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Clonal Complex 258, the Most Frequently Found Multilocus Sequence Type Complex in KPC-2-Producing *Klebsiella pneumoniae* Isolated in Brazilian Hospitals

We read with great interest the article by Andrade et al. (1) describing the predominance of sequence type 258 (ST258) among KPC-2-producing *Klebsiella pneumoniae* isolates in Brazilian hospitals. Recently, we conducted a similar study that evaluated the genetic relationship among KPC-2-producing *K. pneumoniae* isolates from Brazilian hospitals.

A total of 34 KPC-2-producing *K. pneumoniae* isolates were collected from 13 distinct hospitals located at seven Brazilian states during the period of 2008 to 2010 and referred to Laboratório Alerta, UNIFESP, for further characterization. A single isolate per patient was analyzed. Antimicrobial susceptibility testing was performed and interpreted using the CLSI agar dilution (M100-S22) method for all agents except polymyxin B, for which the EUCAST criteria for colistin were applied (2, 4). The presence of the bla_{KPC-2} gene was confirmed by PCR, followed by sequencing (5). Genetic relationship among KPC-2-producing isolates was assessed by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE), using SpeI as the restriction enzyme (3, 7).

The antimicrobial susceptibility profile of the isolates is presented in Table 1. All isolates were resistant to ertapenem, while 44.1% and 23.5% remained susceptible to imipenem and meropenem, respectively. Among the 34 KPC-2 producers, seven STs were observed, including the new ST ST617 (Table 1). In contrast to the results reported by Andrade et al. (1), ST258 itself was not identified among the isolates studied, but STs related to clonal complex 258 (CC258), ST437, and ST11 were observed. ST437 was the most frequently detected ST (18 isolates; 52.9%) and was isolated from seven medical centers in two Brazilian states, while ST11 was the most widely distributed. ST11 was detected in 10 (29.4%) isolates collected from eight hospitals located in five distinct states (Table 1). Interestingly, ST11 isolates showed a greater degree of variability by PFGE (six PFGE patterns) than ST437, which was grouped under a single PFGE pattern.

Single representatives of ST133, ST340, and ST617 were found among isolates collected from distinct hospitals located in the same city. Of notice, only strains belonging to ST11 and ST437 showed imipenem and meropenem resistance, whereas reduced susceptibility to polymyxin B was observed in seven (20.6%) isolates that belonged to three PFGE patterns: A (5 isolates), C, and H. These PFGE patterns were grouped under four STs, ST11 (2 isolates), ST17, ST133, and ST437 (3 isolates).

In the study by Andrade et al. (1), ST258 was the most frequently reported ST among KPC-2-producing *K. pneumoniae* isolates. However, these authors have studied only *K. pneumoniae* isolates collected from two Brazilian states (São Paulo and Rio de Janeiro). In our study, we included strains isolated from São Paulo and six other Brazilian states (Distrito Federal, Mato Grosso do Sul, Minas Gerais, Paraíba, Pernambuco, and Rio Grande do Sul). Despite the identification of novel STs, we observed the predominance of ST437, which is a single-locus variant of ST258, among Brazilian isolates. Our results corroborate the initial findings regarding the predominance of CC258 among KPC-2-producing *K. pneumoniae* isolates (6). These data suggest that KPC-2-producing isolates originated from a common ancestor derived from ST258, which has undergone genetic evolution. These findings point out that horizontal acquisition of bla_{KPC-2} by genetically unrelated strains has contributed to the dissemination of KPC-2-producing isolates throughout Brazilian territory, but hospital outbreaks caused by single clones have also influenced the increased frequency of ST437 isolation observed in this study.

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TABLE 1 Susceptibility profile, sequence types, and PFGE patterns for 34 KPC-2-producing *K. pneumoniae* isolates from Brazil

ST	PFGE pattern(s)	No. of isolates	Location(s)[b]	MIC range (μg/ml) (no. of resistant isolates/total no. of isolates)[a]									
				P/T	CAZ	CRO	FEP	AZT	ERT	IMI	MER	CIP	GENT	POLB
ST11	A, C, E, F, G, I	10	São Paulo, SP; Porto Alegre, RS; Recife, PE; Belo Horizonte, MG; Brasília, DF	64–256 (10/10)	16–256 (10/10)	64–>256 (10/10)	64–>256 (10/10)	128–>256 (10/10)	8–256 (10/10)	2–64 (8/10)	1–64 (9/10)	4–>32 (10/10)	0.5–256 (5/10)	1–64 (1/10)
ST17	H	1	Campo Grande, MS	256 (1/1)	16 (1/1)	16 (1/1)	8 (0/1)	128 (1/1)	4 (1/1)	1 (0/1)	2 (0/1)	4 (1/1)	0.5 (0/1)	32 (1/1)
ST70	B	2	João Pessoa, PB	128 (2/2)	8 (0/2)	8–16 (2/2)	2–4 (0/2)	16–64 (2/2)	2 (2/2)	0.5 (0/2)	1 (0/2)	1 (0/2)	0.5 (0/2)	1 (0/2)
ST133	A	1	São Paulo, SP	128 (1/1)	8 (0/1)	8 (1/1)	64 (1/1)	>256 (1/1)	8 (1/1)	≤1 (0/1)	≤1 (0/1)	≤0.25 (0/1)	1 (0/1)	4 (0/1)
ST340	A	1	São Paulo, SP	128 (1/1)	128 (1/1)	256 (1/1)	64 (1/1)	256 (1/1)	8 (1/1)	1 (0/1)	2 (0/1)	32 (1/1)	128 (1/1)	1 (0/1)
ST437	A	18	João Pessoa, PB; São Paulo, SP	64–256 (17/18)	8–>256 (17/18)	32–>256 (18/18)	8–>256 (17/18)	128–>256 (18/18)	4–>256 (18/18)	≤0.25–64 (6/18)	≤0.5–64 (7/18)	32–>256 (18/18)	0.5–128 (4/18)	1–64 (2/18)
ST617	D	1	São Paulo, SP	256 (1/1)	16 (1/1)	32 (1/1)	16 (0/1)	128 (1/1)	4 (1/1)	0.5 (0/1)	2 (0/1)	0.25 (0/1)	0.5 (0/1)	1 (0/1)
Total % resistance				94.1	88.2	100	85.3	100	100	41.2	47.1	88.2	29.4	11.8

[a] P/T, piperacillin-tazobactam; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; AZT, aztreonam; ERT, ertapenem; IMI, imipenem; MER, meropenem; CIP, ciprofloxacin; GENT, gentamicin; and POLB, polymyxin B.

[b] DF, Distrito Federal; MG, Minas Gerais; MS, Mato Grosso do Sul; PB, Paraíba; PE, Pernambuco; RS, Rio Grande do Sul; and SP, São Paulo.

REFERENCES

1. **Andrade LN, et al.** 2011. Dissemination of bla_{KPC-2} by the spread of *Klebsiella pneumoniae* clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among *Enterobacteriaceae* species in Brazil. Antimicrob. Agents Chemother. **55**:3579–3583.
2. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement. M100-S22. CLSI, Wayne, PA.
3. **Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S.** 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J. Clin. Microbiol. **43**:4178–4182.
4. **European Committee on Antimicrobial Susceptibility Testing.** 5 November 2011, accession date. Breakpoint tables for interpretation of MICs and zone diameters. Version 1. 3, January 2011. http://www.eucast.org/clinical_breakpoints/.
5. **Monteiro J, Santos AF, Asensi MD, Peirano G, Gales AC.** 2009. First report of KPC-2-producing *Klebsiella pneumoniae* strains in Brazil. Antimicrob. Agents Chemother. **53**:333–334.
6. **Seki LM, et al.** 2011. Molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* isolates in Brazil: the predominance of sequence type 437. Diagn. Microbiol. Infect. Dis. **70**:274–277.
7. **Tenover FC, et al.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33:2233–2239.

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