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Longitudinal surveillance for meningitis by *Acinetobacter* in a large urban setting in Brazil

Talita Coelho-Souza[1], **Joice N. Reis**[2], **Natacha Martins**[1], **Ianick Souto Martins**[3], **Ana Paula O. Menezes**[4], **Mitermayer G. Reis**[4], **Neide O. Silva**[5], **Rubens C. S. Dias**[6], **Lee W. Riley**[7], and **Beatriz Meurer Moreira**[1,*]

[1]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

[2]Universidade Federal da Bahia, Salvador, Brazil

[3]Universidade Federal Fluminense, Niterói, Brazil

[4]Fundação Oswaldo Cruz, Salvador, Brazil

[5]Hospital Couto Maia, Salvador, Brazil

[6]Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

[7]University of California, Berkeley, California, USA

Abstract

The study aim was to describe the emergency of carbapenem resistance and clonal complexes (CC), defined by multilocus sequence typing (MLST), in *Acinetobacter baumannii* in a surveillance system for meningitis. Starting in 1996 at an urban setting of Brazil, surveillance detected meningitis by *Acinetobacter* sp for the first time in 2002. Until 2008, 35 isolates were saved. Carbapenem resistance emerged in 2006, reaching 70% of *A. baumannii* isolates in 2008, including one colistin-resistant. *A. baumannii* belonged to CC113/79 (University of Oxford/ Institute Pasteur schemes), CC235/162 and CC103/15. Dissemination of infections resistant to all antimicrobial agents may occur in the future.

Keywords

Acinetobacter baumannii; bacterial meningitis; carbapenem-resistance; multilocus sequence typing; clonal complexes

Acinetobacter baumannii has become increasingly recognized as a cause of multidrug resistant central nervous system infections [1]. *A. baumannii* clones are classified by multilocus sequence typing (MLST) by protocols hosted at Institut Pasteur (IP, www.pasteur.fr) and the University of Oxford (UO, www.pubmlst.org), and grouped in clonal complexes (CC). Typing an isolate by both schemes is useful as there is no link between IP and UO databases. To date, little is known about the population structure of *A. baumannii* from cases of meningitis worldwide [2]. In 1996, a hospital-based active-surveillance for bacterial meningitis was established at Hospital Couto Maia, a state

*Corresponding author: Beatriz Meurer Moreira, M.D., Instituto de Microbiologia, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, 21 941-902 Cidade Universitária, Rio de Janeiro, RJ, Brazil, Phone 5521-25608344, bmeurer@micro.ufrj.br.

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infectious disease reference hospital in Salvador, Brazil [3]. The main purpose of this system was to investigate classical pathogens *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae*. Non-classical agents were also sought since all CSF specimens from public hospitals in the city are processed at this hospital. The aim of the present study was to describe the emergency of carbapenem resistance in *Acinetobacter* spp and the distribution of *A. baumannii* CCs in isolates recovered from meningitis in this system. A case of culture-proven bacterial meningitis was a patient with typical symptoms and *Acinetobacter* sp isolated from CSF. From 2002 to 2008, 57 cases of hospital-acquired *Acinetobacter* sp meningitis were detected; 35 isolates (one/per patient) were saved. Species were identified by sequence analysis of 350-bp *rpoB* gene fragments [4] and defined by at least 97% similarity with one in a set of reference strains and by BLAST [5].

Antimicrobial susceptibility was determined by disk diffusion [6] for: amikacin, gentamicin, tobramycin, ampicillin-sulbactam, cefepime, ceftazidime, ciprofloxacin, imipenem, meropenem, minocycline, tetracycline, piperacillin-tazobactam, trimethoprim-sulfamethoxazole. Minimum inhibitory concentrations (MICs) of cefepime, imipenem, meropenem and tigecycline were defined by Etest following the manufacturer's instructions (bioMérieux, Solna, Sweden). Colistin MICs were determined by broth microdilution [7]. Susceptibility to all agents was interpreted as recommended by CLSI [8] except for tigecycline, interpreted as proposed by the US Food and Drug Administration (FDA) for Enterobacteriaceae. Isolates were classified as multidrug-resistant (MDR) or extensively drug-resistant (XDR) [9]. Metallo-β-lactamase production was screened by a double-disk test [10]. The following carbapenemase encoding genes was investigated by PCR: $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-143}}$, bla_{KPC}, bla_{NDM}, $bla_{\text{GIM-1}}$, $bla_{\text{IMP-type}}$, $bla_{\text{SIM-1}}$, $bla_{\text{SPM-1}}$, $bla_{\text{VIM-type}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-25}}$ [11–15]. Isolates were typed by pulsed-field gel electrophoresis (PFGE) [16] and included within a pulsotype if band profiles had ≤ five differences. UO and IP MLST schemes were performed [17,18]. CCs were formed by STs with five or more identical alleles by goeBURST (goeburst.phyloviz.net). STs and CCs are here referred by UO/IP scheme.

From 2001 to 2008, 1,398 meningitis cases were detected among ~ 3,000 patients, and 931 (67%) were caused by classical agents. *Acinetobacter* sp, identified for the first time in 2002, increased significantly (R^2= 0.94) from 0.9% in 2001–2002 to 4.3% in 2007–2008. Median age of patients was 25 ± 21.3 (range 3–82) years and 71.4% were men. From 57 stored *Acinetobacter* spp isolates (one per patient), 35 (61%) were available for further characterization. Most (31) were *A. baumannii*, two *Acinetobacter nosocomialis*, and one each *Acinetobacter ursingii* and *Acinetobacter* genomic species 15TU. Non-*A. baumannii* isolates were susceptible to all drugs or resistant only to sulfamethoxazole-trimethoprim. All *A. baumannii* isolates were susceptible to minocycline and tigecycline. One isolate from 2008 was colistin resistant (MIC = 64 mg/L), and susceptible only to minocycline, tetracycline, tigecycline and tobramycin. $MICs_{50}$/ $MICs_{90}$ were 32/ >256 mg/L for cefepime, 1/ >32 mg/L for imipenem, 4/ >32 mg/L for meropenem, 0.5/ 1 mg/L for colistin, and 0.38/ 1 mg/L for tigecycline. Thirteen *A. baumannii* isolates were MDR and fourteen XDR. Carbapenem resistance emerged in May 2006 and became endemic (Figure 1). All carbapenem-resistant isolates carried the $bla_{\text{OXA-23-like}}$ gene, and the natural $bla_{\text{OXA-51-like}}$ gene, detected in all *A. baumannii* isolates. $bla_{\text{CTX-M-2}}$ was detected in one MDR *A. baumannii* from 2004. No other carbapenemase encoding-genes or metallo-β-lactamase production was observed.

A. baumannii formed fifteen pulsotypes, and one isolate was not typeable. Nineteen of 30 typeable *A. baumannii* isolates were included in four pulsotypes (A–D). Fourteen isolates of main pulsotypes were selected for MLST. Ten STs (all new) were identified by UO scheme

and five (three new) by IP scheme (Table 1). STs formed four CCs by UO, and three by IP scheme, unrelated to international clones I, II and III. ST164 by IP scheme was not assigned to a CC because this is a double locus variant (DLV) of just one other ST. Each of the seven CCs was isolated over more than twenty months during the six years surveillance (Figure 1). CC113/79 included the colistin resistant isolate (ST237/79). Although 22 of the 57 cases detected in the surveillance system could not have the species determined, we believe all these cases were indeed caused by the genus *Acinetobacter* since such identification is simple. No further data about *Acinetobacter* infections in the study hospital are available, however, these infections are likely part of hospital dissemination of this pathogen, previously noted in Brazil [19].

Carbapenem resistance was first detected by the system in 2006 and increased over time to affect 16 of the 31 study *A. baumannii* isolates, associated with the presence of the $bla_{\text{OXA-23}}$ gene. Alarmingly, 14 of *A. baumannii* isolates were XDR. High susceptibility to colistin, minocycline and tigecycline was observed. Colistin has been recommended for meningitis by carbapenem resistant *A. baumannii* [20], but resistance should become increasingly frequent. Use of tigecycline has been described as effective in a few case reports [20], however, the pharmacodynamic profile of this drug does not seem adequate for this purpose [20].

CC113/79, CC235/162 and CC103/15 were important causes of meningitis in the present study and prone to develop resistance to multiple agents. Except for ATCC 17978 strain, no other isolate from patients with meningitis could be related to the CCs of the present study. This finding suggests meningitis is not caused preferentially by isolates with a specific tropism for the central nervous system, but by clones circulating in hospitals.

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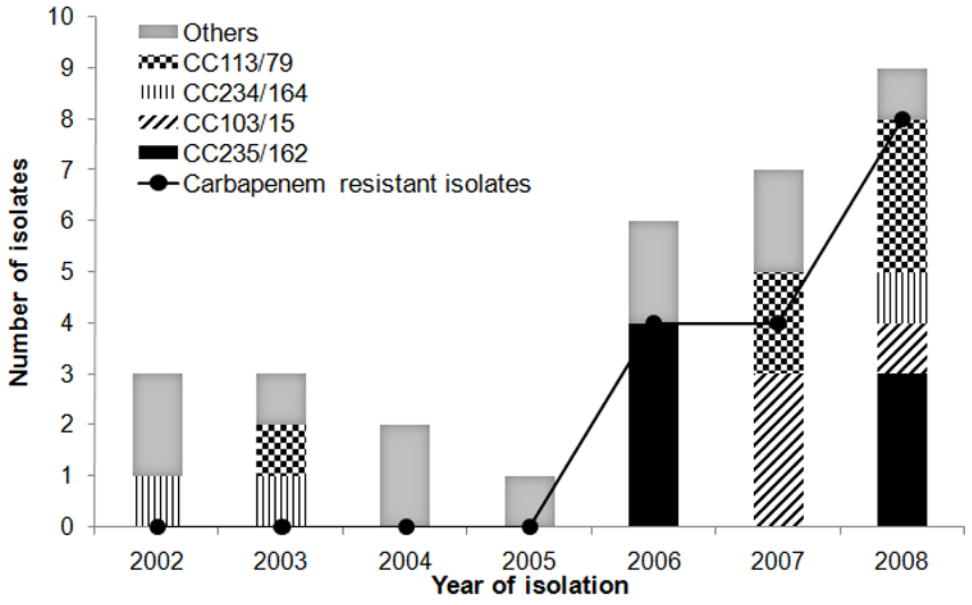

Figure 1.

Temporal distribution of *Acinetobacter baumannii* clonal complexes (CCs) and carbapenem-resistant *A. baumannii* isolates over seven years of study. “Others” include single pulsotypes and one not typeable isolate not selected for MLST analysis. CCs are described according to University of Oxford / Institute Pasteur schemes.

Table 1

Characteristics of *Acinetobacter baumannii* isolates from 31 patients with meningitis

MLST-UO		MLST-IP		Characteristic (number of isolates)		
CC	**ST (isolates sequenced)**	**CC**	**ST (isolates sequenced)**	**Pulsotype**	***bla*$_{OXA-23}$**	**Susceptibility phenotype**
113	237[a] (2)	79	79 (5)	A (6)	+ (3)	COL MIN TET TGC TOB (1) COL MIN TGC TOB (1) MIN TET TGC TOB (1)
	258[a] (1)				+ (1)	COL MIN TET TGC TOB (1)
	259[a] (1)				− (1)	AMS COL GEN IPM MEM MIN TET TGC TOB (1)
	233[a] (1)				− (1)	AMS COL GEN IPM MEM MIN TET TGC TOB (1)
235	235[a] (3)	162	162[a] (4)	B (6)	+ (3)	COL MIN TET TGC (1) COL MIN TGC (2)
					− (1)	AMI AMS COL GEN IPM MEM MIN SXT TGC TOB (1)
	415[a] (1)				+ (2)	COL MIN TET TGC (1) COL MIN TGC (1)
	416[a] (1)		163[a] (1)	L (1)	+ (1)	AMS COL GEN MIN TET TGC TOB (1)
103	236[a] (1)	15	15 (1)	C (4)	+ (4)	COL GEN MIN TET TGC TOB (1) COL MIN TET TGC (1) COL MIN TGC (2)
234	234[a] (2)	NA	164[a,b] (3)	D (3)	− (3)	AMS CIP COL FEP IPM MEM MIN PTZ TET TGC (1) AMS COL IPM MEM MIN TET TGC (1)
	232[a] (1)					AMS CIP COL FEP IPM MEM MIN PTZ TET TGC (1)
ND	ND	ND	ND	E – K, M - N (9)	− (9)	Various[c]
				O (1)	+ (1)	AMS COL MIN TGC TOB (1)
				Not typeable (1)	+ (1)	AMI AMS CAZ COL GEN MIN SXT TGC TOB (1)

CC: clonal complex, ST: sequence type, NA: not assigned, ND: not determined, AMI: amikacin, AMS: ampicillin-sulbactam, CAZ: ceftazidime, CIP: ciprofloxacin, COL: colistin, FEP: cefepime, GEN: gentamicin, IPM: imipenem, MEM: meropenem, MIN: minocycline, PTZ: piperacillin-tazobactam, SXT: trimethoprim-sulfamethoxazole, TET: tetracycline, TGC: tigecycline, TOB: tobramycin.

[a] ST described in the present study.

[b] ST164 was not assigned to neither clonal complex because this one is DLV of other published ST;

[c] Includes susceptibility to all drugs (3 isolates); 1 isolate each of AMI AMS CAZ CIP COL FEP GEN IPM MEM MIN PTZ TET TGC TOB, AMS CIP COL FEP IPM MEM MIN TET TGC, COL GEN IPM MEM MIN TET TGC TOB, COL CIP IPM MEM MIN TET TGC TOB, AMS COL IPM MEM MIN TGC and COL IPM MEM MIN TET TGC.