Clinical and Molecular Description of a High-Copy IncQ1 KPC-2 Plasmid Harbored by the International ST15 Klebsiella pneumoniae Clone

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ABSTRACT This study provides the genomic characterization and clinical description of bloodstream infections (BSI) cases due to ST15 KPC-2 producer Klebsiella pneumoniae. Six KPC-K. pneumoniae isolates were recovered in 2015 in a tertiary Brazilian hospital and were analyzed by whole-genome sequencing (WGS) (Illumina MiSeq short reads). Of these, two isolates were further analyzed by Nanopore MinION sequencing, allowing complete chromosome and plasmid circularization (hybrid assembly), using Unicycler software. The clinical analysis showed that the 30-day overall mortality for these BSI cases was high (83%). The isolates exhibited meropenem resistance (MICs, 32 to 128 mg/liter), with 3/6 isolates resistant to polymyxin B. The conjugative properties of the \textit{bla}_{KPC-2} plasmid and its copy number were assessed by standard conjugation experiments and sequence copy number analysis. We identified in all six isolates a small (8.3-kb), high-copy-number (20 copies/cell) non-self-conjugative IncQ plasmid harboring \textit{bla}_{KPC-2} in a non-Tn4401 transposon. This plasmid backbone was previously reported to harbor \textit{bla}_{KPC-2} only in Brazil, and it could be comobilized at a high frequency (10^{10}) into \textit{Escherichia coli} J53 and into several high-risk \textit{K. pneumoniae} clones (ST258, ST15, and ST101) by a common IncL/M helper plasmid, suggesting the potential of international spread. This study thus identified the international \textit{K. pneumoniae} ST15 clone as a carrier of \textit{bla}_{KPC-2} in a high-copy-number IncQ1 plasmid that is easily transmissible among other common \textit{Klebsiella} strains. This finding is of concern since IncQ1 plasmids are efficient antimicrobial resistance determinant carriers across Gram-negative species. The spread of such carbapenemase-encoding IncQ1 plasmids should therefore be closely monitored.

IMPORTANCE In many parts of the world, carbapenem resistance is a serious public health concern. In Brazil, carbapenem resistance in Enterobacterales is mostly driven by the dissemination of KPC-2-producing \textit{K. pneumoniae} clones. Despite being endemic in this country, only a few reports providing both clinical and genomic data are available in Brazil, which limit the understanding of the real clinical impact caused by the dissemination of different clones carrying \textit{bla}_{KPC-2} in Brazilian hospitals. Although several of these KPC-2-producer \textit{K. pneumoniae} isolates belong to the

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clonal complex 258 and carry Tn4401 transposons located on large plasmids, a concomitant emergence and silent dissemination of small high-copy-number blaKPC-2 plasmids are of importance, as described in this study. Our data identify a small high-copy-number IncQ1 KPC plasmid, its clinical relevance, and the potential for conjugative transfer into several K. pneumoniae isolates, belonging to different international lineages, such as ST258, ST101, and ST15.

KEYWORDS Gram-negative bacteria, IncQ1, KPC-2, Klebsiella pneumoniae, ST15, bloodstream infections, carbapenemase, plasmid-mediated resistance

Carbapenem resistance in Enterobacterales represents a serious threat to modern medicine and the global health system, as stressed by international agencies (1). KPC-producing Klebsiella pneumoniae infections are responsible for a severe burden in health care systems, particularly in North America, Latin America, Southern and Eastern Europe, Israel, and China (2). K. pneumoniae sepsis rates have been rising in recent years; according to PHE (Public Health England, including Wales and Northern Ireland), the rate of Klebsiella species bacteremia increased from 12 cases in 2009 to 17 cases in 2018 per 100,000 population (3).

The Brazilian Health Surveillance Agency (ANVISA) ranked K. pneumoniae as the most frequent pathogen (19.0%) causing central catheter-related bloodstream infections (CR-BSI) among adult intensive care unit (ICU) patients in 2017, with an increasing carbapenem resistance rate of 44.1% (4). This high rate is mostly due to the dissemination in Brazilian hospitals of various KPC-2-producing K. pneumoniae clones, belonging to the clonal complex (CC) 258, such as ST437 (a tonB31 single-allele variant of ST258), ST11, and ST340. Recently, the international KPC clone ST258 (clade 2, KL107, a hybrid clone resulting from genomic recombination events between ST11 and ST442) has been identified as a main driver of KPC-2 dissemination (5–8). Other lineages include non-CC258 KPC-producing clones such as ST101, ST307, and ST16 (8). KPC-3-producing clones have been reported in Latin America, mainly in Colombia, but are not disseminated in Brazil (9).

In contrast, the K. pneumoniae ST15 clone (CC15) has rarely been associated with KPC in Latin America (10, 11). K. pneumoniae CC15 is a global clone associated with both human and animal infections, identified as an important carrier of extended-spectrum β-lactamases (ESBLs) and carbapenemases, particularly metallo-β-lactamases and OXA-48-like enzymes, worldwide (12–14). There are several reports of ST15 harboring NDM-1 in both Nepal and Pakistan (15, 16); OXA-48-like (OXA-48 and OXA-232) in China, Vietnam, Pakistan, and Spain (17–20); KPC-3 in Portugal; and KPC-2 in Bulgaria and China (21–23). The diversity of resistance determinants and plasmid backbones acquired by ST15 clones in the different study locations suggests a high capacity for horizontal acquisition of resistance. This high-risk clone has been described as a strong candidate for convergence of antimicrobial resistance (AMR) and hypervirulence, through the acquisition of hybrid plasmids, carrying both AMR and hypervirulence determinants (24).

In this study, we report the clinical and molecular characterization of a K. pneumoniae ST15 clone, associated with high mortality rates in a Brazilian hospital, including its blaKPC-2-bearing IncQ1 plasmid.

(This study was presented in part at the European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, The Netherlands, 13 to 16 April 2019, abstract O0917 [25].)

RESULTS

Clinical description. Within a retrospective cohort of 165 KPC-2-producing K. pneumoniae BSI cases in a tertiary Brazilian hospital during the 2014 to 2016 period, six cases were due to isolates displaying a clonal pulsotype (data not shown) and were assigned to ST15 group by in silico multilocus sequence typing (MLST). The clinical description of these six cases is provided in Table 1. The patients were hospitalized in

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TABLE 1 Clinical description of the six ST15 KPC-2- K. pneumoniae BSI cases

| Case | Bacterial isolate name | Patient age, yr (sex)* | Underlying disease | Mo/yr of infection | Source of bacteremiaa | Ward(s) during hospital stay | Length of stay at bacteremia onset (days) | Septic shock | Pitt score | Empirical treatment/targeted treatment | In vitro active antimicrobials (n) | 30-day outcomeb |
|------|------------------------|------------------------|--------------------|-------------------|-----------------------|----------------------------|------------------------------------------|-------------|-----------|------------------------------------------|---------------------------------|-----------------|
| 1    | P35                    | 45 (M)                 | Endocarditis       | May 2015          | Lungs (VAP)           | Cardiac surgery ICU       | 38                                        | No          | MD        | PMB + MEM + AMK                         | 2                               | Died (18 days) |
| 2    | P02                    | 81 (M)                 | Cholangitis        | May 2015          | Abdominal Urinary (indwelling catheter) | Emergency room ICU | 19                                        | Yes         | 6         | PMB + MEM                               | 0                               | Died (3 days)  |
| 3    | P45                    | 69 (M)                 | Urosepsis          | September 2015    | Abdominal             | Emergency room            | 1                                        | No          | 2         | PTZ/PMB + MEM + ERT                      | 1*                              | Survived       |
| 4    | P16                    | 72 (M)                 | Acute abdomen      | September 2015    | Abdominal             | General ICU               | 23                                        | Yes        | 6         | PMB + MEM + AMK                         | 1                               | Died (1 day)  |
| 5    | P51                    | 48 (M)                 | Multiple myeloma   | December 2015     | Lungs (VAP)           | Internal medicine         | 16                                        | Yes         | 2         | PMB + MEM + AMK                         | 1                               | Died (6 days)  |
| 6    | P49                    | 76 (M)                 | Acute myocardial infarction | December 2015 | CR-BSI                | Cardiac surgery          | 49                                        | No          | 1         | CEF/PMB + AMK                           | 2                               | Died (13 days) |

*aAbbreviations: M, male; F, female; CR-BSI, catheter-related bloodstream infection; PMB, polymyxin B; MEM, meropenem; ERT, ertapenem; AMK, amikacin; PTZ, piperacillin-tazobactam; CEF, cefepime; VAP, associated pneumonia; MD, missing data. Bold drug abbreviations indicate in vitro nonsusceptibility.

*bNumber of days after bacteremia onset.

*cIn case 3, ERT and MEM were individually tested resistant, and in vitro double synergy was not tested.
diverse wards throughout the hospital, and five out of six were admitted initially at the Emergency Department ICU. The overall 3-day and 30-day crude mortality was 20% (2/6 patients) and 85% (5/6 patients), respectively. Half of these patients presented with septic shock. There was one primary catheter-related BSI, and in the remaining cases the BSI were secondary to ventilator-acquired pneumonia ($n$/) or urinary ($n$/). Four out of six patients were treated with a triple antibiotic combination irrespective of in vitro susceptibility. In all six cases, the combination included polymyxin B, but the median number of in vitro active antimicrobials given to these patients was 1 (interquartile range [IQR], 1;2). The only surviving patient (case three), who had been admitted at the hospital with a urinary sepsis complicating an indwelling urethral catheter, was initially empirically treated with meropenem and ertapenem (dual carbapenem therapy) in association with polymyxin B.

**Antimicrobial susceptibility testing.** Antimicrobial susceptibility results revealed that all six KPC-2-producing ST15 isolates were highly resistant to meropenem (MICs, 32 to 128 mg/liter) but remained 100% susceptible to amikacin (MICs, 2 to 4 mg/liter) and ceftazidime-avibactam (MICs at 0.5 mg/liter). All isolates had tigecycline MICs of 1 mg/liter, while three isolates showed resistance to polymyxin B (MICs, 0.125 to 64 mg/liter; 50% susceptible).

**Genomic analysis of AMR and virulence determinants.** The six ST15 BSI isolates (P02, P16, P35, P45, P49, and P51) and the two ST15 comparator strains (P21 and HSP32) were whole-genome sequenced. Genes related to resistance, virulence determinants, and plasmid replicons are shown in Fig. 1. In the six ST15-KP isolates $\beta$-lactamases bla$\text{KPC-2}$, bla$\text{CTX-M-15}$, and bla$\text{SHV-28}$ were identified. The porin-encoding genes ompK35 and ompK36 as well as their promoter regions did not show any mutations or disruptions compared to wild-type $K.\text{pneumoniae}$ strains, suggesting that these porins were normally expressed. The aminoglycoside resistance genes aac($6{\prime}$)-Ib-cr, aacA4, aph(3’)-Ib, aph(6)-Id, and aadA2 were also identified. No polymyxin resistance mcr gene or responsible mutations ($\text{mgrB}$, $\text{phoPQ}$, $\text{pmrAB}$, and $\text{crrAB}$) could be identified in the three polymyxin-resistant isolates.

The ST15 isolate genomes had type 1 (fimA to -H) and type 3 (mrkABCDF) fimbrial adhesion genes as well as urease (ureA to -G), outer membrane protein (ycFM), entero-bactin siderophore (entA to -F), and wabGHN (lipopolysaccharide [LPS] synthesis) virulence genes. These ST15 genomes also carried the iron uptake system $\text{kuABC}$, as previously reported for this clone. Salmochelin, yersiniabactin, aerobactin, colibactin, and $\text{mpmA}$/$\text{mpmA2}$ hypermucoviscosity factor were not found. The six ST15 KPC- $K.\text{pneumoniae}$ outbreak isolates harbored the KL112 (waz93) capsule. The AMR and virulence determinants of the KPC-negative isolates are also displayed in Fig. 1.
KPC-2 IncQ1 plasmid and additional plasmids. We identified the following plasmid replicons: IncQ1, IncL/M, IncFIA, IncFII, and IncFIB (in all isolates); Col440I (in 5 isolates); and ColRNAI (in one isolate) (Fig. 1). The two KPC-negative ST15 isolates lacked IncQ1 and IncL/M replicons. The hybrid sequencing strategy (short and long reads) of isolate P35 identified 5 plasmids. By size, they were (i) pP35-IncFIB-IncFII of 248.7 kb which harbored *aadA2*, *mphA*, *catA1*, *sul1*, and *dfrA12*; (ii) pP35-IncFIA, an 85.2-kb plasmid, harboring *bla*_{TEM-1B}, *bla*_{CTX-M-15}, *bla*_{OXA-1}, *qnrB1*, *aac(6\^{\text{I}})\text{-Ib-cr, aph(3\^{\text{I}})}\text{-Ib, sul2, catB3, and dfrA14}.* (iii) a 53.3-kb pP35-IncL/M carrying no AMR determinant; (iv) the 8.3-kb plasmid, pP35-KPC-IncQ1, carrying *bla*_{KPC-2}; and (v) a 4.1-kb pP35-Col440I lacking AMR genes.

FIG 2 (A) Genetic context of *bla*_{KPC-2} gene. (B) Circular map of pP35-KPC-IncQ1 plasmid. (C) Alignment of IncQ1 plasmids harboring *bla*_{KPC-2} or *bla*_{BKC-1} in ST15 *K. pneumoniae* pP35-KPC-IncQ1 (accession number CP053039), *Pseudomonas aeruginosa* pCCBH17348 (accession number NOX01000029.1), *K. quasipneumoniae* pKQPS142b (accession number CP023480), BKC-1-K. pneumoniae pA60136 (accession number KP689347), and ST340 K. pneumoniae pKPN535a (accession number MH59533).

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The 8.3-kb IncQ1 plasmid harboring *bla*_{KPC-2} was identified in all isolates (depicted in Fig. 2A and B). In this plasmid, *bla*_{KPC-2} is flanked by the Tn3 resolvase and by ISKpn6 (IS1182 family) and thus belongs to NTE (non-Tn4401) group NTE_{KPN}-Ic. pP35-KPC-IncQ1 shares a common backbone with other IncQ1 plasmids, such as pKQPS142b, identified in KPC-2-producing *Klebsiella quasipneumoniae* isolate KPC-142; p60136 (on BKC-1-producing *K. pneumoniae* A60136); and pKPN535a (on KPC-2-producing *K. pneumoniae* KPN535), as depicted in Fig. 2C. The IncQ1 plasmid identified in this study lacks almost all the genes necessary for self-conjugation (mating pair formation [Mpf] genes and DNA transfer and replication [Dtr] genes). The IncQ1 plasmid and *bla*_{KPC-2} were assessed at 20 copies per cell in isolate P35.

**bla**_{KPC-2} mobilization. To test the mobilization of *bla*_{KPC-2}-IncQ1 plasmids, we performed mating-out assays first into *Escherichia coli* J53 and then into various *K. pneumoniae* recipients, belonging to high-risk clones. It showed *bla*_{KPC-2}-IncQ1 conjugation at high frequency (5 × 10^{-4}) into J53. Both IncQ1 and IncL/M plasmids were transferred, as verified by PCR of 10 independent transconjugants, suggesting comobilization of the IncQ plasmid. Indeed, pP35-IncL/M (and the 100% similar pP16-IncL/M) contains a complete repertoire of genes belonging to the type IV secretion system.
TABLE 2 Mating-out assays using _bla_{KPC-2} donors into several recipients^a

| Donor strain | Recipient name | Recipient species | Recipient ST | Recipient origin | Recipient isolation site | Transconjugant name | Frequency | MEM MIC change (R/TC) |
|--------------|----------------|-------------------|--------------|------------------|--------------------------|--------------------|-----------|----------------------|
| P16          | J53            | _E. coli_         | 10           | Lab strain       | P16-KPC-TC               | 5 × 10^{-4}        | 3 log^2 dilutions (≤0.03/0.25) |
| P16-KPC-TC   | PS2            | _K. pneumoniae_   | 258          | Brazil           | P16-KPC-TC               | 1.13 × 10^{-6}     | 4 log^4 dilutions (2/32) |
| P16-KPC-TC   | HSP65          | _K. pneumoniae_   | 101          | Human blood      | HSP65-TC                 | 3 × 10^{-6}        | 3 log^2 dilutions (1/8) |
| P16-KPC-TC   | HSP32          | _K. pneumoniae_   | 15           | Human blood      | HSP32-TC                 | 8.96 × 10^{-9}     | 5 log^6 dilutions (≤0.03/1) |
| P16-KPC-TC   | 78623          | _K. pneumoniae_   | 185          | Human carriage   | 78623-TC                 | 1.62 × 10^{-7}     | 5 log^6 dilutions (≤0.03/1) |
| P16-KPC-TC   | 45             | _K. pneumoniae_   | 43 (SLV)     | India            | 45-TC                    | 3.5 × 10^{-7}      | 9 log^2 dilutions (≤0.03/16) |
| P16-KPC-TC   | 22             | _K. pneumoniae_   | 858 (SLV)    | India            | 22-TC                    | 3.94 × 10^{-8}     | 4 log^2 dilutions (≤0.03/0.5) |
| P16-KPC-TC   | 4W             | _K. pneumoniae_   | 35           | UK               | 4W-TC                    | 3.94 × 10^{-8}     | 4 log^2 dilutions (≤0.03/0.5) |

^aAbbreviations: ST, sequence type; SLV, single locus variant; MEM, meropenem; R, recipient; TC, transconjugant.

(T4SS), with both Dtr and Mpf genes (pP16-IncL/M accession number CP053039), suggesting that this 53-kb plasmid provides the Mpf machinery (T4SS) allowing comobilization of the IncQ plasmid. Subsequently, we assessed the transmissibility of the _bla_{KPC-2}-IncQ1 plasmid (using P16-KPC-TC as donor) into clinical isolates belonging to ST258, ST101, and ST15 (Table 2). Interestingly, the higher conjugation frequency (10^{-6}) was observed in ST258 and ST101, in accordance with the predominant role of these clones in the global acquisition and dissemination of KPC. The expected increase in the meropenem MICs ranged from 3 to 9 log_{10} dilutions dependent upon the recipient isolate (Table 2). Altogether, these data confirm the potential for comobilization of this IncQ1 plasmid into _E. coli_ and into several epidemiologically important _K. pneumoniae_ clones.

DISCUSSION

To date, few ST15 isolates carrying the _bla_{KPC-2} gene have been reported (21, 22). This study reinforces our knowledge of _K. pneumoniae_ ST15 as a multidrug-resistant clone facilitating the spread of carbapenemase genes worldwide. The clinical characteristics of the KPC-K. pneumoniae_ ST15-infected patients were similar to those encountered for other KPC-K. pneumoniae infections: mainly severely ill patients (high Charlson score) predominantly from ICUs. Though most isolates retained susceptibility to at least one antimicrobial prescribed for Gram-negative BSI treatment, a fatal outcome was observed in 85% of cases. The analysis of virulence factors identified the accessory iron uptake system _kfuABC_, a known invasiveness determinant generally found in ST15 lineage. Currently, there is little information available on the role of the KL112 capsule in virulence.

These ST15 isolates harbored _bla_{KPC-2} on a small IncQ1 mobilizable high-copy-number plasmid. Interestingly _bla_{KPC-2}-bearing IncQ1 plasmids have been described only on rare occasions (8, 26–28). We show here that this plasmid carries _bla_{KPC-2} embedded within an NTE_{KPC} element of class Ic that has successfully established itself within _K. pneumoniae_ ST15 and spread silently in tertiary Brazilian hospitals.

Over the last 5-year period, IncQ1 plasmids carrying _bla_{KPC-2} have been reported in several different pathogens in Brazil including _Klebsiella quasipneumoniae_ (1 isolate, BSI), _K. pneumoniae_ ST340 (CC258) (1 isolate, no clinical data), and _Pseudomonas aeruginosa_ ST2584 (1 isolate, BSI), as shown in Fig. 3 (29–31). This current outbreak added a further six additional cases and suggests that IncQ1 plasmids can act as efficient _bla_{KPC-2} carriers. The comparison of the genetic organization of IncQ1 plasmids found in geographically and temporally unrelated isolates (Fig. 2C) suggests indepen-
dent parallel events rather than clonal horizontal dissemination of a unique clone-plasmid pair.

These small IncQ1 plasmids (5.1 to 14.0 kb) have been shown to have the broadest host range of any known plasmids in both Gram-negative and Gram-positive bacteria; they typically replicate independently of the host chromosome and have high copy number \(32–34\). This combination of high copy number, broad host range, and common comobilization means that IncQ1 plasmids are typically highly promiscuous \(35\). Recently, IncQ1 plasmids were reported to be involved in the tet(X4)-mediated tigecycline resistance dissemination in farm animals in China \(36\), as well as in the spread of \(bla_{CMY-4}\), \(bla_{GES-1}\), \(bla_{IMP-27}\), \(strA\)-\(strB\), and \(sul2\) gene clusters \(37–40\). At the same tertiary hospital, an IncQ1 plasmid was previously described carrying the carbapenemase \(bla_{BKC-1}\) in \(K. pneumoniae\) isolates belonging to ST11 and ST442 (2010 to 2012) (unpublished data). We also identified a common IncL/M coresident helper plasmid that was responsible for the mobilization of these IncQ plasmids \(34\). Besides IncL/M plasmids, IncP, IncF, IncI, IncX, IncN, and IncW plasmids have also been described aiding IncQ1 mobilization \(35\).

In conclusion, we have presented here a cryptic outbreak of a \(K. pneumoniae\) ST15 clone that was carbapenem resistant due to an IncQ1 plasmid-carried \(bla_{KPC-2}\) gene. The outbreak resulted in several fatalities and highlights the importance of IncQ1 plasmids in the spread of the KPC carbapenemase gene. The ubiquitous presence of IncQ plasmids among both enteric and nonfermentative Gram-negative bacteria together with acquisition of KPC-2 suggests this combination of carbapenemase gene...
and promiscuous plasmid deserves particular attention and should be closely monitored.

MATERIALS AND METHODS

Study population. The present study involves a 3-year (2014 to 2016) retrospective cohort of KPC-producing K. pneumoniae bloodstream infections (BSIs), from a Brazilian public teaching hospital located in the city of São Paulo, published by our collaborative group (8). This cohort included the microbiological and genetic characterization of unique KPC-K. pneumoniae BSI adult cases. The study was approved by the Hospital São Paulo/Federal University of São Paulo (UNIFESP) Ethics Committee for Clinical Research (protocol number 1.814.158). Epidemiological and clinical data were extracted from the medical records in a standardized case form, as previously described (8).

Isolates selection and microbiological analysis. Six clonally related isolates, belonging to ST15, were selected for the detailed analysis presented here. In addition, two carbapenem-susceptible K. pneumoniae ST15 isolates from the same hospital collection, blaKPC negative (HSP32 and P21), were selected for comparative genomic analysis. Isolate identification was confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using a Microflex LT mass spectrometer and Biotype 3.3 software (Bruker Daltonics) according to the manufacturer’s recommendations. MICs of meropenem, amikacin, gentamicin, tigecycline, and ceftazidime-avibactam were determined by agar dilution, while the broth microdilution technique was used to determine the polymyxin B MICs. Susceptibility testing results were performed and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (41).

WGS and bioinformatics analysis. The isolates were sequenced using the Illumina MiSeq platform (Illumina Inc.). DNA libraries were prepared for paired-end sequencing (2 × 300 cycles) using Nextera XT (Illumina Inc.). Quality control of raw sequence reads included FastQC (0.11.2), and adaptor trimming was performed using Trimm Galore (0.4.3). K. pneumoniae genome assembly was performed using Spades (version 3.8.0), with the k-mer length increased to 127 (42). Multilocus sequence type (MLST), antimicrobial resistance (AMR) determinants, and plasmid replicons were identified using the MLST 2.0, ResFinder 3.1, and PlasmidFinder online tools (Center for Genomic Epidemiology) setting cutoff values of 90% identity and 80% minimum coverage (10 September 2018 database) (43). Virulence genes were analyzed with GeneFinders 10.6.1 using an in-house data set (80% minimal coverage, 75% identity) (8). Assembled genomes were submitted to the Kaptive platform, and capsular loci (KL) were determined using Klebsiella K loci primary as a reference (44). In addition, two isolates (P35 and P16) were selected for complete assembly (chromosome and plasmids). For these, total genomic DNA was extracted and sequenced using long-read (MinION; Oxford Nanopore Technologies), in combination with MiSeq Illumina raw short-read, hybrid de novo assembly using Unicycler (v0.4.0). This strategy enabled the generation of complete circularized sequences of both chromosomes and plasmids (45). Plasmid copy number was obtained based on the ratio of long reads containing blaKPC-2 divided by the mean of chromosomal single-copy tandB- and gapA-containing reads.

Mating-out (conjugation) experiments. To evaluate and compare the transferabilities of plasmid-borne blaKPC-2-conjugation assays were carried out with an ST15 donor isolate into the E. coli J53 azide-resistant strain. Subsequently, a sequence-verified J53-derived transconjugant, named P16-KPC-TC, was used as donor for a secondary conjugation set into selected K. pneumoniae isolates. Briefly, mid-log cultures of donor and recipient strains were mixed in LB broth. The mating culture was then incubated overnight at 37°C, appropriately diluted in physiological saline, and plated onto UTI agar (16636 HiCrome UTI agar; Sigma-Aldrich) containing 0.5 mg/liter meropenem for assessing the colony count. After incubation, for each conjugation, at least 5 (when available) putative transconjugant colonies were tested by restreaking onto meropenem 0.5-mg/liter UTI agar plates and the putative transconjugants were further tested by PCR for blaKPC-2. Conjugative frequency was calculated as the ratio of transconjugant CFU per donor. Isolates were considered unable to transfer blaKPC-2 into the recipient species if the transfer frequency was 10⁻³ or lower (46-48).

Data availability. Whole-genome sequences of the studied K. pneumoniae ST15 isolates have been deposited in the NCBI database under nucleotide accession numbers CP053035 to CP053041 and JABEPK0000000000, JABEPK0000000000, JABEPX0000000000, JABEPY0000000000, JABEPZ0000000000, and JABEOA0000000000.

ACKNOWLEDGMENTS

We thank the staff at the medical microbiology of Hospital São Paulo, Uttapoln Tansawai, Qiu E. Yang, and Mélanie Roch for their technical support in this study.

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior provided a grant to W.M.B.S.M. (88881.133245/2016-01). M.F.N. is a grant recipient of the National Council for Scientific and Technological Development (process number 306894/2019-0) and CAPES (process no. 88887.368759/2019-00). The National Council for Science and Technological Development provided a grant to A.C.G. (process number 312066/2019-8) and E. A. Medeiros (process number 307784/2018-5). A.T.R.V. is supported by CNPq (303170/2017-4) and FAPERJ (26/202.903/20). D.O.A. is the recipient of a Swiss National Science Foundation Mobility Postdoctoral Research Fellowship (APM P300PB_171601), a Geneva University Hospitals Training Grant, and a grant provided by The Sir...
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