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Letter to the Editor

Hyperendemic clone of KPC producing *Klebsiella pneumoniae* ST 258 in Buenos Aires hospitals

Since KPC-type carbapenemases were first reported in *Klebsiella pneumoniae* in 2001 in the USA (Yigit et al., 2001), they have become a frequent resistant marker encountered also in other *Enterobacteriaceae* and *Pseudomonads* from the Americas, Europe, Asia and Middle East (Villegas et al., 2007; Nordmann et al., 2009; Walsh, 2010). Their wide spread across multiple continents and species has been associated with a mobile genetic element, Tn4401 (Naas et al., 2008). More recently, the molecular epidemiology of KPC-producing *K. pneumoniae* isolates has revealed the successful dissemination of a single sequence type 258 clone (Kitchel et al., 2009). Although in Argentina KPC-2 producing *K. pneumoniae* were first detected in 2006 (Pasterán et al., 2008), a substantial increase was observed in 2010, in Buenos Aires (http://www.ine.gov.ar/publi_pdfs/Carbapenemasas.pdf).

To characterize this occurrence, single, non-repetitive *K. pneumoniae* isolates obtained from 57 patients from May 2009 through April 2010 in six hospitals in Buenos Aires were included (Hosp 1:3, Hosp 2:5, Hosp 3:30, Hosp 4:3, Hosp 5: 2, Hosp 6:14).

Antimicrobial susceptibility tests were conducted by disk diffusion according to CLSI recommendations (CLSI, 2010). Taking into account the local resistance prevalence in hospital-acquired *K. pneumoniae* infections, the selected antibiotic disks were placed on two primary 90 mm Mueller Hinton agar plates as follows: plate 1: ampicillin, cephalotin, gentamicin, amikacin, ciprofloxacin and trimethoprime/sulfamethoxazole; plate 2: amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, ceftazidime, imipenem, and meropenem. As it has been previously reported, phenyl boronic acid is a good inhibitor of both AmpC and KPC β-lactamases (Yagi et al., 2005; Pasteran et al., 2009), we included a disk containing 300 μg phenyl boronic acid in the center of the second plate (distance between disks was 25 mm center to center). An enhancement in the inhibition zone of the carbapenem-containing disks adjacent to the one containing the inhibitor was considered a positive screening for KPC. All the isolates displayed inhibition zones for imipenem and/or meropenem $\leqslant$21 mm and a positive synergy with phenyl boronic acid. Minimal inhibitory concentrations (MICs) were determined according to CLSI. The isolates showed a multidrug-resistant phenotype with varying levels of carbapenem resistance (Table 1). MIC_{50} and MIC_{90} values were as follows (μg/ml) ampicillin: >256 and >256, cephalotin: >256 and >256, ceftazidime: 256 and >256, cefotaxime: 128 and >256, imipenem: 2 and 16, meropenem: 2 and 32, colistin: 4 and 8, amikacin: >128 and >128 and gentamicin 2 and 4, respectively. Three isolates displayed MICs of colistin of 128 μg/ml. The presence of bla_{KPC} was confirmed by PCR amplification using the following primers: KPC-F: 5′ATGTCAC TGTATCGCCGTCT 3′ and KPC-R: 5′TTTT CAGAGCCTTACTGCCC 3′ on heat-extracted DNA as template (Bradford et al., 2004). In all cases, the 893-bp amplicon sequence corresponded to bla_{KPC-2}.

As the *Xba*I (Fermentas Inc., Burlington Ontario) PFGE patterns generated were indistinguishable (Fig. 1), multilocus sequence typing (MLST) with seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB*) was performed on selected isolates representing the different hospitals (Diancourt et al., 2005). Allele sequences and STs were verified at http://www.pasteur.fr, corresponding to ST 258 (allelic profile 3–3-1–1-1–1-79).

The spread of KPC-producing clones constitutes a serious infection control concern. Prevention of their dissemination requires prompt, accurate and proactive laboratory detection systems followed by strong reinforcement of contact isolation precautions and hygiene measures. The outbreak was able to be easily controlled only at hospital 1 where the suspicious index case was detected and informed from the preliminary antibiotic tests, performed as mentioned previously. Control measurements were applied on these preliminary data, even before genetic confirmation.

Patients were placed in contact precautions where all persons were required to wear gowns and gloves in every contact with the patient, environment or medical equipment next to him/her. Patients were identified with bracelets and a warning was attached to their medical record so that these patients would automatically be placed in contact isolation in any other hospital ward. Cleaning and disinfection measures of the area were strengthened and terminal disinfection was performed after each patient was transferred.

The inclusion of phenyl boronic acid disks in the primary susceptibility agar diffusion tests close to the carbapenem-containing disks constituted a useful tool for the rapid detection of KPC-producing isolates, especially in those isolates that did not display high level resistance to imipenem and/or meropenem. This disk diffusion test is easy to handle and can be performed as a routine test in any clinical microbiology laboratory, especially before KPC reaches the hospital. It is much less expensive and time-consuming than any other alert system. Inclusion of ertapenem disks was not appropriate for the detection of KPC producers in our region, as many cefotaximase-producing isolates display reduced inhibition zones to this drug.

Although by applying the new interpretative CLSIs criteria (CLSI, 2011) all these isolates could be considered as resistant or intermediate to carbapenems (which may be enough to decide the individual patient therapy), the synergy effect of phenyl boronic is indicative of the resistance mechanism involved (decisive for epidemiologically sounded resistance contention), and negative in impermeable/extended spectrum β-lactamase producing *K. pneumoniae*.

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Table 1
Epidemiological data and antimicrobial susceptibility of KPC producing *Klebsiella pneumoniae*.

	Isolate number	Culture source	Date of isolation	Antimicrobial Susceptibility (MICs: μg/ml)										Therapeutic treatment	Outcome
				AM	FOX	CEF	CAZ	CTX	IMI	MER	COL	AKN	GEN		
H1	0048.5	Urine	7-1-09	>256	⩽64	>256	>256	256	8	8	8	>128	2	COL	Died
	93,45.20	Aspiration of paranasal sinuses	11-12-09	>256	⩽64	>256	256	256	4	4	8	>128	2	COL	Favorable
	37.3045	Catheter	17-02-10	>256	⩽64	>256	256	256	4	4	8	>128	2	COL, TIG, IMI	Favorable
H2	815	Urine	13-02-10	>256	⩽64	>256	256	>256	8	4	2	>128	2	–	Favorable
	P1	Urine	25-02-10	>256	⩽64	>256	256	⩽64	1	2	2	>128	2	–	Favorable
	876	Urine/intraabdominal collection	16-02-10	>256	⩽64	>256	>256	>256	8	4	2	>128	4	COL	Favorable
	145	Bone/muscle	26-01-10	>256	⩽64	>256	256	⩽64	1	2	2	>128	2	COL, TIG	Favorable
	I18	Stool	24-02-10	>256	⩽64	>256	>256	256	4	4	2	>128	2	–	Favorable
H3	13,3351	Tracheal aspirate	2-03-10	>256	⩽64	>256	256	⩽64	1	2	4	>128	4	TIG	Died
	13,3504	Soft tissues and phrotesis	24-02-10	>256	⩽64	>256	256	256	4	4	8	>128	2	TIG	Died
	13,4064	Subphrenic abscess	10-03-10	>256	⩽64	>256	256	256	4	4	8	>128	2	TIG	Died
	13,5570	Rectal swab	29-04-10	>256	⩽64	>256	128	128	1	2	4	>128	2	COL	Favorable
	13,5790	Cerebroespinal fluid	6-04-10	>256	⩽64	>256	256	128	2	2	4	>128	4	COL	Favorable
	13,6009	Catheter	10-04-10	>256	⩽64	>256	256	256	16	16	8	>128	2	IMP	Died
	13,6080	Rectal swab	10-04-10	>256	⩽64	>256	256	⩽64	1	2	4	>128	2	–	Favorable
	13,6645	Rectal swab	17-04-10	>256	128	>256	256	⩽64	2	8	8	128	2	–	Favorable
	13,8195	Rectal swab	10-05-10	>256	128	>256	>256	64	16	32	4	>128	16	–	Favorable
	13,8266	Rectal swab	11-05-10	>256	⩽64	>256	256	128	2	2	8	>128	2	–	Favorable
	13,8332	Catheter	11-05-10	>256	⩽64	>256	256	256	4	4	8	>128	4	COL	Died
	13,9745	Rectal swab	1-06-10	>256	⩽64	>256	256	>256	2	2	4	>128	4	–	Favorable
	14,1156	Blood	20-06-10	>256	⩽64	>256	256	128	2	2	4	>128	2	PTZ/VAN	Died
	14,4359	Urine	25-07-10	>256	⩽64	>256	⩽64	⩽64	1	2	>128	>128	8	COL/TIG/MER	Favorable
H4	25E	Soft tissues	16-10-09	>256	⩽64	>256	256	256	256	256	4	>128	2	COL	Favorable
	B1	Urine	23-02-10	>256	⩽64	>256	>256	128	2	4	4	>128	4	COL	Favorable
	B2	Urine	3-06-10	>256	⩽64	>256	256	⩽64	1	2	4	>128	2	COL	Favorable
H5	36E	Urine	6-03-10	>256	⩽64	>256	>256	⩽64	4	2	4	>128	2	COL	Favorable
	79.045	Rectal swab	8-03-10	>256	⩽64	>256	256	⩽64	2	2	4	>128	2	COL	Favorable
H6	1	Urine	29-04-09	>256	⩽64	>256	>256	>256	8	8	8	>128	4	–	Favorable
	2	Urine	21-05-09	>256	⩽64	>256	256	128	2	2	8	>128	2	–	Favorable
	4	Blood and urine	04-07-09	>256	⩽64	>256	256	256	4	2	4	>128	4	PTZ/VAN/IMP	Died
	5	BAL	23-08-09	>256	⩽64	>256	256	256	4	2	4	>128	2	–	Died
	6	Blood and urine	01-09-09	>256	⩽64	>256	256	256	4	4	8	>128	2	COL	Favorable
	7	Urine	04-09-09	>256	⩽64	>256	128	⩽64	2	4	128	>128	2	–	Died
	8	Urine	15-09-09	>256	⩽64	>256	256	128	2	2	8	>128	2	–	Died
	9	BAL	15-09-09	>256	⩽64	>256	256	⩽64	2	2	>128	>128	4	PTZ/VAN/IMP	Died
	11	Abdominal fluid	24-09-09	>256	⩽64	>256	256	128	1	2	4	>128	2	AMS/CIPRO	Died
	12	Sputum	24-09-09	>256	⩽64	>256	256	256	4	2	4	>128	4	–	Favorable
	13	Urine	24-09-09	>256	⩽64	>256	256	256	4	4	4	>128	2	–	Favorable
	14	Catheter	05-10-09	>256	⩽64	>256	256	128	2	2	8	>128	2	–	Favorable
	15	Urine	07-10-09	>256	⩽64	>256	256	128	2	2	4	>128	2	—	Favorable
	16	BAL	13-10-09	>256	⩽64	>256	256	⩽64	1	2	8	>128	2	COL	Died
	17	Urine	12-10-09	>256	⩽64	>256	256	256	16	32	8	>128	4	–	Favorable
	18	Urine	15-10-09	>256	⩽64	>256	256	128	2	2	4	>128	2	COL	Favorable
	19	Urine	19-10-09	>256	⩽64	>256	256	128	2	4	4	>128	2	–	Favorable
	22	Urine	05-11-09	>256	⩽64	>256	128	128	2	4	4	>128	4	–	Favorable
	23	Blood	09-11-09	>256	⩽64	>256	256	128	2	2	4	>128	2	PTZ	Died
	25	Urine	11-11-09	>256	⩽64	>256	>256	256	8	8	4	>128	2	–	Favorable
	26	Sputum	11-11-09	>256	⩽64	>256	>256	256	4	4	8	>128	2	–	Died
	27	Urine	22-11-09	>256	⩽64	>256	256	128	2	2	8	>128	2	–	Favorable
	28	Soft tissues	11-12-09	>256	⩽64	>256	256	128	2	4	4	>128	2	VAN/IMP/COL	Died
	31	Urine	08-01-10	>256	⩽64	>256	256	256	16	16	8	>128	2	PTZ/COL	Died
	32	Urine	12-01-10	>256	⩽64	>256	256	128	2	2	4	>128	4	–	Favorable
	33	Blood	28-01-10	>256	⩽64	>256	256	128	2	2	4	>128	4	VAN/PTZ/COL	Died
	34	Blood	03-02-10	>256	⩽64	>256	256	128	2	2	4	>128	2	AMS/CIPRO	Died
	36	Blood	25-02-10	>256	⩽64	>256	256	256	8	32	8	>128	2	PTZ/COL	Died
	37	Urine	24-02-10	>256	⩽64	>256	256	256	4	4	4	>128	2	–	Favorable
	39	Urine	01-03-10	>256	⩽64	>256	256	256	16	32	4	>128	2	COL	Died

H: hospital, BAL: bronchoalveolar lavage, AM: ampicillin, FOX: cefoxitin, CEF: cephalotin, CAZ: ceftazidime, CTX: cefotaxime, IMI: imipenem, MER: meropenem, COL: Colisitin, AKN: amikacin, GEN: gentamicin, TIG: tigecyclin, IMI: imipenem, PTZ: piperacillin/tazobactam, VAN: Vancomycin, (–): not treated.

Competing interests

None declared.

Ethical approval

Not required.

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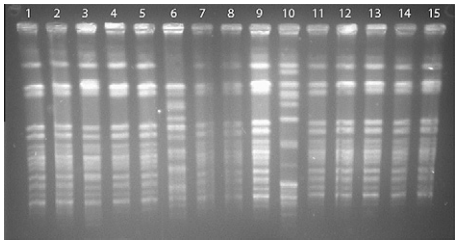

Fig. 1. *Xba*I generated PFGE patterns of representative isolates. Line 1: 9345.20 (H1), line 2: 0048.5 (H1), line 3: 815 (H2), line 4: 145 (H2), line 5: 36E (H5), line 7: 1 (H3), line 8: 9 (H3), line 9: 37 (H3), line 11: B1 (H4), line 12: B2 (H4), line 13: 133351 (H6), line14: 138195 (H6), line 15: 1444359 (H6), line 6 and 10: two unrelated KPC-*K. pneumoniae* previously isolated in Colombia (Villegas et al., 2006; Espinal et al., 2007).

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