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

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Abstract

The study aim was to describe the emergence 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 in an urban setting of Brazil, surveillance detected meningitis by *Acinetobacter* sp for the first time in 2002. Up to 2008, 35 isolates were saved. Carbapenem resistance emerged in 2006, reaching 70% of *A. baumannii* isolates in 2008, including one that was 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

Original Submission: 13 September 2012; **Revised Submission:** 15 December 2012; **Accepted:** 28 December 2012

Editor: R. Cantón

Article published online: 12 January 2013

Clin Microbiol Infect 2013; **19:** E241–E244

10.1111/1469-0691.12145

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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 into clonal complexes (CCs). 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 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 because all CSF specimens from public hospitals in the city are processed at this hospital. The aim of the present study was to describe the emergence 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 and trimethoprim-sulphamethoxazole. 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 were 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 ≤ 5 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 the UO/IP scheme.

From 2001 to 2008, 1398 meningitis cases were detected among *c.*3000 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. The 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 sulphamethoxazole-trimethoprim. All *A. baumannii* isolates were susceptible to minocycline and tigecycline. One isolate from 2008 was colistin resistant (MIC = 64 mg/L),

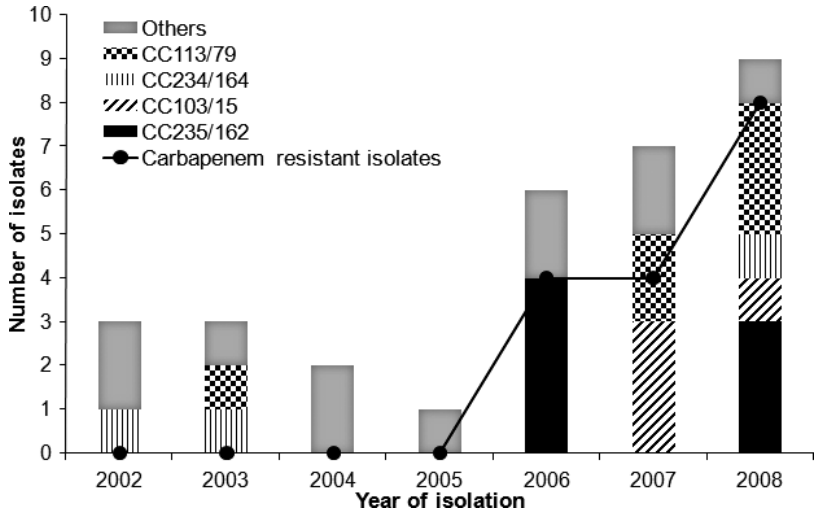

FIG. 1. Temporal distribution of *Acinetobacter baumannii* clonal complexes (CCs) and carbapenem-resistant *A. baumannii* isolates over 7 years of study. 'Others' include single pulsotypes and one not typeable isolate not selected for MLST analysis. CCs are described according to University of Oxford/Institute 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-sulphamethoxazole; TET, tetracycline; TGC, tigecycline; TOB, tobramycin.
[a]ST described in the present study.
[b]ST164 was not assigned to either clonal complex because this one is DLV of other published ST.
[c]Includes susceptibility to all drugs (three isolates); one 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.

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 14 XDR. Carbapenem resistance emerged in May 2006 and became endemic (Fig. 1). All carbapenem-resistant isolates carried the $bla_{OXA\text{-}23\text{-}like}$ gene and the natural $bla_{OXA\text{-}51\text{-}like}$ gene, detected in all *A. baumannii* isolates. $bla_{CTX\text{-}M\text{-}2}$ was detected in one MDR *A. baumannii* from 2004. No other carbapenemase-encoding gene or metallo-β-lactamase production was observed.

Acinetobacter baumannii formed 15 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 the UO scheme and five (three new) by the IP scheme (Table 1). STs formed four CCs by UO, and three by the IP scheme, unrelated to international clones I, II and III. ST164 by the 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 20 months during the 6 years of surveillance (Fig. 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* because such identification is simple. No further data about *Acinetobacter* infections in the study hospital are available; however, these infections are likely to be 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 $bla_{OXA\text{-}23}$ gene. Alarmingly, 14 of *A. baumannii* isolates were XDR. High susceptibility to colistin, minocycline and tigecycline was observed. Colistin has been recommended for meningitis caused 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 the ATCC 17978 strain, no other isolate from patients with meningitis could be related to the CCs of the present study. This finding suggests that meningitis is not caused preferentially by isolates with a specific tropism for the central nervous system, but by clones circulating in hospitals.

Acknowledgements

Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/Comissão Fullbright-Brasil, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) of Brazil and Fogarty International Program in Global Infectious Diseases of the National Institute of Health (TW006563). 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). The development of this site has been funded by the Wellcome Trust. 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.

Transparency Declarations

No conflicts of interest to declare.

References

1. Kim BN, Peleg AY, Lodise TP *et al.* Management of meningitis due to antibiotic-resistant *Acinetobacter* species. *Lancet Infect Dis* 2009; 9: 245–255.
2. Bayramoglu G, Kaya S, Besli Y *et al.* Molecular epidemiology and the clinical significance of *Acinetobacter baumannii* complex isolated from cerebrospinal fluid in neurosurgical intensive care unit patients. *Infection* 2012; 40: 163–172.
3. Lima JB, Ribeiro GS, Cordeiro SM *et al.* Poor clinical outcome for meningitis caused by *Haemophilus influenzae* serotype A strains containing the IS*1016-bexA* deletion. *J Infect Dis* 2010; 202: 1577–1584.
4. Nemec A, Krizova L, Maixnerova M *et al.* Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). *Res Microbiol* 2011; 162: 393–404.
5. Nemec A, Musílek M, Maixneróva M *et al. Acinetobacter beijerinckii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov., haemolytic organisms isolated from humans. *Int J Syst Evol Microbiol* 2009; 59: 118–124.
6. CLSI. *Performance standards for antimicrobial disk susceptibility tests; approved standard – eleventh edition, M02-A11*. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
7. CLSI. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard – ninth edition, M07-A9*. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
8. CLSI. *Performance standards for antimicrobial susceptibility testing; twenty-second information supplement, M100-S22*. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
9. Magiorakos AP, Srinivasan A, Carey RB *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an

international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18: 268–281.
10. Picão RC, Andrade SS, Nicoletti AG *et al.* Metallo-β-lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. *J Clin Microbiol* 2008; 46: 2028–2037.
11. Hossain A, Ferraro MJ, Pino RM *et al.* Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 in an *Enterobacter* sp. *Antimicrob Agents Chemother* 2004; 48: 4438–4440.
12. Woodford N, Ellington MJ, Coelho JM *et al.* Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006; 27: 351–353.
13. Mendes RE, Kiyota KA, Monteiro J *et al.* Rapid detection and identification of metallo-beta-lactamase-enconding genes by multiplex real-time PCR assay and melt curve analysis. *J Clin Microbiol* 2007; 45: 544–547.
14. Poirel L, Revathi G, Bernabeu S, Nordmann P. Detection of NDM-1-producing Klebsiella pneumoniae in Kenya. *Antimicrob Agents Chemother* 2011; 55: 934–936.
15. Wang XR, Chen JC, Kang Y, Jiang N, An SC, Gao ZC. Prevalence and characterization of plasmid-mediated bla_{ESBL} with their genetic environment in *Escherichia coli* and *Klebsiella pneumoniae* in patients with pneumonia. *Chin Med J* 2012; 125: 894–900.
16. Seifert H, Dolzani L, Bressan R *et al.* Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; 43: 4328–4335.
17. Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodriguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; 43: 4382–4390.
18. Nemec A, Krízová L, Maixnerová M *et al.* Emergence of carbapenem resistance in *Acinetobacter baumannii* in the Czech Republic is associated with the spread of multidrug-resistant strains of European clone II. *J Antimicrob Chemother* 2008; 62: 484–489.
19. Sader HS, Gales AC, Pfaller MA *et al.* Pathogen frequency and resistance patterns in Brazilian hospitals: summary of results from three years of the SENTRY antimicrobial surveillance program. *Braz J Infect Dis* 2001; 5: 200–214.
20. van de Beek D, Brouwer MC, Thwaites GE, Tunkel AR. Advances in treatment of bacterial meningitis. *Lancet* 2012; 380: 1693–1702.