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

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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 spp 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 determined 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: blaOXA-23-like, blaOXA-24-like, blaOXA-51-like, blaOXA-58-like, blaOXA-143, blaKPC, blalqdm, blalqsm, blalqse, blalqse, blalqsp-1, blalqsp-1, blalqse, blaCTX-M-1, blaCTX-M-2, blaCTX-M-8, blaCTX-M-9 and blaCTX-M-25 [11–15]. Isolates were typed by pulsed-field gel electrophoresis (PFGE) [16] and included within a pulsortype 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.phylotviz.net). STs and CCs are here referred by the UO/IP scheme.
From 2001 to 2008, 1398 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. 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

<table>
<thead>
<tr>
<th>MLST-UO</th>
<th>MLST-IP</th>
<th>Characteristic (number of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>ST (isolates sequenced)</td>
<td>CC</td>
</tr>
<tr>
<td>113</td>
<td>237 (2)</td>
<td>79</td>
</tr>
<tr>
<td>258</td>
<td>235</td>
<td>79</td>
</tr>
<tr>
<td>235</td>
<td>235</td>
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<td>415</td>
<td>415</td>
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<td>103</td>
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<td>79</td>
</tr>
<tr>
<td>234</td>
<td>234</td>
<td>79</td>
</tr>
<tr>
<td>ND</td>
<td>ND</td>
<td>79</td>
</tr>
</tbody>
</table>

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 COL FEP IPM MEM MIN PTZ TET TGC TOB, AMS COL GEN MEM MIN TET TGC, COL GEN IPM MEM MIN TET TGC TOB, COL CIP IPM MEM MIN TET TGC TOB, AMS COL GEN IPM MEM MIN TET TGC, and COL IPM MEM MIN TET TGC.
and susceptible to minocycline, tetracycline, tigecycline and tobramycin. MICsM/ICsM 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 blaOXA-23-like gene and the natural blaOXA-51-like gene, detected in all A. baumannii isolates. blaCTX-M-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 I). 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 (three new) by the IP scheme (Table I). 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 (three new) by the IP scheme (Table I). 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 (three new) by the IP scheme (Table I). 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 (three new) by the IP scheme (Table I).

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 blaOXA-23 gene. Alarmingl, 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.

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Transparency Declarations

No conflicts of interest to declare.

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