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EPIDEMIOLOGY

Imported and Intensive Care Unit-Born *Acinetobacter baumannii* Clonal Complexes: One-Year Prospective Cohort Study in Intensive Care Patients

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The main objective of this study was to assess the frequency and possible sources of colonization and infection by *Acinetobacter* in the intensive care unit (ICU) of a university hospital in Rio de Janeiro, Brazil, and characterize the isolates for relatedness to internationally and locally disseminated lineages. Patients consecutively admitted to the ICU from April 2007 to April 2008 were screened for colonization and infection. Species were identified by *rpo*B sequencing. The presence of acquired and intrinsic carbapenemase genes was assessed by polymerase chain reaction (PCR). Strains were typed by random amplification of polymorphic DNA (RAPD)-PCR, pulsed-field gel electrophoresis, and multilocus sequence typing (MLST) using the schemes hosted at the University of Oxford (UO) and Institut Pasteur (IP). Of 234 patients, 98 (42%) had at least one specimen positive for the *Acinetobacter* isolate, and 24 (10%) had infection. A total of 22 (92%) infections were caused by *Acinetobacter baumannii* and one each (4%) by *Acinetobacter nosocomialis* and *Acinetobacter berezinae. A. baumannii* isolates from 60 patients belonged to RAPD types that corresponded to MLST clonal complexes (CCs) 109/1 (UO/IP scheme, known as International Clone I), CC 110/110 (UO/IP), CC 113/79 (UO/IP), and CC 104/15 (UO/IP). Most CCs were carbapenem resistant and carried the $bla_{\text{OXA-23}}$-like gene. Strains were introduced by patients transferred from other wards of the same hospital (11 patients, 18%) or acquired from cross-transmission within the ICU (49 patients, 82%). *A. nosocomialis* lineage sequence type 260 colonized 10% of the whole study population. *A. baumannii* have become established in this hospital as a part of a global epidemic of successful clones. Once introduced into the hospital, such clones have become entrenched among patients in the ICU.

Introduction

Acinetobacter baumannii is a major human pathogen and a leading cause of healthcare-associated infections; its primary source is not well understood, and the role of community sources is a matter of speculation. A rapid global expansion of a limited number of related lineages of *A. baumannii* resistant to several antimicrobial agents has been observed.[6] Several of these strains carry the $bla_{\text{OXA-23}}$-like, $bla_{\text{OXA-58}}$-like, and $bla_{\text{OXA-24/40}}$-like genes that may confer carbapenem resistance. In Latin America, high frequencies of *Acinetobacter*-related infections have been described since the late 1990s.[8] In Brazil, as observed in Europe and other parts of the world, the dissemination *A. baumannii* clones characterized by typical pulsed-field gel electrophoresis (PFGE) patterns was described in 2004.[9] This organism caused about 11% of the bloodstream infections (BSIs) detected in a surveillance system established at public hospitals in the country from 2007 to 2010.[20]

The development of multilocus sequence typing (MLST) simplified significantly the analysis of relatedness among *A. baumannii* international clones. Three European clones

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previously defined by amplified fragment length polymorphism and PFGE fingerprints were then recognized as International Clone (IC) I, II, and III by MLST.[2,6,24] The successful dissemination of these strains was possibly related to their ability to accumulate resistance genes. However, other species, such as *Acinetobacter nosocomialis*, previously known as genomic species (gen. sp.) 13TU,[25] may also accumulate resistance genes.[4,17] MLST schemes developed for *A. baumannii* allow definition of *A. nosocomialis* lineages, though no international clone of this species has been described to date.[30]

There are currently two MLST schemes in use to define *A. baumannii* sequence types (STs) and clonal complexes (CCs): one hosted at Institut Pasteur (IP, www.pasteur.fr) and another at the University of Oxford (UO, www.pubmlst.org). In 2011, the spread of two multidrug-resistant OXA-23-producing *A. baumannii* CCs was described throughout eight hospitals in Rio de Janeiro: CC 113/79 (UO/IP) and CC 104/15 (UO/IP).[3,10] Recently, transmission of an IC I $bla_{\text{OXA-23}}$-positive *A. baumannii* isolate from a donor lung to a transplant recipient was reported in Rio de Janeiro.[22]

Published data about the isolation frequency of *Acinetobacter* in Brazil are restricted to case–series studies, and refer main to collections of carbapenem-resistant isolates.[7,8,21,27] Such studies are unlikely to provide estimates of the distribution of the *Acinetobacter* lineages causing colonization and infection. This prospective cohort study was carried out at a university-affiliated hospital in Rio de Janeiro, Brazil, where, in recent years, *Acinetobacter* isolates became endemic agents of healthcare-associated infections. Its primary aim was to describe the distribution of *Acinetobacter* species isolated from patients admitted to the intensive care unit (ICU) of this hospital and to characterize the relatedness of local strains to internationally circulating clones. We hypothesized that patients staying in the ICU served as an active ground for *Acinetobacter* species multiplication and spread.

Materials and Methods

Setting

Hospital Universitário Clementino Fraga Filho (HUCFF) is a 474-bed public university hospital in Rio de Janeiro, Brazil. *Acinetobacter*-related infections were rare at HUCFF until 2000 when *A. baumannii* emerged as a main agent of hospital-acquired primary BSI (data not shown) and, ever since, has become endemic. Four years later, carbapenem resistance was first detected in *A. baumannii* at HUCFF. The 16-bed medical-surgical ICU, where the study was performed, had about 49 admissions per month during the study period. Hand hygiene facilities were conveniently located with six sinks, chlorhexidine liquid soap, and paper towels. Alcohol-based gel dispensers were available at the bedside. The environment was cleaned as suggested by the Infection Control Committee using alcohol- or chlorine-based solutions. The study was approved by the Institutional Review Board of HUCFF (protocol No. 120/06).

Study design and definitions

This report analyzes data from a prospective cohort study of colonization or infection by *Acinetobacter* species among patients admitted to the ICU from April 17, 2007, through April 14, 2008. Subjects staying in the ICU for ≥72 h were included in the study. Patients admitted to the ICU were screened for colonization with *Acinetobacter* isolates by cultures of respiratory (tracheal or oropharyngeal) secretions and rectal swab specimens at admission, on the third day after admission, and then weekly until discharge or death. A case of colonization was defined as a patient with the isolation of an *Acinetobacter* isolate from a nonsterile site, with no evidence of infection. A case of infection was defined as any patient with symptoms or signs of infection, according to the judgment of the Infection Control Committee medical staff and isolation of *Acinetobacter*, following standard infection definitions.[15] Clinical specimens for diagnosis of infection were collected according to the attending physician's judgment and were routinely cultured at the clinical microbiology laboratory of HUCFF, with no interference of the study team. *Acinetobacter* isolate detection was stratified by occurring within or after 24 h of admission to the ICU. When the first *Acinetobacter* isolate was detected within 24 h of admission, the strain was classified as imported to the ICU, and when detected after 24 h of admission and preceded by a negative screening specimen, the strain was classified as acquired in the ICU.

Microbiological procedures

Clinical specimens were collected with moistened swabs (screening specimens) or by standard techniques (for investigation of infectious episodes). Blood samples were inoculated into BacT/ALERT (BioMerieux, Askim, Sweden) bottles and subcultured onto blood agar and chocolate agar. Samples were subcultured onto MacConkey agar at 36°C for 48 h. After phenotypic identification, isolates were stored as 10% skim milk–10% glycerol suspensions at −20°C. Isolates identified as *Acinetobacter* species in the clinical microbiology laboratory of HUCFF were also stored. Susceptibility to 11 antimicrobial agents was determined for all isolates by disk diffusion; minimum inhibitory concentrations (MICs) were determined in triplicate by the broth microdilution test to colistin, and by Etest (bioMérieux, Solna, Sweden) to imipenem and meropenem for *A. baumannii* isolates that caused infection.[5] Multidrug resistance (MDR) was defined as resistance to drugs belonging to at least three of the following drug classes: aminoglycosides (amikacin, gentamicin, and tobramycin), ampicillin-sulbactam, carbapenems (imipenem and meropenem), fluoroquinolone (ciprofloxacin), colistin, extended-spectrum cephalosporin (cefepime and ceftazidime), piperacillin-tazobactam, and trimethoprim-sulfamethoxazole.[19] Extensively, drug-resistant (XDR) isolates were those susceptible to drugs in only one or two of these antimicrobial categories.[19]

Species identification

A partial *rpo*B gene sequence was analyzed as proposed.[12] Polymerase chain reaction (PCR) amplicons were purified with the QIAquick DNA kit (Qiagen, CA), sequenced, and 351-bp overlapping sequences were compared to a set of reference strains.[12]

Detection of carbapenemase encoding genes

Production of metallo-β-lactamase was screened by a double-disk synergy test as described previously.[26] The

presence of the following carbapenemase-encoding genes was investigated by a multiplex PCR: bla_{OXA-23}-like, bla_{OXA-24}-like, bla_{OXA-51}-like, bla_{OXA-58}-like, $bla_{OXA-143}$, bla_{GIM-1}, bla_{IMP}-type, bla_{SIM-1}, bla_{SPM-1}, and bla_{VIM}-type.[14,23] The nucleotide sequences were determined for all representative bands and compared to reference sequences in the GenBank database.

Strain typing

All *Acinetobacter* isolates were typed by random amplification of polymorphic DNA (RAPD)-PCR with primer M-13, as described.[11] Band patterns were analyzed with GelCompar version 4.01 (Applied Maths, Kortrijk, Belgium) using the Dice coefficient and unweighted pair group method with arithmetic average. Isolates with 100% level of similarity were included in a single RAPD genotype. To validate RAPD-PCR results, a sample of 15 *A. baumannii* and 3 *A. nosocomialis* isolates of the predominant RAPD types were selected for PFGE, performed with *Apa*I.[28]

Multilocus sequence typing

Isolates from the predominant *A. baumannii* RAPD types were selected for MLST analysis. MLST schemes hosted at IP (www.pasteur.fr) and at the UO (PubMLST, www.pubmlst .org) were performed as suggested.[2,24] CCs (referred from now on by the UO/IP scheme) were defined for isolates with five or more identical alleles by eBURST software (http:// eburst.mlst.net) and minimum spanning tree analysis (www .pasteur.fr), for data obtained with UO and IP schemes, respectively.

Statistical analysis

Fisher or chi-square test was used to compare resistance prevalence with EPI Info 6.0 software. Statistical significance was defined as $p < 0.05$.

Results

Study population, detection of Acinetobacter, and identification of species

During the study period, 502 patients were admitted to the ICU: 235 (46.8%) stayed ≥72 h in the ICU, and 1 patient did not consent to participate. Of the 234 patients included in the study, 98 (41.9%) had at least one specimen positive for *Acinetobacter* species. In 32 patients (13.7% of the 234), the first screening was positive within 24 h of ICU admission: 22 were from other wards in HUCFF, 7 from other hospitals, and 3 from the emergency room. Among 202 patients who had a negative screening at admission, 66 were positive after 24 h of ICU hospitalization (late acquisition), with an overall cumulative incidence of 32.7 case–patients/100 admissions. Infections caused by *Acinetobacter* species occurred in 24 patients (prevalence: 10.2/100 admissions), including BSI ($n = 14$), ventilator-associated pneumonia ($n = 7$), infection of a vascular access ($n = 2$), and intra-abdominal infection ($n = 1$). In 5 cases, infection was present at admission; other 19 cases occurred after 24 h, with a cumulative incidence of 9.4/100 admissions.

A total of 255 *Acinetobacter* isolates were obtained from the 98 patients (1–14 per patient), distributed into five species. The predominant species was *A. baumannii*: 177 isolates obtained from 70.4% ($n = 69$) of the patients. The second most frequent species was *A. nosocomialis*: 72 isolates from 48% ($n = 47$) of the patients. Other species isolated were as follows: *Acinetobacter soli*, obtained from three patients; *Acinetobacter berezinae*, obtained from two patients; and one *Acinetobacter* gen. sp. close to 13TU. Eighteen patients (18.3%) had *A. baumannii* and *A. nosocomialis* isolates concomitantly. Positive specimens were mostly tracheal secretions and rectal swabs. *A. soli* and *Acinetobacter* gen. sp. close to 13TU were obtained only from oropharyngeal swabs. Of the 24 infections, 22 (91.7%) were caused by *A. baumannii*, 1 by *A. nosocomialis*, and 1 by *A. berezinae*. The monthly prevalence and distribution of cases of colonization or infection caused by *A. baumannii* and *A. nosocomialis* are shown in Figure 1. The prevalence of patients with *A. baumannii* during December 2007 (37.8%) was higher than the maximum expected value of 35.5% (mean monthly prevalence $\pm 1.96 \times$ standard deviation $= 15\% \pm 20.5$); thus, a cluster of cases occurred during this month. An elevated number of *A. baumannii* infections diagnosed after 24 h in the ICU contributed for this increase. The time-based distribution of *A. nosocomialis* (Fig. 1) did not show clusters.

Strain types

RAPD typing of *A. baumannii* identified 28 types. Representative RAPD fingerprints are shown in Supplementary Figure S1 (Supplementary Data are available online at www.liebertpub.com/mdr). PFGE performed for 18 isolates showed equivalent clusters, with up to four band differences among isolates of the same type. Ten representative isolates of the five predominant types (A to E, two of each) were selected for MLST. For internal analysis of the transmission dynamics of clones within the study ICU, isolates with an RAPD band profile indistinguishable from the one selected for MLST were considered as belonging to the same CC.

Both MLST schemes clustered the representative isolates of Rio de Janeiro into four CCs, including IC I, as shown in Table 1. Clusters formed by the UO scheme and by the IP scheme were congruent. CCs corresponded to seven STs in the UO scheme, and none of them previously deposited in the PubMLST database, and five STs in the IP scheme, including a new one from a single isolate. The most common RAPD types B and E, corresponding to CC (113/79), affected 24 (34.8%) of the patients with *A. baumannii*, 10 of whom (42%) had infections, and was detected in 11 of the 13 study months, as shown in Figure 2. The second most common RAPD type A (CC 104/15) affected 13 (18.8%) of the patients.

RAPD typing of *A. nosocomialis* identified 12 types; a single type, called A, affected 24 (24.4%) of the patients (Fig. 2) and caused the single infection by this species. The two *A. nosocomialis* RAPD type-A isolates selected for MLST were included in a single ST by both MLST schemes: ST 260 of UO scheme and a new ST by IP scheme.

In Figure 3, cases caused by mainly *A. baumannii* and *A. nosocomialis* types are stratified by the supposed place of acquisition: within the ICU, which included most isolates; or imported to the ICU. Additionally, imported cases are further classified by the place of patient's origin—the hospital ward where patient was admitted before the ICU. *A. baumannii* strains were mostly introduced into the ICU with patients transferred from other HUCFF wards, while *A. nosocomialis* was introduced with subjects admitted from the emergency room and other hospitals.

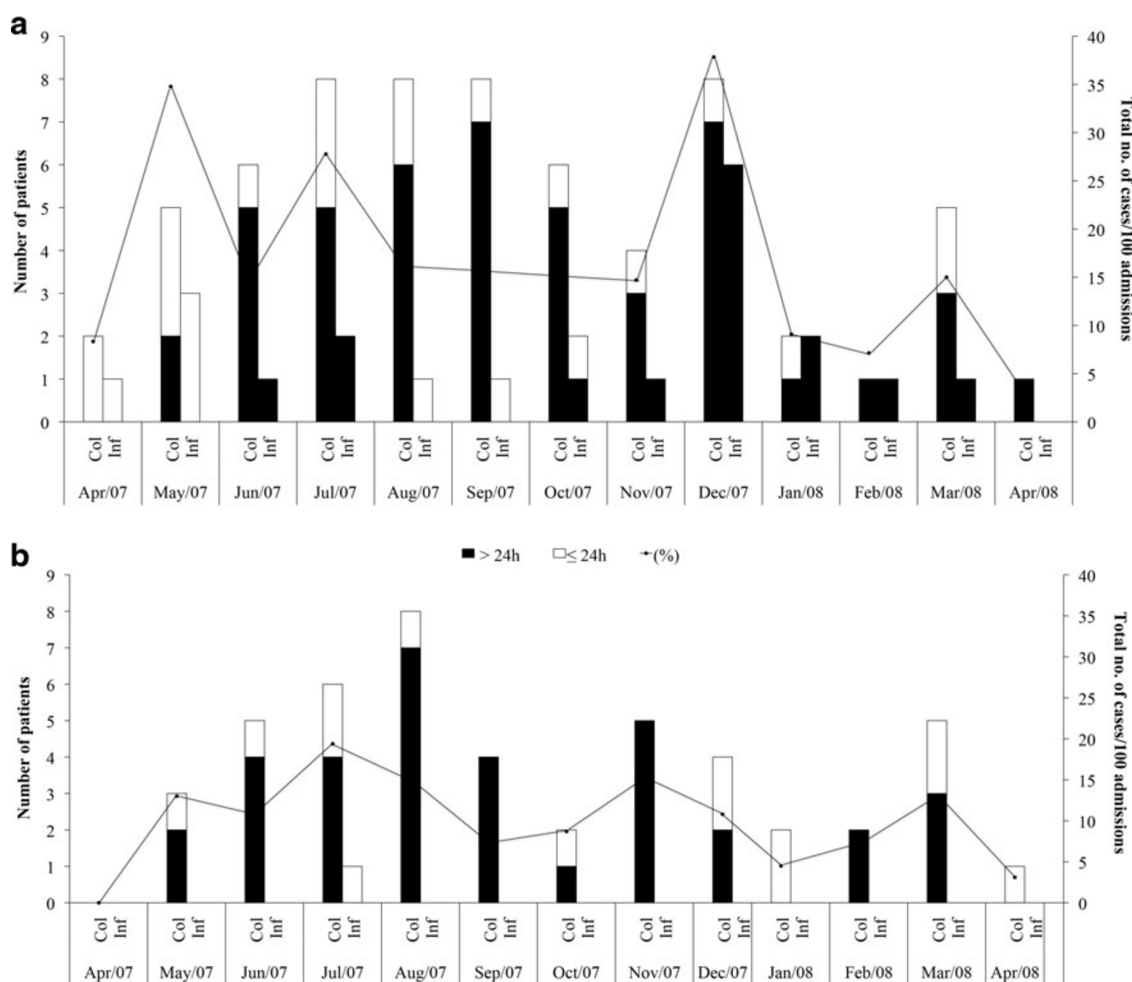

FIG. 1. Prevalence curve and distribution of cases of infection (Inf) and colonization (Col) detected >24 h (gray bar) or ≤24 h (white bar) after admission by *A. baumannii* **(a)** and *A. nosocomialis* **(b)**. Line indicates number of cases per 100 admissions.

Antimicrobial susceptibility and presence of carbapenemase genes

Most isolates included in the major *A. baumannii* lineages were XDR. The 17 (25%) carbapenem-susceptible *A. baumannii* isolates were diverse, belonging to 13 RAPD types. A resistance prevalence of >50% to an antimicrobial agent and an MDR or XDR phenotype were significantly more frequent in *A. baumannii* than in *A. nosocomialis* ($p < 0.05$, Table 2). All 22 *A. baumannii* isolates that caused infections had MICs of meropenem and imipenem >32 μg/ml. The six isolates of *A. soli*, *A. berezinae*, and *A.* gen. sp. close to 13TU were susceptible to all antimicrobial agents tested.

All *A. baumannii* isolates had the natural β-lactamase-encoding $bla_{oxa\text{-}51}$-like gene. The $bla_{OXA\text{-}23}$-like gene was detected in 144 (81%) of the *A. baumannii* and in two

Table 1. *Acinetobacter baumannii* Multilocus Sequence Types and Clonal Complexes

Genotype[a]/number (%) of patients	*Date of isolation*	*City*	*ST-UO (isolates sequenced)*	*ST-IP (isolates sequenced)*	*CC-UO/CC-IP (International Clone)*
B/14 (20.3)	Sep/07	Rio de Janeiro	227 (2)	79 (2)	113/79
E/10 (14.5)	Jul/07		227 (1)	79 (1)	
	Jan/08		230 (1)	156 (1)	
A/13 (18.8)	Jul/07	Rio de Janeiro	225 (1)	15 (2)	104/15
	Mar/08		226 (1)		
C/12 (17.4)	May/07	Rio de Janeiro	228 (1)	25 (2)	110/110
			229 (1)		
D/11 (15.9)	Apr/07, Aug/07	Rio de Janeiro	231 (2)	160 (2)	109/1 (I)

[a]Defined by RAPD and/or PFGE.

ST, sequence type; UO, University of Oxford MLST scheme; IP, Institut Pasteur scheme; CC, MLST clonal complex; RAPD, random amplification of polymorphic DNA; PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing.

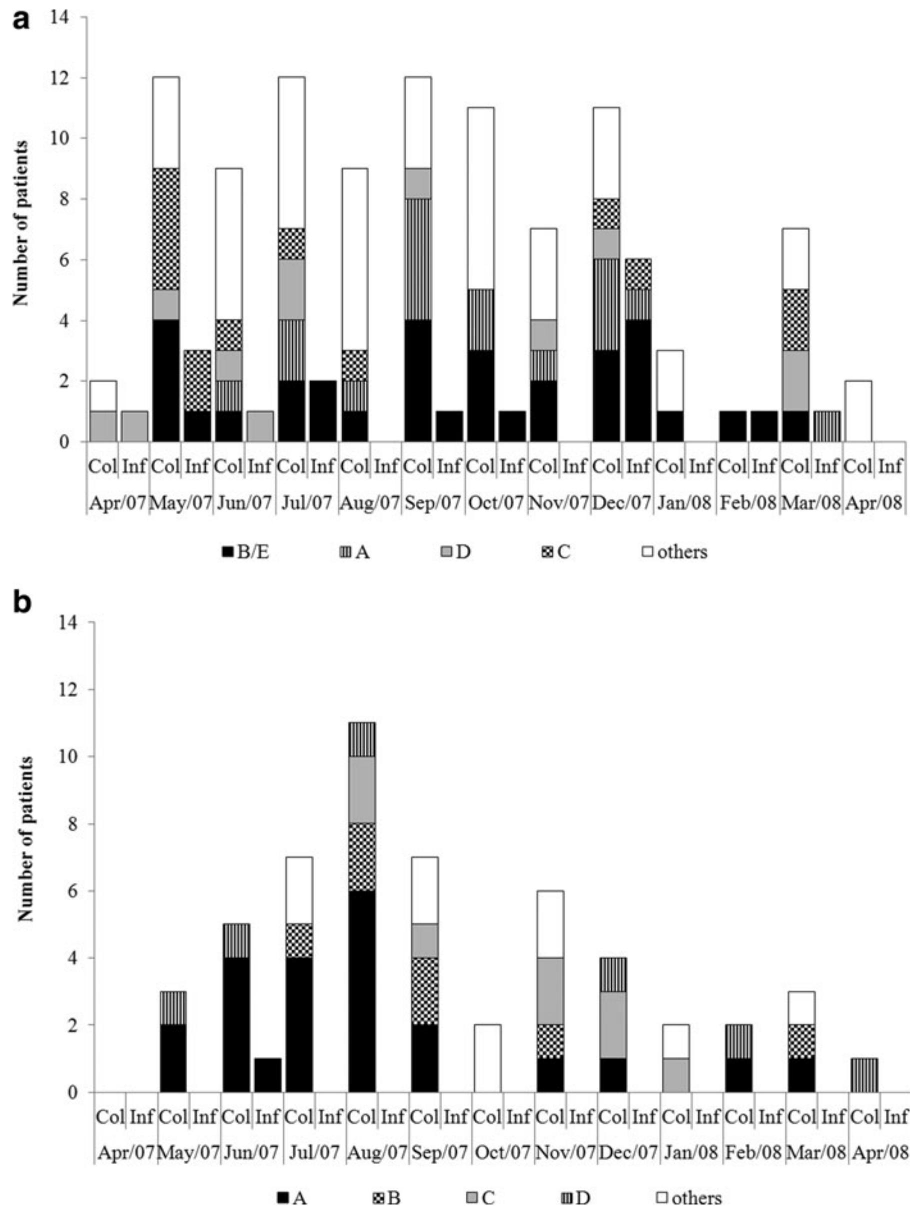

FIG. 2. Temporal distribution of *A. baumannii* **(a)** and *A. nosocomialis* **(b)** random amplification of polymorphic DNA (RAPD) types affecting ≥5 patients. RAPD types B/E, A, C, and D corresponded to following clonal complexes (University of Oxford and Institut Pasteur multilocus sequence typing scheme), 113/79, 104/15, 110/110, and 109/1, respectively. Other RAPD types affected <5 patients.

A. nosocomialis isolates. Resistance prevalence was significantly higher ($p < 0.05$) in *A. baumannii* containing the bla_{OXA-23}-like gene than in the bla_{OXA-23}-like gene negative for amikacin, ampicillin-sulbactam, cefepime, ciprofloxacin, ceftazidime, piperacillin-tazobactam, carbapenems, and trimethoprim-sulfamethoxazole. The bla_{OXA-23}-like gene-positive *A. baumannii* isolates were distributed in all CCs; no other bla_{OXA} genes or metallo-β-lactamase-encoding genes were found.

Discussion

This is the first cohort study of patients presenting colonization and infection by *Acinetobacter* species, including susceptible and resistant isolates with strain typing data from Brazil. We describe a high incidence of colonization and infection by *Acinetobacter* isolates in the ICU of a large urban public referral hospital over 1 year; most of these were related to hospital transmission of highly successful clones. Prospective surveillance led to the observation that imported and ICU-acquired colonization by *Acinetobacter* species occurred, respectively, in 13.7% and 32.7% of patients. Those numbers are very high, twice as many as those detected in a similar study by Arvaniti and collaborators in a Greek ICU, where 5.6% and 15.7% of patients had imported and ICU-acquired colonization by *Acinetobacter* species.[1] Notably, about 24% of the colonized patients in the present study had infections.

Most of the isolates included in the main CCs were ICU born or came from other wards of the same hospital. The *A. baumannii* polyclonal outbreak in December 2007, with a high number of infections, suggests a deterioration in the quality of healthcare.

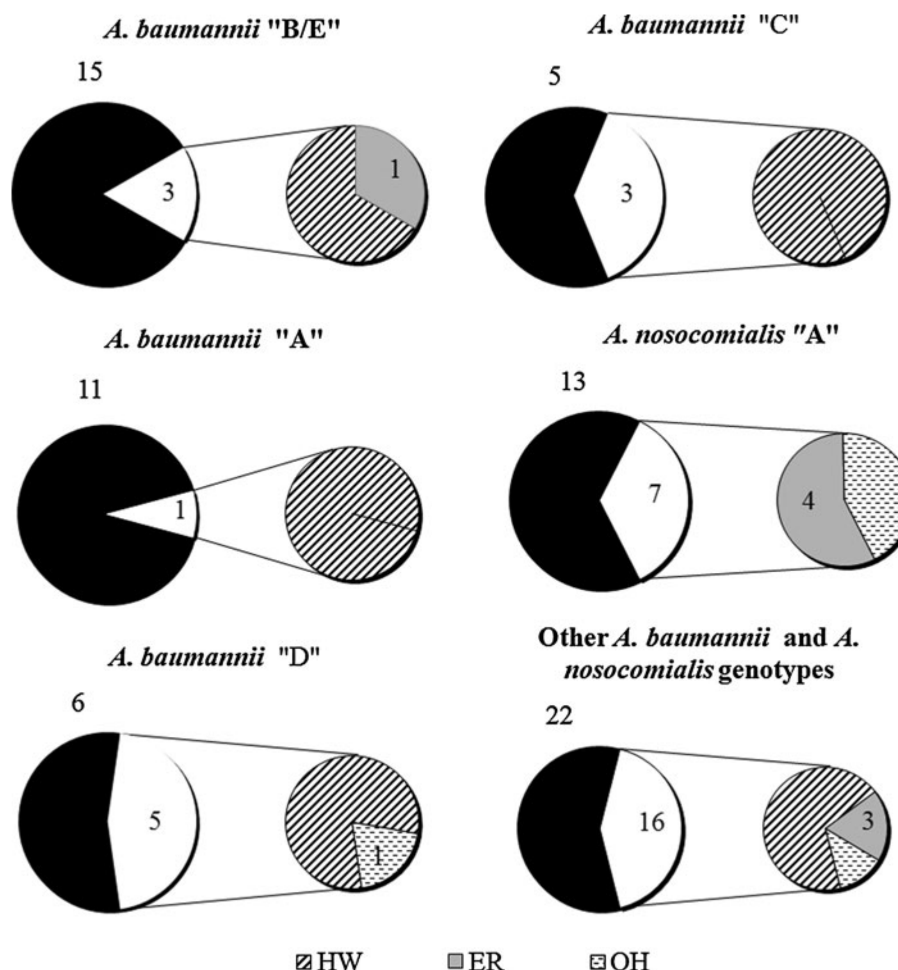

FIG. 3. Representation of patients with main *A. baumannii* RAPD types (University of Oxford/Institute Pasteur [UO/IP] schemes) and *A. nosocomialis* genotype A (UO scheme) imported to the intensive care unit (ICU). RAPD types B/E, A, C, and D corresponded to following clonal complexes (UO/IP multilocus sequence typing scheme), 113/79, 104/15, 110/110, and 109/1, respectively. Only one isolate of each RAPD genotype per patient was included. White areas are cases diagnosed <24 h of admission. Black areas are cases diagnosed >24 h of admission. HW: patients from non-ICU wards of HUCFF; ER: patients from emergency room; and OH: patients from other hospitals.

Table 2. Antimicrobial Resistance Prevalence in *Acinetobacter baumannii* and *Acinetobacter nosocomialis* Isolates

	Number and (%) of resistant isolates[b]		
Antimicrobial agent[a]	A. baumannii *(n = 69)*	A. nosocomialis *(n = 47)*	*Total (N = 116)*
Trimethoprim-sulfamethoxazole	63 (91)	31 (66)	94 (81)
Ciprofloxacin	64 (92)	11 (23)	75 (64)
Cefepime	61 (88)	11 (23)	72 (62)
Piperacillin-tazobactam	59 (85)	4 (8)	63 (54)
Carbapenems[c]	52 (75)	5 (10)	57 (49)
Ampicillin-sulbactam	52 (75)	4 (8)	56 (48)
Ceftazidime	51 (74)	5 (10)	56 (48)
Tobramycin	41 (59)	10 (21)	51 (44)
Gentamicin	39 (56)	11 (23)	50 (43)
Amikacin	36 (52)	6 (13)	42 (36)

[a]$p < 0.05$ for all comparisons between resistance in *A. baumannii* and *A. nosocomialis*.
[b]A single isolate of each RAPD genotype of each species per patient is included.
[c]Isolate resistant to meropenem or imipenem.

RAPD typing, validated by PFGE and complemented with MLST, was convenient and allowed classification of isolates into international clones. Strain typing detected a highly clonal population of *A. baumannii* isolates in the ICU, indicating intense cross-transmission of strains. The frequent admission of patients carrying successful clones from other wards or hospitals and poor staffing (not measured in the study) might have facilitated this scenario.

All STs detected by the UO scheme were new, but just one of the IP schemes was not described previously. The UO MLST scheme was more discriminatory than the IP scheme, possibly due to significant variations in the *gyr*B and *gpi* genes, as observed previously.[13] The predominant CC 113/79 was originally described in Argentina, and is highly successful in Rio de Janeiro.[10] This CC affected the largest number of patients, followed by CC 104/15. These data are in line with the study by Grosso and collaborators, performed with 96 *A. baumannii* isolates from Rio de Janeiro; CC 113/79 was also the predominant (70%), followed by CC 104/15 (25%), present in seven and five hospitals of the city, respectively.[10] In contrast with those authors, the present study also found that CC 110 and IC I strains disseminated in 17% and 16% of the patients with *A. baumannii*-positive cultures, respectively. The oldest IC I strain (HK302) was isolated in 1977 in Switzerland[18] and subsequently disseminated widely in Europe, East Asia, and Australia (http://pubmlst.org/abaumannii/).

Although *A. nosocomialis* was frequently isolated from screening specimens, it caused only one case of BSI in the present study. Recently, *A. nosocomialis* was reported as more prevalent than *A. baumannii* in blood cultures from patients admitted to hospitals in Norway.[16] Unfortunately, the distribution of *Acinetobacter* species by ward was not described in that report. In the present study, a single lineage of *A. nosocomialis* affected more than 10% of all patients. In fact, either this species has a clonal structure, or *A. nosocomialis* ST260 (UO scheme) is a highly successful clone in this hospital. Moreover, two $bla_{OXA\text{-}23}$ gene-positive *A. nosocomialis* isolates recovered could indicate that this *Acinetobacter* species may provide, indeed, an efficient reservoir of resistance determinants. Another study documented the dissemination of a single *A. nosocomialis* strain, characterized by ribotyping, PFGE and amplified ribosomal DNA restriction analysis, over 23 patients, causing an outbreak in an ICU in the Netherlands.[29]

In conclusion, the intense input of patients carrying *Acinetobacter* species observed in the ICU showed that subjects from other wards of the same hospital served as reservoirs of the pathogen.

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Author Disclosure Statement

All authors report no conflicts of interest relevant to this article.

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