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PREVALENCE OF EXTREMELY DRUG RESISTANT (XDR) *ACINETOBACTER BAUMANNII* AT A NORTHEAST BRAZILIAN EMERGENCY HOSPITAL

Laís Rocha Lima[1], Lucas Vale Soares[2], Eliezer Erbe Freitas[3], Ceres Lima Batista[3], Avilnete Belém de Souza Mesquita[3], Girlene Soares de Figueirêdo[3], Humberto Medeiros Barreto[3], Josie Haydée Lima Ferreira[1,3*] and Viriato Campelo[1,3]

[1]Graduate Program in Science and Health, Federal University of Piauí, Teresina (PI), Brazil
[2]Microbiology Laboratory of the Hospital Med Imagem, Teresina (PI), Brazil
[3]Laboratory of Microbiology Research, Federal University of Piauí, Teresina (PI), Brazil

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Corresponding author:
Josie Haydée Lima Ferreira

ABSTRACT

Acinetobacter baumannii is an opportunistic pathogen that causes healthcare-associated infections, especially in intensive care units (ICUs). This study aimed to analyze the epidemiological and resistance profile of 106 patients hospitalized at an emergency hospital from Teresina, Piauí, Brazil, as well as, to investigate the resistance profile of *A. baumannii* strains isolated from these patients. To determine the occurrence of resistance mediated by efflux pump mechanism, minimum inhibitory concentration values of different antibiotics were determined in the presence or absence of carbonylcyanidem-chlorophenylhydrazone, a known efflux pump inhibitor. Many of the patients with healthcare-associeted infections (HAI) caused by A. baumannii in analizeted hospital were male (69%), aged20-60 years old (56.6%), with respiratory tract infections (77.3%). A high percentage of extremely drug resistant (XDR) strains (81.1%) was verified among A. baumannii clinicalisolates, with greater prevalence of resistance to gentamicin (98.0%), ceftriaxone (94.3%), ceftazidime (92.0%), ciprofloxacin(90.5%) and levofloxacin (90.5%). On the other hand, all strains were sensitive to colistin. Resistance mediated by efflux pump was verified for ceftazidime (26.4%), amikacin (16.0%) and ciprofloxacin (8.5%). These results point to a need for implementing preventive measures aiming to reduce the prevalence of multidrug-resistant A. baumannii strains in hospital environments.

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INTRODUCTION

Infections caused by multidrug-resistant (MDR) bacteria have been currently acquired in community or hospital environments from around the world representing a serious public health problem. Health care-associated infections (HAI) are infectious diseases acquired following 72 hours of hospitalization, when the incubation period of the etiological agent is unknown, and no clinical or laboratory data are available to prove infection at the time of hospitalization, including cases in which a patient diagnosed with a community infection manifests aggravation, or isolates a new etiological agent (OLIVEIRA *et al*., 2016). Most HAIs are caused by non-fermenting Gram-negative bacilli (NFGNB), including *Pseudomonas aeruginosa, Stenotrophomonas maltofilia, Burkholderia cepacia* and *Acinetobacter baumannii* species (EL CHAKHTOURA *et al*, 2018).

These pathogens are of great clinical and epidemiological relevance as they frequently cause opportunistic infections with high morbidity and mortality rates (OSAWA *et al*., 2018; BAGHERI-NEMASI *et al*., 2017). *A. baumannii* is an opportunistic pathogen commonly associated with nosocomial infectious outbreaks, presenting great relevance as an etiological agent in intensive care units (ICUs) of various diseases, such as infections of the urinary tract, soft tissue, and surgical site, as well as pneumonia and septicemia (ANTUNES *et al*., 2014). Studies have shown that this bacterium possesses mechanisms that facilitate the colonization of patients and hospital equipment through biofilm production and the action of bacterial outer membrane proteins (CHOI *et al*., 2005). These mechanisms may also be associated with their survival capacity in environmental conditions and their persistence over long periods of time in surfaces, making it an important cause of infectious hospital outbreaks (CHAPARTEGUI-GONZÁLEZ *et al*., 2018). Another *A. baumannii* characteristic which contributes to its elevated prevalence in the

hospital environment is its high potential for acquisition of resistance to antimicrobials (IMPERI *et al*., 2011). Antimicrobial resistance can occur through different mechanisms, including mutation in genes encoding the antibiotic targets, through the production of drug-inactivating enzymes, or by overproduction of efflux pumps (BARKER, 1999). Efflux pumps act by increasing antibiotic efflux into the extracellular medium, leading to an increase in the minimum inhibitory concentration (BARKER, 1999). These transmembrane proteins perform different functions, including substance transport, nutrient, ion assimilation and metabolite, and toxic substance excretion (BARKER, 1999; LI AND NIKAIDO, 2009). In addition to being causally related to host colonization (BARKER, 1999; LI AND NIKAIDO, 2009). Some substances such as carbonyl cyanide m-chlorophenylhydrazone (CCCP) are known to interfere with the action of these pumps, by dissipating the cytoplasmic membrane proton gradient, thus reducing the energy available for the antibiotic extrusion (XING *et al*., 2014). The objectives of this study were to evaluate the epidemiological profile of infections caused by *A. baumannii* in an emergency hospital of the Northeast of Brazil, as well as, to analyze the resistance profile of the isolated strains to antimicrobial agents.

MATERIALS AND METHODS

The epidemiological data from 106 patients with HAIs caused by *Acinetobacter baumannii*, hospitalized at the Teresina Emergency Hospital (HUT) between July 2016 and July 2017, were analyzed. Epidemiological data on the patients (age, sex, and clinical specimen from the infected site) were collected from their respective medical records. Bacterial strain isolation was performed in blood agar followed by subculture in MacConkey Agar using duplicates. Bacterial strain identification, as well as determination of their respective antimicrobial resistance profiles, were performed using the BD PHOENIX 5.1 automation method (Becton Dickison Sparks, MD 21152, USA). The resistance profile of the isolated strains was confirmed by the diffusion method according to Clinical and Laboratory Standards Institute - CLSI (CLSI, 2016). An extensively drug-resistant (XDR) *Acinetobacter baumannii* was defined as any isolate not susceptible by European Committee on Antimicrobial Susceptibility Test (EUCAST) criteria to ≥1 drug of ≥3 of the following classes: aminoglycosides, carbapenems, fluoroquinolones, and polymyxins (13). The tested antimicrobials were: gentamycin (GEN), ceftriaxone (CRO), ciprofloxacin (CIP), ceftazidime (CAZ), levofloxacin (LEV), amikacin (AMI), cefepime (CPM), piperacicline-tazobactam (PPT), meropenem (MER), imipenem (IPM), sulfamethoxazole-trimethoprim (SUT), tigecycline (TIG), colistin (COL).

To verify the occurrence of efflux pump-mediated resistance, minimal inhibitory concentration (MIC) values of antibiotics were determined in the presence or absence of a carbonyl cyanide m-chlorophenylhydrazone (CCCP) solution at subinhibitory concentration. For determination of the CCCP subinhibitory concentrations to be used in all tests, a preliminary assay using 10 randomly selected strains was carried out. These strains were seeded in Brain Heart Infusion broth (BHI, Himedia, India) containing CCCP at different concentrations (4 to 10 µg/ml) and then incubated at 37 °C for 24 hours. The results showed that the 4 to 7 µg/mL concentrations did not affect the viability of the tested strains, with these concentrations being used for all isolated strains. For the assays, the tested strains were inoculated into BHI followed by incubation at 37°C for 24 hours. Following the incubation period, a standard bacterial suspension was prepared in saline solution with a turbidity equivalent to 0.5 on the MacFarland scale (approximately 1.5 x 10^8 CFU/mL). Then, 150 µL of this bacterial suspension was transferred to an eppendorf tube containing 1350 µL BHI with or without CCCP at a subinhibitory concentration. Then, 100 µL aliquots from the medium were distributed into the wells of a microtiter plate in a numerical sense. MIC values for amikacin, ciprofloxacin and ceftazidime were determined by transferring 100 µL of each antibiotic solution into the first well of the plate, followed by 1:2 serial microdilution up to the penultimate well (512 to 0.25 µg/mL). In the last well, no antibiotic solution was added, this serving as a positive control of the bacterial growth.

Microtitulation plates were incubated at 37 °C for 24 hours and following this time 20 µl of a 0.01% (w/v) aqueous Resazurin sodium solution was added to each well. This plates were incubated for 1 hour at room temperature where following this period a reading was performed taking into account that a change in coloration from blue to pink indicated the occurrence of bacterial growth due to resazurin reduction (EUCAST, 2018). As a criterion for classifying occurrence of resistance mediated by eflux pumpphenotype, a minimum of a 2-fold antibiotic MIC reduction in the presence of CCCP was necessary (ARDEBILI *et al*., 2014). The data were organized using the software GraphPad Prism 5.0 worksheets where they were submitted to Pearson's Chi-square correlation test with 95% Confidence Interval and significance at $p < 0.05$, with the results being presented in graphs and tables.

RESULTS

The epidemiological analyzes results can be seen in Table 1. Most patients hospitalized at the HUT who acquired infection caused by *A. baumannii* were male subjects (68.9%), in the age group 20 to 60 years old (56.6%). Respiratory tract infections were the most prevalent, with 77.4% of *A. baumannii* strains being isolated from tracheal secretions. In addition, was verified a high prevalence of MDR *A.* b*aumannii* strains (90.6 %). The antimicrobial resistance frequencies of the isolated strains from HUT patients are presented in Figure 1. The strains showed high resistance rates to ceftriaxone (94.3%), cefepime (87.7%), ceftazidime (92.0%), meropenem (83.0%), imipenem (82.0%), piperacicline-tazobactam (90.5%), ciprofloxacin (90.5%), levofloxacin (90.5%), gentamicin (98%) and amikacin (89%). Intermediate resistance frequencies were evidenced for sulfamethoxazole-trimethoprim (71.6%) and tigecycline (57.5%). On the other hand, all isolated strains were sensitive to colistin.
All strains which showed resistance to amikacin, ceftazidime and ciprofloxacin were subjected to a phenotypic test to verify if the resistance mechanism was mediated by efflux pumps (Table 2). Efflux pump-mediated resistance was most frequently verified among ceftazidime-resistant strains (26.4%), followed by amikacin-resistant strains (16.0%). On the other hand, a low frequency of efflux pump-mediated resistance was observed among ciprofloxacin-resistant strains (8.5%).

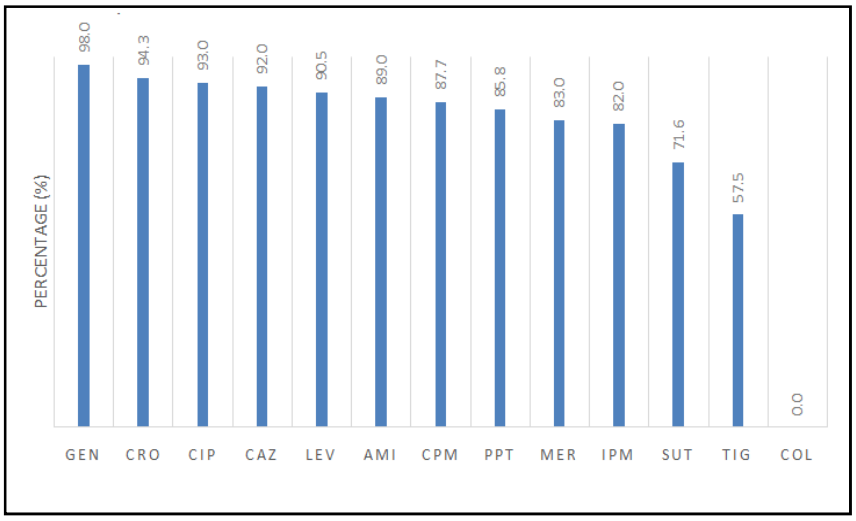
DISCUSSION

A higher prevalence of male individuals, in the age group 20 to 60 years of age, was observed among the 106 patients that comprised the sample for this investigation (Table 1). One hypothesis which may justify this epidemiological profile would be the relationship between young men and traffic accidents, since many injured patients in the several municipalities from the state of Piauí are referred to the HUT for care. An earlier study, conducted between September 2011 and February 2012, showed that 60.2% of the polytrauma patients hospitalized at the HUT orthopedic clinic had been victims of traffic accidents, with a higher prevalence of men (81.0%) in the age group 18 to 38 years of age (61.9%), of which 89.8% of the cases had surgical indication (SANTOS *et al*., 2016). Patients hospitalized for prolonged periods in an ICUs, who have undergone invasive procedures, as well as polytraumatized patients, frequently acquire nosocomial infections caused by *A. baumannii* (HUANG *et al*., 2018; BARAN *et al*., 2008). A study carried out in the HUT between October and November 2010 found that *A. baumannii* infections were the most prevalent (20.3%) among patients admitted to the ICUs (20.8%), who were predominantly male (75.0%), in the age range 15 to 30 years (33.3 %) (RODRIGUES *et al*., 2013). In the present study, *A. baumannii* strains were predominantly isolated from tracheal secretion (77.4%), followed by catheter tip (8.5%), surgical site (5.7%), blood and urine (2.8%). These results agree with a study carried out in the south of the country, which found that most *A.*

Table 1. Sex, age and clinical isolate distribution of patients with HAIs

VARIABLES	N[a]	%[b]	P[c]
Age (years)			
< 20	9	8.5	
20 – 60	60	56.6	<0.001[d]
> 60	37	34.9	
Sex			
Male	73	68.9	0.001 [d]
Female	33	31.1	
Source of clinical specimen material			
Tracheal secretion	82	77.4	
Catheter tip	9	8.5	
Surgical site	6	5.7	
Urine	3	2.8	
Blood	3	2.8	<0.001[d]
Peritoneal fluid	1	0.9	
Diabetic foot	1	0.9	
Scalp secretion	1	0.9	
Clinical isolate antibacterialdrugs[e]			
Sensitive	4	3.8	
Not multi – resistant	6	5.7	
Multi - resistant	10	9,4	<0.001[d]
Extremely drug resistant[e]	86	81.1	
Total	106	100.0	

a: absolute frequency; b: relative frequency; c: probability for Pearson's Chi-square test with 95% CI and significance level at p<0.05; d: highly significant difference; e: EUCAST criteria.



Gentamycin (GEN), ceftriaxone (CRO), ciprofloxacin (CIP), ceftazidime (CAZ), levofloxacin (LEV), amikacin (AMI), cefepime (CPM), piperacycline-tazobactam (PPT), meropenem (MER), imipenem (IPM), sulfamethoxazole-trimethoprim (SUT), tigecycline (TIG), colistin (COL).

Figure 1. *Acinetobacter baumannii* resistance profiles from patients with HAIs admitted to the Teresina Emergency Hospital (HUT) - PI between 2016-2017.Teresina 2021.

Table 2. Presence of efflux pump-mediated resistance mechanisms

Antibiotics	Resistant	Efflux		P[d]
	N[a]	N[b]	%[c]	
Ceftazidime	85	28	26.42	<0.001***
Amikacin	81	17	16.04	<0.001***
Ciprofloxacin	85	9	8.49	<0.001***

a: absolute universal frequency; b: absolute sample rate; c: relative frequency; d: probability for Pearson's Chi-square test with 95% CI and significance level at p <0.05.c%, relative frequencydp, probability for Pearson's Chi-square test with 95% CI and significance level at p <0.05.

baumannii strains were isolated from tracheal secretion samples (DELIBERALI *et al*., 2011). Similar results were also observed in patients hospitalized in ICUs hospitals in China, where a higher prevalence of respiratory tract infections in relation to other sites was also observed (HUANG *et al*., 2018, JIA *et al*., 2015). The upper respiratory tract has been reported as the preferred site for *A. baumannii* colonization as this pathogen frequently causes mechanical ventilation-associated infections (LIM *et al*., 2019). High resistance percentages were observed for the aminoglycosides, cephalosporins, fluroquinolones and carbapenems (Figure 1). These antibiotics have traditionally been used in the treatment of HAIs caused by *A. baumannii*, which may favor the selection of resistant strains to such antibiotics in a hospital environment. A study with ICUs patients from a hospital in Serbia found that all urine isolated

strains were resistant to third generation cephalosporins and fluoroquinolones (DJORDJEVIC *et al*., 2016). A survey on Gram-negative bacilli resistance rates isolated from medicais centers enrolled in the SENTRY Antimicrobial Surveillance Program, from 2008 to 2010, showed high *Acinetobacter* resistance rates to ciprofloxacin (87.2%), piperacillin/tazobactan (86.3%), ceftazidime (81.7%), cefepime (76.6%), amikacin (62.6%) and gentamicin (53.3 %) (GALES *et al*., 2012). Carbapenems have been considered as the most appropriate agents for the treatment of infections caused by multidrug-resistant *A. baumannii* strains. However, a large increase in carbapenem resistance has been observed in several parts of the world, driven mainly by the spread of international clones. Increasing carbapenem resistance percentages and cross-resistance to several other antimicrobial agents in *A. baumannii* strains causing HAIs are of great concern in clinical medicine, especially in intensive care units worldwide (SCHUERTZ *et al*., 2018; HIDRON *et al*., 2008). The strains isolated in this study also revealed a high carbapenem resistance percentage: meropenem (83.0%) and imipenem (82.0%). In the Latin American, the *A. baumannii* resistance rates to the carbapenems imipenem e meropenem in period of the 2013 to 2016 were the 85,6% and 86,3%, respectively, second results from the SENTRY Antimicrobial Surveillance Program (GALES *et al*., 2019). In Goiânia, Brazil, 78.0 % of *A. baumannii* strains isolated from patients in intensive care units were resistant to meropenem and imipenem (CASTILHO *et al*., 2017).

Another study carried out in São Luís, Brazil, found that 100.0 % of the 128 *A. baumannii* strains isolated from hospitalized patients were resistant to imipenem and meropenem (RIBEIRO *et al*., 2016). Therefore, the results obtained in the present study corroborate with data previously obtained in other Brazilian states which show high rates of carbapenem resistance. In this study, 57.5% of *A. baumannii* strains causing HAIs in the Teresina Emergency Hospital were resistant to tigecycline. A study in South Africa reported a 2% resistance incidence to this antibiotic among *A. baumannii* clinical isolates (LOWINGS *et al*., 2015). In Goiânia, Brazil, the incidence of *A. baumannii* strains involved in HAIs was 7.1 % (CASTILHO *et al*., 2017). In another study including 16 hospitals in São Luís, Brazil, a 0.8% tigecycline resistance rate by *A. baumannii* was reported, despite the strains showing 100.0 % resistance to carbapenems (RIBEIRO *et al*., 2016). Thus, the tigecycline resistance incidence obtained in this study can be considered high and raises concerns regarding the indiscriminate use of this drug in the hospital environment. Differently from the other antibiotics, all analyzed strains were sensitive to colistin. Currently, colistin appears to be the most reliable drug for MDR *A. baumannii* treatment, however the use of colistin has been associated with various side effects and is not suitable for the treatment of all types of infections. Despite studies have described the emergence of resistance to colistin in various regions the word and the discovery of the news mechanisms of resistance for this drug (EL-SAYED AHMED *et al*., 2020). Even so result from the SENTRY Antimicrobial Surveillance Program (1997-2016) show that colistin is the most active agent against *Acinetobacter calcoaceticus- Acinetobacter baumannii* (Acb) complex, presenting 95,1% of the susceptible all regions (GALES *et al*., 2019). In this study, almost all strains isolated (81.1%) were not susceptible by EUCAST criteria to ≥1 drug of ≥3 of the following classes: aminoglycosides, carbapenems, fluoroquinolones, and polymyxins (EUCAST, 2018; GALES *et al*., 2012). *A. baumannii* clinical isolates with this resistance profile have been classified as extremely drug resistant (XDR) strains (EUCAST, 2018; GALES *et al*., 2012).

When analyzing the frequency of XDR Acb isolates obtained in the 2013-2016 period in various regions of the world, the SENTRY Antimicrobial Surveillance Program describes that the highest rates were collected in Latin America (86.6%), followed by Europe (79.0%) and Asia-Pacific region (72.7%) (TAVARES *et al*., 2019). In Brasil, a study that analyzed total of 107 carbapenem-resistant *A. baumannii* isolates from patients with bacteraemia attended at a teaching hospital, from 2008 to 2014, showed that 60.7% of isolates were considered XDR (TAVARES *et al*., 2019). In addition, 40.0 % (42/106) of the analyzed strains were resistant to all the drugs evaluated in this study, with the exception colistin alone. Therefore, the results obtained in the present study are alarming indicating a need for urgent preventive measures aimed at controlling resistance levels in the hospital environment. Previous studies have shown that the presence of efflux pumps is a resistance mechanism frequently seen in *A. baumannii* clinical isolates (JIA *et al*., 2015, ZHU *et al*., 2017). In the present study, 26.4% (28/85) of *A. baumannii* strains resistant to ceftazidime were found to have a phenotype compatible with a resistance mediated by efflux pump overproduction (Table 2). A higher percentage of efflux-mediated ceftazidime resistance (72.7%) was obtained in a study analyzing food-grade *Acinetobacter spp* in Taiwan, carried out using a phenotypic modulation test with the CCCP inhibitor. It was further observed that when adeD and adeIJK expression levels were evaluated in four representative isolates, significantly increased expression levels of the adeI gene were found suggesting that ceftazidime resistance in *Acinetobacter spp*. may be attributed to adeI expression levels (LIN *et al*., 2017a).

The involvement of the AdeABC, another RND-type efflux pump, is not only associated with ceftazidime resistance in *A. baumannii* but is also involved in the level of susceptibility to many antibiotics β-lactam and of other classes, contributing to multidrug resistance in *A. baumannii* (LIN *et al*., 2017b, CARDOSO *et al*., 2018). From the amikacin-resistant strains isolated in the present study, 16.0% (17/81) of them had an efflux pump-mediated resistance phenotype (Table 2). A study using eleven amikacin resistant *A. baumannii*s trains isolated from Chinese patients with HAIs showed that ten of the eleven (91.0 %) tested strains showed a minimal inhibitory concentration decrease ≥ 4-fold in the presence of the CCCP inhibitor, characterizing the presence of an efflux pump mechanism in these strains. Moreover, the tested strains showed increased expression of the adeB gene, showing that amikacin resistance could be mediated by overexpression of the AdeABC efflux pump (LIN *et al*., 2017a).

Another study showed that the increased expression of the efflux pump encoded by the *abeS*gene is strongly associated with the amikacin resistance in this species (ZHU *et al*, 2017). Only 8.5 % (9/85) of the ciprofloxacin-resistant strains isolated in the present study showed an efflux pump-mediated resistance mechanism (Table 2). This percentage is below a percentage obtained in a previous study, which observed the presence of an efflux pump in 66.2% of ciprofloxacin-resistant *A. baumannii* strains (ARDEBILI *et al*., 2014). Another study obtained an 86.0% percentage of norfloxacin-resistant *A. baumannii* strains caused by efflux pump overexpression (JIA *et al*., 2015). In *A. baumannii*, fluoroquinolone efflux is mediated by RND family members represented by the proteins AdeABC and AdeIJK (LARI *et al*., 2018; DAMIER-PIOLLEL *et al*., 2008). Due to the presence of *A. baumannii* strains which are extremely resistant to antimicrobials used in clinical practice in the hospital environment and the relevance of the efflux pump mechanism, the constant surveillance of the *A. baumannii*resistance profile is necessary, with the aim of reducing the incidence of infections caused by multiresistant*A. baumannii*strains and elucidating the resistance mechanisms involved.

CONCLUSION

Patients admitted to the HUT who acquired infections caused by *Acinetobacter* were predominantly male individuals in the 20-60 age group with respiratory tract impairment. From the *Acinetobacter* clinical isolates, high resistance percentages were detected for most of the tested antimicrobial classes, with high predominance of XDR strains. Efflux pump-mediated resistance was also found to be more prevalent for ceftazidime and amikacin antibiotics. These results are extremely concerning and serve as warning for the need to develop new antimicrobial agents and to monitor the resistance mechanisms developed by microorganism involved with HAIs. In addition to the implementation of successful infection control measures to contain XDR *Acinetobacter baumannii*.

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