Carbapenem-Resistant *Acinetobacter baumannii* Producing the OXA-23 Enzyme: Dissemination in Southern Brazil

Acinetobacter baumannii has emerged as an important nosocomial pathogen due to its ability of long-term survival in the hospital environment, which facilitate spreading, causing institutional outbreaks, and due to its acquired multiple mechanisms of antimicrobial resistance [1]. A number of factors such as immunosuppression, use of invasive devices, and the use of antimicrobial agents have been reported as increasing the risk of infection or colonization by this opportunistic pathogen [2].

The carbapenems used to be an effective therapeutic option for the treatment of *Acinetobacter* spp. infections. However, in the last few years, carbepenem-resistant *Acinetobacter* species have been recovered worldwide. Loss of porins, alterations in the penicillin-binding protein (PBP) affinity, and different class B (metalloenzymes) and D (OXA enzymes) β-lactamases have been associated with resistance to carbapenems in *A. baumannii* [3].

The OXA carbapenemases of *Acinetobacter* are divided into four phylogenetic subgroups: OXA-23, OXA-24 and OXA-58 (acquired enzymes), and OXA-51 that is intrinsic to *A. baumannii* [4]. In Brazil, the first report of acquired oxacillinase has been described in Curitiba [5] in 2003, but carbapenem resistance seems to constitute an emerging problem in other regions of the country and in Latin America [6].

In Porto Alegre, a city with 1.4 million inhabitants and 7,700 beds situated in 25 hospitals, the first isolate of carbapenem-resistant *A. baumannii* (CRAB) was identified only in 2004, but, further, CRAB has been reported to the local health department in an unprecedented outbreak involving 16 hospitals and more than 500 detected cases from 2004 to 2008 [7].

The goal of this study was to investigate the mechanisms of emerging resistance to carbapenems in multiresistant *A. baumannii* isolates and to characterize the molecular type using pulsed-field gel electrophoresis (PFGE).

A total of 53 CRAB isolates were obtained from patients attending two university tertiary teaching hospitals in Porto Alegre from July to December 2007 (one isolate per patient). Three isolates were obtained from the environment in hospital 2 through surveillance culture during an outbreak investigation. Isolates were identified using the GN32 card using the API 20NE system (bioMérieux, Basingstoke, United Kingdom).

Antimicrobial susceptibility testing was performed by the disk diffusion method, as described by the Clinical Laboratory Standards Institute (CLSI) [8]. The minimum inhibitory concentrations (MICs) of imipenem and meropenem were established using the E-test.

The presence of genes coding for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$, and $bla_{\text{OXA-51-like}}$ were detected by multiplex polymerase chain reaction (PCR) as described previously with minor modifications [9]. Positive controls included *Acinetobacter* spp. strains known to produce OXA-23-like, OXA-24-like, and OXA-58-like enzymes [5], and the type strain of *A. baumannii* ATCC 19606 (negative control) for these genes and positive for OXA-51-like (intrinsic to *A. baumannii*).

All three isolates from the hospital environment, as well as a set of 13 clinical isolates of CRAB, randomly selected from all clinical isolates, which were stored at –80 °C, were selected for molecular typing. Macrorestriction analysis of chromosomal DNA with SmaI (Invitrogen, Paisley,

Infection 2009; 37: 474–476
DOI 10.1007/s15010-009-9003-9

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Received: December 19, 2008 · Revision accepted: April 23, 2009
Published online: September 18, 2009

United Kingdom) was carried out by PFGE using CHEF-DRII apparatus. The band patterns were interpreted according to the recommendations of *Tenover* et al. [10].

The 53 clinical isolates were recovered from the lower respiratory tract (n = 39), blood (n = 9), urine (n = 2), and other clinical specimens (n = 3). These isolates were obtained from adult patients in intensive care units (ICUs; n = 42) and from a clinical ward (n = 11). Three CRAB isolates from the environment were obtained from a mobile RX machine, from a shelf at the infirmary, and from the surface of a table surrounding the bed from a colonized patient.

All clinical and environmental isolates proved to be resistant to carbapenems by the disk diffusion method as well by E-test (MIC > 16 μg/ml). All clinical isolates of CRAB were also resistant to ciprofloxacin, gentamicin, piperacillin/tazobactam, and trimethoprim/sulphametoxazole. Only a minority of CRAB was susceptible to ampicillin/sulbactam and ceftazidime (data not shown).

All CRAB isolates were positive for the $bla_{\text{OXA-23-like}}$ gene but none presented $bla_{\text{OXA-58-like}}$ or $bla_{\text{OXA-24-like}}$ genes. The presence of the gene $bla_{\text{OXA-51-like}}$ in all isolates confirmed that they were *A. baumannii* [9].

A total of 16 CRAB isolates were randomly selected for molecular typing (Table 1). Five distinct PFGE profiles were identified among the 16 CRAB isolates from both hospitals. Two major types were identified: type ''A'' (five isolates) and type ''B'' (seven isolates). Type ''A'' was found only in hospital 1 in contrast to type ''B'', which was found in both hospitals (one isolate in hospital 1 and six isolates in hospital 2). All three CRAB obtained from the environment from hospital 2 were proved to belong to major type ''B''. There was no correlation between the susceptibility profile and the PFGE type of CRAB isolates.

The emergence of resistance to carbapenems in *A. baumannii* has limited the therapeutic options to treat infections caused by these bacteria. In fact, CRAB tend to be multiresistant [11] and, in this study, the multiresistance was confirmed, as only a minority of CRAB were susceptible to other antibiotics. It has been, therefore, suggested that colistin may be the only antibiotic effective against CRAB [11]. We did not evaluate the polymyxins in the present study.

Although penicillin-binding protein modification and/or porin loss have been reported as mechanisms of resistance in *Acinetobacter* spp., it is the carbapenemase production that is the most worrying mechanism of β-lactamase resistance in the genus [12]. In fact, the combination of β-lactamase production and porin loss is the most synergic resistance mechanism to carbapenems.

The OXA-23 enzyme was originally reported in *Acinetobacter* from Scotland in 1985 and was termed ARI-1 [13]. OXA-23 was further reported in patients from England, South America, and Asia [13]. In the present report, we were able to identify the *A. baumannii*-producing OXA-23 enzyme in all clinical isolates, and this is the first report of OXA-23 in the city of Porto Alegre.

We were able to type 16 CRAB isolates by PFGE and we found that there were two main types and a few minor

Table 1
Molecular typing of *Acinetobacter baumannii* OXA-23 isolates.

Hospital	Isolate number	Susceptibility profiles								PFGE group
		AMPS	CAZ	CIP	GEN	IMI	MER	PIP	SUT	
1	9	I	R	R	R	R	R	R	R	A1
1	10	S	R	R	R	R	R	R	R	A
1	12	R	R	R	R	R	R	R	R	B1
1	20	R	R	R	R	R	R	R	R	A
1	28	R	R	R	R	R	R	R	R	A
1	31	R	R	R	R	R	R	R	R	C
1	41	S	R	R	R	R	R	R	R	A
2	70	S	S	R	R	R	R	R	R	B
2	56[a]	R	I	R	R	R	R	R	R	B1
2	71[a]	R	I	R	R	R	R	R	R	B
2	72[a]	R	I	R	R	R	R	R	R	B
2	105	S	I	R	R	R	R	R	R	B
2	120	R	S	R	R	R	R	R	R	B
2	122	S	R	R	R	R	R	R	R	D
2	127	S	R	R	R	R	R	R	R	E
2	129	R	R	R	R	R	R	R	R	E

AMPS: ampicillin/sulbactam; CAZ: ceftazidime; CIP: ciprofloxacin; GEN: gentamicin; IMI: imipenem; MER: meropenem; PIP: piperacillin–tazobactam; SUT: trimethoprim–sulfamethoxazole [a]Environmental isolates

types disseminated in both hospitals. Noteworthy, CRAB clinical isolates from the same type were found in both hospitals and CRAB from the environment also belonged to this major type. This indicates that the OXA-23 CRAB dissemination may occur in both ways: by transmission of the same strain among different patients and from the environment, as well as by horizontal transmission of the genetic element carrying this gene. In fact, the polyclonal dissemination of CRAB has been described in other studies around the world [14, 15].

In conclusion, our work indicates that the OXA-23 enzyme is probably the major factor leading to carbapenem resistance in *A. baumannii* in the two teaching hospitals from Porto Alegre, although other resistance mechanisms may be involved. Moreover, the CRAB dissemination may be complex, as it may be related to both patient-to-patient transmission and/or the spreading of the genetic element containing the gene.

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Acknowledgments

This study has received financial support from the Fundo de Incentivo à Pesquisa e Eventos (FIPE), Hospital de Clínicas de Porto Alegre. We thank Dr. Libera Dalla-Costa, who kindly provided the *Acinetobacter* spp. which were used as positive controls in this study.

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