

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

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Background. Carbapenemase-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* carbapenemase–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 multilocus 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*_{KPC-2} 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 BSI 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. carbapenem-resistant Enterobacteriaceae; KPC; bloodstream infections; CC258; *Klebsiella pneumoniae*.

The widespread prevalence of carbapenem-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 carbapenemase–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)

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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 χ^2 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 bacteremia 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%)

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

Characteristic	MLST						Total Typed (n = 151)	Total Cohort (N = 165)	P Value ^a
	CC258								
	ST16 (n = 20)	CC258 (n = 109)	ST11 (n = 48)	ST258 (n = 46)	ST437 (n = 15)	Other STs (n = 22)			
Baseline epidemiological and clinical characteristics ^b									
Age, y, median (IQR)	55 (43–67)	59 (46–68)	59 (50–64)	60 (50–72)	56 (38–69)	59 (46–69)	58 (45–68)	59 (45–69)	.535
Elderly (age >65 y)	6 (30)	30 (28)	9 (19)	17 (37)	4 (27)	7 (32)	43 (29)	48 (29)	1.000
Female sex	13 (65)	46 (42)	19 (40)	20 (44)	7 (47)	8 (36)	67 (44)	75 (46)	.055
CCI score, median (IQR)	4 (3–7)	6 (3–8)	6 (4–8)	6 (4–8)	6 (4–8)	4 (3–7)	5 (2–7)	5 (3–7)	.174
Solid malignant tumor	3 (15)	21 (19)	12 (25)	7 (15)	2 (14)	5 (23)	29 (19)	30 (18)	.618
Hematological malignancy	7 (35)	16 (15)	6 (13)	8 (17)	2 (13)	6 (27)	29 (19)	34 (21)	.075
Corticosteroids 1 mg/kg	3 (15)	15 (14)	10 (21)	3 (7)	2 (13)	2 (9)	20 (13)	24 (15)	.736
Transplantation	3 (15)	13 (12)	8 (17)	4 (9)	1 (7)	1 (5)	17 (11)	19 (12)	.750
HIV	0	7 (6)	2 (5)	3 (7)	2 (13)	0	7 (5)	7 (4)	.594
Hepatobiliary disorders	8 (40)	19 (17)	10 (21)	8 (17)	1 (7)	6 (27)	33 (22)	35 (21)	.079
Autoimmune/rheumatological disorder	1 (5)	11 (10)	5 (10)	4 (9)	2 (13)	2 (9)	14 (9)	14 (9)	.693
Cardiac failure	7 (35)	22 (20)	13 (27)	7 (15)	2 (13)	4 (18)	33 (22)	40 (24)	.126
Chronic renal failure	2 (10)	30 (28)	16 (33)	11 (24)	3 (20)	2 (9)	34 (23)	38 (23)	.306
Diabetes	4 (20)	34 (31)	17 (35)	16 (35)	1 (7)	6 (27)	44 (29)	50 (30)	.740
COPD	3 (15)	13 (12)	6 (13)	6 (13)	1 (7)	3 (14)	19 (13)	20 (12)	.931
Exposure to antimicrobial (last 3 mo)	20 (100)	105 (96)	46 (96)	44 (96)	15 (100)	21 (96)	146 (97)	160 (97)	.934
Healthcare exposure in last 3 mo ^c	20 (100)	108 (99)	48 (100)	45 (98)	15 (100)	20 (91)	148 (98)	160 (97)	.985
LOS prior to bacteremia, d, median (IQR)	15 (12–27)	24 (14–39)	19 (10–35)	28 (16–42)	26 (18–46)	23 (17–46)	22 (13–39)	22 (14–39)	.079
ICU (prior to bacteremia onset)	14 (70)	57 (52)	24 (50)	27 (59)	6 (40)	6 (27)	77 (51)	84 (51)	.092
Sepsis characteristics and severity scores									
Source of bacteremia									.299
CLABSI	5 (25)	36 (33)	14 (29)	18 (39)	4 (27)	7 (32)	48 (32)	53 (32)	
Mucosal barrier injury	2 (10)	5 (5)	2 (4)	3 (7)	0	0	7 (5)	10 (6)	
Lungs	4 (20)	31 (28)	14 (29)	11 (24)	6 (40)	6 (27)	41 (27)	45 (27)	
Urinary	0	7 (6)	3 (6)	2 (4)	2 (13)	2 (9)	9 (6)	9 (6)	
Abdominal	7 (35)	17 (16)	11 (23)	4 (9)	2 (13)	6 (27)	30 (20)	32 (19)	
Central nervous system	1 (5)	2 (2)	0	2 (4)	0	0	3 (2)	3 (2)	
Skin and soft tissue	1 (5)	9 (8)	3 (6)	5 (11)	1 (7)	0	10 (7)	10 (6)	
ICU (following bacteremia onset)	15 (75)	60 (55)	25 (52)	29 (63)	6 (40)	9 (41)	84 (56)	92 (56)	.089
Mechanical ventilation	13 (65)	50 (46)	22 (46)	22 (48)	6 (40)	9 (41)	72 (48)	77 (47)	.151
Septic shock	14 (70)	53 (49)	26 (54)	21 (46)	6 (40)	15 (68)	82 (54)	88 (53)	.227
Pitt bacteremia score, median (IQR)	4.5 (3–6)	3.5 (2–6)	4 (2–5)	4 (2–6)	2 (2–6)	5 (2–6)	4 (2–6)	4 (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)	45 (27)	.114
All-cause death at 30 d postbacteremia	19 (95)	62 (57)	29 (60)	27 (59)	6 (40)	15 (68)	96 (64)	100 (60)	.002 ^e
Treatment									
No. of gram-negative-spectrum antibiotics, median (IQR) ^f	3 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)	3 (2–3)	3 (2–3)	2 (2–3)	2 (2–3)	.021 ^g
At least 1 in vitro–active antibiotic ^{a,h}	11 (58)	34 (37)	13 (35)	17 (41)	4 (31)	4 (29)	49 (39)	NA	.080
At least 2 in vitro–active antibiotics ^{a,h}	3 (17)	7 (8)	3 (9)	2 (5)	2 (17)	1 (7)	11 (9)	NA	.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.

^cDialysis, day-care hospital, ward admission.

^dFor 2 patients (1 ST258 and 1 untyped case), 30-day outcome data could not be retrieved (censored) due to early hospital discharge.

^eMeans it was considered statistically significant = *P* < .05.

^fIrrespective of antimicrobial susceptibility testing (AST) results.

^gIn vitro activity was established for 125 cases where AST following European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations could be performed a posteriori because the strains were viable for growth (see [Methods](#)).

^hAccording to EUCAST breakpoints [19].

compared with any other group ($P < .0056$; 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

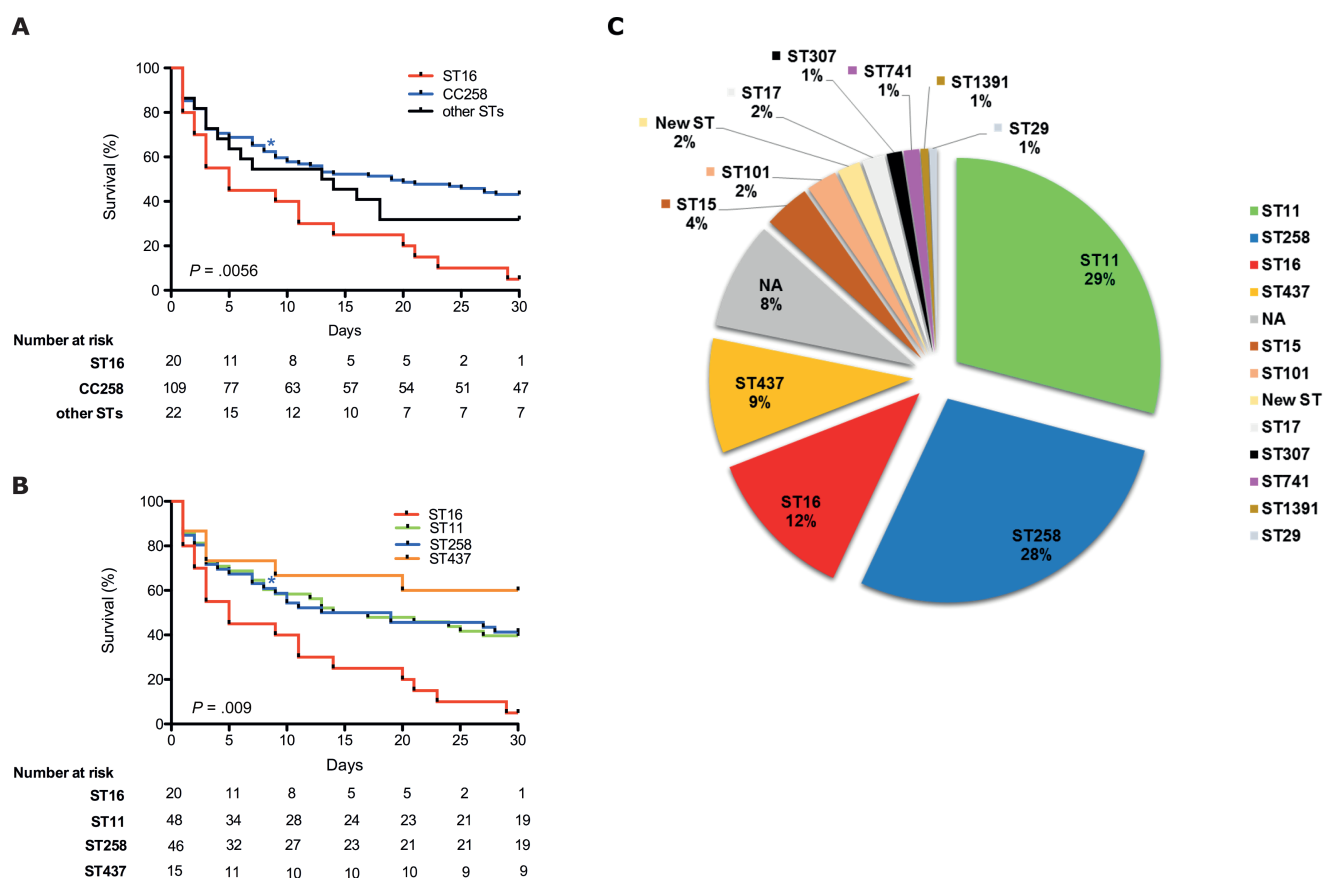


Figure 1. Kaplan-Meier plots showing the survival of patients following bacteremia due to *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* (KPC-KP) belonging to sequence type (ST) 16 vs clonal complex (CC) 258 vs other STs (A) and ST16 vs ST11, ST258, and ST437 (B), over 30 days. C, Pie chart showing multilocus sequence typing distribution of KPC-KP isolates. Survival curves were plotted using the Kaplan-Meier method (GraphPad Prism version 5 software), and P values were calculated by log-rank (Mantel-Cox) test. One patient (ST258 group) was censored. One patient (ST258 group) was censored and is signaled on by *. Abbreviations: CC, clonal complex; NA, isolates unavailable for multilocus sequence typing analysis; ST, sequence type.

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^a

Covariate	Univariate Analysis				Multivariate Analysis			
	Coefficient (<i>b</i>)	OR [exp(<i>b</i>)]	(95% CI)	<i>P</i> Value	Coefficient (<i>b</i>)	OR [exp(<i>b</i>)]	(95% CI)	<i>P</i> Value
ST16 (vs non-ST16)	2.59	13.35	(1.73–102.88)	.013 ^b	3.06	21.41	(2.26–202.82)	.008 ^b
Age >65 y	−0.02	0.98	(.46–2.08)	.964	
Female sex	−0.17	0.85	(.43–1.67)	.627	
Charlson comorbidity index	0.16	1.18	(1.04–1.33)	.012 ^b	0.20	1.23	(1.03–1.46)	.022 ^b
Hepatobiliary condition	1.17	3.21	(1.23–8.37)	.017 ^b	0.79	2.20	(.55–8.88)	.267
Hematological condition	0.49	1.64	(.67–4.02)	.279	
Septic shock	2.32	10.40	(4.69–23.08)	<.001 ^b	2.48	11.90	(4.15–34.13)	<.001 ^b
Mechanical ventilation	1.08	2.93	(1.44–5.96)	.003 ^b	−0.08	0.92	(.31–2.73)	.879
BSI source: MBI-BSI ^c	1.79	6.00	(.67–53.68)	.109	2.84	17.05	(1.36–214.14)	.028 ^b
Lungs	1.20	3.33	(1.31–8.49)	.012 ^b	1.05	2.87	(.87–9.46)	.083
Abdominal	0.85	2.33	(.89–6.12)	.085 ^b	0.06	1.06	(.25–4.39)	.940
Others (urinary, SSTI, CNS)	0.18	1.20	(.44–3.30)	.724	0.44	1.56	(.43–5.59)	.499
Anti-gram-negative agents, No.	−0.14	0.87	(.56–1.35)	.526	
Length of stay (prior to BSI)	0.00	1.00	(.98–1.01)	.576	

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.

disease, pulmonary disease, and abdominal BSI source to be risk factors. Of note, the multivariable model confirmed ST16 KPC-KP infection (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), among other covariables, to be independently associated with 30-day fatal outcome (Table 2).

ST16 BSI Case Descriptions

The clinical description and temporal distribution of the 20 ST16 BSI cases are presented in Table 3. Notably, these 20 cases were scattered across the 2014–2016 period and among several wards, suggesting that the ST16 clone had already spread in the hospital and persisted throughout the study period. Meropenem in combination with polymyxin B (*n* = 18), often associated with amikacin (*n* = 15), was the most frequent empirical treatment prescribed. A significant number of patients did not have their definitive treatment adjusted due to early death (ie, before AST results became available) or due to unavailability of certain antimicrobials. Exact time from bacteremia onset to death was shown for the 19 deceased patients, showing a fatal outcome within 1 week after blood culture were drawn in the majority of the cases (55%).

Isolates Genetic Relatedness, AST, Antibiotic Resistance Genes, and Plasmid Replicons

Whole-genome sequence–based relatedness analysis indicated that ST16 was not genetically related to CC258, suggesting the introduction of a novel clone in the CC258-endemic context (Supplementary Figure 3). Antimicrobial susceptibility profiles are shown in Table 4 and Supplementary Table 2. KPC-KP ST16

isolates were highly resistant to meropenem (90% MIC [MIC₉₀], 128 mg/L; 100% resistance) and polymyxin B (MIC₉₀, 64 mg/L; 68.4% resistance). Although CC258 isolates showed slightly different resistance rates to meropenem (MIC₉₀, 128 mg/L; 98% resistance) and polymyxin B (MIC₉₀, 128 mg/L; 51% resistance), when compared to those of ST16, these differences were not statistically significant. In contrast, 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 *bla*_{KPC-2}, found in all genomes as expected, *bla*_{CTX-M-15}, *bla*_{CTX-M-14}, and *bla*_{CTX-M-2} were often present, as was *bla*_{SHV-like}. 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 *rmtB* 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 *bla*_{KPC-2} on a 113-kb IncFIBpQIL plasmid. The genetic context of *bla*_{KPC-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).

Table 3. Description of the 20 Patients Diagnosed With Bloodstream Infections Caused by *Klebsiella pneumoniae* Carbapenemase 2–Producing *K. pneumoniae* Sequence Type 16

Case	Age, y (Sex)	Underlying Disease	Month/Year of Infection	Source of Bacteremia	Ward	Treatment ^a	Outcome at 30 d	Time From Bacteremia Onset to Death, d
1	33 (M)	NHL	July 2014	CLABSI	Hematology	PMB + MEM + AMK/ TGC + FOS	Alive	NA
2	70 (F)	NHL	August 2014	MBI	ED, hematology	PMB + MEM + AMK	Died	3
3	41 (M)	Promyelocytic leukemia	September 2014	CLABSI	Hematology	PMB + MEM + AMK + TGC	Died	9
4	54 (F)	Multiple myeloma	September 2014	CLABSI	ED, ICU	PMB + MEM + AMK	Died	3
5	77 (F)	Duodenal papillary neoplasia	December 2014	Abdominal	GI surgery, medical ICU	MEM/PMB + GEN	Died	5
6	64 (M)	Burns	February 2015	Skin and soft Tissue	Burns ICU	PMB + MEM	Died	14
7	50 (F)	Chronic myeloid leukemia	March 2015	Lungs	Hematology, medical ICU	PMB + MEM + AMK	Died	21
8	42 (F)	Postsurgical infection (meningitis)	June 2015	CNS	Transplant unit, medical ICU	PMB + MEM + AMK	Died	20
9	37 (M)	Pulmonary neoplasia	July 2015	Lungs	ED, ICU	PMB + MEM + AMK	Died	1
10	57 (F)	Kidney transplant	July 2015	Lungs	Medical ICU	PMB + MEM	Died	2
11	66 (M)	Liver cirrhosis	August 2015	Abdominal	Medical ICU	PMB + MEM + AMK	Died	3
12	71 (F)	Bacterial peritonitis	September 2015	Abdominal	Nephrology ICU	MEM	Died	1
13	65 (F)	Endocarditis	May 2016	CLABSI	ED, ICU	PMB + MEM/AMK	Died	11
14	68 (F)	Biliary fistula	October 2016	Abdominal	GI surgery	PMB + MEM	Died	29
15	44 (F)	AML	October 2016	CLABSI	Hematology	PMB + MEM + AMK	Died	3
16	56 (M)	Cardiac failure, transplant	October 2016	Lungs	Cardiac surgery ICU	PMB + MEM + AMK	Died	2
17	79 (M)	Liver cirrhosis	October 2016	Abdominal	ED, ICU	PMB + MEM/AMK	Died	11
18	22 (F)	AML	November 2016	MBI	Hematology, pneumology ICU	PMB + MEM + AMK	Died	5
19	53 (F)	Biliary tract neoplasia	November 2016	Abdominal	GI surgery	PMB + MEM + AMK	Died	23
20	50 (F)	Cholangitis	December 2016	Abdominal	General ICU	PMB + MEM + AMK	Died	1

Abbreviations: AMK, amikacin; AML, acute myeloid leukemia; CLABSI, central line–associated bloodstream infection; CNS, central nervous system; ED, emergency department; F, female; FOS, fosfomycin trometamol; GI, gastrointestinal; ICU, intensive care unit; M, male; MBI, mucosal barrier injury; MEM, meropenem; NA, not applicable; NHL, non-Hodgkin lymphoma; PMB, polymyxin B; TGC, tigecycline.

^aIn cases 1, 5, 13, and 17, additional targeted antimicrobial treatment was added to the initial empirical regimen and are shown after “/”. Bold characters indicate in vitro susceptibility. In case 1, meropenem MIC was 16 mg/L.

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

Table 4. Percentage of Resistance to Selected Antimicrobial Agents of *Klebsiella pneumoniae* Carbapenemase 2–Producing *K. pneumoniae* Isolates According to Sequence Type

Antimicrobial Resistance ^{a,b}	MLST						Total Typed (N = 125)	PValue ^b
	ST16 (n = 19)	CC258 (n = 92)	CC258			Other STs (n = 14)		
			ST11 (n = 37)	ST258 (n = 42)	ST437 (n = 13)			
High meropenem (≥32 mg/L)	94	98	94	100	100	72	94	1.000
Polymyxin B	68	51	35	64	54	29	51	.211
Amikacin	21	49	8	95	16	0	39	.191
Gentamicin	16	86	81	98	61	43	70	<.001 ^c
Tigecycline	5	26	43	2	54	0	20	.075

Abbreviations: CC, clonal complex; MLST, multilocus sequence typing; ST, sequence type.

^aAntimicrobial susceptibility testing following European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations was performed for 125 cultivable isolates (see Methods).

^bAccording to EUCAST breakpoints [19].

^cIndicates statistical significance as defined by P value <0.05.

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 *kvgAS* 2 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).

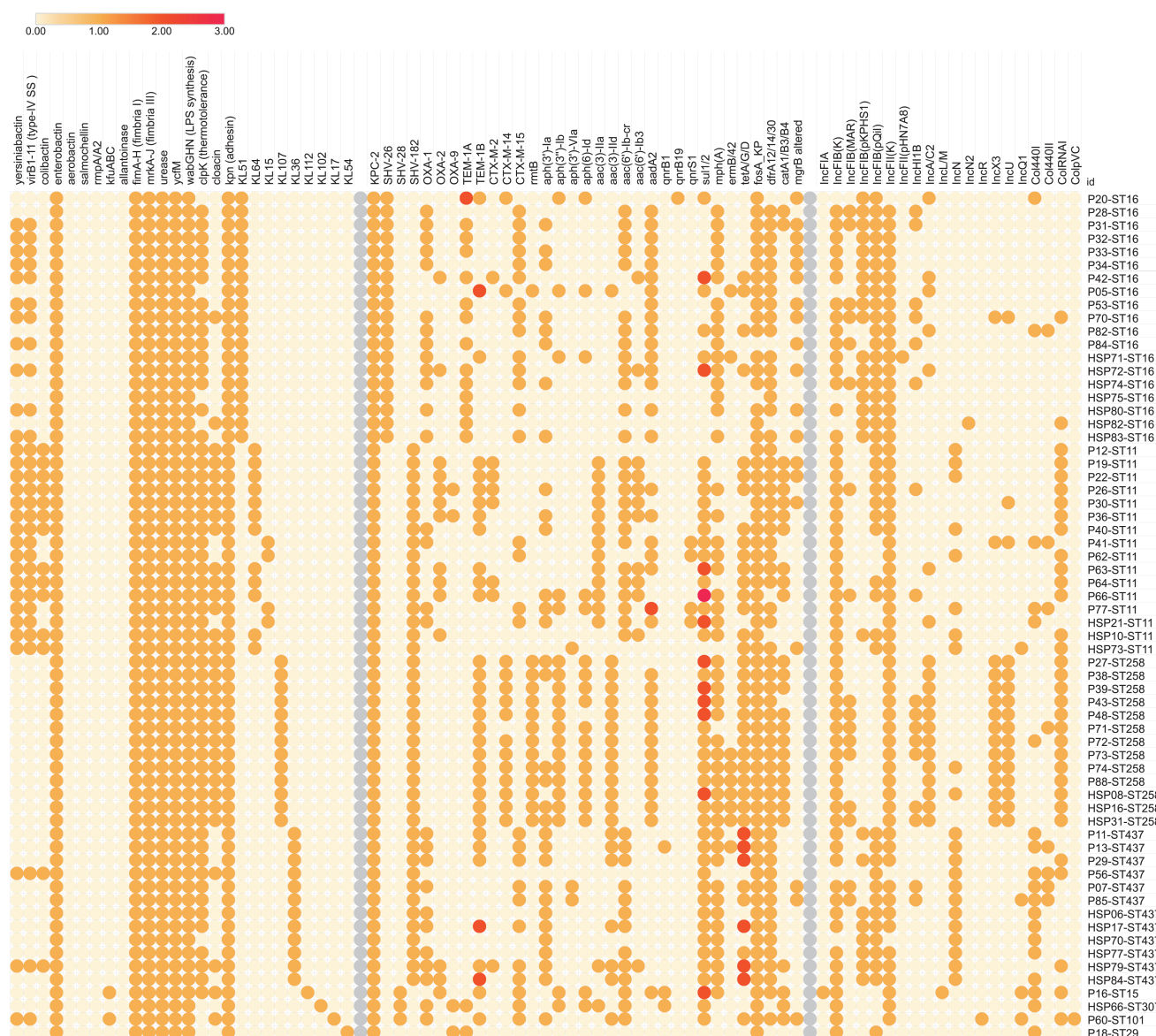


Figure 2. Virulence, antibiotic resistance genes, and plasmid replicons of 64 sequenced *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* strains. The heatmap was generated after aligning the contigs of sequenced genomes of each strain to our virulence gene dataset, to Resfinder, and to PlasmidFinder. Chromosomally encoded *ogxAB* 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.

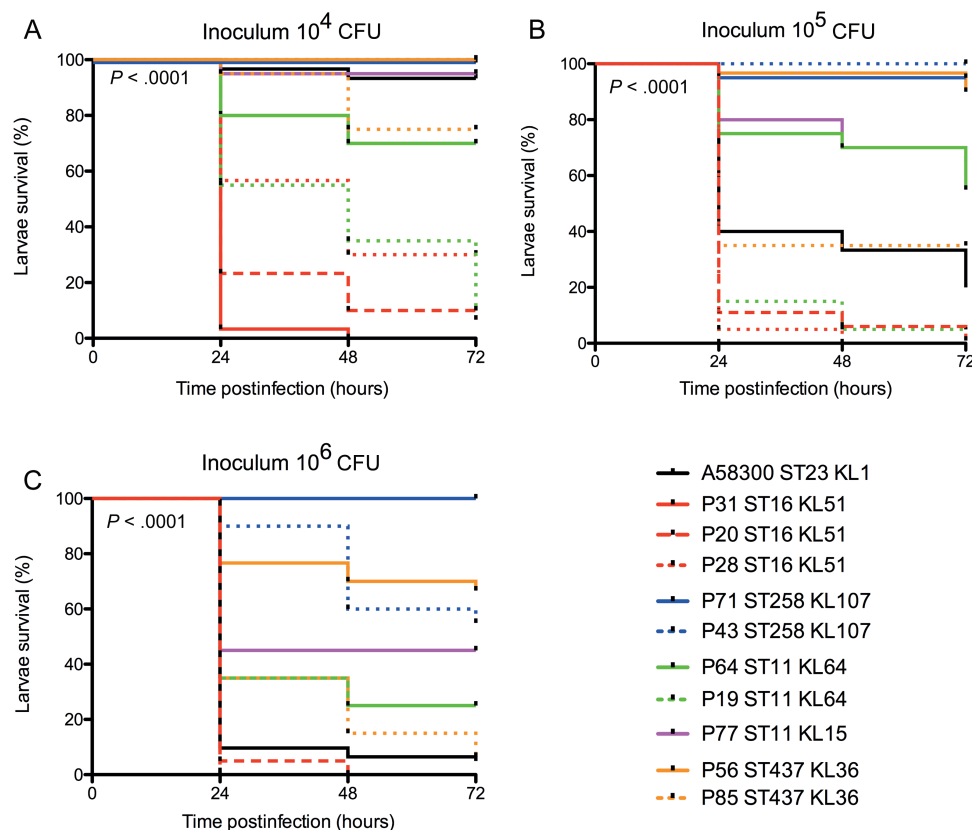


Figure 3. A, Kaplan-Meier plots showing the percentage survival of *Galleria mellonella* over 72 hours postinfection with clinical isolates representative of sequence type (ST) 16 (KL51), ST11 (KL64 or KL15), ST258 (KL107), and ST437 (KL36), at 10^4 (A), 10^5 (B), and 10^6 CFU (C). A58300 is a ST23 K1 *rmpA2*-positive hypermucoviscous positive control. Survival curves were plotted using the Kaplan-Meier method, and *P* values were calculated by log-rank (Mantel-Cox) test. Each line represents a single isolate. Data on all tested isolates are available in [Supplementary Figure 6](#); a subset is shown here for clarity. Abbreviation: CFU, colony-forming units.

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>) [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 multidrug 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 anti-gram-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 multivariate 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 copyedited 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. P. 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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