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An Emerging Clone, *Klebsiella pneumoniae* Carbenpenemase 2–Producing *K. pneumoniae* Sequence Type 16, Associated With High Mortality Rates in a CC258-Endemic Setting

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**Background.** Carbenpenemase-producing *Klebsiella pneumoniae* has become a global priority, not least in low- and middle-income countries. Here, we report the emergence and clinical impact of a novel *Klebsiella pneumoniae* carbenpenemase–producing *K. pneumoniae* (KPC-KP) sequence type (ST) 16 clone in a clonal complex (CC) 258–endemic setting.

**Methods.** In a teaching Brazilian hospital, a retrospective cohort of adult KPC-KP bloodstream infection (BSI) cases (January 2014 to December 2016) was established to study the molecular epidemiology and its impact on outcome (30-day all-cause mortality). KPC-KP isolates underwent mutilocus sequence typing. Survival analysis between ST/CC groups and risk factors for fatal outcome (logistic regression) were evaluated. Representative isolates underwent whole-genome sequencing and had their virulence tested in a *Galleria* larvae model.

**Results.** One hundred sixty-five unique KPC-KP BSI cases were identified. CC258 was predominant (66%), followed by ST16 (12%). The overall 30-day mortality rate was 60%; in contrast, 95% of ST16 cases were fatal. Patients’ severity scores were high and baseline clinical variables were not statistically different across STs. In multivariate analysis, ST16 (odds ratio [OR], 21.4; 95% confidence interval [CI], 2.3–202.8; \(P = .008\)) and septic shock (OR, 11.9; 95% CI, 4.2–34.1; \(P < .001\)) were independent risk factors for fatal outcome. The ST16 clone carried up to 14 resistance genes, including *bla*<sub>KPC</sub> in an IncFIBpQIL plasmid, KL51 capsule, and yersiniabactin virulence determinants. The ST16 clone was highly pathogenic in the larvae model.

**Conclusions.** Mortality rates were high in this KPC-KP BS cohort, where CC258 is endemic. An emerging ST16 clone was associated with high mortality. Our results suggest that even in endemic settings, highly virulent clones can rapidly emerge demanding constant monitoring.

**Keywords.** carbenpenem-resistant Enterobacteriaceae; KPC; bloodstream infections; CC258; *Klebsiella pneumoniae*.

The widespread prevalence of carbenpenem-resistant Enterobacteriaceae (CRE) bacteria is a major health challenge, as stressed by several national and international health organizations, including the World Health Organization, the Centers for Disease Control and Prevention, and Public Health England [1–3]. CRE are often resistant to last-resort antibiotics, including polymyxins, aminoglycosides, and tigecycline, hereby restricting therapeutic options available for treatment of serious infections [4]. New antibiotic options have become commercially available; however, these medicines are rarely available in low- and middle-income countries due to regulatory agencies’ approval delay and to high cost [5, 6].

*Klebsiella pneumoniae* carbenpenemase–producing *K. pneumoniae* (KPC-KP) has emerged as a leading cause of hospital outbreaks and has become endemic in several hospitals in Southern Europe, North America, Latin America, Israel, and China [7, 8], with most KPC-KP isolates belonging to clonal complex (CC) 258. Within CC258, sequence type (ST)
11 and ST258 are the most prevalent STs [9]. ST258 is a hybrid clone mostly composed of the ST11 genome and a minor part of ST442, which includes the capsule operon [10]. In general, ST258 has disseminated in North America, Latin America, and several European countries, whereas in Asia and particularly in China, ST11 is still the dominant clone [11, 12]. KPC-2-producing K. pneumoniae isolates are endemic in several Brazilian hospitals, mostly belonging to CC258, particularly ST437, ST258 (clade II), and ST11 [13–15]. Other STs such as ST101, ST340, and ST442, have been sporadically reported [16–18]. Recently, an increase in the number of rapidly fatal outcomes of patients infected by KPC-KP was noticed by the infectious diseases medical team in a 740-bed public teaching hospital located in the city of São Paulo, Brazil, where CC258 is endemic. Accordingly, this study was undertaken to understand the epidemiology of KPC-KP infections. Herein, we described the emergence of a new KPC-2 clone belonging to ST16 and associated with high bloodstream infection (BSI) mortality rates.

MATERIALS AND METHODS

Study Population

We conducted a retrospective cohort study of adult KPC-KP BSI cases in a 740-bed public teaching hospital located in São Paulo, Brazil. We retrieved the cases of KPC-KP BSIs by searching the routine microbiology laboratory database, selecting for hospitalized adult patients (>18 years old) who had positive blood cultures with KPC-KP, from January 2014 to December 2016. Unique cases of BSI with their corresponding KPC-KP isolates were included in the cohort, to avoid epidemiological bias. In case of sustained or recurrent bacteremia, only the first episode isolate was included. Polymicrobial BSIs were excluded (Supplementary Figure 1). This study was approved by the Hospital São Paulo/Universidade Federal de São Paulo) 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. Definitions of variables are available in the Supplementary Materials.

Microbiological Analysis and Galleria Testing

Descriptions of the initial identification, antimicrobial susceptibility testing (AST), and KPC polymerase chain reaction (PCR) at the routine microbiology laboratory are available in the Supplementary Materials. Frozen KPC-KP isolates were cultured, identification was confirmed by matrix-assisted laser desorption/ionization–time of flight, and minimum inhibitory concentrations (MICs) were determined by either European Committee on Antimicrobial Susceptibility Testing agar dilution or broth microdilution [19]. Genetic relatedness was established by Spe-I pulsed-field gel electrophoresis (PFGE) and interpreted using Tenover criteria [20]. A total of 64 isolates including representatives of each CC258 PFGE pattern, and all culturable ST16 isolates were sequenced using the MiSeq Illumina platform. Remaining isolates underwent multilocus sequence typing (MLST) by PCR [21]. When frozen isolates did not grow after subculturing, only MLST was carried out (Supplementary Figure 1). In vivo pathogenicity studies were carried out in the Galleria mellonella model following previously published protocols [22]. Details on the sequencing pipeline are available in the Supplementary Materials and the virulence determinants dataset in Supplementary Table 1.

Statistical Analysis

Kaplan-Meier survival curves (log-rank test) were obtained with GraphPad Prism version 5 software (GraphPad, San Diego, California). Group comparisons were performed using χ² or Fisher exact test for categorical variables and t test or Mann-Whitney U test for continuous variables. The multivariable analysis to determine the impact of covariables on 30-day mortality was carried out by binary logistic regression, adjusting for confounders, using IBM SPSS Statistics 25.0 (Armonk, New York). A cutoff of P = .1 in the univariate analysis was used to select covariables entering the multivariate model and the Horner-Lemeshow goodness-of-fit test was applied. The total number of cases included in each analysis (taking into account missing values) is indicated in each table or figure. Exact P values are indicated throughout the study (2-tailed). Statistical significance was established at P < .05.

RESULTS

Overall Cohort Clinical Data

One hundred sixty-five unique cases were included in this 3-year retrospective cohort of KPC-KP BSIs. The patients’ overall epidemiological and clinical characteristics (including outcome and treatment) are depicted in Table 1. Patients’ median age was 59 years, with 54% male. The median Charlson comorbidity index score at admission was 5 (interquartile range [IQR], 3–7), with 39% of patients diagnosed with cancer (solid tumor or hematological malignancies). At the time of bacteraemia onset, 56% of patients were hospitalized in intensive care units (ICUs). Overall, 53% of patients developed septic shock. Only 9% and 38% of patients received at least 2 and at least 1 in vitro–active antibiotic, respectively. All-cause mortality rates at 3 and 30 days were 27% and 60%, respectively.

Clinical Analysis According to Sequence Type

MLST typing was determined for 151 KPC-KP isolates. One hundred nine patients (66%) were infected by KPC-KP isolates belonging to CC258 (ST258, ST11, ST437), 20 patients (12%) by ST16, and 22 patients (13%) by other STs (including ST307, ST15, and ST101), as shown in Figure 1. To establish whether the circulating clones could be associated with different mortality rates, survival analysis was performed comparing patients infected by ST16, compared with CC258 and other STs. Strikingly, survival after 30 days was lower for ST16-infected patients (5%)

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Table 1. Characteristics of Patients With Bloodstream Infections Caused by *Klebsiella pneumoniae* Carbapenemase-2-Producing *K. pneumoniae* According to Multilocus Sequence Typing

| Characteristic | ST16 (n = 20) | CC25B8 (n = 109) | ST11 (n = 48) | ST25B8 (n = 46) | ST437 (n = 15) | Other STs (n = 22) | Total Typed (n = 151) | Total Cohort (N = 165) | P-Valuea |
|---------------|--------------|------------------|--------------|-----------------|----------------|------------------|----------------------|----------------------|---------|
| **Baseline epidemiological and clinical characteristics**b | | | | | | | | | |
| Age, y, median (IQR) | 55 (43–67) | 59 (46–68) | 60 (50–72) | 56 (38–69) | 59 (46–69) | 58 (45–68) | 59 (45–69) | .935 |
| Elderly (age >65 y) | 6 (30) | 30 (28) | 17 (37) | 4 (27) | 7 (32) | 43 (29) | 48 (29) | 1.000 |
| Female sex | 13 (65) | 46 (42) | 20 (44) | 7 (47) | 8 (36) | 67 (44) | 75 (46) | .055 |
| CCI score, median (IQR) | 4 (3–7) | 6 (3–8) | 6 (4–8) | 4 (3–7) | 5 (2–7) | 5 (3–7) | .174 |
| Solid malignant tumor | 3 (15) | 21 (19) | 12 (25) | 14 (27) | 23 (14) | 29 (19) | 30 (18) | .618 |
| Hematological malignancy | 7 (35) | 16 (15) | 6 (13) | 8 (17) | 2 (12) | 27 (17) | 29 (19) | .075 |
| Corticosteroids 1 mg/kg | 3 (15) | 15 (14) | 10 (21) | 7 (13) | 3 (18) | 14 (9) | 19 (12) | .736 |
| Transplantation | 3 (15) | 13 (12) | 8 (17) | 4 (9) | 7 (12) | 11 (7) | 19 (12) | .750 |
| ICU (following bacteremia onset) | 15 (75) | 60 (55) | 25 (52) | 29 (54) | 29 (68) | 84 (56) | 92 (56) | .089 |
| Hepatobiliary disorders | 8 (40) | 19 (17) | 10 (21) | 8 (17) | 2 (12) | 27 (17) | 30 (19) | .079 |
| Autoimmune/rheumatological disorder | 1 (5) | 11 (10) | 5 (10) | 4 (9) | 2 (12) | 6 (4) | 9 (6) | .693 |
| Cardiac failure | 7 (35) | 22 (20) | 13 (27) | 7 (13) | 4 (18) | 33 (22) | 40 (24) | .126 |
| Chronic renal failure | 2 (10) | 10 (9) | 6 (13) | 11 (24) | 3 (15) | 20 (13) | 24 (15) | .736 |
| Diabetes | 4 (20) | 34 (31) | 17 (35) | 18 (35) | 1 (7) | 6 (4) | 10 (6) | .340 |
| COPD | 3 (15) | 13 (12) | 6 (13) | 6 (13) | 7 (13) | 3 (2) | 19 (12) | .931 |
| Liver failure | 20 (100) | 105 (96) | 46 (96) | 44 (96) | 15 (100) | 21 (100) | 33 (22) | .079 |
| Healthcare exposure in last 3 mo^c | 8 (40) | 19 (17) | 10 (21) | 8 (17) | 2 (12) | 27 (17) | 30 (19) | .079 |
| LOS prior to bacteremia, d, median (IQR) | 4 (3) | 7 (6) | 2 (5) | 3 (7) | 2 (13) | 7 (5) | 7 (4) | .594 |
| ICU (prior to bacteremia onset) | 14 (70) | 57 (52) | 24 (50) | 27 (59) | 6 (40) | 6 (27) | 77 (51) | .021 |
| Septic characteristics and severity scores | | | | | | | | | |
| Source of bacteremia | Carbapenemase 2–Producing | 5 (25) | 36 (33) | 14 (29) | 18 (39) | 4 (27) | 7 (32) | 48 (32) | .533 |
| CLABSI | 2 (10) | 5 (5) | 2 (4) | 3 (7) | 0 | 7 (5) | 10 (6) | .371 |
| Lungs | 4 (20) | 31 (28) | 14 (29) | 11 (24) | 4 (20) | 6 (27) | 41 (27) | .740 |
| Urinary | 0 | 7 (6) | 3 (6) | 2 (4) | 2 (13) | 9 (6) | 9 (6) | .306 |
| Abdominal | 7 (35) | 17 (16) | 11 (23) | 4 (9) | 2 (13) | 6 (27) | 30 (19) | .092 |
| Central nervous system | 1 (5) | 2 (2) | 0 | 2 (4) | 0 | 3 (2) | 3 (2) | .227 |
| Skin and soft tissue | 1 (5) | 9 (8) | 3 (6) | 5 (11) | 1 (7) | 10 (7) | 10 (6) | .151 |
| ICU (following bacteremia onset) | 15 (75) | 60 (55) | 25 (52) | 29 (63) | 6 (40) | 9 (41) | 84 (56) | .089 |
| Mechanical ventilation | 13 (65) | 50 (46) | 22 (46) | 22 (48) | 6 (40) | 9 (41) | 72 (48) | .151 |
| Septic shock | 14 (70) | 53 (49) | 26 (54) | 21 (46) | 6 (40) | 15 (68) | 82 (54) | .227 |
| Pitt bacteremia score, median (IQR) | 4.5 (3–6) | 3.5 (2–6) | 3.5 (2–6) | 3.5 (2–6) | 3.5 (2–6) | 3.5 (2–6) | 3.5 (2–6) | .168 |
| Mortality^d | All-cause death at 3 d postbacteremia | 9 (45) | 29 (27) | 13 (27) | 12 (26) | 4 (27) | 6 (27) | 44 (29) | .114 |
| All-cause death at 30 d postbacteremia | 19 (95) | 62 (57) | 29 (60) | 27 (59) | 6 (40) | 15 (68) | 96 (64) | .022 |
| Treatment | | | | | | | | | |
| No. of gram-negative-spectrum antibiotics, median (IQR) | 3 (2–3) | 2 (2–3) | 2 (2–3) | 2 (2–3) | 3 (2–3) | 3 (2–3) | 2 (2–3) | .021 |
| At least 1 in vitro-active antibiotic | 11 (58) | 34 (37) | 13 (35) | 17 (41) | 4 (31) | 4 (29) | 49 (39) | .080 |
| At least 2 in vitro-active antibiotic | 3 (17) | 7 (8) | 3 (9) | 2 (5) | 2 (17) | 1 (17) | 11 (9) | .371 |

Data are presented as no. (%) unless otherwise indicated.

Abbreviations: CC, clonal complex; CCI, Charlson comorbidity index; CLABSI, central line–associated bloodstream infection; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; ICU, intensive care unit; IQR, interquartile range; LOS, length of stay; MLST, multilocus sequence typing; NA, not available; ST, sequence type.

aStatistical analysis was performed comparing ST16 vs non-ST16 data (any other typed isolate). P-values were calculated by Fisher exact test (categorical variables) or Mann-Whitney test (continuous variables).

bData were available for 160 cases for every variable, except for age, elderly age, sex, healthcare exposition in the 3 previous months, LOS, ICU prior to bacteremia onset, ICU following BSI, and all-cause death, where data for all 165 cases were collected.

cBaseline epidemiological and clinical characteristics, where data for all 165 cases were collected.

dMortality refers to all-cause death at 30 d post-bacteremia.

Median (IQR).

fAt least 2 in vitro-active antibiotics were required to be active according to EUCAST recommendations.

eAt least 1 in vitro-active antibiotic was required to be active according to EUCAST recommendations.

fStatistical analysis was performed comparing ST16 vs non-ST16 data (any other typed isolate). P-values were calculated by Fisher exact test (categorical variables) or Mann-Whitney test (continuous variables).
compared with any other group \((P < .0056; \text{Figure 1})\). Indeed, 30-day all-cause mortality rate was 95% for patients infected by ST16 vs 57% and 68% for those infected by CC258 and other STs, respectively \((P < .002)\). The 3-day all-cause mortality rates of ST16 group tended to be higher than those for CC258 and other STs (45% vs 27% and 27%, respectively), although it did not reach statistical significance (Table 1). Three-day survival curves are shown in Supplementary Figure 2. Scores reflecting BSI severity tended to be higher in the ST16 group compared to CC258, but not compared to other STs. The median Pitt bacteremia score was 4.5 vs 3.5 and 5 for patients infected by ST16, CC258, and other STs, respectively \((P = .168)\). Septic shock was observed in 70% of ST16 patients compared to 49% and 68% with CC258 and other STs, respectively (Table 1). Baseline comparison between patient groups showed that the median Charlson comorbidity index score was 4 (IQR, 3–7), 6 (IQR, 3–8), and 4 (IQR, 3–7) for ST16, CC258, and other ST groups, respectively \((P = .174)\). The median patient age and proportion of patients >65 years old were comparable between groups. The proportion of patients in ICU prior to the BSI event (not influenced by the KPC-KP septic event) tended to be higher in the ST16 group (not statistically significant). Trends to higher proportion of hepatobiliary disease \((P = .075)\) and hematological malignancies underlying disease \((P = .079)\) were seen in the ST16 group. The antimicrobial therapy prescribed for the ST16 group showed a higher number of anti-gram-negative antimicrobial drugs prescribed \((P = .021)\) and more in vitro active antibiotics \((P = .080)\) (Table 1). Overall, ST16-infected patients’ variables were in most cases statistically comparable across groups. Taken together, these comparisons raise the possibility that the worse outcome for ST16-infected patients was due to a virulent clone, rather than infection of more severely ill patients.

### Mortality Predictor Analysis

To test this hypothesis, we performed an analysis for factors expected to influence 30-day mortality, including ST16 KPC-KP BSIs (Table 2). The univariate analysis identified ST16 KPC-KP (vs non-ST16), Charlson comorbidity index, septic shock, mechanical ventilation, hepatobiliary underlying disease, hematological malignancy, and increased number of anti-gram-negative antimicrobial drugs prescribed as mortality predictors. The multivariate analysis identified ST16 KPC-KP BSIs, Charlson comorbidity index, septic shock, and mechanical ventilation as independent predictors of mortality. Furthermore, a higher number of anti-gram-negative antimicrobial drugs prescribed tended to be associated with increased mortality, although this did not reach statistical significance. These findings suggest that the worse outcome for ST16-infected patients was due to a virulent clone, rather than infection of more severely ill patients.
Table 2. Univariate and Multivariate Analyses of Risk Factors Associated With a 30-day Fatal Outcome in Patients With Klebsiella pneumoniae Carbapenemase 2–Producing K. pneumoniae Bloodstream Infections

| Covariate                          | Univariate Analysis          | Multivariate Analysis          |
|------------------------------------|------------------------------|--------------------------------|
|                                    | Coefficient (b) | OR [exp(b)] (95% CI) | PValue | Coefficient (b) | OR [exp(b)] (95% CI) | PValue |
| ST16 (vs non-ST16)                 | 2.59             | 13.35 (1.73–102.88) | .013b  | 3.06             | 21.41 (2.26–202.82) | .008b  |
| Age >65 y                          | −0.02            | 0.98 (0.46–2.08)    | .964   | ...              | ...               | ...    |
| Female sex                         | −0.17            | 0.85 (0.43–1.67)    | .227   | ...              | ...               | ...    |
| Charlson comorbidity index         | 0.16             | 1.18 (1.04–1.33)    | .012a  | 0.20             | 1.23 (1.03–1.46)   | .022a  |
| Septic shock                       | 1.17             | 3.21 (1.23–8.37)    | .017b  | 0.79             | 2.20 (0.55–8.88)   | .267   |
| Septic shock                       | 0.49             | 1.64 (0.67–4.02)    | .279   | ...              | ...               | ...    |
| Charlson comorbidity index         | 1.79             | 6.00 (0.67–53.68)   | .109   | 2.84             | 17.05 (1.36–214.14) | .028b  |
| Septic shock                       | 1.20             | 3.33 (1.31–8.49)    | .012b  | 1.05             | 2.87 (0.87–9.46)   | .083   |
| Septic shock                       | 0.85             | 2.33 (0.89–6.12)    | .085b  | 0.06             | 1.06 (0.25–4.39)   | .940   |
| Septic shock                       | 0.18             | 1.20 (0.56–3.60)    | .724   | 0.44             | 1.56 (0.43–5.59)   | .499   |
| Septic shock                       | 0.00             | 1.00 (0.98–1.01)    | .576   | ...              | ...               | ...    |
| Length of stay (prior to BSI)      | ...              | ...               | ...    | ...              | ...               | ...    |
| Septic shock                       | ...              | ...               | ...    | ...              | ...               | ...    |
| Septic shock                       | ...              | ...               | ...    | ...              | ...               | ...    |

Abbreviations: BSI, bloodstream infection; CI, confidence interval; CNS, central nervous system; MBI, mucosal barrier injury; OR, odds ratio; SSTI, skin and soft tissue infection; ST, sequence type.

aThe analysis (binary logistic regression) was performed on 146 BSI cases where the complete dataset was available. Variables showing P value < .1 in the univariate analysis were further included in the multivariate model.

bIndicates statistical significance as defined by P value < .05.

cCompared to central line–associated BSI.

dThe antibiotic resistance genes (ARGs) identified in the ST16 isolates were less resistant to aminoglycosides, amikacin (37% vs 55% resistance), and gentamicin (16% vs 86% resistance) than isolates belonging to the CC258. The antibiotic resistance genes (ARGs) identified in the ST16 and CC258 genomes are depicted in Figure 2. Besides blaKPC-2 found in all genomes as expected, blaCTX-M-15 and blaCTX-M-14, and blaCTX-M-2 were often present, as was blaSHV-12. Several aminoglycoside modifying enzymes were identified including a subset of aac(6’)-Ib-cr, providing resistance to aminoglycoside and certain quinolones. Interestingly, all sequenced ST258 isolates carried mrtB 16S-methylase, explaining the elevated proportion of high level of aminoglycoside resistance in this subgroup. No MCR encoding genes were identified. In contrast, mgrB alterations responsible for polymyxin resistance were found in 63% of ST16 isolates. Plasmid replicons were also shown in Figure 2. Within ST16 group, 2 genomes (P20 and P31) with distinct ARG and replicon profiles were fully assembled for further plasmid analysis. Both isolates carried blaKPC-2 on a 113-kb IncFIBpQIL plasmid. The genetic context of blaKPC-2 in a Tn4401 transposon was compared to the context of ST258 isolates (Supplementary Figure 4). Interestingly, no virulence plasmid was found in these ST16 isolates. A description of the ST16 plasmids is provided in Supplementary Table 3.)
Virulence Factor Determinants

Virulence determinants in ST16 genomes and in CC258 clones (ST258, ST11, ST437) in silico are shown in Figure 2. All genomes had type 1 (fim) and type 3 (mrk) fimbrial adhesins as well as the urease gene cluster (ure), outer membrane protein (ycfM), enterobactin, wabGHN (lipopolysaccharide synthesis), and kpn. Yersiniabactin siderophore and the virB1–11 type IV secretion system were present in all ST11 and in a
subset of ST16 isolates (n = 11), but not in the ST258 and ST437 isolates. Colibactin was present in all the ST11 genomes, whereas cloacin was present mainly in the ST11 and ST258 isolates. Aerobactin, salmochelin, hyperviscosity factors rmpA/rmpA2, the iron uptake system kfuABC, and the kvgA2 component system were not identified in any of these 60 isolates. Distinct capsule loci (KL) were noticed for the KPC-KP isolates classified under different STs as shown in Figure 2 and Supplementary Figure 5. A single KL was observed within ST16, ST258, and ST437 isolates that displayed KL51, KL107, and KL36 capsular types, respectively. In contrast, 2 distinct KL variants were detected in ST11 isolates. Twelve of the 16 ST11 isolates showed KL64, while the remaining 4 isolates showed the KL15 genotype.

**G. mellonella Virulence Testing**

To test virulence properties of these strains, larvae were infected by representative isolates of ST16 (KL51), ST258 (KL107), ST11 (KL64 and KL15), and ST437 (KL36). Survival of ST16-infected larvae were inferior to all other tested isolates even at the lowest tested inoculum (10^4 colony-forming units). Of all tested isolates, the isolates P31 and P20 ST16/KL51 were the most virulent strains in this model at all tested inocula (see Figure 3 and Supplementary Figure 6 for complete strains set at each inoculum).

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**Figure 2.** Virulence, antibiotic resistance genes, and plasmid replicons of 64 sequenced *Klebsiella pneumoniae* carbapenemase–producing *K. pneumoniae* strains. The heatmap was generated after aligning the contigs of sequenced genomes of each strain to our virulence gene dataset, to Resfinder, and to PlasmidFinder. Chromosomally encoded *qpxAB* genes were found in all *K. pneumoniae* genomes and were not displayed. None of these genomes was found to harbor MCR genes. Abbreviation: MCR, mobile colistin resistance.
**DISCUSSION**

Despite its retrospective and single-center nature, this study provides a uniquely detailed clinical and microbiological description of a KPC-2–producing *K. pneumoniae* BSI cohort from a Brazilian hospital setting. No previous study has reported Brazilian data in such a comprehensive manner, even though the endemicity of KPC-KP in Brazil is well established. In this study, central line–associated BSI (primary BSI), lower respiratory tract infections, and intra-abdominal infections were the most frequent sources of KPC-KP BSI, in accordance with data from other Brazilian hospitals provided by the Brazilian Health Surveillance Agency (ANVISA; [http://www20.anvisa.gov.br](http://www20.anvisa.gov.br)) [23, 24]. Although the optimal treatment for KPC-KP BSI infections remains controversial, most experts recommend at least 2 in vitro active antimicrobials, especially when new therapeutic options such as ceftazidime-avibactam and meropenem-vaborbactam are not commercially available, as occurred in the present study period [23, 25]. Importantly, this study was not meant to evaluate KPC-KP therapeutic success. In fact, most patients did not receive 2 in vitro–active drugs because (1) the isolates were multirdrug resistant, showing high levels of resistance to meropenem (median MIC largely above the 32 mg/L threshold), aminoglycosides, and polymyxin B; (2) new β-lactam/β-lactamase inhibitor combinations and tigecycline (despite its debated efficacy for BSI) were not readily available; and (3) susceptibility results were often reported after patient death. For these reasons, it was difficult to infer the role of adequate antimicrobial therapy.

Here we report 20 cases due to a novel KPC-KP ST16 clone, of which 19 were fatal. The ST16 clone was not related to the CC258 isolates as shown by our phylogenetic analysis. It is not known when and how precisely this clone was introduced into this hospital, but our data show its maintenance during the study period. The potential for clonal dissemination of KP ST16 beyond this institution remains uncertain. Previous reports exist of NDM-5–, OXA-48–, and CTX-M-5–producing KP ST16 in Denmark, the United Kingdom, and Spain, respectively [26–28]. Scarce reports of isolated cases in the Netherlands (NDM-5) [29], Italy (NDM-1 and OXA-232) [30], and Rio de Janeiro, Brazil (OXA-370) were also documented [31]. In contrast, ST16 KP has rarely been described harboring *bla*KPC, except for 2 isolates reported in Rio de Janeiro in 2008 and 2009 (*bla*KPC-2) and 1 isolate in Israel. More recently, KPC-2–producing *K. pneumoniae* ST16 was detected in another teaching hospital also located in São Paulo [32].
This study thus depicts the largest outbreak caused by KPC-ST16 [16, 17, 33], which is a poorly characterized clone, in contrast to the successful ST11, ST258, ST307, or ST15 clones (also found in this study) [9, 12, 34–37].

Based on the group comparisons of baseline characteristics and on the multivariable analysis, it does not appear that ST16 infected more severely ill patients. Even treatment strategies of ST16-infected patients were not statistically different compared to patients infected with CC258 and other STs (non-ST16, non-CC258), with the exception of a higher number of antibiotic-negative antibiotics used in the ST16 group. Altogether, this suggested that the ST16 clone had a high virulence potential. Survival curves showed lower survival of patients infected by ST16 compared to those infected by CC258 or other nonrelated CC258 clones. Both ST16 KPC-KP and septic shock were identified as independent risk factors for all-cause 30-day death in the multivariable analysis. The absence of collinearity between these covariables suggested that death may not be consequent to severe sepsis only. Finally, there was a trend for more hepatobiliary comorbidity and abdominal BSI source in the ST16 patient group, indicating that ST16 KPC-KP might have a particular tropism for hepatobiliary anatomical structures. These findings must be further addressed in prospective studies.

In this study, the ST16 clone exhibited higher virulence in the G. mellonella pathogenicity model, killing more larvae than CC258 counterparts, including ST11, a clone harboring colibactin and yersiniabactin encoding genes, at all tested inocula. Genomic analysis of virulence determinants showed that the ST16 isolates possessed a wide array of virulence genes such as the KL51 capsule. In addition, a ST16 subset also possessed the yersiniabactin siderophore. However, it remains unclear if unknown key virulence factors were involved in the ST16 virulence profile because neither rmpA/rmpA2 nor any known hypervirulence genes were identified.

Some studies have reported that the ST258 clone itself (but not every CC258-related clone) exhibited a low virulence profile in animal models [38]. This finding was corroborated by our Galleria results. In this cohort, the differences in mortality across STs might be the result of a high virulence of the ST16 clone combined with the lower virulence displayed by ST258 clones.

In conclusion, we described a virulent KPC-KP ST16 clone. This clone has emerged and disseminated in a hospital setting where KPC-KP CC258 is endemic. It has been identified as an independent risk factor for fatal outcome at 30 days. These results show that even in endemic settings, the epidemiological scenario can change and highly virulent clones can rapidly emerge, demanding constant monitoring.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
Author contributions. D. O. A., E. A. M., T. R. W., and A. C. G. contributed to the study concept. P. D. and A. C. G. collected clinical data. D. O. A., W. B. S. M., and E. F. performed laboratory work. D. O. A., F. M. C., M. R. N., L. A. G., A. R. V., and K. S. performed whole-genome sequencing and bioinformatics analysis. D. O. A., A. C. G., R. C., E. A. M., M. R. N., J. S., and T. R. W. analyzed clinical and microbiological data. D. O. A., A. C. G., and T. R. W. prepared the manuscript.

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