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Clinical and microbiological characteristics of OXA-23- and OXA-143-producing *Acinetobacter baumannii* in ICU patients at a teaching hospital, Brazil

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ABSTRACT

Background: Carbapenem-resistant *Acinetobacter baumannii* (CRAb) is an important cause of nosocomial infections especially in intensive care units. This study aimed to assess clinical aspects and the genetic background of CRAb among ICU patients at a Brazilian teaching hospital.

Methods: 56 critically ill patients colonized or infected by CRAb, during ICU stay, were prospectively assessed. Based on imipenem MIC $\geq 4\,\mu g/mL$, 28 CRAB strains were screened for the presence of genes encoding metallo-β-lactamases and OXA-type β-lactamases. The blaOXA-type genes were characterized by PCR using primers targeting ISAba-1 or -3. Genetic diversity of blaOXA-positive strains was determined by ERIC-PCR analysis.

Results: Patient's mean age (±SD) was 61 (±15.1), and 58.9% were male. Eighty-percent of the patients presented risk factors for CRAb colonization, mainly invasive devices (87.5%) and previous antibiotic therapy (77.6%). Thirty-three patients died during hospital stay (59.0%). Resistance to carbapenems was associated with a high prevalence of blaOXA-23 (51.2%) and/or blaOXA-143 (18.6%) genes. ERIC-PCR genotyping identified 10 clusters among OXA-producing CRAb. Three CRAb strains exhibited additional resistance to polymyxin B (MIC $\geq 4\,\mu g/mL$), whereas 10 CRAb strains showed tigecycline MICs $> 2\,\mu g/mL$.

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Conclusions: In this study, clonally unrelated OXA-123- and OXA-143-producing *A. baumannii* strains in ICU patients were strongly correlated to colonization with infected patients being associated with a poor outcome.

Introduction

Acinetobacter baumannii is a Gram-negative non-fermentative coccobacillus that has gained growing notoriety as a nosocomial pathogen, showing patterns of increasing resistance to antimicrobial drugs and disinfectants. These organisms are implicated in a diverse array of infections, especially in intensive care units (ICUs) associated with countless outbreaks.[1,2] Classically, carbapenems plays a crucial role in the treatment of serious nosocomial infections due to *A. baumannii*, but this scenario has changed substantially in the last decade, with an increasing prevalence of carbapenem-resistant and multidrug-resistant isolates.[3]

Carbapenem resistance in *A. baumannii* is mainly due to the presence of β-lactamases (class B metallo-β-lactamases – MBL, or class D OXA-type β-lactamases – oxacillinases – OXA), to the loss of outer membrane proteins,[4] and to altered penicillin-binding proteins (PBP).[5] OXA-type β-lactamases are chromosomal enzymes that in *A. baumannii* can be intrinsic (e.g., OXA-51-like) or, more frequently, acquired (e.g., OXA-23-like, OXA-24-like, OXA-58-like).[6] Outbreaks of OXA-23-producing *A. baumannii* have been reported in some Brazilian hospitals. Moreover, a new oxacillinase, OXA-143-like, was recently described in this country.[3,7–9]

Only few studies concerning the molecular basis of carbapenem resistance in *A. baumannii*, as well as the clinical impact of these resistant strains on patient outcomes, have been reported, despite the growing importance of these strains in ICUs of Brazilian hospitals.[3,7,8]

Thus, the aim of this study was to investigate the genetic determinants of carbapenem resistance and the clonal relatedness of carbapenem-resistant *A. baumannii* (CRAb) strains isolated from patients admitted to a Brazilian intensive care unit.

Materials and methods

Study setting and population

This study was conducted in a medical ICU of a 500-bed tertiary care teaching hospital, affiliated to the Federal University of Minas Gerais (UFMG), in Brazil. All adult (≥18 years) patients colonized or infected by CRAb during ICU stay between December 2009 and December 2010 were assessed for potential inclusion in the study. Demographic and clinical data were retrospectively obtained from a data bank prospectively filled, which is used for research and administrative issues. Colonization was defined by the presence of CRAb on skin, on mucous membranes, in open wounds, or in excretions or secretions obtained during routine surveillance for multiresistant bacteria. According to the protocol followed in our ICU, surveillance swabs are obtained upon admission and weekly up to discharge from the unit.[10] Infection was defined as the presence of CRAb identified in specimens associated with infection reported according to NHSN criteria. For both cases, colonization and infection, only the first isolate was considered.

Ethical aspects

This study was approved by the Institutional Research Ethical Committee. Privacy was guaranteed and patients were identified by their hospital registration numbers. Only researchers had access to the information of the enrolled patients.

Variables and definitions

Baseline demographic and clinical data were obtained from medical charts. Colonization was defined as the presence of microorganisms on skin, on mucous membranes, in open wounds, or in excretions or secretions, unassociated with clinical signs or symptoms of infection during routine surveillance or specimens not associated to infections.[10] Infection was defined as identification of CRAb in clinical specimens associated with infection reported according to NHSN criteria site. Baseline demographic and clinical data were obtained from medical charts.

Laboratory tests

CRAb strains and antimicrobial susceptibility testing

CRAb isolates were identified using the VITEK 2 system (bioMérieux, France®). Antimicrobial screening was carried out using the Kirby-Bauer disk diffusion method.[11] The confirmatory susceptibility test was performed using the agar dilution method for imipenem, tigecycline, and polymyxin B, as recommended by the Clinical and Laboratory Standards Institute and the Food and Drug Administration (FDA).[11] FDA-approved tigecycline breakpoints for Enterobacteriaceae, were applied to all isolates (susceptible ≤2 μg/mL; resistant ≥8 μg/mL).

Detection of carbapenemases

A screening test for MBL production was performed using the double-disk synergy test (DDST), as recommended by Arakawa et al. Phenotypic detection of MBLs was performed using

Etest® MBL strips, following the manufacturer's recommendations (AB bioMérieux, St. Louis, MO, USA).

Polymerase chain reaction (PCR) amplification and DNA sequencing

Multiplex PCR was performed to investigate the genes encoding the following carbapenemases: $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-143-like}}$, $bla_{\text{VIM-like}}$, $bla_{\text{IMP-1}}$, and $bla_{\text{IMP-like}}$. Specific primers were used to identify the insertion sequences (ISAba1 and ISAba3).[12]

ERIC-PCR typing

The genetic similarity of *A. baumannii* isolates was determined using Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR). A similarity matrix was calculated using the DICE coefficient with 2% tolerance. Percent similarity determination, cluster calculation, and subsequent dendrogram construction were performed using the Bio Numerics software.[13]

Statistical analysis

Clinical and epidemiological characteristics were described. The distribution of continuous variables was tested, and their normal or non-normal distributions presented as mean ± standard deviation or median (IQR), respectively. Categorical variables were analyzed using frequencies and percentages. Associations between β-lactamases and ICU mortality were tested using chi-square test. The Statistical Package for the Social Sciences (SPSS), version 15.0 (Chicago, IL), was used for all analyses. Statistical significance was determined as $p \leq 0.05$ and comparative analyses are presented with the 95% confidence interval (95% CI).

Results

Patients characteristics

Overall, 867 patients were admitted on the ICU during the study period, 56 of whom had positive results for carbapenem-resistant *A. baumannii* cultures in clinical samples (e.g., blood, tracheal aspirate, urine, or wound secretion) or surveillance swabs. Forty-three out of the 56 strains were subjected to quantitative testing, as well as phenotypic and genotypic resistance evaluation. The characteristics of patients infected or colonized by CRAb are shown in Table 1. From a total of 56 patients, 38 (67.9%) were colonized and 18 (32.1%) were infected with CRAb. Table 2 presents the type of CRAb isolation (colonization or infection), site of infection, and patient outcome. The majority of patients (83.9%) had comorbidities, and the most common condition was diabetes mellitus (33.9%). Eighty-seven percent of the patients had previously received invasive devices and 78.6% had received antimicrobial therapy, mainly cephalosporins (58.9%), carbapenems (33.9%), and glycopeptides (26.8%). Among the 18 infected patients, 10 (55.5%) had pneumonia, six (33.3%) had bloodstream infections, one (5.6%) had urinary tract infection, and one (5.6%) presented an intra-abdominal infection. Overall, 33 out of the 56 patients (59%) died during hospital stay.

Table 1 – Demographic data of ICU patients with CRAb.

Parameters	*n* (±SD)	%
Patient characteristics		
Age (years)	61.0 ± 15.1	–
Male/female	33/23	–
ICU stay (days)	18.7 ± 16.5	–
APACHE II score	14.3 ± 6.0	–
Co-morbidities (%)	47	83.9
Underlying pathology		
Hematological malignancies	3	5.3
Solid tumor	2	3.6
Steroid use	5	8.9
Diabetes mellitus	19	33.9
Chronic kidney disease	13	23.2
Cardiovascular disease	14	25.0
Chronic obstructive pulmonary disease	10	17.8
Liver failure (Child B or C)	6	10.7
Previous invasive devices	49	87.5
Central venous catheter	38	67.9
Foley catheter	46	82.1
Mechanical ventilation	46	82.1
Previous antimicrobial use	44	78.6
Carbapenems	19	33.9
Cephalosporins 3rd Gen.	18	32.1
Cephalosporins 4th Gen.	15	26.8
Quinolones	9	16.0
β-Lactamase inhibitors	12	21.4
Glycopeptides	15	26.8

Table 2 – Classification of the patients according to CRAb colonization or infection.

Parameters	*n*	%
Type of isolation		
Colonization	38	67.9
Nosocomial infection	18	32.1
Site of infection		
Intra-abdominal	1	5.6
Lungs (pneumonia)	10	55.5
Bloodstream (catheter-associated)	5	27.7
Bloodstream (primary)	1	5.6
Urinary tract	1	5.6
Progression of infection		
Discharge	23	41.0
Death	33	59.0

Phenotypic antimicrobial susceptibility of isolates

The antimicrobial susceptibility profiles of the CRAb isolates, defined by disk diffusion and agar dilution methods, are presented in Table 3. Gentamicin turned out to be the best antimicrobial in terms of susceptibility (33.9%), followed by sulfamethoxazole/trimethoprim (21.4%). Resistance to imipenem was confirmed for only 58.1% of all *A. baumannii* strains. Polymyxin and tigecycline resistance was seen in three (7.0%) and 10 (23.3%) out of all the 43 strains studied, respectively.

Table 3 – Clinical and microbiological characteristics of OXA-producing *Acinetobacter baumannii* strains isolated from ICU patients.

CRAb strain	Sample	Resistance profile				*bla*$_{OXA}$-type gene[a]	ERIC profile[b]	S/Age (y)	Admission (m/d/y)	Apache II	Clinical status/ATM	Isolation data	Clinical outcome	Invasive devices
		MIC (μg/mL)		Kirby-Bauer										
		IMP	POL	TIG	Antimicrobial drugs									
288	Nasal	>32	1.0	1.0	AK, SAM, CFP, CTZ, CIP, MER, PTZ	*bla*$_{OXA-51}$, *bla*$_{OXA-143}$	G2	M/71	12/02/09	23	Colonized/No	12/13/09	Death	CVC + IUC + MV
295	Nasal	>32	1.0	2.0	AK, SAM, CFP, CFZ, CIP, MER, PTZ	*bla*$_{OXA-51}$, *bla*$_{OXA-143}$	G4	M/56	01/03/10	15	Colonized/Yes	01/19/10	Survival	CVC + IUC + MV
296	Perianal	>32	1.0	2.0	AK, SAM, CFP, CFZ, CIP, MER, PTZ	*bla*$_{OXA-51}$, *bla*$_{OXA-143}$	H	M/49	01/19/10	16	Colonized/No	01/19/10	Death	–
297	Perianal	>32	1.0	1.0	AK, SAM, ATM, CFP, CTZ, CIP, GEN MER	*bla*$_{OXA-51}$, *bla*$_{OXA-143}$	I	F/77	12/08/09	12	Colonized/Yes	01/19/10	Death	CVC + IUC + MV
356	Nasal	4.0	0.5	<0.5	AK, SAM, ATM CFP, CTZ, CIP, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-143}$, *bla*$_{OXA-23}$	F	M/63	05/19/10	15	Colonized/Yes	05/19/10	Survival	MV
384	Nasal	>32	2.0	2.0	AK, SAM, CFP, CTZ, CIP, GEN, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-143}$, *bla*$_{OXA-23}$	F	M/58	05/13/10	9	Colonized/Yes	06/10/10	Survival	CVC + IUC + MV
385	Blood	>32	2.0	4.0	AK, SAM, CFP, CTZ, CIP, GEN, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-143}$, *bla*$_{OXA-23}$	F	M/75	06/11/10	10	NI-sepse/Yes	06/23/10	Death	CVC + IUC + MV
390	Perianal	8.0	4.0	4.0	SAM, ATM, CFP, CTZ, CIP, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	J1	F/45	07/20/10	15	Colonized/Yes	07/29/10	Survival	CVC + IUC + MV
394	Nasal	16	1.0	4.0	AK, SAM, CFP, CTZ, CTX, CIP, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	J1	F/83	07/10/10	15	Colonized/Yes	07/16/10	Death	CVC + IUC + MV
395	Perianal	16	0.5	2.0	AK, SAM, CFP, CTZ, CIP, MER, PTZ	*bla*$_{OXA-51}$, *bla*$_{OXA-143}$, *bla*$_{OXA-23}$	I	M/59	07/16/10	15	Colonized/Yes	07/28/10	Death	CVC + IUC + MV
399	Perianal	>32	8.0	4.0	SAM, ATM, CFP, CTZ, CIP, GEN, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	F	F/76	07/23/10	15	Colonized/No	07/24/10	Survival	IUC
407	Perianal	16	2.0	4.0	AK, SAM, CFP, CTZ, CIP, GEN, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	B	M/20	07/27/10	10	Colonized/Yes	08/02/10	Survival	CVC + IUC
408	Nasal	16	1.0	<0.5	AK, SAM, CFP, CTZ, CIP, GEN, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	H	M/68	07/28/10	18	Colonized/Yes	08/02/10	Death	CVC + IUC + MV
414	Nasal	>32	1.0	2.0	AK, SAM, ATM, CFP, CTZ, CIP, MER, PTZ, SUT	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	H	F/71	08/02/10	11	NI-sepse/No	08/07/10	Death	CVC + IUC + MV
416	Perianal	16	1.0	4.0	AK, CFP, CTZ, CIP, GEN, MER, PTZ	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	B	F/68	08/02/10	20	Colonized/Yes	08/06/10	Death	CVC + IUC + MV
417	Catheter	16	2.0	4.0	AK, SAM, ATM, CFP, CTZ, CIP, PTZ	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	A	M/47	08/03/10	20	NI-PNU/Yes	08/09/10	Survival	CVC + IUC + MV
421	Catheter	16	1.0	4.0	AK, SAM, ATM, CFP, CTZ, CIP, GEN, MER, PTZ	*bla*$_{OXA-51}$, *bla*$_{OXA-23}$	B	M/41	06/16/10	17	NI-sepse/Yes	08/17/10	Death	CVC + IUC + MV

Table 3 – (*Continued*)

CRAb strain	Sample	Resistance profile				bla_{OXA}-type gene[a]	ERIC profile[b]	S/Age (y)	Admission (m/d/y)	Apache II	Clinical status/ATM	Isolation data	Clinical outcome	Invasive devices
		MIC (μg/mL)		Kirby-Bauer										
		IMP	POL	TIG	Antimicrobial drugs									
436	Nasal	16	0.5	2.0	AK, ATM, CFP, CTZ, CIP, MER, PTZ, SUT	bla_{OXA-51}, bla_{OXA-23}	K	M/85	08/25/10	13	NI-PNU/No	09/01/10	Death	CVC + IUC + MV
461	Nasal	16	0.5	<0.5	AK, SAM, CFP, CTZ, CIP, MER, PTZ, SUT	bla_{OXA-51}, bla_{OXA-23}	K	M/79	09/16/10	13	NI-PNU/Yes	13/10/10	Death	CVC + IUC + MV
464	Nasal	16	1.0	2.0	AK, SAM, CFP, CTZ, CTX, CIP, MER, PTZ	bla_{OXA-51}, bla_{OXA-23}	K	F/85	08/27/10	–	Colonized/Yes	10/03/10	Death	CVC + IUC + MV
470	Nasal	>32	0.5	2.0	AK, SAM, ATM, CFP, CTZ, CTX, CIP, GEN, MER, PTZ, SUT	bla_{OXA-51}, bla_{OXA-23}	D	M/47	10/08/10	16	Colonized/Yes	10/15/10	Death	CVC + IUC + MV
479	Catheter	16	4.0	2.0	AK, ATM, CFP, CTZ, CTX, CIP, GEN, MER, PTZ, SUT	bla_{OXA-51}, bla_{OXA-23}	O	F/65	09/30/10	13	Colonized/Yes	10/20/10	Survival	CVC + IUC + MV
487	Perianal	16	0.5	4.0	AK, SAM, ATM, CFP, CTZ, CIP, GEN, MER, PTZ, SUT	bla_{OXA-51}, bla_{OXA-23}	G	M/53	10/17/10	9	Colonized/Yes	10/23/10	Survival	CVC + IUC + MV
489	Nasal	4.0	1.0	4.0	AK, SAM, ATM, CFP, CTZ, CIP, GEN, MER, PTZ	bla_{OXA-51}, bla_{OXA-23}	O	M/64	10/22/10	10	NI-sepse/No	10/26/10	Death	CVC + IUC + MV
503	Nasal	16	0.5	2.0	AK, SAM, ATM, CFP, CTZ, CIP, MER, PTZ, SUT	bla_{OXA-51}, bla_{OXA-23}	O	M/77	09/29/10	21	Colonized/Yes	11/18/10	Death	CVC + IUC + MV
511	Nasal	16	1.0	2.0	SAM, ATM, CFP, CTZ, CIP, GEN, MER, PTZ	bla_{OXA-51}	O	M/56	11/08/10	13	Colonized/No	11/21/10	Death	–
515	Perianal	16	0.5	2.0	AK, SAM, ATM, CFP, CTZ, CIP, MER, PTZ, SUT	bla_{OXA-51}, bla_{OXA-23}	K1	F/72	11/12/10	11	NI-PNU/Yes	11/22/10	Death	–
544	Nasal	16	1.0	2.0	AK, SAM, ATM, CFP, CTZ, CIP, GEN, MER, PTZ, SUT	bla_{OXA-51}	D1	F/20	12/06/10	9	Colonized/No	12/24/10	Survival	CVC + IUC + MV

IMP, imipenem; POL, polymyxin; TIG, tigecycline; AK, amikacin; SAM, ampicillin/sulbactam; ATM, aztreonam; CFP, cefepime; CTZ, ceftazidime; CTX, ceftriaxone; CIP, ciprofloxacin; GEN, gentamicin; MER, meropenem; PTZ, piperacillin/tazobactam; SUT, sulfamethoxazole/trimethoprim; NI, nosocomial infection; PNU, pneumonia; ATM, previous antimicrobial therapy; CVC, central venous catheter; IUC, indwelling urinary catheter; MV, mechanical ventilation.

[a] IS*Aba-1* was found upstream of the bla_{OXA-23} gene.

[b] ERIC-PCR fingerprint patterns were analyzed using the Dice similarity coefficient and the unweighted-pair group method using average linkages cluster method (BioNumeric software, Applied Maths, Kortrijk, Belgium).

The MBL enzyme was not found in any of the 43 CRAb strains by the double-disk synergy test (DDST).

Molecular characterization of CRAb strains

The bla_{OXA-51} gene was detected in all studied strains, whereas bla_{OXA-23} was found in 22 (51.2%) and $bla_{OXA-143}$ was detected in eight (18.6%) isolates. Of the 17 strains susceptible to imipenem in the confirmatory phenotypic tests, i.e., agar dilution method, two presented bla_{OXA-23} and one presented $bla_{OXA-143}$. Table 3 depicts the main clinical and microbiological characteristics of the 56 patients harboring (colonized or infected by) OXA producing CRAb strains. Other resistance-encoding genes such as VIM and IMP-like were not found among the tested strains. Finally, two imipenem-resistant strains did not display any of the acquired oxacillinases or metallo-β-lactamases during the investigation.

Molecular typing

Genotypic analysis of the CRAb isolates from 43 patients, using the ERIC-PCR, identified 15 different patterns, with 90% similarity according to dendrogram analysis. The main clusters (B, F, H, J, K, O) were observed in 25 (58.1%) CRAb isolates. Sensitive and resistant strains were found for the same genotype (Table 3).

Discussion

Carbapenem-resistant *A. baumannii* has emerged over the last decades as an important infection-causing microorganism in intensive care patients.[1,2] Despite being considered opportunistic, many authors underline the negative impacts stemming from CRAb infections, probably due to the delay to initiate appropriate antimicrobial therapy.[14] However, in spite of the high prevalence of this microorganism in hospitals around the world, especially in Brazil, little is known about the relationship between the presence of resistance genes and the outcome of CRAb infected patients.

CRAb is an important microorganism that colonizes and infects patients in our intensive care unit. During this study CRAb was isolated from ~10% of the patients and the main determinant of carbapenem resistance among *A. baumannii* isolates in this population was the production of oxacillinases OXA-23 and OXA-143. Moreover, a polyclonal pattern was observed among the studied strains.

This study presents limitations that are inherent to retrospective analysis and the quality of data depended on the clinical records. Patient enrollment occurred during a limited period of time, with small sample of patients evaluated. Additionally, molecular tests were done in ~77% of cases (43/54) and even though carbapenem resistance was an inclusion criteria for the study, only ~60% of *A. baumannii* samples had this resistance confirmed by microdilution tests. The disk diffusion is a method of screening, with potential limitations as inadequate drug concentration and lability of the drug on the disk. Thus, transportation and disk storage in less than ideal conditions may interfere in the results.[15]

In a study conducted in Brazil showed that none of the evaluated brands distributed in Brazil presented satisfactory performance.[15] It is worth mentioning that some strains even presenting a sensible profile, but with elevated minimum inhibitory concentration, had already genetic mutations (e.g. OXA 23) related to resistance. In this case series only enzymatic mechanisms of resistance were investigated.

Patients colonized or infected by CRAb strains presented a high hospital mortality rate (59%), although mortality could not be attributed only to the presence of CRAb. This feature, however, was not the focus of our study. Recently, Lemos et al. (2013) observed in a meta-analysis that CRAb patients had an increased mortality risk (OR-2.22; 95% CI: 1.66–2.98). These authors included 16 studies and most of them referred to infections due to CRAb, mainly bacteremia. Only one study considered infected and colonized patients. Nevertheless, most patients also presented a severe underlying illness and received inappropriate empirical antimicrobial treatment.[16]

Treatment options for CRAb infections are scarce and generally employ polymyxins or tigecycline.[17,18] In our study, 76.7% of the strains were susceptible to tigecyline, a lower percentage than the 97.1% found by Rossi et al. in samples from Latin America.[19] Regarding polymyxins (polymyxin B and E), resistance to polymyxin B was observed in three (7%) of the analyzed strains in this study (C> 4 μg/mL, CLSI 2011). SENTRY – 2006 found 2.1% resistance to polymyxin in Latin America.[20] This finding is worrisome since polymyxins are regarded as the major therapy options for treating CRAB infections, despite their toxicity.[21] Another important issue is the variable therapeutic effectiveness of polymyxin on CRAB (50–80%).[18,22]

In the present study, we found that the bla_{OXA-51} gene was present in all strains studied, confirming the *A. baumannii* species. OXA-51 has weak carbapenemase activity, but potentially increases the minimum inhibitory concentration (MIC) when overproduced.[23] Carbapenem resistance in *A. baumannii* frequently results from the production of acquired oxacillinases, such as OXA-23, OXA-24, and OXA-58, besides the less frequent OXA-143 and OXA-72.[24] The production of the carbapenemase OXA-23 has been frequently identified in CRAB strains from patients admitted to Brazilian hospitals.[25] In this study, OXA-23 was the most commonly acquired carbapenemase (51.2%), followed by OXA-143, which was identified in eight strains (18.6%). It is worth noting that OXA-143 was described in 2009 by Higgins et al., and has so far only been found in Brazilian hospitals.[3,7,8]

In our study, almost all CRAB strains presented at least one acquired oxacillinase (OXA-23 or OXA-143), and only two resistant strains did not display any of the investigated enzyme mechanisms. In this case, other resistance mechanisms may be involved, such as efflux pumps, changes in outer membrane proteins (OMPs) or changes in affinity or expression of penicillin-binding proteins (PBPs).[1]

Among the analyzed strains, 15 distinct clones were identified in six main related clusters, which characterizes a polyclonal distribution but includes groupings with >90% similarity. This fact is probably related to cross-transmission. CRAb strain was first identified in our institution in 2003, and since then there has been an exponential increase in number of cases. HC/UFMG is a hospital of high complexity and

public reference center. Thus, the hospital always receives many patients who are possibly colonized/infected with CRAb from many other services.

In the present study, three out of the four CRAb patients with the K clone ultimately passed away. Moreover, the bla_{OXA-23} gene was identified in all these CRAb strains. Due to the small sample of patients and the heterogeneity of their clinical and microbiological presentations, associations regarding the production of oxacillinases and patient mortality could not be performed in this study.

Conclusions

Despite the limitations, this study showed that CRAb colonized or infected patients presented a high frequency of comorbidities, with long hospital stays and increased hospital morbidity. The strains studied showed a wide genetic diversity with polyclonal dissemination pattern. The most frequent resistance mechanism was the production of carbapenemases, notably OXA-23, but with cases of OXA-143 expression. Although the production of metallo-β-lactamases was not identified, other mechanisms may be involved, as there were resistant strains that did not express any gene related to carbapenemases. Strains drug resistance profile was discordant in 40% of cases when using disk diffusion and agar dilution methods. We also need to underscore that polymyxin B resistance was higher than described in literature and this may be a challenge for patient management, due to the limitations of therapeutic options. In our view, prevention for MDR infection is the key for better assisting patients in intensive care unit and antimicrobial susceptibility tests need to present more rigorous quality control. The increasing of polymixin resistance also warrants a better approach.

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Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21:538–82.
2. Falagas ME, Karveli EA, Siempos II, Vardakas KZ. Acinetobacter infections: a growing threat for critically ill patients. Epidemiol Infect. 2008;136:1009–19.
3. Higgins PG, Poirel L, Lehmann M, Nordmann P, Seifert H. OXA-143, a novel carbapenem-hydrolyzing class D beta-lactamase in *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2009;53:5035–8.
4. Clark BR. Imipenem resistance among *Acinetobacter baumannii*: association with reduced expression of a 33–36 kDa outer membrane protein. J Antimicrob Chemother. 1996;38:245–51.
5. Gehrlein M, Leying H, Cullmann W, Wendt S, Opferkuch W. Imipenem resistance in *Acinetobacter baumannii* is due to altered penicillin-binding proteins. Chemotherapy. 1991;37:405–12.
6. Kusradze I, Diene SM, Goderdzishvili M, Rolain J-M. Molecular detection of OXA carbapenemase genes in multidrug-resistant *Acinetobacter baumannii* isolates from Iraq and Georgia. Int J Antimicrob Agents. 2011;38:164–8.
7. Antonio CS, Neves PR, Medeiros M, Mamizuka EM, Elmor de Araujo MR, Lincopan N. High prevalence of carbapenem-resistant *Acinetobacter baumannii* carrying the blaOXA-143 gene in Brazilian hospitals. Antimicrob Agents Chemother. 2011;55:1322–3.
8. Mostachio AK, Levin AS, Rizek C, Rossi F, Zerbini J, Costa SF. High prevalence of OXA-143 and alteration of outer membrane proteins in carbapenem-resistant *Acinetobacter* spp. isolates in Brazil. Int J Antimicrob Agents. 2012;39:396–401.
9. Mostachio AK, van der Heidjen I, Rossi F, Levin AS, Costa SF. Multiplex PCR for rapid detection of genes encoding oxacillinases and metallo-beta-lactamases in carbapenem-resistant *Acinetobacter* spp. J Med Microbiol. 2009;58 Pt 11:1522–4.
10. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36:309–32.
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twenty-third informational supplement M100-S23. Wayne, PA, USA: CLSI; 2013.
12. Higgins PG, Lehmann M, Seifert H. Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents. 2010; 35:305.
13. Silbert S, Pfaller MA, Hollis RJ, Barth AL, Sader HS. Evaluation of three molecular typing techniques for nonfermentative Gram-negative bacilli. Infect Control Hosp Epidemiol. 2004;25:847–51.
14. Lee YT, Kuo SC, Yang SP, et al. Impact of appropriate antimicrobial therapy on mortality associated with *Acinetobacter baumannii* bacteremia: relation to severity of infection. Clin Infect Dis. 2012;55:209–15.
15. Sejas LL. Avaliação da qualidade dos discos com antimicrobianos para testes de disco-difusão disponíveis comercialmente no Brasil. Revista Brasileira de Patologia e Medicina Laboratorial. 2003;39:27–35.
16. Lemos EV, la Hoz FP, Einarson TR, et al. Carbapenem resistance and mortality in patient with *Acinetobacter baumannii* infections: systematic review and meta-analysis. Clin Microbiol Infect. 2013:1–7.
17. Stein GE, Babinchak T. Tigecycline: an update. Diag Microbiol Infect Dis. 2013:331–6.
18. Zavascki AP, Goldani LZ, Li J, Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. J Antimicrob Chemother. 2007;60:1206–15.
19. Rossi F, Garcia P, Ronzon B, Curcio D, Dowzicky MJ. Rates of antimicrobial resistance in Latin America (2004–2007) and in vitro activity of the glycylcycline tigecycline and of other antibiotics. Braz J Infect Dis. 2008;12:405–15.
20. Gales AC, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram-negative bacilli: report from the SENTRY

antimicrobial surveillance programme (2001–2004). Clin Microbiol Infect. 2006;12:315–21.
21. Fishbain J, Peleg AY. Treatment of Acinetobacter infections. Clin Infect Dis. 2010;51:79–84.
22. Oliveira MS, Prado GV, Costa SF, Grinbaum RS, Levin AS. Polymyxin B and colistimethate are comparable as to efficacy and renal toxicity. Diag Microbiol Infect Dis. 2009;65:431–4.
23. Brown S, Young HK, Amyes SG. Characterization of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. Clin Microbiol Infect. 2005;11:15–23.
24. Walsh TR. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents. 2010;36 Suppl. 3:S8–14.
25. Carvalho KR, Carvalho-Assef AP, Peirano G, Santos LC, Pereira MJ, Asensi MD. Dissemination of multidrug-resistant *Acinetobacter baumannii* genotypes carrying bla(OXA-23) collected from hospitals in Rio de Janeiro, Brazil. Int J Antimicrob Agents. 2009;34:25–8.