Published in final edited form as:

Infect Genet Evol. 2013 December ; 20: . doi:10.1016/j.meegid.2013.09.028.

Emergence of *Acinetobacter baumannii* international clone II in Brazil: reflection of a global expansion

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Abstract

The aim of this study was to investigate the occurrence of carbapenem-resistant *Acinetobacter baumannii* international clones (IC) in Curitiba, Brazil, using multilocus sequence typing and trilocus PCR-based typing schemes. IC2 was the first emerging clone. This IC was detected in an isolate from 2003 of a PFGE type spread in at least two hospitals since 1999. Subsequently, IC2 waned while IC1 and clonal complex 15/104 prevailed. This is the first description of IC2 in Brazil and Latin America.

Keywords

Acinetobacter baumannii; multilocus sequence typing; trilocus PCR-based typing; international clone; molecular epidemiology

1. Introduction

During the last three decades, *Acinetobacter baumannii* has become a remarkable opportunistic pathogen. Multilocus sequence typing (MLST), a major strain typing tool hosted by Institute Pasteur (IP) (Nemec et al., 2008) and University of Oxford (UO) (Bartual et al., 2005), led to the unambiguous recognition of several widely disseminated *A. baumannii* clones. Initially, three successful “international clones” (IC) with high capacity to acquire resistance to antibiotics were recognized: IC1, IC2 and IC3 (Karah et al., 2012), corresponding in MLST IP/UO schemes to clonal complexes (CC) 1/109, 2/118 and 3/187, respectively. Currently, eighteen clones spreading in more than one continent have been described, a strong evidence of *A. baumannii* global expansion (Karah et al., 2012). IC2 is by far the most widely dispersed, already found in at least 34 countries (Karah et al., 2012). To facilitate the recognition of isolates belonging to IC clones, a trilocus PCR-based typing scheme (3LST) was developed to detect groups of *ompA*, *csuE* and *bla*_{OXA51-like} alleles specific to IC1, IC2 and IC3 (Turton et al., 2007).

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Infections caused by carbapenem-resistant *A. baumannii* (CRAB) have increased dramatically in Latin America. In Brazil, most CRAB isolates have been associated with CC79/113 and CC15/104, reported in Rio de Janeiro and Salvador (Grosso et al., 2010, Coelho-Souza et al., 2013, Martins et al., 2013). Although most *A. baumannii* isolates from Rio de Janeiro, Brazil, clustered with IC2 by 3LST, the corresponding STs were unrelated to IC2 by eBURST analysis (Grosso et al., 2010). These results have put into question the convenience of 3LST to identify local isolates belonging to ICs.

The first description of CRAB in Brazil was from Curitiba, where a single PFGE clone caused infections in two hospitals in 1999 (Dalla-Costa et al., 2003). The persistence of this PFGE clone was documented in one of these hospitals, together with other dominant clones, during a period of 3 years in the 2000s (Schimith Bier et al., 2010). The aim of the present study was to use MLST and 3LST techniques in order to characterize seven key *A. baumannii* isolates from this time in Curitiba (Schimith Bier et al., 2010), and to investigate the potential emergence of ICs in the country.

2. Material and methods

From a total of 172 CRAB isolates previously studied (Schimith Bier et al., 2010), seven were selected, representing the most frequent PFGE types. The isolates were typed by MLST schemes hosted at IP (www.pasteur.fr) and UO (PubMLST, www.pubmlst.org), as previously described (Bartual et al., 2005, Nemec et al., 2008). STs were included in CCs when five or more identical alleles were detected by eBURST (<http://eburst.mlst.net>) and minimum spanning tree analysis (www.pasteur.fr). The isolates were also typed by 3LST as previously described using two sets of multiplex-PCR (Turton et al., 2007). Amplification of the three target alleles in multiplex PCR-group 1 (G1) or in group 2 (G2) includes isolates in IC2 or IC1, respectively.

3. Results and discussion

MLST data analysis showed that the isolates belonged to three CCs corresponding to IC1, IC2 and CC15/104 (Table 1). By IP scheme, each ST was the founder of the respective CC. By UO scheme, STs were single locus variants (SLV) or double locus variants (DLV) of the founder ST. 3LST results were completely concordant with MLST analysis, as shown in Table 1. However, the isolates included by MLST into CC15/104 were not assigned to any of the previously described 3LST groups.

This was the first report of the presence of *A. baumannii* IC2 in Brazil and in Latin America. The IC2 of the present study corresponded to the PFGE genotype identified as the first emergence of CRAB in the country, which caused infections that contributed to the death of five in eight affected patients in two hospitals (Dalla-Costa et al., 2003). A second study was conducted by the same authors due to an increase of CRAB isolates at one of the hospitals between 2002–2005 (Schimith Bier et al., 2010). Interestingly, IC2 was restrained and affected only 20% of the 172 studied patients, while IC1 and CC15/104 affected each 39% and 41%, respectively. Apparently, IC2 was waning while the study was performed, because it was not found in the last year of the study period (Schimith Bier et al., 2010). IC2 was not reported among a total collection of 287 *A. baumannii* isolates later typed by MLST in two other Brazilian cities, Rio de Janeiro and Salvador (Grosso et al., 2010, Coelho-Souza et al., 2013, Martins et al., 2013), and no IC2 isolates from Brazil have been deposited in any of the MLST databases, except for the isolate from this study.

The two other ICs found in Curitiba (CC15/104 and IC1) have been well-established in Brazil and in other countries of Latin America (Coelho-Souza et al., 2013; Martins et al., 2013; Stietz et al., 2013). IC1 became one of the predominant clones in Brazil, isolated

during 2003–2004 in Curitiba, and 2007–2008 in Rio de Janeiro (Martins et al., 2013). In Brazil, CC15/104 was found in Rio de Janeiro and Salvador between 2007–2008 as the second most common clone among patients (Coelho-Souza et al., 2013, Martins et al., 2013). Based on previous reports of 3LST analysis, this IC belonged to G4 and G5 (Grosso et al., 2010; Karah et al., 2010, Zarrilli et al., 2013), though this relationship was not observed in the present study. It is worth to mention that the correct 3LST classification depends on the combination of PCR group 1 and group 2 results, as recommended (Turton et al., 2007). If a single PCR group is performed, misclassifications can occur.

4. Conclusions

In the present report, we described that IC2 was a major cause of infection when it first emerged in Brazil, in the late 1990s. Surprisingly, IC2 isolates were short lived and surpassed by several other clones well succeeded locally. IC1 remained from 2003 to 2004. The reasons for such predominance and changes over time are unknown; the ability of particular strains to acquire antibiotic resistance and virulence determinants has most likely been part of the process (Zarrilli et al., 2013).

Acknowledgments

Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/Comissão Fulbright-Brasil, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) of Brazil and Fogarty International Program in Global Infectious Diseases (TW006563) of the National Institute of Health. This publication made use of the *Acinetobacter baumannii* MLST website (<http://pubmlst.org/abaumannii/>) developed by Keith Jolley and sited at the University of Oxford (Jolley & Maiden 2010, *BMC Bioinformatics*, 11:595). We thank also platform Genotyping of Pathogens and Public Health (Institut Pasteur, Paris, France) for coding MLST alleles and profiles and making them available at www.pasteur.fr/mlst.

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Highlights

- First report of international *A. baumannii* clone II in Brazil.
- Increasing information about global spread of multidrug-resistant *A. baumannii*
- Multilocus sequence typing supported new data about hospital spread of international *A. baumannii* clone II since 1999.

Table 1
Acinetobacter baumannii multilocus sequence types (MLST), clonal complexes (CC) and PCR 3LST based-groups

Isolate number ^a	Date of Isolation	MLST		PCR groups 3LST (<i>csuE</i> ; <i>ompA</i> ; <i>bla_{OXA5}</i>)		PCR 3LST based-group (allelic profile)
		ST (IP) ^b (allelic profile)	ST (UO) ^c (allelic profile)	CC (IP/UO) ^d	SG1	SG2
7894	Nov/03	15 (6-6-8-2-3-5-4)	236 (104 SLV) (12-17-12-1-29-102-39)	15/104	+, +, +	+, -, -
2681	Apr/04					
7718	Jan/05					
1198	May/03	1 (1-1-1-1-5-1-1)	231 (109 SLV) (10-12-4-11-4-98-5)	1/109	-, -, -	+, +, +
2280	May/04					
6505	Jun/04					
546A1	Oct/02	2 (2-2-2-2-2-2-2)	238 (118 DLV) (1-3-3-2-38-97-3)	2/118	+, +, +	-, -, -

SLV: single locus variant; DLV: double locus variant; 3LST: three-locus sequence typing; SG1: sequence group 1; SG2: sequence group 2; +: PCR band present; -: PCR band absent; IC1: International Clone I; IC2: International Clone II.

^aData from reference (Schmith Bier et al. 2010).

^bSequence type by Institut Pasteur MLST scheme.

^cSequence type by University of Oxford MLST scheme.

^dCC: MLST clonal complex defined by IP and UO scheme.