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1 **Silent Circulation of BKC-1-producing *Klebsiella pneumoniae* ST442: Molecular**
2 **and Clinical Characterisation of an Early and Unreported Outbreak**

3

4 Willames M. B. S. Martins^{1,2*}; Michael H. Lenzi¹; Ana C. Narciso¹; Priscila Dantas³;
5 Diego O. Andrey⁴; Qiu E. Yang⁵; Kirsty Sands²; Eduardo A. Medeiros³; Timothy R.
6 Walsh⁶; Ana C. Gales^{1*}

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8

9 ¹Division of Infectious Diseases, Department of Internal Medicine. Escola Paulista de
10 Medicina/Universidade Federal de São Paulo - UNIFESP, São Paulo, Brazil.

11 ²Department of Medical Microbiology, Division of Infection and Immunity, Cardiff University,
12 Cardiff, United Kingdom.

13 ³Universidade Federal de São Paulo, Hospital Epidemiology Committee, Hospital São Paulo,
14 Division of Infectious Diseases, Department of Internal Medicine, Escola Paulista de Medicina,
15 São Paulo, Brazil.

16 ⁴Service of Infectious Diseases, Department of Medicine, Geneva University Hospitals and
17 Medical School, Geneva, Switzerland.

18 ⁵Fujian Provincial Key Laboratory of Soil Environmental Health and Regulation, College of
19 Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou, 350002,
20 China.

21 ⁶Department of Zoology, University of Oxford, United Kingdom.

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25 **Running Title:** Early Outbreak of BKC-1-producing *K. pneumoniae*

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29 *Corresponding Authors: **Willames M. B. S. Martins**
30 **E-mail: willamesbrasileiro@hotmail.com**
31 **Ana C. Gales**
32 **Email: ana.gales@unifesp.br**

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35

36 **Abstract**

37 **Objective:** This study aimed to describe the undetected circulation of an epidemic
38 *Klebsiella pneumoniae* ST442 clone, producer of BKC-1, occasioning the first reported
39 outbreak of the infrequent carbapenemase BKC-1.

40 **Methods:** Six hundred forty-seven *K. pneumoniae* isolates (2008-2017) with reduced
41 susceptibility to carbapenems were screened for *bla*_{BKC-1}. BKC-1 positive isolates were
42 typed using PFGE and MLST. Susceptibility profiles were determined by broth
43 microdilution and additional antimicrobial resistance genes (ARG) investigated by
44 PCR. Some isolates were submitted to the full-genomic characterization by WGS
45 (Illumina MiSeq and MinIon) and virulence assays as biofilm detection and *in vivo*
46 infection by *Galleria mellonella* model evaluated.

47 **Results:** Sixteen (2.5%) *K. pneumoniae*, from 15 patients, between 2010 to 2012 were
48 found carrying *bla*_{BKC-1}. Among these patients, all cause mortality rate was 54.5%. A
49 major clone A1-ST442 (13/16) was isolated during the time period. The BKC-
50 producing isolates had a multidrug resistance phenotype, remaining susceptible only to
51 gentamicin (87.5%) and ceftazidime-avibactam (100%). The presence of two
52 carbapenemases, *bla*_{BKC-1} and *bla*_{KPC-2}, was detected in six isolates increasing
53 significantly the β -lactam MICs. Additionally, other ARG were identified on A1-ST442
54 and B1-ST11 clones. The B1-ST11 clone was more virulent than A1-ST442 clone.

55 **Conclusion:** An undetected outbreak predominantly caused by a BKC-1 positive A1-
56 ST442 clone between 2010-2012 was retrospectively captured ten years later in a
57 Brazilian Hospital. The misidentification of BKC-1 might have triggered worsening
58 events to antimicrobial resistance, which reinforces the need of correct and rapid
59 identification of antimicrobial resistance mechanisms in our hospitals.

60

61

62 **Key-words:** infection, carbapenem resistance, carbapenemase, outbreak.

63 1. Introduction

64 Carbapenemases are the most versatile β -lactamase enzymes, representing the
65 main mechanism responsible by carbapenem resistance, and are generally able to
66 hydrolyse most clinical available β -lactams [1]. In South America, the carbapenemases
67 KPC-like and NDM-like are frequently reported in *K. pneumoniae* isolates, with KPC-2
68 specifically endemic in Argentina, Brazil, and Colombia [2]. The endemicity of KPC-2,
69 especially in Brazil, is attributed to the clonal expansion of clonal complex 258
70 (CC258), also responsible for the rapid dissemination and stability of KPC-enzymes in
71 the world [2,3]. In the last few years, new carbapenemases have been characterized such
72 as BKC-1 [4], FRI-1 [5], and VCC-1 [6]; however, few studies have been reported the
73 clinical significance of infections caused by pathogens producing these enzymes [5,7-9].

74 Brazilian *Klebsiella* Carbapenemase 1 (BKC-1) is a serine carbapenemase with
75 weak hydrolytic activity against carbapenems initially described in carbapenem-
76 resistant *K. pneumoniae* isolates from 2008 in São Paulo, Brazil [4]. To date, two
77 variants of BKC were described, BKC-1 and BKC-2. While BKC-1 has been described
78 in few *K. pneumoniae* isolates and, more recently, in *Citrobacter freundii*, BKC-2 was
79 detected in *Enterobacter hormaechei* subsp. *xiangfangensis* [4,7,10]. In *K. pneumoniae*,
80 BKC-1 seems to be related to CC442 with all reports linked to ST442 or ST1781.
81 *bla*_{BKC-1} is plasmid-mediated and associated with the insertion sequence *ISKpn23* and a
82 phosphotransferase, *aph(3')-VII* (also called *aph(3')-VIa*), which is able to confer
83 amikacin and kanamycin resistance [4,7]. In addition, other resistance mechanisms have
84 been reported in BKC-1 producers such as ESBL production or OmpK36 loss,
85 increasing β -lactam resistance, or chromosomal mutations occasioning polymyxin
86 resistance [4,7].

87 The aim of this study was to assess the frequency of BKC-producing *K.*

88 *pneumoniae* from clinical isolates cultured from a teaching hospital in São Paulo, Brazil
89 over a 10-year period (2008-2017). In addition, the microbiological, genomic
90 characterization and the *in vivo* virulence were assessed for BKC-positive isolates .

91

92 **2. Material and Methods**

93

94 *2.1 BKC-1-producing Isolates Screening and Identification*

95 A 10-year retrospective surveillance study (January 2008 to December 2017)
96 was conducted by testing 647 *K. pneumoniae* clinical isolates with reduced
97 susceptibility to ertapenem, imipenem or meropenem (MICs \geq 0.5 mg/mL), from a
98 biological collection of a teaching hospital located in São Paulo, Brazil. The isolates
99 were initially screened for *bla*_{BKC-1} by PCR as previously described [4], and
100 subsequently submitted to DNA sequencing by Sanger. All *bla*_{BKC-1} positive *K.*
101 *pneumoniae* isolates were subject to identification confirmation by matrix-assisted laser
102 desorption/ionization-time of flight (MALDI-TOF MS) and selected for further
103 microbiological and genetic characterization.

104

105 *2.2. Clinical Data Collection*

106 Clinical data of patients with BKC-1 positive cultures was extracted from the
107 medical records in a standardized case form. The variables collected included patient
108 demographic data, underlying diseases, Charlson comorbidity index, Pitt bacteremia
109 score (when applicable), antibiotic use, ICU admission, type of ward, presence of septic
110 shock, and outcome (mortality) data at 3 days, 14 days and 30 days.

111

112 *2.3. Genetic Relatedness and Plasmid Analysis*

113 The clonal relationship of BKC-1 positive isolates was initially determined by
114 Pulsed Field Gel Electrophoresis (PFGE) using SpeI as restriction enzyme and
115 interpreted according to Tenover and colleagues (1995) [11]. Multilocus sequence
116 typing (MLST) was performed as previously described [12]. To verify the plasmid
117 profile, S1 nuclease-PFGE was performed [13], followed by direct in-gel P32-
118 radiolabelled hybridisation for location of the carbapenemase-encoding genes.

119

120 2.4. Antimicrobial Susceptibility Testing

121 *In vitro* antimicrobial susceptibility testing was performed to sixteen
122 antimicrobial agents (Table 1) by broth microdilution and interpreted following
123 EUCAST guidelines.

124

125 2.5. Investigation of Additional Antimicrobial Resistance Determinants

126 Antimicrobial resistance genes (ARG) were investigated to identify the presence
127 of other resistance mechanisms to β -lactams, aminoglycosides, polymyxins, and
128 quinolones as previously described [4,7,14]. All antimicrobial resistance genes were
129 investigated by PCR (Table S1) followed by DNA Sanger sequencing.

130

131 2.6. Conjugation experiments

132 Conjugation experiments were carried out in six representative isolates
133 (A45517, A47758, A48120, A48295, A48524, and A48907B). Briefly, 3h-broth
134 cultures of BKC-1-producing *K. pneumoniae* (donor strains) were mixed with the
135 receptor strain *E. coli* J53 AziR (ratio 1:3) and incubated overnight at 37°C without
136 agitation [15]. Transconjugants were selected on MacConkey agar plates supplemented
137 with azide (100 μ g/mL) plus ampicillin (100 μ g/mL) and confirmed by *bla*_{BKC-1} PCR.

138 Conjugation frequency was determined by the ratio between the numbers of
139 transconjugants and donors.

140

141 2.5. *In vivo* Virulence Study using *Galleria mellonella* model

142 Representatives of ST11 harbouring *bla*_{BKC-1} (A48295 and A48906), ST442
143 isolates harbouring only *bla*_{BKC-1} (A45517, A47758, and A52034), or *bla*_{BKC-1} and
144 *bla*_{KPC-2} (A46209 and A48120) were selected for *in vivo* virulence studies using the
145 *Galleria mellonella* wax moth larvae model. An initial standardised suspension
146 containing 1×10^9 bacteria/mL was prepared and 10 μ L of selected dilutions (reaching
147 10^6 , 10^5 and 10^4 bacteria) was injected into the hemocoel of the larvae, through the rear
148 left pro-leg using a Hamilton syringe. Each suspension was injected in 10 larvae, and
149 incubated at 37°C for 72 hours. Experiments were performed in triplicate. Furthermore,
150 the isolates A58300 (K1 strain), KP13 (ST442, KPC-producer, capsular type KL107),
151 P12 (ST11, KPC-producer, capsular type KL64) and, P41 (ST11, KPC-producer,
152 capsular type KL15) were used for comparative purposes [16].

153

154 2.6. *Biofilm Assay*

155 Biofilm measurements were performed using the 96-well plate method of
156 Stepanovic and colleagues [17]. Briefly, 2 μ L of fresh bacteria culture was inoculated
157 on 198 μ L of TSB supplemented with 1% glucose, poured in a well and incubated for
158 24 h at 37°C. After incubation, each well content was washed three times with saline
159 0.85% and posteriorly fixated with 150 μ L of methanol. Biofilm was stained with 2%
160 crystal violet for 15 minutes at room temperature and the Optical Density (OD_{550nm}) was
161 measured. Each assay was performed in biological and technical triplicates.

162

163 2.7. Whole-genome Sequencing

164 Based on previous analysis, the whole-genome sequencing (WGS) was
165 performed on two representative isolates (A52034 and A48295) using the Illumina
166 MiSeq platform (Illumina Inc., San Diego, CA). DNA libraries were prepared for
167 paired-end sequencing (2x300 cycles) using Nextera XT V2. Quality control of raw
168 sequence reads included fastqc (0.11.2), and quality and adaptor trimming were
169 performed using Trim galore (0.4.3). Reads were assembled in contigs using the *de*
170 *novo* assembler SPAdes (3.9.0) and were aligned to the original fastq reads using BWA
171 aligner (0.7.15). The investigation of antimicrobial resistance genes and plasmid
172 replicons were assessed by ResFinder v.3.2 and PlasmidFinder v.2.1
173 (<https://cge.cbs.dtu.dk/services/>) and virulence determinants were investigated using a
174 previous *Klebsiella* virulome dataset [16] and Kaptive ([https://kaptive-](https://kaptive-web.erc.monash.edu/)
175 [web.erc.monash.edu/](https://kaptive-web.erc.monash.edu/)).

176 In addition, a transconjugant TcA48295.9 was sequenced using a hybrid
177 sequencing approach. Total genomic DNA was extracted and libraries were prepared
178 using a PCR free rapid barcoding kit (SQK RBK004) and sequenced with a R9.4 flow
179 cell on a MinION (Oxford Nanopore technologies, UK).

180

181 3. Results

182 3.1. General Microbiological and Epidemiological data

183 Among 647 *K. pneumoniae* isolates screened by PCR, 16 (frequency of 2.5%)
184 harboured *bla*_{BKC-1}, and were all confirmed as *K. pneumoniae* by MALDI-TOF MS. The
185 16 BKC-1-producing *K. pneumoniae* were isolated from 15 patients hospitalized
186 between October 2010 and March 2012. It was not possible to retrieve clinical data of
187 four patients. The clinical description and temporal distribution of infected patients by

188 BKC-1 producers are shown in Figure 1. The most frequent clinical specimens culturing
189 *K. pneumoniae* were blood (10 isolates; 62.5%) and tracheal aspirate (3 isolates;
190 18.8%). The three remaining isolates were recovered from wound skin, urine, and
191 central venous catheter tip (6.3% each) (Figure 1). The average age of patients was 56.8
192 years-old (± 21.6), with 10 males (66.6%) and 5 females (33.4%). Six of eleven patients
193 with clinical data died within 30 days of infection. The first case (index case) was
194 described in October 2010, in a female patient admitted in the neurology ICU, who
195 presented the BKC-1-producing *K. pneumoniae* blood infection after four months of
196 hospitalization.

197

198 3.2. Isolates Genetic Relatedness

199 PFGE analysis revealed the presence of a major clone, named A1, recovered
200 from 13 isolates of 12 patients, distributed along the three years (2010-2012). Besides
201 that, an isolate (A47758) belonged to clone A2, while the two remain isolates recovered
202 from the emergency room (A48295) and nephrology (A48906) belonged to clone B1
203 (Table 1). All clone A isolates were identified as ST442, while the clone B isolates
204 belonged to the ST11.

205

206 3.3. Antimicrobial Susceptibility Profile

207 The BKC-1-producing *K. pneumoniae* isolates showed a multidrug resistance
208 (MDR) profile with high MICs for monobactam, cephalosporins, and carbapenems
209 (Table 1). Polymyxin B resistance was detected in 11 of 16 isolates (68.8%) isolates
210 (MIC_{50/90}, 32 and 64 mg/L). The most potent *in vitro* antimicrobial agents against
211 BKC-1 clone were ceftazidime-avibactam (MIC_{50/90}, 4/4 and 8/4 mg/L) and gentamicin
212 (MIC_{50/90}, 0.5/64 mg/L). Interestingly, five isolates showed elevated MICs for

213 ceftazidime-avibactam (8/4 mg/L), however, no isolates were categorized as resistant.

214

215 3.4. Antimicrobial Resistance Genes and Plasmid Mobilization Analysis

216 The β -lactamase *bla*_{SHV-like} and the phosphotransferase *aph(3')-VII* were
217 detected in all BKC-1-producing *K. pneumoniae* isolates, while *bla*_{CTX-M} and *bla*_{TEM}
218 were only amplified from clone B1-ST11 isolates. The simultaneous presence of *bla*_{BKC-}
219 ₁ and *bla*_{KPC-2} was detected in six isolates (Table 1). The mechanism responsible for
220 polymyxin resistance was not identified; however, our data suggested that *mgrB*
221 alterations were not the mechanism involved in polymyxin resistance in those isolates.
222 S1-PFGE analysis revealed the presence of a single 10-Kb plasmid carrying *bla*_{BKC-1} in
223 all BKC-1 producers. In contrast, *bla*_{KPC-2} was located in a different plasmid on the
224 isolates harbouring *bla*_{BKC-1} and *bla*_{KPC-2} (data not shown).

225 Transconjugants were only obtained for A48295 (ST11), and A48907B (ST442)
226 isolates. The conjugants cells TcA48295.9 and TcA48907B.15 carrying *bla*_{BKC-1}
227 showed conjugation frequency of 1.7×10^{-7} and 4.2×10^{-8} , respectively (Table S2). B-
228 lactams MIC for the transconjugants demonstrated higher values for TcA48295.9 (Table
229 S2). To verify the mechanism of *bla*_{BKC-1} plasmid mobilization and the reason for the
230 higher MICs, we chose the transconjugant TcA48295.9 for MinIon sequencing. The
231 plasmid carrying *bla*_{BKC-1} was the same 10-Kb IncQ1 plasmid detected in previous
232 studies (p60136) [15]. The IncQ1-*bla*_{BKC-1} plasmid was co-mobilized by a 68.6 Kb
233 IncM1-plasmid named pA48295A which also carried other ARG as *bla*_{CTX-M-8}, *bla*_{TEM-}
234 _{1A}, and *qnrE1*.

235

236 3.5. *Galleria mellonella* Virulence Testing and Biofilm production

237 *In vivo* virulence assay analysis revealed that larvae infected by BKC-1 producer

238 ST11 isolates had lower survival rates than those infected by BKC-1 ST442 isolates
239 ($p < 0.0001$) (Figure 2A). Besides, BKC-1 ST11 with capsule serotype KL64 seem to
240 show higher virulence profiles than ST11 isolates harbouring different capsule
241 serotypes (Figure 2A).

242 No difference was noticed on biofilm production of ST442 and ST11. In
243 general, the isolates were classified as weakly adherent ($n = 7$ isolates) and moderately
244 adherent ($n = 9$ isolates), however A47758 (ST442, A2 pulsotype) had a higher capacity
245 for biofilm production (Figure 2B).

246

247 *3.6. Genetic Analysis of the two BKC-1 clones*

248 WGS of two representative isolates A48295 (B1-ST11) and A52034 (A1-
249 ST442) provided the general genomic features of each clone evaluated during this
250 study. Molecular capsule analysis indicated the capsule type KL64 for A48295 and
251 KL107 for A52034. The isolates carried similar virulence-encoding genes, except for
252 the presence of *traT* (human serum resistance) and *clpK* (thermotolerance) only
253 identified on B1-ST11 clone (Table S3). Resfinder analysis resulted in the presence of
254 multiple ARG collaborating to MDR phenotype in both isolates. Acquired antimicrobial
255 resistance genes identified in each isolate as so as the antimicrobial resistance classes
256 are present on Table S3. Mutational analysis on two-component systems (TCS) of
257 A52034 (polymyxin B resistant isolate) revealed mutations in the follow TCS: CrrA
258 (E192V), CrrB (C68S and Q296L), and PmrB (T246A). According to the analysis using
259 PROVEAN, the mutation E192V in CrrA was predicted to be deleterious for the
260 protein function. As expected, no mutations on TCS were found on polymyxin-
261 susceptible A48295 isolate.

262

263 4. Discussion

264 The description of BKC-1 has not gained attention, because this enzyme has
265 been infrequently reported and restricted to clinical isolates recovered from São Paulo
266 [4,7]. However, the identification of multiple *K. pneumoniae* carrying *bla*_{BKC-1} is a cause
267 of concern because reveals a silent and undetected circulation of a resistant clone in the
268 studied hospital.

269 In our study, most BKC-1 producers belonged to ST442 (CC442) as the first
270 reported isolates [4,7]. Moreover, two BKC-1-producing *K. pneumoniae* ST11 were
271 detected, representing the first description of *bla*_{BKC-1} out of the CC442 in *K.*
272 *pneumoniae*. The acquisition of the IncQ1 plasmid carrying *bla*_{BKC-1} by the epidemic
273 ST11 clone, is worrisome since its high virulence capacity is well established [18,19].
274 The frequency of BKC-1 producers in this work was almost ten times higher when
275 compared to the results of our previous study which determined the frequency of BKC-
276 1 from Brazilian isolates in 2016 [7].

277 The presence of two carbapenemases (BKC-1 and KPC-2) had impact on β -
278 lactam MICs, with those isolates being highly resistant to β -lactams compared with
279 isolates producing BKC-1 alone. Interestingly, MICs of 8/4 mg/L to ceftazidime-
280 avibactam were also observed in those isolates carrying *bla*_{BKC-1} and *bla*_{KPC-2}. The co-
281 production of serine carbapenemases has been shown to increase MICs to ceftazidime-
282 avibactam [20]. It is highly probable that the presence of *bla*_{BKC-1} and *bla*_{KPC-2} be the
283 cause of the low inhibitory efficacy of avibactam, although other mechanisms (as
284 permeability defects) could not be discarded [20]. As the genetic background of *bla*_{BKC-1}
285 is highly conserved, with *bla*_{BKC-1} being flanked by the phosphotransferase *aph(3')-VII*
286 (downstream) and *ISKpn23* (upstream), the pattern of resistance to amikacin and
287 kanamycin and the remaining susceptibility to gentamicin was something expected in

288 ST442 clone [7]. In fact, gentamicin was an important therapeutic option for the patients
289 evaluated in this retrospective study. Four of the five discharged patients, who had a
290 favourable outcome, had received gentamicin. However, the clone B1-ST11 was highly
291 resistant to gentamicin (MICs, 64 and >64 mg/L) likely due to the codification of
292 different aminoglycoside modifying enzymes such as *aac(3)-IIa*, *aac(6')-Ib-cr*, *aadA1*,
293 *addA2*, *aph(3')-VII*.

294 Although multiple mechanisms have been shown to cause polymyxin B
295 resistance in our hospital, the inactivation of MgrB is the most common mechanism
296 detected thus far [16,21]. In contrast, our polymyxin-resistant isolates did not show
297 alterations in *mgrB*. Analysis of A52034 (single A1-ST442 polymyxin B resistant)
298 revealed deleterious mutations on the TCS CrrA. Although we have generated WGS
299 data on a single representative isolate for both clones analysed, this finding could
300 evidence that polymyxin resistance in this clone could be mediated by mutations on
301 TCS.

302 Virulence analysis confirmed a high virulence profile of ST11-KL64 isolates
303 with high larvae mortality. Analysis conducted using *K. pneumoniae* ST11 with two
304 types of capsules (KL64 and KL15) showed that the survival rates of ST11-KL64 was
305 lower when compared to that of ST11-KL15. In a recent study conducted in China, a
306 novel sub-clone of carbapenem-resistant *K. pneumoniae* ST11-KL64 carrying
307 *rmpA/rmpA2*, was responsible for increased 30-day mortality rate in Chinese patients
308 [22]. Different of the Chinese isolates, the studied ST11-KL64 clone did not present
309 important virulence-encoding genes such as *rmpA/rmpA2* or aerobactin [22]. We
310 believe that the difference of virulence between the two clones could be explained by
311 different capsule types (KL64 vs KL107).

312 In conclusion, we reported for the first time a silent outbreak caused by BKC-1-

313 producing *K. pneumoniae*, capturing clinical, microbiological and genomic data. The
314 detection of this outbreak almost 10 years after its start reinforces the challenge to
315 monitor and control AMR in real time. In addition, the misidentification of new
316 carbapenemase variants may have worsened the spread of resistant clones and led to
317 dissemination in other hospitals. The impact of this information on the patient outcome
318 remains unclear; however, the role of BKC-1 infections can hardly be determined if
319 these isolates are misidentified as possessing other mechanisms of resistance.

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328

329 **Conflict of interests**

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332 Other authors have nothing to declare.

333

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339

340 **Ethics approval**

341 Ethical approval for this study was obtained from Research Ethics Committee from
342 Universidade Federal de São Paulo - UNIFESP/São Paulo Hospital (Process number:
343 8567211118). Waiver of Informed Consent was granted by REC because this study
344 involved a retrospective chart review with patients' confidentiality and anonymity were
345 preserved.

346

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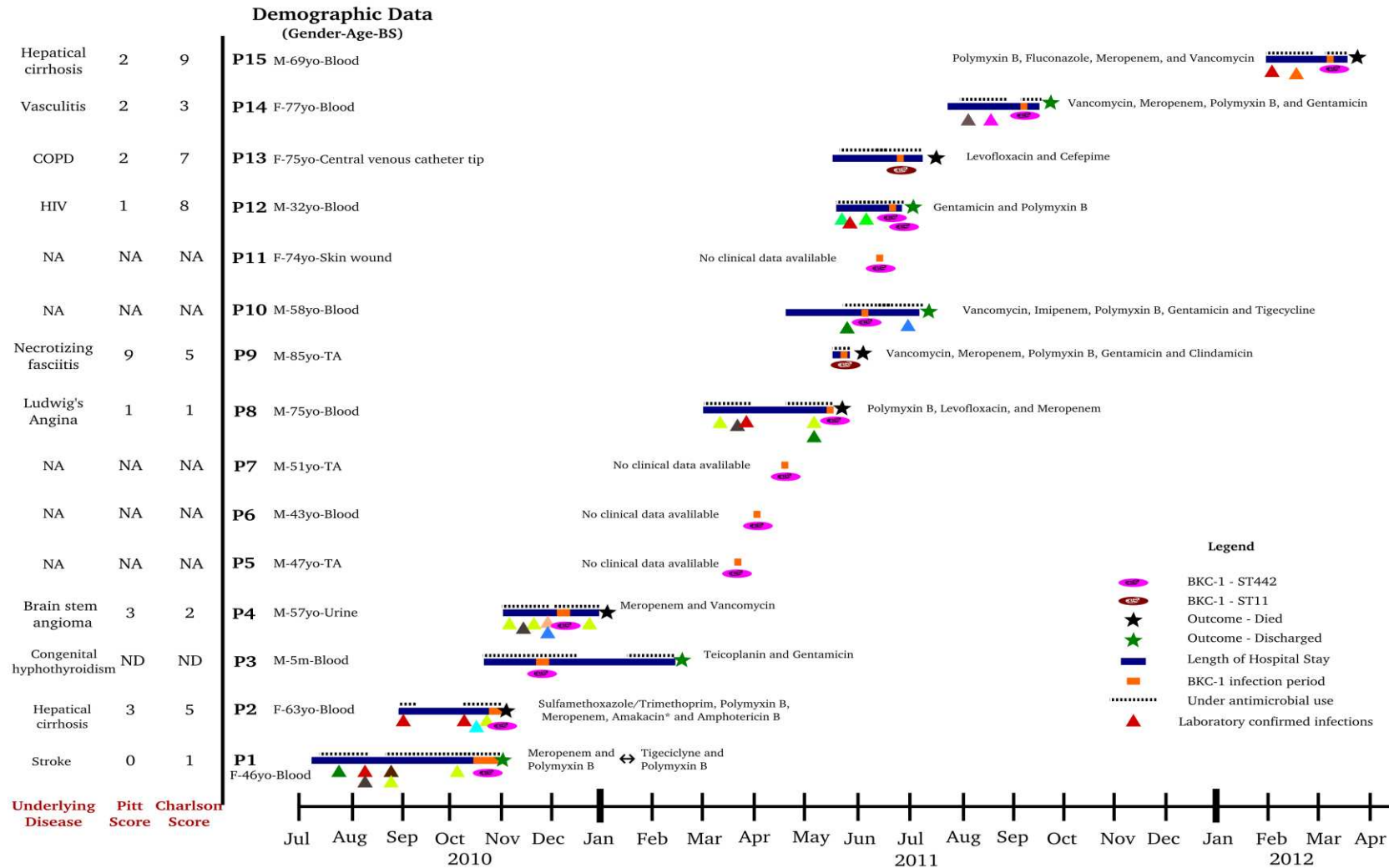
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Table 1. Clinical and microbiological features of BKC-1-producing *K. pneumoniae* isolates recovered in this study.

Isolate	Patient	Ward	PFGE	ST	MIC (mg/L)																	Antimicrobial Resistance genes ^a	
					ATM	CFL	CFX	CPM	CAZ	CRO	ETP	IMI	MER	PTZ	CAZ-AVI	CIP	LEV	AMK	GEN	TOB	PMB		TGC
A45517	Pt1	Neurology ICU	A1	442	128	>512	>64	256	8	>512	1	4	2	16/4	2/4	>64	64	1024	0.5	8	32	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1}
A45843	Pt2	General ICU	A1	442	8	>512	>64	64	2	256	≤1	2	0.5	16/4	4/4	>64	64	512	0.5	8	64	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1}
A46209	Pt3	Pediatrics	A1	442	>512	>512	>64	>256	512	>512	512	256	128	512/4	8/4	>64	>64	128	1	16	≤0.125	2	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1} and <i>bla</i> _{KPC-2}
A46313	Pt4	Neurology ICU	A1	442	256	>512	>64	64	128	256	16	8	8	512/4	4/4	>64	64	512	1	4	64	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1}
A47626	Pt5	-	A1	442	256	>512	64	64	64	>512	32	8	8	512/4	2/4	>64	64	512	0.5	4	32	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1}
A47758	Pt6	-	A2	442	256	>512	>64	128	128	512	8	8	8	512/4	4/4	>64	64	256	0.5	4	32	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1}
A48120	Pt7	-	A1	442	>512	>512	>64	>256	256	>512	512	128	512	>512/4	8/4	>64	64	256	0.5	4	128	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1} and <i>bla</i> _{KPC-2}
A48240	Pt8	Emergence room	A1	442	256	>512	>64	128	128	>512	16	8	8	512/4	2/4	>64	64	512	0.5	4	64	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1}
A48295 ^b	Pt9	Nephrology	B1	11	512	>512	64	>256	128	>512	64	128	32	512/4	4/4	>64	>64	256	>64	32	≤0.125	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-182} ; <i>bla</i> _{BKC-1} ; <i>bla</i> _{CTX-M-8} ; <i>bla</i> _{CTX-M-15} and <i>bla</i> _{TEM-1B}
A48524	Pt10	Nephrology	A1	442	>512	>512	>64	>256	128	>512	512	128	512	>512/4	8/4	>64	64	512	0.5	4	64	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1} and <i>bla</i> _{KPC-2}
A48827	Pt11	-	A1	442	>512	>512	>64	>256	256	>512	256	64	128	>512/4	4/4	>64	32	256	0.5	16	≤0.125	0.25	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1} and <i>bla</i> _{KPC-2}
A48834	Pt12	Pulmonology ICU	A1	442	8	>512	64	64	2	512	≤1	2	1	16/4	4/4	>64	64	512	0.5	4	32	1	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1}
A48906	Pt13	Emergence room	B1	11	>512	>512	64	>256	256	>512	64	128	32	>512/4	2/4	>64	>64	256	64	32	≤0.125	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-182} ; <i>bla</i> _{BKC-1} ; <i>bla</i> _{CTX-M-8} ; <i>bla</i> _{CTX-M-15} and <i>bla</i> _{TEM-1B}
A48907B	Pt12	Pulmonology ICU	A1	442	>512	>512	>64	>256	128	>512	128	64	256	>512/4	8/4	>64	64	256	0.5	4	≤0.125	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1} and <i>bla</i> _{KPC-2}
A49436	Pt14	Emergence	A1	442	>512	>512	>64	>256	256	>512	1024	128	512	>512/4	8/4	>64	64	256	0.5	16	32	0.5	<i>aph(3')-VIIi</i> ; <i>bla</i> _{SHV-110} ; <i>bla</i> _{BKC-1}

420 **Figure 1.** General demographic data and the length of hospital stay of the patients infected by BKC-1-producing *K. pneumoniae* isolates. Underlying
 421 diseases and their respective Pitt and Charlson scores are also provided. Pitt score was just provided in bacteremia cases.
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444 Legend: ND, not determined; NA, not available; P, patient; yo, years-old; COPD, Chronic obstructive pulmonary disease, M, male; F, female; TA,
445 tracheal aspirate.

446 **Figure 2.** Virulence assays performed in BKC-1-producing *K. pneumoniae* isolates. (A) Kaplan-Meier plots showing the survival rates of *G.*
 447 *mellonella* over 72 hours post infection with BKC-1 producers detected in the outbreak described in this study. A58300 strain (K1 – *K. pneumoniae*
 448 ST23) was used as hypervirulent positive control. In addition, some isolates previously tested [5] (Kp13, P12, and P41) were also used to comparative
 449 purposes. Saline solution (NaCl 0.85%) was used as negative control. (B) Biofilm production plot of BKC-1-producing isolates. The isolates were
 450 categorized according biofilm production (non-adherent, weakly adherent, moderately adherent, or strongly adherent) using the OD of negative
 451 control, following recommendation established by Stepanovic and colleagues (2009) [24]. Different colours were used to represent clones and control
 452 isolates. Dark blue, A1-ST442 clone; Light blue, A2-ST442; Red, B1-ST11; salmon, control isolates.
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