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Molecular Epidemiology of Imipenem-Resistant *Acinetobacter haemolyticus* and *Acinetobacter baumannii* Isolates Carrying Plasmid-Mediated OXA-40 from a Portuguese Hospital▽

Major outbreaks of multidrug-resistant *Acinetobacter baumannii* associated with nosocomial infections have been increasingly reported worldwide (1, 10, 12). The endemicity of an OXA-24/40-producing *A. baumannii* clone associated with mortality events in Portugal has been observed at numerous hospitals within the Iberian Peninsula (5, 6, 10). Inversely, *Acinetobacter haemolyticus*, isolated only occasionally from clinical samples (9), usually presents susceptibility to different antibiotics, including β-lactams (13). The isolation of two carbapenem-resistant *A. haemolyticus* strains prompted us to assess the relative contribution of clonal spread to the observed high rate of carbapenem-resistant *Acinetobacter* spp. in a general hospital in Porto, Portugal.

Between January 2001 and October 2004, 224 imipenem-resistant *Acinetobacter* spp. were collected from several specimen sources and different hospital wards, where *A. baumannii* was associated with nosocomial infections and colonizations for several months (Table 1). Imipenem resistance significantly increased from 2001 to 2002 and from 2002 to 2003. Macrorestriction analysis of genomic DNA by pulsed-field gel electrophoresis (5) and 16S rRNA gene sequencing, performed for each clone and species representative, showed that, with the exception of two clonally related *A. haemolyticus* isolates, the remainder were *A. baumannii* isolates, distributed among three different pulsotypes. Clonal dissemination of two major pulsotypes (A and B), widespread throughout the hospital, contributed to the observed *A. baumannii* imipenem resistance, which has persisted since at least 2001 despite several elimination attempts, including the use of polymyxin. Pulsotype B was predominant from 2001 to 2002, after which clone A emerged as the dominant type (Table 1). This clone was found to be identical to the previously described Iberian OXA-24/40-producing clone (5). Pulsotype C, with only two isolates, seemed to represent a sporadic event within the observed prevalence of clones A and B. Antimicrobial susceptibilities varied among isolates according to clones (Table 2). *A. haemolyticus* isolates presented resistance to all β-lactams, with the exception of cefepime, ceftazidime, and aztreonam. All *Acinetobacter* sp. isolates were resistant to ciprofloxacin, whereas susceptibility to aminoglycosides was variable. Only 11 isolates (including the two *A. haemolyticus* isolates) showed a colistin MIC of ≥4 μg/ml (2). However, when the recently updated CLSI susceptible interpretative criterion of ≤2 μg/ml (3, 8) was applied, the susceptibility rate dropped from 96.1% to 92.1%. Detection of carbapenemase production, ulteriorly identified as an OXA-24/40 enzyme, was performed as previously described (5) and was positive only for clone A *A. baumannii* isolates and, for the first time, *A. haemolyticus* isolates. Hybridization assays after

TABLE 1. Clinical data for imipenem-resistant *Acinetobacter* spp.[a]

Yr	% Imipenem resistance (no. of isolates)[d]	Clone (no. of isolates)[b]	Ward(s) (no. of isolates)	Main specimen source(s) (no. of isolates)[c]
2001	32 (47)	A (14)	ICU (8), ICU-P (2), ICU-S (1), NC (2), NK (1)	Respiratory tract (12), urine (1), NK (1)
		B (33)	ICU (6), ICU-P (14), CET (3), surgery 12B (3), Med A, B, and D (3), OBS (2), neurology (1), orthopedics (1)	Respiratory tract (13), urine (9), pus (4), catheter (3), blood (3), CSF (1)
2002	53 (31)	A (6)	ICU (4), Med B and C (2)	Respiratory tract (5), urine (1)
		B (22)	Med B and D (7), ICU (5), ICU-P (6), neurology (2), orthopedics (1), urology (1)	Respiratory tract (8), urine (10), pus (1), blood (2), catheter (1)
		A. haemolyticus (2)	Endocrinology (1), Med B (1)	Pus (1), urine (1)
2003	97 (104)	A (53)	ICU (25), CET (2), ICU-P (1), ICU-S (4), Med D (2), OBS (1), orthopedics (1), NC (5), surgery (9), urology (2), GIN (1)	Respiratory tract (27), pus (4), CSF (4), blood (6), urine (6), ascitic liquid (1), catheter (3), peritoneal liquid (2)
		B (9)	Surgery 2 (2), PED (1), Med (5), ICU (1)	Respiratory tract (3), urine (5), blood (1)
		C (2)	Med D (1), ICU-P (1)	Catheter (1), urine (1)
2004	98 (42)	A (9)	ICU (4), surgery (1), NC (1), Med (1), OBS (1), oncology (1)	Respiratory tract (6), blood (1), NK (2)
		B (1)	GIN (1)	Urine (1)

[a] ICU, intensive care unit; Med, medical unit(s); ICU-P, polivalent ICU; ICU-S, postsurgical ICU; OBS, observation; NC, neurosurgery; PED, pediatrics; GIN, gynecology; CET, cranium-encephalic traumatism; CSF, cerebrospinal fluid; NK, origin not known.
[b] Number of pulsotyped isolates (in 2003 and 2004, only representative isolates from the different hospital units were included). Clones are designated by capital letters and refer to *A. baumannii* isolates.
[c] The respiratory tract includes sputum, bronchial secretions, and tracheal aspirate.
[d] P was <0.01 for the difference between the two values (after Bonferroni's adjustment) for 2001 and 2002, and P was <0.001 for the difference between the two values for 2002 and 2003. No significant differences were observed between 2003 and 2004 ($P = 0.73$)

TABLE 2. In vitro susceptibilities of imipenem-resistant *Acinetobacter* sp. clinical isolates

Species	Clone	Carbapenemase production	MIC range (μg/ml)[a]												
			IPM	MEM	AMX	AMC	CAZ	FEP	FOX	TIC	TIM	PIP	TZP	ATM	CST
A. baumannii	A	bla_{OXA-40}	≥32	≥32	≥256	≥256	≥256	≥32	≥256	≥256	≥256	≥256	≥256	8–>256	<1–≥16
	B	Neg[b]	≥32	2–8	64–>256	64–>256	4–8	8–24	≥256	≥256	≥256	≥256	≥256	16–>256	<1–≥16
	C	Neg	32	4	128	≥256	4	2	≥256	≥256	≥256	64	64	8	2–4
A. haemolyticus		bla_{OXA-40}	≥32	≥32	≥256	≥256	4	4	32	≥256	≥256	≥256	≥256	4	4

[a] IPM, imipenem; MEM, meropenem; AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; TIC, ticarcillin; TIM, ticarcillin-clavulanic acid; PIP, piperacillin; TZP, piperacillin-tazobactam; ATM, aztreonam; CST, colistin. The MICs of β-lactams were determined by the Etest method and those of colistin by the agar dilution method (11).

[b] Neg, absence of carbapenemase production.

both S1 nuclease digestion and I-CeuI digestion, performed as previously described (7), revealed that although some clone A *A. baumannii* isolates showed a chromosome-positive signal (ca. 150 kb) for the $bla_{OXA-24/40}$ probe, most also presented a positive hybridization in plasmidic bands of ca. 180 kb and ca. 30 kb. Similar hybridization signals were observed in the *A. haemolyticus* isolates. Further studies on plasmid characterization, assessing the homology among different plasmids, are ongoing.

We describe, for the first time, the presence of an OXA-24/40 enzyme in an *A. haemolyticus* clinical isolate. Although the spread of OXA-24/40, both in the Iberian Peninsula and in France, has been correlated with the progressive dissemination of a single *A. baumannii* clone, the observation of this enzyme in a different, previously unreported, genomic species, *A. haemolyticus*, poses new questions on OXA-24/40 dissemination. It now seems reasonable to suspect a horizontal dissemination of the bla_{OXA-40} gene between different species, an ability supported by the observation of this enzyme, previously described as chromosomally encoded (7), in a plasmid. Notwithstanding, the dissemination of "successful" clones may possibly contribute to the high rates and persistence of imipenem-resistant *A. baumannii* isolates (4).

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Sandra Quinteira
REQUIMTE
Instituto Politécnico da Saúde do Norte-ESSVA
VN Famalicão
Porto, Portugal

Filipa Grosso
REQUIMTE
Laboratório de Microbiologia
Faculdade de Farmácia
Universidade do Porto
Porto, Portugal

Helena Ramos
Hospital Geral de Santo António
Porto, Portugal

Luísa Peixe*
REQUIMTE
Laboratório de Microbiologia
Faculdade de Farmácia
Universidade do Porto
Rua Aníbal Cunha
164 4050-047 Porto, Portugal

*Phone: 351-222078946
Fax: 351-222003977
E-mail: lpeixe@ff.up.pt

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