





## Article

# Prevalence of *bla*<sub>KPC-2</sub>, *bla*<sub>KPC-3</sub> and *bla*<sub>KPC-30</sub>—Carrying Plasmids in *Klebsiella pneumoniae* Isolated in a Brazilian Hospital

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**Abstract:** *Klebsiella pneumoniae* carbapenemase (KPC) actively hydrolyzes carbapenems, antibiotics often used a last-line treatment for multidrug-resistant bacteria. KPC clinical relevance resides in its widespread dissemination. In this work, we report the genomic context of KPC coding genes *bla*<sub>KPC-2</sub>, *bla*<sub>KPC-3</sub> and *bla*<sub>KPC-30</sub> in multidrug-resistant *Klebsiella pneumoniae* isolates from Brazil. Plasmids harboring *bla*<sub>KPC-3</sub> and *bla*<sub>KPC-30</sub> were identified. Fifteen additional carbapenem-resistant *K. pneumoniae* isolates were selected from the same tertiary hospital, collected over a period of 8 years. Their genomes were sequenced in order to evaluate the prevalence and dissemination of *bla*<sub>KPC</sub>—harboring plasmids. We found that *bla*<sub>KPC</sub> genes were mostly carried by one of two isoforms of transposon Tn4401 (Tn4401a or Tn4401b) that were predominantly located on plasmids highly similar to the previously described plasmid pKPC\_FCF3SP (IncN). The identified pKPC\_FCF3SP-like plasmids carried either *bla*<sub>KPC-2</sub> or *bla*<sub>KPC-30</sub>. Two *K. pneumoniae* isolates harbored pKpQIL-like (IncFII) plasmids, only recently identified in Brazil; one of them harbored *bla*<sub>KPC-3</sub> in a Tn4401a transposon. Underlining the risk of horizontal spread of KPC coding genes, this study reports the prevalence of *bla*<sub>KPC-2</sub> and the recent spread of *bla*<sub>KPC-3</sub>, and *bla*<sub>KPC-30</sub>, in association with different isoforms of Tn4401, together with high synteny of plasmid backbones among isolates studied here and in comparison with previous reports.

**Keywords:** antimicrobial resistance; carbapenem; healthcare-associated infection; KPC; plasmid; *Klebsiella pneumoniae*

## 1. Introduction

Infections caused by carbapenem-resistant *Enterobacteriaceae* represent a significant global health threat [1]. *Enterobacteriaceae* become resistant to carbapenems by three major mechanisms: porin alteration, hyper-expression of efflux pumps, and  $\beta$ -lactamase production [2]. There are three main groups of  $\beta$ -lactamases associated with resistance to carbapenems (carbapenemases): *Klebsiella pneumoniae* carbapenemase (KPC), metallo- $\beta$ -lactamases, and OXA-48-like  $\beta$ -lactamase. Mobile genetic elements, such as plasmids and

transposons, are the main vectors enabling and facilitating the spread of these resistance determinants [3,4].

KPC is a plasmid-encoded enzyme which occurs in several different variants, and its clinical relevance is associated with its worldwide spread [5]. The occurrence of carbapenemases varies according to the geographic region; the KPC-2 variant has been reported as the most frequent carbapenemase in Latin America [6,7]. In Brazil, the first report of KPC-2 occurred in 2006, and soon, numerous reports attested to its widespread dissemination [8–11]. KPC-2 and KPC-3 differ by only one amino acid (H272Y) and are the most prevalent carbapenemases worldwide [12–15]. Only KPC-2 is currently considered endemic in Brazil [10,16]. The treatment of infections by carbapenem-resistant *Enterobacteriaceae* is complicated, with severe clinical and socioeconomic consequences [4,17,18].

Despite the worldwide spread of KPC, genomic studies including KPC-harboring isolates from Latin America, and in particular from Brazil, are underrepresented in the literature. For instance, a recent study evaluated all KPC-harboring plasmids described in the NCBI database, and only 12 out of 257 representative KPC-plasmids evaluated were from Brazil, all harboring *bla*<sub>KPC-2</sub> [19].

Our hospital is one of the Brazilian medical centers enrolled in a longitudinal surveillance study (Study for Monitoring Antimicrobial Resistance Trends, SMART) [20]. Due to our participation, two KPC variants not previously reported in *Enterobacteriaceae* in Brazil, *bla*<sub>KPC-3</sub>, and *bla*<sub>KPC-30</sub>, were identified in *K. pneumoniae* isolated in Brazil [21]. Both isolates showed resistance to colistin, a last-resort therapeutic drug against multidrug-resistant *Enterobacteriaceae*.

In this work, we report the genomic characterization of these two isolates by whole genome sequencing. In addition, 15 *K. pneumoniae* isolates, collected in the same tertiary hospital over a period of 8 years, were genome-sequenced. We found *bla*<sub>KPC</sub>-harboring plasmids in most isolates; these originated from two different plasmids and contained *bla*<sub>KPC-2</sub>, *bla*<sub>KPC-3</sub>, or *bla*<sub>KPC-30</sub>, mostly on one of two isoforms of transposon Tn4401 (Tn4401a or Tn4401b).

## 2. Results

Genome characteristics and MLST types for the *K. pneumoniae* isolates harboring *bla*<sub>KPC-2</sub>, *bla*<sub>KPC-30</sub>, and *bla*<sub>KPC-3</sub> are summarized in Table 1. All isolates belonged to the Clonal Complex 258 (CC258), except for Kp326 (ST16). Capsule polysaccharide synthesis (*cps*) locus analysis demonstrated that isolates belonged to five distinct K-loci groups, the most recurrent being KL36, as previously reported [22].

All isolates harbored the *bla*<sub>KPC-2</sub> gene, except for Kp391, harboring *bla*<sub>KPC-30</sub>, and Kp392, harboring *bla*<sub>KPC-3</sub>. We selected three isolates harboring distinct *bla*<sub>KPC</sub> variants for a complete genomic characterization: Kp90 (*bla*<sub>KPC-2</sub>), Kp391 (*bla*<sub>KPC-30</sub>), and Kp392 (*bla*<sub>KPC-3</sub>). Major differences between the three genomes are depicted in Figure 1A. We then looked for the genomic loci of the KPC genes; in all strains, the genes were located on contigs that were parts of plasmids (Figure 1B). This is in agreement with previous observations, that is, the *bla*<sub>KPC</sub> gene is mostly carried by a multireplicon IncFIIk-IncFI plasmid [23,24]. In fact, in Kp392, the *bla*<sub>KPC-3</sub> gene was located on a region that is 100% identical to a region of the plasmid pKpQIL (113,637 bp) found in *K. pneumoniae* ST258 strains [25] and to a region of plasmid pKPC (113,639 bp) found in a ST512 strain [26] (Figure 1B). Kp392 from this work is also a ST512 strain, and the plasmid in strain Kp392 is a multireplicon IncFIIk-IncFI plasmid (Figure 1B). Next, we looked into the *bla*<sub>KPC-30</sub>-harboring strain Kp391 that belongs to the well-known ST11 [27–30]. The *bla*<sub>KPC-30</sub> gene was located on a 49 kb contig that showed high similarity with the 54 kb plasmid pKPC\_FCF3SP that carries *bla*<sub>KPC-2</sub> in strain FCF3SP (ST442) isolated from a blood culture of a patient in Brazil [31]. The entire plasmid in strain Kp391 could be reconstructed using pKPC\_FCF3SP as template. The assembled plasmid is designated pKPC30\_Kp391 (Figure 1C). All genes present in the IncN plasmid backbone were found in pKPC30\_Kp391 (Figure 1D). The plasmids from strains FCF3SP and Kp391 share 99% nucleotide identity, with a total of 76 SNPs. The strain Kp90 carries a

*bla*<sub>KPC-2</sub>-encoding plasmid that shares high similarity with pKPC30\_Kp391, pKPC\_FCF3SP, as well as pKPC\_FCF1305. Figure 1E describes the genomic region of the three plasmids.

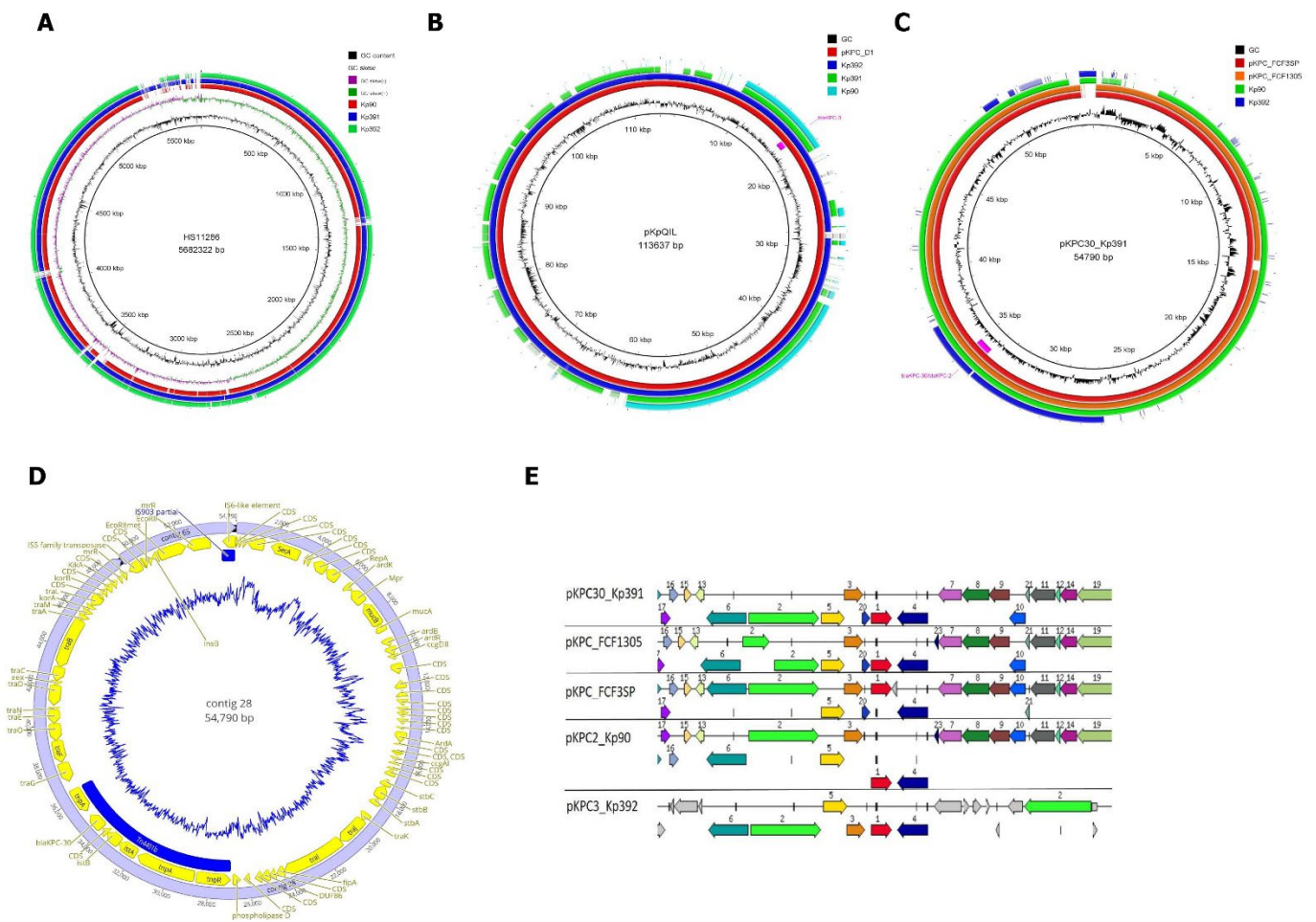
**Table 1.** Features of sequenced genomes of *K. pneumoniae*.

Isolate	Year of Isolation	Origin of Specimen	<i>bla</i> <sub>KPC</sub> Variant	Mlst Type (ST)	Clonal Group (CG)	CPS Locus (KL)	Genome Size (kb)	G+C (%)	Coverage (Fold)	Contigs (n <sup>1</sup> )	CDS (n)	Genbank Accession
Kp90	2015	Blood	<i>bla</i> <sub>KPC-2</sub>	ST437	258	36	5429	57.4	77	76	5496	JACBOR000000000
Kp391	2016	Abdominal abscess	<i>bla</i> <sub>KPC-30</sub>	ST11	258	64	5871	56.9	77	67	5956	JACBOQ000000000
Kp392	2017	Urine	<i>bla</i> <sub>KPC-3</sub>	ST512	258	107	5681	57.1	103	81	5787	JACBOP000000000
Kp121	2014	Blood	<i>bla</i> <sub>KPC-2</sub>	ST437	258	36	5554	57.3	84	55	5601	JAIEVGO000000000
Kp177	2013	Blood	<i>bla</i> <sub>KPC-2</sub>	ST437	258	36	5588	57.3	83	79	5679	JAIEVGN000000000
Kp225	2011	Blood	<i>bla</i> <sub>KPC-2</sub>	ST437	258	36	5777	57.1	64	81	5847	JAIEVGM000000000
Kp326	2016	Blood	<i>bla</i> <sub>KPC-2</sub>	ST16	-	51	5568	57.2	91	73	5648	JAIEVGL000000000
Kp329	2015	Bronchoalveolar lavage	<i>bla</i> <sub>KPC-2</sub>	ST11	258	64	5887	57.1	66	127	6049	JAIEVGK000000000
Kp384	2014	Blood	<i>bla</i> <sub>KPC-2</sub>	ST437	258	36	5569	57.3	66	79	5672	JAIEVGJ000000000
Kp387	2013	Ascitic fluid	<i>bla</i> <sub>KPC-2</sub>	ST11	258	27	5852	57.0	51	105	5967	JAIEVGI000000000
Kp389	2013	Bronchoalveolar lavage	<i>bla</i> <sub>KPC-2</sub>	ST437	258	36	5473	57.3	88	75	5524	JAIEVGH000000000
Kp373	2013	Rectal surveillance swabs	<i>bla</i> <sub>KPC-2</sub>	ST11	258	64	5900	57.0	81	68	6021	JAIEVGG000000000
Kp374	2013	Rectal surveillance swabs	<i>bla</i> <sub>KPC-2</sub>	ST437	258	36	5604	57.3	84	78	5702	JAIEVGF000000000
Kp376	2015	Rectal surveillance swabs	<i>bla</i> <sub>KPC-2</sub>	ST11	258	27	6162	56.6	82	135	6385	JAIEVGE000000000
Kp377	2015	Rectal surveillance swabs	<i>bla</i> <sub>KPC-2</sub>	ST11	258	64	5808	56.8	91	115	5953	JAIEVGD000000000
Kp378	2016	Rectal surveillance swabs	<i>bla</i> <sub>KPC-2</sub>	ST11	258	64	5872	56.9	87	62	5965	JAIEVGC000000000
Kp381	2017	Rectal surveillance swabs	<i>bla</i> <sub>KPC-2</sub>	ST437	258	36	6095	56.5	78	128	6344	JAIEVGB000000000
Kp382	2017	Rectal surveillance swabs	<i>bla</i> <sub>KPC-2</sub>	ST11	258	64	5877	56.9	94	97	6023	JAIEVGA000000000

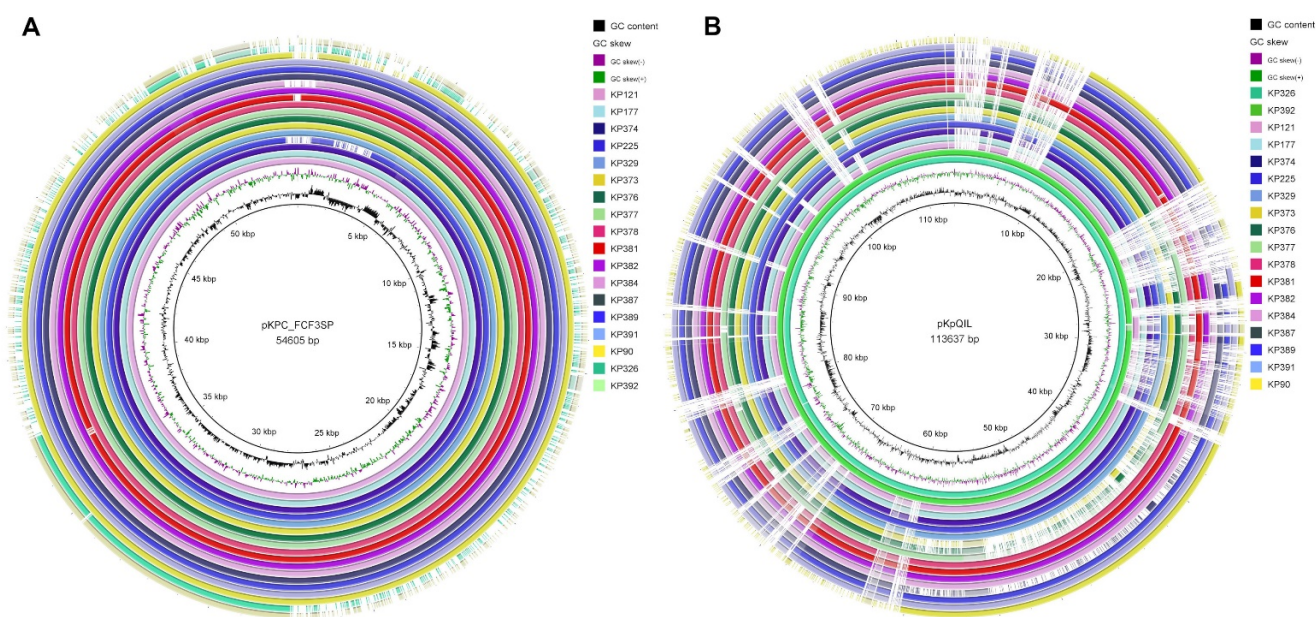
<sup>1</sup> N: number.

Fourteen additional *K. pneumoniae* isolates that were collected in the same tertiary hospital over a period of 8 years were sequenced and analyzed. We searched for the genomic region where the *bla*<sub>KPC</sub> genes were located in these 14 strains; in all strains, the genes were located on contigs that were parts of plasmids belonging to two distinct incompatibility groups: IncN or IncFII. Among the 17 sequenced isolates, 15 harbored the IncN backbone, showing high similarity with pKPC\_FCF3SP (Figure 2A). On this plasmid, except for Kp391, harboring *bla*<sub>KPC-30</sub>, all isolates harbored *bla*<sub>KPC-2</sub>. Isolates Kp326 (*bla*<sub>KPC-2</sub>) and Kp392 (*bla*<sub>KPC-3</sub>) contained plasmids highly similar to the plasmid pKpQIL (IncFII) (Figure 2B). pQIL-like (IncFIB) plasmids have just recently been reported in Brazil, in clones belonging to ST16, such as Kp326, and associated with high mortality rates [32]. However, the sequence of the pQIL-like plasmid identified in Brazil is not publicly available, so we were not able to compare ours to it and, to our knowledge, the first sequence made publicly available.

Three isoforms of the Tn4401 transposon were found in the isolates described in this study. Two isoforms are well-known, Tn4401a and Tn4401b. These isoforms differ by a 100 bp deletion in the region upstream of *bla*<sub>KPC</sub> in Tn4401a (Figure 3A). The isoform Tn4401a was carried by pKpQIL-like plasmids (strains Kp326 and Kp392), while the isoform Tn4401b was carried by pKPC\_FCF3SP-like plasmids, as previously reported [31]. It has been shown that isolates harboring Tn4401a present higher resistance to carbapenems due to alterations in the promoter region of *bla*<sub>KPC</sub> [33,34]. A third isoform of Tn4401 was detected in isolate Kp381. This variant lacks 253 bp downstream from the *bla*<sub>KPC</sub> gene, leading to a shorter *tnpA/ISKpn6* region (1320 bp/439 aa to 963 bp/320 aa) (Figure 3B).



**Figure 1.** Genomic characteristics of *Klebsiella pneumoniae* isolates. **(A)** Genome comparison of three *Klebsiella pneumoniae* isolates using strain HS11286 as the reference genome. Genome sizes were similar to the reference genome *K. pneumoniae* strain HS11286 (5682 kb) and differences are mostly associated with acquired genomic regions. **(B)** The plasmid in strain Kp392 is highly similar to plasmids pKpQIL and pKPC\_D1. The analysis compared the plasmid pKpQIL with the plasmid pKPC\_D1 and the genomes of the three strains sequenced here. Only the genome of strain Kp392 contains contigs (including the 21 kb contig that carries *bla*<sub>KPC-3</sub>) that match almost entirely the plasmid pKpQIL. The strain Kp391 carries a different plasmid that shares some homology with pKpQIL. The *bla*<sub>KPC-3</sub> gene is highlighted in pink. **(C)** The *bla*<sub>KPC-30</sub> carrying plasmid pKPC30\_Kp391 in strain Kp391 is highly similar to *bla*<sub>KPC-2</sub> carrying plasmids pKPC\_FCF3SP and pKPC\_FCF1305. The analysis compared the assembled plasmid sequence in strain Kp391 with the plasmids pKPC\_FCF3SP and pKPC\_FCF1305 and the genomes of the other strains sequenced here (Kp90 and Kp392). pKPC30\_Kp391 almost entirely matches the plasmids pKPC\_FCF3SP and pKPC\_FCF1305. The strain Kp90 also carries a *bla*<sub>KPC-2</sub> encoding plasmid that shares high homology with pKPC30\_Kp391, pKPC\_FCF3SP and pKPC\_FCF1305. The location of *bla*<sub>KPC-2</sub> or *bla*<sub>KPC-30</sub> is highlighted in pink. **(D)** General structure of pKPC30\_Kp391 based on the IncN conserved backbone and the two acquired regions Tn4401b and IS903B. The conserved IncN region carries genes involved in replication and replication regulation (*repA*, *ardB*, *ardR*, *ardK*, *ccgEIII*, *ccgD*, *ccgC*, *ccgAI*), genes responsible for plasmid stability (*stbA*, *stbB*, *stbC*, *fipA*, *eex*, *korA*, *korB*, *kikA*), in DNA repair (*mpr*, *mucA*, *mucB*), inhibition of type I restriction enzymes (*ardA*), and conjugative transfer (*tra* gene region). Transposon Tn4401b comprises *tnpR*, *tnpA*, *istA*, *istB*, *bla*<sub>KPC</sub>, and *tnpA*. **(E)** Plasmid gene organization in the vicinity of *bla*<sub>KPC</sub> gene in pKPC\_Kp90, pKPC\_Kp391, pKPC\_Kp392, and closely related plasmids. The colors indicate high homology of the genes. 1, *bla*<sub>KPC</sub> gene; 2 (*tnpA*) and 4 (*tnpA*) (transposase); 3 (*istB*) and 5 (*istA*) (mobile element); 6 (*tnpR*) site-specific recombinase; 7–14 (*tra* genes) components of a type IV conjugative transfer system; 15–17, hypothetical proteins.



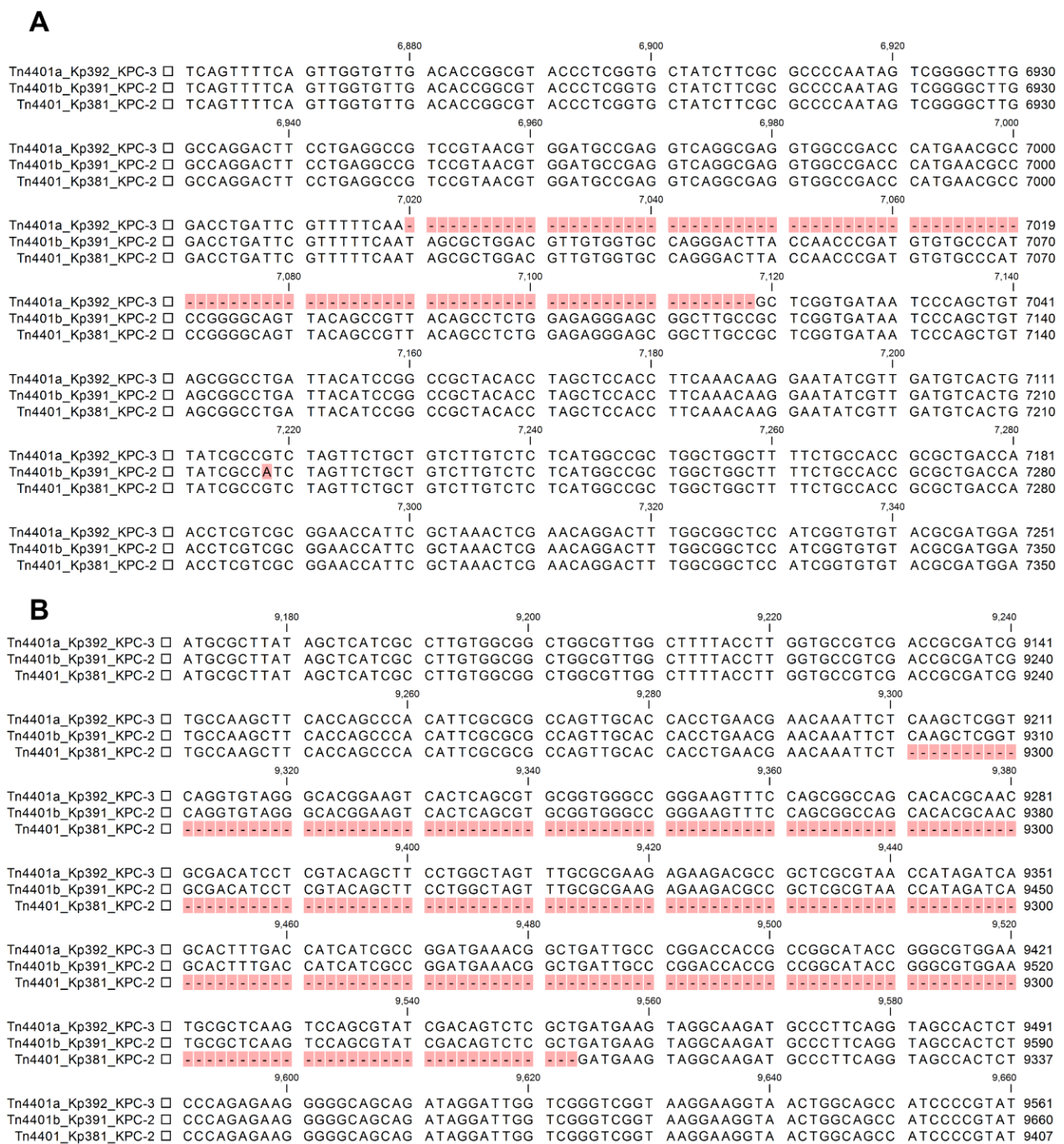
**Figure 2.** Presence of plasmids in 17 sequenced *Klebsiella pneumoniae* isolates. (A) Comparison using pKPC\_FCF3SP as the reference plasmid identified 15 strains that contained highly identical plasmids. (B) A comparison using pKpQIL as the reference plasmid identified two strains (Kp326 and Kp392) with highly similar plasmids.

As a means to understand the impact of the different *bla*<sub>KPC</sub> genes in the susceptibility profile of *K. pneumoniae*, the susceptibility profiles of Kp391 (*bla*<sub>KPC-30</sub>), Kp392 (*bla*<sub>KPC-3</sub>), and Kp90 (*bla*<sub>KPC-2</sub>) are reported in Table 2. All three isolates showed resistance to cephalosporins, quinolones, colistin, and carbapenems, and were susceptible to the combination ceftazidime/avibactam. Kp392, harboring the pKpQIL-like plasmid with Tn4401a and *bla*<sub>KPC-3</sub>, displayed a MIC of 6 µg/mL for the combination ceftazidime/avibactam (breakpoint for resistance: ≥8 µg/mL); this is higher than for the other two isolates which were clearly susceptible (2 µg/mL) [35].

**Table 2.** Antibiotic susceptibility of three *K. pneumoniae* isolates with different *bla*<sub>KPC</sub> genes.

Antimicrobial	Kp90		Kp391		Kp392	
	MIC µg/mL	Profile	MIC µg/mL	Profile	MIC µg/mL	Profile
Ampicillin/Sulbactam	≥32	R	≥32	R	≥32	R
Piperacilin/Tazobactam	≥128	R	≥128	R	≥128	R
Cefuroxime	≥64	R	≥64	R	≥64	R
Cefoxitin	≥64	R	≥64	R	≥64	R
Ceftazidime	16	R	≥64	R	≥64	R
Ceftriaxone	≥64	R	≥64	R	≥64	R
Cefepime	≥64	R	≥64	R	≥64	R
Ertapenem	≥8	R	≥8	R	≥8	R
Imipenem	≥16	R	≥16	R	≥16	R
Meropenem	≥16	R	≥16	R	≥16	R
Amikacin	≤2	S	≤2	S	≥64	R
Gentamicin	≤1	S	≤1	S	8	R
Ciprofloxacin	≥4	R	≥4	R	≥4	R
Tigecycline	≥8	-	≤0.5	-	2	-
Imipenem	>32	R	>32	R	32	R
Meropenem	>32	R	>32	R	>32	R
Ceftazidime/avibactam	2	S	2	S	6	S
Colistin	≥16	R	≥16	R	≥16	R

MIC: minimum inhibitory concentration. Breakpoints for Tigecycline are not available for *K. pneumoniae* [36].



**Figure 3.** Comparison of the three Tn4401 isoforms found in *K. pneumoniae* isolates in this work. (A) Tn4401a\_Kp392 shows a deletion of 100 bp in the region upstream of the *bla*<sub>KPC</sub> gene when compared with Tn4401b\_Kp391 and Tn4401\_Kp381. (B) Downstream from the *bla*<sub>KPC</sub> gene Tn4401\_Kp381 shows a deletion of 253 bp when compared with Tn4401a\_Kp392 and Tn4401b\_Kp391.

### 3. Discussion

KPC-producing *Enterobacteriaceae* have been considered a pandemic in the history of Gram-negative bacteria [37]. According to gene sequences deposited in Genbank (<https://www.ncbi.nlm.nih.gov/pathogens/refgene/#kpc>, accessed on 4 February 2021) at the time of this submission (date: 4 February 2021), a total of 66 *bla*<sub>KPC</sub> gene variants had been reported.

As a result of the antibiotic resistance surveillance study, SMART, two new KPC variants were detected in *K. pneumoniae* in Brazil: *bla*<sub>KPC-3</sub> and *bla*<sub>KPC-30</sub> [21]. This work

was conducted to gain an understanding of the genetic context surrounding *bla*<sub>KPC</sub> genes in the tertiary hospital where the new variants were detected, using this information to discuss the scenario in Brazil.

KPC-3, still not endemic in Brazil, differs by one amino acid substitution (H272Y) from KPC-2 [38]. This single amino acid change has been reported to increase the catalytic efficiency of the enzyme by up to nine times when degrading ceftazidime [14], and, most recently, it has been described to increase the MIC for ceftazidime/avibactam [14,15,39]. This is particularly worrisome since, in Brazil, this combination has only recently been approved as a treatment option for multidrug-resistant bacteria (June 2018). Strains Kp90 (harboring *bla*<sub>KPC-2</sub>), Kp391 (harboring *bla*<sub>KPC-30</sub>), and Kp392 (harboring *bla*<sub>KPC-3</sub>) showed very similar susceptibility profiles for carbapenems and ceftazidime. However, Kp392 displayed a MIC of 6 µg/mL for ceftazidime/avibactam (breakpoint for resistance: ≥8 µg/mL) [35], compared to Kp90 and Kp391, which were clearly susceptible (2 µg/mL). We suggest that the increased resistance to ceftazidime/avibactam found for Kp392 (MIC of 6 mg/mL) is mostly associated with the presence of KPC-3, in agreement with previous studies [14,40]. Additionally, in Kp392, the pKpQIL-like plasmid harbored the *bla*<sub>KPC-3</sub> gene in a Tn4401a transposon; this transposon has been associated with increased resistance to carbapenems due to modifications in the promoter region that increase the expression of the *bla*<sub>KPC-3</sub> gene (a 100 bp deletion upstream of this gene) [33,34].

Plasmid pKpQIL belongs to the IncFII incompatibility group and has often been reported in association with antibiotic resistance and epidemic isolates. It was first reported in Israel in 2006, but a retrospective study revealed that it had been carried by a *K. pneumoniae* isolate from a patient in New York in 2004 [41–44]. This plasmid was the first *bla*<sub>KPC</sub>-bearing plasmid identified in epidemic ST258 strains, becoming well-known for the early dissemination of KPC-encoding genes [25,45,46]. Despite its worldwide spread, pKpQIL-like plasmids were only recently reported in Brazil in *K. pneumoniae* ST16 [25,32,41,43,44,47,48]. In this work, isolates harboring this plasmid were collected in 2016 and 2017 in strains belonging to ST16 and ST512, suggesting a recent, and possibly local, spread.

Plasmids belonging to IncN, such as pKPC\_FCF3SP, and highly similar plasmids identified in this study (in strains isolated since 2011), have been reported in Brazil since 2005; the earliest report of an IncN plasmid carrying *bla*<sub>KPC-2</sub> was in 1997 [19,31,49]. Even though pKPC\_FCF3SP is associated with the *bla*<sub>KPC-2</sub> gene, here we report an isolate carrying a pKPC\_FCF3SP-like plasmid with *bla*<sub>KPC-30</sub>. The *bla*<sub>KPC-30</sub> gene sequence has been previously reported in one strain from Brazil, but without any associated publication (strain 1472816, GenBank accession number KY646302.1). The *bla*<sub>KPC-30</sub> variant shows a single-point mutation compared to *bla*<sub>KPC-2</sub>, leading to one amino acid change (R6H) in the region coding for the signal peptide. In this regard, a high degree of synteny between *bla*<sub>KPC-2</sub> and *bla*<sub>KPC-30</sub>-carrying plasmids was noted and, as matter of fact, highly similar plasmids have often been identified in epidemic strains, such as those belonging to CC258 [45,50,51].

In conclusion, in this work we reported the genetic background for *bla*<sub>KPC</sub> found in carbapenem-resistant *K. pneumoniae* isolates from Brazil. Plasmids highly similar to pKpQIL and pKPC\_FCF3SP harbored *bla*<sub>KPC-2</sub>, *bla*<sub>KPC-3</sub>, *bla*<sub>KPC-30</sub>, in association with different isoforms of Tn4401, an active transposon encoding insertion sequences (ISKpn7 and ISKpn6) capable of efficiently mobilizing the *bla*<sub>KPC</sub> gene to random targets. The diversity and structural complexity of genetic elements carrying *bla*<sub>KPC</sub> genes suggest that they play major roles in actively promoting transposition of these genes to various genetic locations in the bacterial genome, but also suggest that they may increase the genetic plasticity of plasmids, leading to improved ability to coexist with bacterial hosts [45]. However, we also observed high synteny of plasmid backbones among isolates studied here and in comparison with previous reports from Brazil and the rest of the world. This highlights the importance of surveillance for early detection and implementation of control measures to prevent the rapid dissemination of *bla*<sub>KPC</sub> in the clinical environment.

## 4. Materials and Methods

### 4.1. Clinical Isolates

*K. pneumoniae* isolates harboring *bla*<sub>KPC</sub> genes were identified as *K. pneumoniae* by MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA) between 2011 and 2017. All patients had hospital-acquired infections, with a history of previous hospitalization and antimicrobial use (carbapenem, quinolone, cephalosporin, or piperacillin-tazobactam) during the 30 days prior to *K. pneumoniae* isolation.

### 4.2. Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined using the Vitek 2 XL System (bioMérieux, Craponne, France). Additionally, susceptibility to ceftazidime/avibactam, imipenem, and meropenem was also carried out by the epsilometric (Etest<sup>®</sup>) method. Broth microdilution was performed for MIC testing of polymyxin B with *Pseudomonas aeruginosa* ATCC 27853 used as a reference for susceptibility. Antimicrobial susceptibility results were interpreted according to BrCAST/EUCAST guidelines [52].

### 4.3. Genome Sequencing

Genomic DNA isolation of *K. pneumoniae* isolates was performed as previously described [53]. Concentration and purity of the isolated DNA was first checked with a NanoDrop ND-1000 (Peqlab, Erlangen, Germany), and the exact concentration was determined using the Qubit<sup>®</sup> dsDNA HS Assay Kit, as recommended by the manufacturer (Life Technologies GmbH, Darmstadt, Germany). Illumina shotgun libraries were prepared using the Nextera XT DNA Sample Preparation Kit and subsequently sequenced on a MiSeq system using the reagent kit v3 with 600 cycles (Illumina, San Diego, CA, USA), as recommended by the manufacturer. Quality filtering was done with version 0.36 of Trimmomatic [54]. Assembly was performed using the SPAdes genome assembler software version 3.13.0 [55], using an average of 2,009,581 paired-end reads (range: 1,716,540–2,544,588). Qualimap version 2.2.1 [56] was used to validate the genome assembly and determine the sequence coverage. The average coverage was 81-fold (range: 69–103). Comparative genome and plasmid analyses were done using RAST and visualized with BRIG [57]. Insertion sequences were identified by IS finder [58].

### 4.4. MLST and Capsule Synthesis Loci (K-loci) Analysis

WGS data were also used to determine the sequence types (STs) using the multi-locus sequencing typing (MLST) scheme available at ([https://bigsdbs.pasteur.fr/cgi-bin/bigsdbs/bigsdbs.pl?db=pubmlst\\_klebsiella\\_seqdef&page=sequenceQuery/](https://bigsdbs.pasteur.fr/cgi-bin/bigsdbs/bigsdbs.pl?db=pubmlst_klebsiella_seqdef&page=sequenceQuery/); accessed on 2 April 2020) sited at the Institut Pasteur MLST. Capsule synthesis loci (K-loci or KL) analysis was carried out using the software Kaptive available at <http://kaptive.holtlab.net/>, accessed on 2 April 2020 [59].

### 4.5. NCBI Database Data Extraction for *K. pneumoniae* KPC-Harboring Plasmids

The KPC-2, -3, and -30 sequences (Accession Numbers NC\_019161.1, NG\_049257.1 and NG\_054685.1) were used for a nucleotide-nucleotide BLAST search in the NCBI database (expected threshold  $10 \times 10^{-70}$ ), following the protocol described by Brandt et al. [19]. The resulting sequence hits (accessed on February 2021) were then filtered for sequences reported only in *K. pneumoniae* and for plasmid structures (with at least 1000 bp) carrying KPC-2, -3, and -30 nucleotide sequences.

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**Data Availability Statement:** All genome sequences were deposited in the GenBank with the following accession numbers: JACBOR000000000, JACBOQ000000000, JACBOP000000000, JAEVGO000000000, JAEVGN000000000, JAEVGM000000000, JAEVGL000000000, JAEVKG000000000, JAEVGJ000000000, JAEVGI000000000, JAEVGH000000000, JAEVGG000000000, JAEVGF000000000, JAEVGE000000000, JAEVGD000000000, JAEVGC000000000, JAEVGB000000000, JAEVGA000000000.

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## References

- Durante-Mangoni, E.; Andini, R.; Zampino, R. Management of carbapenem-resistant Enterobacteriaceae infections. *Clin. Microbiol. Infect.* **2019**, *25*, 943–950. [[CrossRef](#)] [[PubMed](#)]
- Tooke, C.L.; Hinchliffe, P.; Bragginton, E.C.; Colenso, C.K.; Hirvonen, V.H.A.; Takebayashi, Y.; Spencer, J. beta-Lactamases and beta-Lactamase Inhibitors in the 21st Century. *J. Mol. Biol.* **2019**, *431*, 3472–3500. [[CrossRef](#)]
- Ambler, R.P.; Coulson, A.F.; Frère, J.M.; Ghuysen, J.M.; Joris, B.; Forsman, M.; Levesque, R.C.; Tiraby, G.; Waley, S.G. A standard numbering scheme for the class A beta-lactamases. *Biochem. J.* **1991**, *276 Pt 1*, 269–270. [[CrossRef](#)]
- Queenan, A.M.; Bush, K. Carbapenemases: The Versatile  $\beta$ -Lactamases. *Clin. Microbiol. Rev.* **2007**, *20*, 440. [[CrossRef](#)] [[PubMed](#)]
- Nordmann, P.; Poirel, L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin. Microbiol. Infect.* **2014**, *20*, 821–830. [[CrossRef](#)]
- Logan, L.K.; Weinstein, R.A. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. *J. Infect. Dis.* **2017**, *215*, S28–S36. [[CrossRef](#)]
- Maya, J.J.; Ruiz, S.J.; Blanco, V.M.; Gotuzzo, E.; Guzman-Blanco, M.; Labarca, J.; Salles, M.; Quinn, J.P.; Villegas, M.V. Current status of carbapenemases in Latin America. *Expert Rev. Anti-Infect. Ther.* **2013**, *11*, 657–667. [[CrossRef](#)]
- Monteiro, J.; Santos, A.F.; Asensi, M.D.; Peirano, G.; Gales, A.C. First report of KPC-2-producing *Klebsiella pneumoniae* strains in Brazil. *Antimicrob. Agents Chemother.* **2009**, *53*, 333–334. [[CrossRef](#)] [[PubMed](#)]
- Seki, L.M.; Pereira, P.S.; de Souza Mda, P.; Conceição Mde, S.; Marques, E.A.; Porto, C.O.; Colnago, E.M.; Alves Cde, F.; Gomes, D.; Assef, A.P.; et al. Molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* isolates in Brazil: The predominance of sequence type 437. *Diagn. Microbiol. Infect. Dis.* **2011**, *70*, 274–277. [[CrossRef](#)] [[PubMed](#)]
- Sampaio, J.L.M.; Gales, A.C. Antimicrobial resistance in Enterobacteriaceae in Brazil: Focus on  $\beta$ -lactams and polymyxins. *Braz. J. Microbiol.* **2016**, *47*, 31–37. [[CrossRef](#)] [[PubMed](#)]
- Ribeiro, P.C.S.; Monteiro, A.S.; Marques, S.G.; Monteiro, S.G.; Monteiro-Neto, V.; Coqueiro, M.M.M.; Marques, A.C.G.; de Jesus Gomes Turri, R.; Santos, S.G.; Bomfim, M.R.Q. Phenotypic and molecular detection of the blaKPC gene in clinical isolates from inpatients at hospitals in São Luis, MA, Brazil. *BMC Infect. Dis.* **2016**, *16*, 737. [[CrossRef](#)] [[PubMed](#)]
- Woodford, N.; Tierno, P.M., Jr.; Young, K.; Tysall, L.; Palepou, M.-F.I.; Ward, E.; Painter, R.E.; Suber, D.F.; Shungu, D.; Silver, L.L.; et al. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York Medical Center. *Antimicrob. Agents Chemother.* **2004**, *48*, 4793–4799. [[CrossRef](#)] [[PubMed](#)]
- Kitchel, B.; Rasheed, J.K.; Patel, J.B.; Srinivasan, A.; Navon-Venezia, S.; Carmeli, Y.; Brolund, A.; Giske, C.G. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: Clonal expansion of multilocus sequence type 258. *Antimicrob. Agents Chemother.* **2009**, *53*, 3365–3370. [[CrossRef](#)] [[PubMed](#)]
- Mehta, S.C.; Rice, K.; Palzkill, T. Natural Variants of the KPC-2 Carbapenemase have Evolved Increased Catalytic Efficiency for Ceftazidime Hydrolysis at the Cost of Enzyme Stability. *PLoS Pathog.* **2015**, *11*, e1004949. [[CrossRef](#)] [[PubMed](#)]
- Manageiro, V.; Ferreira, E.; Almeida, J.; Barbosa, S.; Simões, C.; Bonomo, R.A.; Caniça, M. Predominance of KPC-3 in a survey for carbapenemase-producing Enterobacteriaceae in Portugal. *Antimicrob. Agents Chemother.* **2015**, *59*, 3588–3592. [[CrossRef](#)]
- Campos, A.C.; Albiero, J.; Ecker, A.B.; Kuroda, C.M.; Meirelles, L.E.; Polato, A.; Tognim, M.C.; Wingeter, M.A.; Teixeira, J.J. Outbreak of *Klebsiella pneumoniae* carbapenemase-producing K pneumoniae: A systematic review. *Am. J. Infect. Control* **2016**, *44*, 1374–1380. [[CrossRef](#)] [[PubMed](#)]
- Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States. 2019. Available online: [www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf](http://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf) (accessed on 20 November 2020).

18. Tzouveleakis, L.S.; Markogiannakis, A.; Psychogiou, M.; Tassios, P.T.; Daikos, G.L. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: An evolving crisis of global dimensions. *Clin. Microbiol. Rev.* **2012**, *25*, 682–707. [CrossRef] [PubMed]
19. Brandt, C.; Viehweger, A.; Singh, A.; Pletz, M.W.; Wibberg, D.; Kalinowski, J.; Lerch, S.; Müller, B.; Makarewicz, O. Assessing genetic diversity and similarity of 435 KPC-carrying plasmids. *Sci. Rep.* **2019**, *9*, 11223. [CrossRef] [PubMed]
20. Villegas, M.V.; Blanco, M.G.; Sifuentes-Osornio, J.; Rossi, F. Increasing prevalence of extended-spectrum-beta-lactamase among Gram-negative bacilli in Latin America—2008 update from the Study for Monitoring Antimicrobial Resistance Trends (SMART). *Braz. J. Infect. Dis.* **2011**, *15*, 34–39. [PubMed]
21. Beirão, E.M.; Rodrigues, S.D.S.; Andrade, T.K.; Serra, F.B.; Paula, M.D.N.; Polis, T.J.B.; Gales, A.C. Activity of ceftolozane-tazobactam and comparators against gram-negative bacilli: Results from the study for monitoring antimicrobial resistance trends (SMART–Brazil; 2016–2017). *Braz. J. Infect. Dis.* **2020**. [CrossRef] [PubMed]
22. Wyres, K.L.; Gorrie, C.; Edwards, D.J.; Wertheim, H.F.L.; Hsu, L.Y.; Van Kinh, N.; Zadoks, R.; Baker, S.; Holt, K.E. Extensive Capsule Locus Variation and Large-Scale Genomic Recombination within the *Klebsiella pneumoniae* Clonal Group 258. *Genome Biol. Evol.* **2015**, *7*, 1267–1279. [CrossRef]
23. Qu, D.; Shen, Y.; Hu, L.; Jiang, X.; Yin, Z.; Gao, B.; Zhao, Y.; Yang, W.; Yang, H.; Han, J.; et al. Comparative analysis of KPC-2-encoding chimera plasmids with multi-replicon IncR:Inc(pA1763-KPC):IncN1 or IncFII(pHN7A8):Inc(pA1763-KPC):IncN1. *Infect. Drug Resist.* **2019**, *12*, 285–296. [CrossRef] [PubMed]
24. Rodrigues, C.; Bavlovič, J.; Machado, E.; Amorim, J.; Peixe, L.; Novais, A. KPC-3-Producing *Klebsiella pneumoniae* in Portugal Linked to Previously Circulating Non-CG258 Lineages and Uncommon Genetic Platforms (Tn4401d-IncFIA and Tn4401d-IncN). *Front. Microbiol.* **2016**, *7*, 1000. [CrossRef] [PubMed]
25. Leavitt, A.; Chmelnitsky, I.; Carmeli, Y.; Navon-Venezia, S. Complete nucleotide sequence of KPC-3-encoding plasmid pKpQIL in the epidemic *Klebsiella pneumoniae* sequence type 258. *Antimicrob. Agents Chemother.* **2010**, *54*, 4493–4496. [CrossRef] [PubMed]
26. Jousset, A.B.; Bonnin, R.A.; Takissian, J.; Girlich, D.; Mihaila, L.; Cabanel, N.; Dortet, L.; Glaser, P.; Naas, T. Concomitant carriage of KPC-producing and non-KPC-producing *Klebsiella pneumoniae* ST512 within a single patient. *J. Antimicrob. Chemother.* **2020**, *75*, 2087–2092. [CrossRef] [PubMed]
27. Yu, F.; Hu, L.; Zhong, Q.; Hang, Y.; Liu, Y.; Hu, X.; Ding, H.; Chen, Y.; Xu, X.; Fang, X.; et al. Dissemination of *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in integrated and emergency intensive care units in a Chinese tertiary hospital. *J. Med. Microbiol.* **2019**, *68*, 882–889. [CrossRef]
28. Chen, C.M.; Guo, M.K.; Ke, S.C.; Lin, Y.P.; Li, C.R.; Vy Nguyen, H.T.; Wu, L.T. Emergence and nosocomial spread of ST11 carbapenem-resistant *Klebsiella pneumoniae* co-producing OXA-48 and KPC-2 in a regional hospital in Taiwan. *J. Med. Microbiol.* **2018**, *67*, 957–964. [CrossRef] [PubMed]
29. Liu, J.; Yu, J.; Chen, F.; Yu, J.; Simner, P.; Tamma, P.; Liu, Y.; Shen, L. Emergence and establishment of KPC-2-producing ST11 *Klebsiella pneumoniae* in a general hospital in Shanghai, China. *Eur. J. Clin. Microbiol. Infect. Dis.* **2018**, *37*, 293–299. [CrossRef] [PubMed]
30. Zhao, Y.; Zhang, X.; Torres, V.V.L.; Liu, H.; Rocker, A.; Zhang, Y.; Wang, J.; Chen, L.; Bi, W.; Lin, J.; et al. An Outbreak of Carbapenem-Resistant and Hypervirulent *Klebsiella pneumoniae* in an Intensive Care Unit of a Major Teaching Hospital in Wenzhou, China. *Front. Public Health* **2019**, *7*, 229. [CrossRef] [PubMed]
31. Pérez-Chaparro, P.J.; Cerdeira, L.T.; Queiroz, M.G.; de Lima, C.P.S.; Levy, C.E.; Pavez, M.; Lincopan, N.; Gonçalves, E.C.; Mamizuka, E.M.; Sampaio, J.L.M.; et al. Complete nucleotide sequences of two blaKPC-2-bearing IncN Plasmids isolated from sequence type 442 *Klebsiella pneumoniae* clinical strains four years apart. *Antimicrob. Agents Chemother.* **2014**, *58*, 2958–2960. [CrossRef] [PubMed]
32. Andrey, D.O.; Pereira Dantas, P.; Martins, W.B.S.; Marques De Carvalho, F.; Almeida, L.G.P.; Sands, K.; Portal, E.; Sausser, J.; Cayô, R.; Nicolas, M.F.; et al. An Emerging Clone, *Klebsiella pneumoniae* Carbapenemase 2-Producing K. pneumoniae Sequence Type 16, Associated With High Mortality Rates in a CC258-Endemic Setting. *Clin. Infect. Dis.* **2020**, *71*, e141–e150. [CrossRef] [PubMed]
33. Huang, J.; Hu, X.; Zhao, Y.; Shi, Y.; Ding, H.; Wu, R.; Zhao, Z.; Ji, J. Comparative Analysis of bla (KPC) Expression in Tn4401 Transposons and the Tn3-Tn4401 Chimera. *Antimicrob. Agents Chemother.* **2019**, *63*. [CrossRef]
34. Gootz, T.D.; Lescoe, M.K.; Dib-Hajj, F.; Dougherty, B.A.; He, W.; Della-Latta, P.; Huard, R.C. Genetic organization of transposase regions surrounding blaKPC carbapenemase genes on plasmids from *Klebsiella* strains isolated in a New York City hospital. *Antimicrob. Agents Chemother.* **2009**, *53*, 1998–2004. [CrossRef] [PubMed]
35. Nichols, W.W.; Stone, G.G.; Newell, P.; Broadhurst, H.; Wardman, A.; MacPherson, M.; Yates, K.; Riccobene, T.; Critchley, I.A.; Das, S. Ceftazidime-Avibactam Susceptibility Breakpoints against Enterobacteriaceae and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **2018**, *62*. [CrossRef]
36. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint Tables for Interpretation of MICs and Zone Diameters. 2020. Available online: [https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Breakpoint\\_tables/v\\_10.0\\_Breakpoint\\_Tables.pdf](https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf) (accessed on 30 April 2020).
37. Walsh, F. The multiple roles of antibiotics and antibiotic resistance in nature. *Front. Microbiol.* **2013**, *4*, 255. [CrossRef]
38. Alba, J.; Ishii, Y.; Thomson, K.; Moland, E.S.; Yamaguchi, K. Kinetics study of KPC-3, a plasmid-encoded class A carbapenem-hydrolyzing beta-lactamase. *Antimicrob. Agents Chemother.* **2005**, *49*, 4760–4762. [CrossRef]

39. Stillwell, T.; Green, M.; Barbadora, K.; Ferrelli, J.G.; Roberts, T.L.; Weissman, S.J.; Nowalk, A. Outbreak of KPC-3 Producing Carbapenem-Resistant *Klebsiella pneumoniae* in a US Pediatric Hospital. *J. Pediatric Infect. Dis. Soc.* **2015**, *4*, 330–338. [[CrossRef](#)] [[PubMed](#)]
40. Shields, R.K.; Clancy, C.J.; Hao, B.; Chen, L.; Press, E.G.; Iovine, N.M.; Kreiswirth, B.N.; Nguyen, M.H. Effects of *Klebsiella pneumoniae* carbapenemase subtypes, extended-spectrum  $\beta$ -lactamases, and porin mutations on the in vitro activity of ceftazidime-avibactam against carbapenem-resistant *K. pneumoniae*. *Antimicrob. Agents Chemother.* **2015**, *59*, 5793–5797. [[CrossRef](#)] [[PubMed](#)]
41. Navon-Venezia, S.; Leavitt, A.; Schwaber, M.J.; Rasheed, J.K.; Srinivasan, A.; Patel, J.B.; Carmeli, Y.; Israeli, K.P.C.K.S.G. First report on a hyperepidemic clone of KPC-3-producing *Klebsiella pneumoniae* in Israel genetically related to a strain causing outbreaks in the United States. *Antimicrob. Agents Chemother.* **2009**, *53*, 818–820. [[CrossRef](#)] [[PubMed](#)]
42. Leavitt, A.; Chmelnitsky, I.; Ofek, I.; Carmeli, Y.; Navon-Venezia, S. Plasmid pKpQIL encoding KPC-3 and TEM-1 confers carbapenem resistance in an extremely drug-resistant epidemic *Klebsiella pneumoniae* strain. *J. Antimicrob. Chemother.* **2010**, *65*, 243–248. [[CrossRef](#)] [[PubMed](#)]
43. Doumith, M.; Findlay, J.; Hirani, H.; Hopkins, K.L.; Livermore, D.M.; Dodgson, A.; Woodford, N. Major role of pKpQIL-like plasmids in the early dissemination of KPC-type carbapenemases in the UK. *J. Antimicrob. Chemother.* **2017**, *72*, 2241–2248. [[CrossRef](#)]
44. Chen, L.; Chavda, K.D.; Melano, R.G.; Jacobs, M.R.; Koll, B.; Hong, T.; Rojtmán, A.D.; Levi, M.H.; Bonomo, R.A.; Kreiswirth, B.N. Comparative genomic analysis of KPC-encoding pKpQIL-like plasmids and their distribution in New Jersey and New York Hospitals. *Antimicrob. Agents Chemother.* **2014**, *58*, 2871–2877. [[CrossRef](#)] [[PubMed](#)]
45. Yang, X.; Dong, N.; Chan, E.W.-C.; Zhang, R.; Chen, S. Carbapenem Resistance-Encoding and Virulence-Encoding Conjugative Plasmids in *Klebsiella pneumoniae*. *Trends Microbiol.* **2020**. [[CrossRef](#)]
46. Holt, K.E.; Wertheim, H.; Zadoks, R.N.; Baker, S.; Whitehouse, C.A.; Dance, D.; Jenney, A.; Connor, T.R.; Hsu, L.Y.; Severin, J.; et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E3574–E3581. [[CrossRef](#)]
47. O’Hara, J.A.; Hu, F.; Ahn, C.; Nelson, J.; Rivera, J.I.; Pasculle, A.W.; Doi, Y. Molecular epidemiology of KPC-producing *Escherichia coli*: Occurrence of ST131-fimH30 subclone harboring pKpQIL-like IncFIIk plasmid. *Antimicrob. Agents Chemother.* **2014**, *58*, 4234–4237. [[CrossRef](#)]
48. Papagiannitsis, C.C.a. Characterization of KPC-encoding plasmids from two endemic settings, Greece and Italy. *J. Antimicrob. Chemother.* **2016**, *71*, 2824–2830. [[CrossRef](#)] [[PubMed](#)]
49. Eilertson, B.; Chen, L.; Chavda, K.D.; Kreiswirth, B.N. Genomic Characterization of Two KPC-Producing *Klebsiella* Isolates Collected in 1997 in New York City. *Antimicrob. Agents Chemother.* **2017**, *61*. [[CrossRef](#)] [[PubMed](#)]
50. Andrade, L.N.; Curiao, T.; Ferreira, J.C.; Longo, J.M.; Clímaco, E.C.; Martinez, R.; Bellissimo-Rodrigues, F.; Basile-Filho, A.; Evaristo, M.A.; Del Peloso, P.F.; et al. Dissemination of blaKPC-2 by the spread of *Klebsiella pneumoniae* clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among Enterobacteriaceae species in Brazil. *Antimicrob. Agents Chemother.* **2011**, *55*, 3579–3583. [[CrossRef](#)] [[PubMed](#)]
51. Reyes, J.A.; Melano, R.; Cárdenas, P.A.; Trueba, G. Mobile genetic elements associated with carbapenemase genes in South American Enterobacteriales. *Braz. J. Infect. Dis.* **2020**, *24*, 231–238. [[CrossRef](#)] [[PubMed](#)]
52. Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST). *Breakpoint Tables for Interpretation of MICs and Zone Diameters—Tabelas de Pontos de Corte PARA Interpretação de CIMs e Diâmetros de Halos*; Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST): São Paulo, Brazil, 2020.
53. Salvà-Serra, F.; Svensson-Stadler, L.; Busquets, A.; Jaén-Luchoro, D.; Karlsson, R.; Moore, E.R.B.; Gomila, M. A protocol for extraction and purification of high-quality and quantity bacterial DNA applicable for genome sequencing: A modified version of the Marmur procedure. *Protoc. Exch.* **2018**. [[CrossRef](#)]
54. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [[CrossRef](#)] [[PubMed](#)]
55. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [[CrossRef](#)]
56. García-Alcalde, F.; Okonechnikov, K.; Carbonell, J.; Cruz, L.M.; Götz, S.; Tarazona, S.; Dopazo, J.; Meyer, T.F.; Conesa, A. Qualimap: Evaluating next-generation sequencing alignment data. *Bioinformatics* **2012**, *28*, 2678–2679. [[CrossRef](#)] [[PubMed](#)]
57. Alikhan, N.-F.; Petty, N.K.; Ben Zakour, N.L.; Beatson, S.A. BLAST Ring Image Generator (BRIG): Simple prokaryote genome comparisons. *BMC Genom.* **2011**, *12*, 402. [[CrossRef](#)] [[PubMed](#)]
58. Siguier, P.; Perochon, J.; Lestrade, L.; Mahillon, J.; Chandler, M. ISfinder: The reference centre for bacterial insertion sequences. *Nucleic Acids Res.* **2006**, *34*, D32–D36. [[CrossRef](#)] [[PubMed](#)]
59. Wick, R.R.; Heinz, E.; Holt, K.E.; Wyres, K.L. Kaptive Web: User-Friendly Capsule and Lipopolysaccharide Serotype Prediction for *Klebsiella* Genomes. *J. Clin. Microbiol.* **2018**, *56*, e00197-00118. [[CrossRef](#)] [[PubMed](#)]