC}$-carrying isolates. All studies that reported dissemination platforms or MGE related with *bla*$_{KPC}$, and/or studies that reported sequence types (STs) associated with the isolates were included. In addition, we included some articles that did not describe the dissemination platforms but were considered relevant since they provided information related to the emergence of CRPA in new geographical locations.

We excluded studies in which the isolates were from environmental or animal samples, and studies solely reporting *P. aeruginosa* without KPC. Other reviews, duplicate reports and articles which were not fully accessible were also excluded. The quality of the studies was not considered as an exclusion criterion.

2.3. Data Extration and Analysis

The following information was entered into an Excel® database: (i) study-related variables (authors, year of publication and country), (ii) isolates-related variables (collection date, strain's name, KPC variant and STs), (iii) genetic location (plasmid or chromosome) and genetic structures surrounding *bla*$_{KPC}$ (transposons, NTE$_{KPC}$, insertion sequences). Information not available was classified as 'not specified'. The data obtained in this review were grouped into evidence tables and a descriptive analysis of the data was carried out to observe the factors associated with the dissemination of *bla*$_{KPC}$-harboring *P. aeruginosa*.

3. Results and Discussion

3.1. Search Results

The literature search resulted in a total of 153 articles with a publication date range from 2007 to 2021. After removing duplicate, 137 articles were eligible for full text review. Ten additional articles were obtained via hand searching. Finally, 42 studies were included in the systematic review after evaluating the inclusion/exclusion criteria (Figure 1). The detailed characteristics of the articles are presented in Table 1.

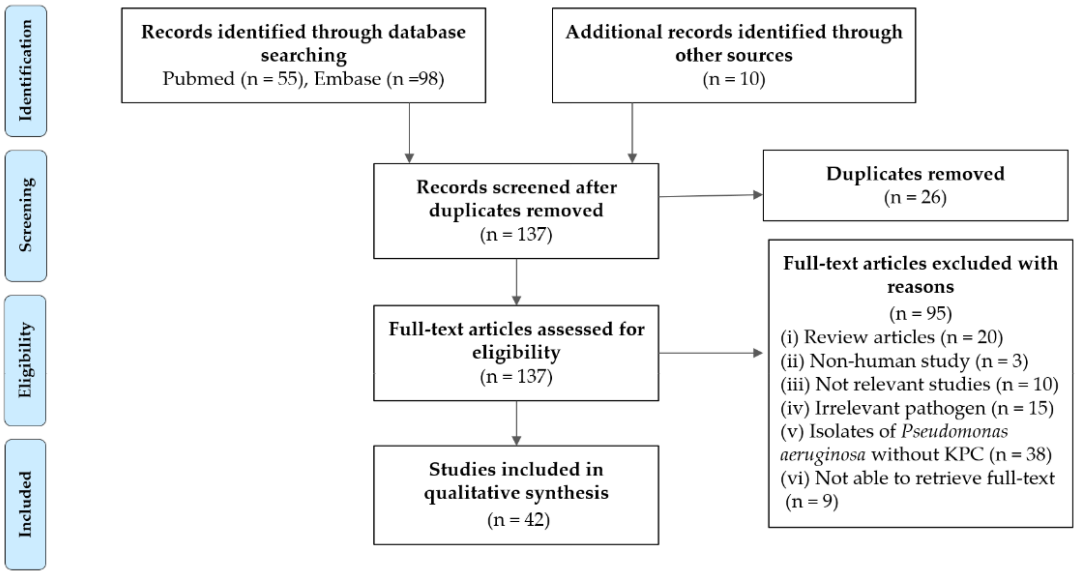

Figure 1. Flow diagram of study selection.

3.2. Characteristics of the Studies

The studies were conducted in American, European and Asian countries (42 studies); Colombia (10 studies) [1,5,6,10–16], Brazil (9 studies) [8,17–24], China (8 studies) [3,25–31], Puerto Rico (4 studies) [32–35], Chile (1 study) [36], Argentina (1 study) [37], Germany (1 study) [38], India (1 study) [39], Spain (1 study) [2], Trinidad and Tobago (1 study) [40], Vietnam (1 study) [41], and United States (EE.UU) (1 study) [42], contributed with studies. Two articles were conducted in an unclear location and one article was a worldwide surveillance study with isolates collected from Colombia, China, Argentina and Chile [7]. Although the reports of KPC continue to be more frequent in species of *Enterobacteriaceae* [36], the emergence and expansion of *bla*$_{KPC}$-carrying CRPA, suggests that this pathogen has had epidemiological success spreading in different countries, mainly in America and Asia [43], contributing with 42.9% and 37.4% of the reported isolates, respectively.

Table 1. Characteristics of studies included in the systematic review.

Country	Author, Year	Collection Date	No. Patients/ Isolates [a]	KPC Variants	Sequence Type	Ref
Colombia	Villegas, 2007	2006	3/3	KPC, KPC-2	NS	[16]
	Naas, 2008	NE	NS/1	KPC-2	NS	[14]
	Cuzon, 2011	2006–2010	NS/10	KPC-2	ST235, ST308, ST1006, ST1060	[12]
	Correa, 2012	2010	1/1	KPC-2	ST111	[11]
	Buelvas Doria, 2013	2008	1/1	KPC-2	NS	[10]
	Naas, 2013	NE	NS/2	KPC-2	NS	[13]
	Vanegas, 2014	2012–2014	25/25	KPC	ST111, ST235, ST362, ST1801, ST1803	[15]
	Abril, 2019	2014-2016	NS/4	KPC-2	ST235	[1]
	Pacheco, 2019	2017	5/5	KPC-2	NE	[6]
	Rada, 2021	2013–2015	10/12	KPC-2	ST308, ST309, ST313, ST699, ST3512	[5]
Puerto Rico	Wolter, 2009	2006	NS/2	KPC-2, KPC-5	NS	[34]
	Wolter, 2009	2006–2007	NS/25	KPC, KPC-2, KPC-5	NS	[35]
	Robledo, 2011	2009	NS/89	KPC	NS	[33]
	Martínez, 2012	2009	1/1	KPC-2	NS	[32]
Brazil	Jácome, 2012	2010	2/2	KPC-2	NS	[22]
	Cavalcanti, 2015	2008–2010	3/3	KPC-2	ST244, ST235	[17]
	Galetti, 2016	2011	1/1	KPC-2	ST244	[20]
	de Paula-Petroli, 2018	2008	1/1	KPC-2	ST235	[19]
	de Oliveira, 2018	2014	1/1	KPC-2	ST2584	[8]
	Galetti, 2019	2011	1/1	KPC-2	ST381	[21]
	Souza, 2021	2015–2016	3/3	KPC-2	NS	[23]

	Tartari, 2021	2018	1/1	KPC-2	ST312	[24]
	Costa-Júnior, 2021	2018–2019	11/11	KPC	NS	[18]
China	Ge, 2011	2009	3/3	KPC-2	ST463	[27]
	Hu, 2015	2013	NS/39	KPC-2	ST209, ST244, ST357, ST463, ST836, ST850, ST1755, ST1076	[28]
	Dai, 2016	2013	1/1	KPC-2	NS	[26]
	Shi, 2018	2016	1/1	KPC-2	NS	[31]
	Hu, 2019	2010	1/1	KPC-2	ST463	[29]
	Li, 2020	2018	21/21	KPC-2	ST664	[30]
	Cai, 2021	2019	1/4	KPC-2	ST463	[25]
	Hu, 2021	2007–2018	105/105	KPC-2	ST9, ST209, ST244, ST274, ST277, ST360, ST377, ST463, ST836, ST1076, ST1212, ST1642, ST2235	[3]
Vietnam	Tran, 2021	2011–2015	7/7	KPC-1	ST3151	[41]
Chile	Wozniak, 2021	2015	2/2	KPC-2	ST654	[36]
Argentina	Pasteran, 2012	2006–2011	NS/65	KPC-2	ST162, ST654	[37]
Germany	Hagemann, 2018	NS	1/1	KPC-2	ST235	[38]
Spain	Pérez-Vázquez, 2020	2016	2/2	KPC-2	ST244	[2]
India	Paul, 2015	2012–2013	2/2	KPC-2	NS	[39]
Trinidad and Tobago	Akpaka, 2009	NS	1/1	KPC-2	NS	[40]
EE.UU	Poirel, 2010	2009	1/1	KPC-2	NS	[42]
Several countries	Kazmierczak, 2016	2012–2014	NS/29	KPC-2	NS	[7]
Unclear location	Roth, 2011	NS	NS/3	KPC-2, KPC-5	NS	[44]
	Roth, 2013	NS	NS/1	KPC-2	NS	[45]

Notes: [a], number of clinical isolates obtained by the total number of patients. **Abbreviations:** NS: Not specified.

KPC Variants and Sequence Types

From the evaluated studies, 494 *bla*KPC-*P. aeruginosa* isolates (KPC-*Pa*) were found, of which 340 were associated with *bla*KPC-2 (69%) and only 3 isolates with *bla*KPC-5 (1%), the remaining isolates do not specify the KPC variant. Based on these results, the predominant variant among the analyzed isolates was KPC-2.

Thirty-five different STs were reported in 296 KPC-*Pa*. The most predominant STs were ST463 with 110 reports (37.16%), ST654 with 66 (22.30%), ST664 with 21 (7.09%) and ST235 with 15 (5.07%). However, most of the reports associated with ST463 were obtained from two different studies conducted in Zhejiang City, China [25,28]. Likewise, for ST654, 97% of the reports were part of the same study that evaluated isolates located in different hospitals in the Patagonia region in Argentina [37]. The predominance of specific clonal groups among populations with different geographical locations is evident in both studies.

The ST209 and ST274 reported in two studies conducted in Zhejiang, China [3,28], were the only two STs with single locus variations (SLV). The great diversity of STs identified in this systematic review reveals an alarming increase of unrelated clones that may have significant public healthcare importance.

*3.3. Dissemination Platform and bla*KPC*-Harboring MGE*

From all isolates, only 250 KPC-*Pa* (26 studies) described dissemination platforms and/or MGE associated with *bla*KPC. Regarding its genetic location, 228 of these isolates (91%) harbored *bla*KPC in a plasmid structure, and only 5 isolates (2%) contained the gene in the chromosome. 17 KPC-*Pa* (7%) did not emphasize in the genetic location (chromosomal or plasmid) but did report the genetic environment adjacent to the *bla*KPC (Figure 2).

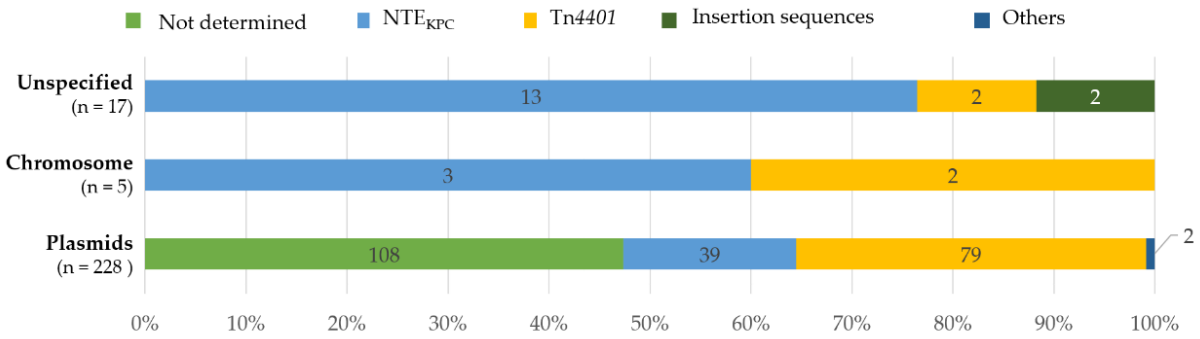

Figure 2. Genetic elements associated with the *bla*KPC according to genetic location: plasmids, chromosome or unspecified. The insertion sequences (IS) reported correspond to a ΔIS *Ec33* element [2].

Of the 228 reports of isolates harboring *bla*KPC in a plasmid structure, it was possible to identify that 79 of these isolates (34.6%) reported the gene as part of a Tn*4401* element (mainly in its b isoform), 39 isolates (17.1%) reported a non-Tn*4401* element (NTEKPC), and 2 isolates (0.9%) reported KPC within a genetic structure similar to an integron [39]. There was information of plasmid size for 87 KPC-harboring plasmids, most of them determined by gel electrophoresis. KPC-associated plasmids varied widely in their size, finding small plasmids with sizes less than 4 Kbp [20], and mega plasmids with sizes greater than 400 kbp [31]. In addition, it was possible to identify six different incompatibility groups: IncP-3-like (IncA/C) [30], IncU [13,24], IncP-6 [13,26], IncF-like [39], IncQ1 [8], and IncHI1 [38]. According to the information collected, 12 *P. aeruginosa* plasmids carrying *bla*KPC were fully sequenced and available in the NCBI public database and in the published literature (Table 2).

Table 2. *Pseudomonas aeruginosa* plasmids carrying *bla*KPC completely sequenced and reported in the literature.

Author, Year	Strain	ST	Plasmid (bp)	Inc [a]	Access Number	Ref
Naas, 2013	COL-1	NR	pCOL (31,529 bp)	IncP-6	KC609323	[13]
Naas, 2013	PA-2	NR	pPA-2 (7995 bp)	IncU	KC609322	[13]
Dai, 2016	10265	NR	p10265-KPC (38,939 bp)	IncP-6	KU578314	[26]
Galetti, 2016	BH6	ST244	pBH6 (3652 bp)	ND	LGVH01000782.1	[20]
Shi, 2018	14057	NR	p14057A (51,663 bp)	ND	KY296095	[31]
Galetti, 2019	BH9	ST381	pBH6::Phage (41.024 bp)	NR	CP029714	[21]
Hu, 2019	PA1011	ST463	pPA1011 (62.793 bp)	ND	MH734334	[29]
Li, 2020	NK546	ST664	pNK546a (475,027 bp)	IncP-3-like (IncA/C)	MN433457	[30]
Tartari, 2021	MIMA_PA2.1	ST312	pMIMA_PA2.1 (7975 bp)	IncU	MT683857	[24]
Cai, 2021	P23	ST463	pP23 (40,937 bp)	NR	CP065418	[25]
Cai, 2021	P33	ST463	pP33-2 (48,306 bp)	ND	CP065414	[25]
Wozniak, 2021	Pae-13	ST654	pPae-13 (35,034 bp)	ND	MT949191	[36]

Notes: [a], Incompatibility group. **Abbreviations:** NR: Not reported (Plasmid incompatibility groups were not evaluated in these studies); ND, Incompatibility group not determined.

In the case of the five isolates that harbor *bla*KPC on the chromosome: 3 of these reports (60%) were associated with an NTEKPC element, and the other 2 (40%) with a Tn*4401b* structure. Although remaining 17 isolates did not specify location of this gene on the genome: 13 of them (76.5%) were associated with an NTEKPC and three (17.6%) with a Tn*4401* structure. Even though KPC mobilization among *P. aeruginosa* isolates has been associated primarily with the Tn*4401* transposon variants and plasmids with different incompatibility groups [1]; in this systematic review, we observed a high incidence of NTEKPC structures [46,47]. In fact, in recent years the *bla*KPC gene has been reported within these non-

conventional genetic elements in several bacterial species, including *P. aeruginosa* [46,47]; the presence of NTE$_{KPC}$ in several of the isolates, shows these elements are contributing to the successful dissemination of *bla*$_{KPC}$-harboring *P. aeruginosa.* Likewise, it has been shown that the wide circulation of plasmids with *bla*$_{KPC,}$ has brought increased in the appearance of resistant isolates in hospital environments [48]. Finally, the great diversity of unrelated clones may imply a greater risk of dissemination at global level [1].

4. Conclusions

In conclusion, this is the first systematic review that consolidates information about *bla*$_{KPC}$ dissemination and genetic platforms in carbapenem-resistant *P. aeruginosa* strains worldwide. The results presented in this review provide a general overview of the epidemiology of this carbapenemase at a global level; the Tn*4401* is the main element associated with the dissemination of the *bla*$_{KPC}$ gene, however, the results of this systematic review indicate that in *P. aeruginosa* the presence of NTE$_{KPC}$ elements is being increasingly prevalent in this species.

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Conflicts of Interest:

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