

Prevalence of Gram-negative rods antibiotic resistance in public Brazilian hospital

Prevalência de bastonetes Gram negativos em um hospital público brasileiro

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Abstract: Introduction: Non-fermenters Gram negative and family bacteria Enterobacteriaceae are closely related to hospital infections, and with decreased sensitivity to antimicrobials. Objective: This study aims to evaluate the prevalence of resistance to carbapenems in *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacteriaceae. Material and methods: The samples and antimicrobial susceptibility test recovered by the laboratory accredited by the hospital, with care exclusively by Unified Health System, from 2013 to 2017. Results and discussion: Was observed that among the serious infections, pneumonia were the most prevalent in both *P. aeruginosa* and *A. baumannii*, with Enterobacteriaceae in the bloodstream, in men, over 61 years old in more than half of study time, predominantly in the ICU. Furthermore, the main phenotype for non-fermenters MBL and Enterobacteriaceae KPC. Final considerations: Multiple drugs resistance reflects the combination of resistance mechanisms and the use of epidemiological surveillance to identify the problems and risks of infection combined with the practice of prevention and control measures can help in the reduction of infections.

Keywords: *Acinetobacter baumannii*, enterobacteriaceae, hospital infection, *Pseudomonas aeruginosa*, resistance mechanism.

Resumo: Introdução: As bactérias Gram negativas não fermentadoras e as da família Enterobacteriaceae estão intimamente relacionadas às infecções hospitalares, e com diminuição da sensibilidade aos antimicrobianos. Objetivo: Este estudo tem como objetivo avaliar a prevalência de resistência aos carbapenêmicos em *Pseudomonas aeruginosa*, *Acinetobacter baumannii* e Enterobacteriaceae. Material e métodos: As amostras e teste de suscetibilidade antimicrobiana recuperados pelo laboratório credenciado pelo hospital, com atendimento exclusivo pelo Sistema Único de Saúde, nos anos de 2013 a 2017. Resultados e discussão: Observou-se que dentre as infecções graves, as pneumonias foram as mais prevalentes tanto em *P. aeruginosa*, quanto em *A. baumannii* e Enterobacteriaceae na corrente sanguínea, em homens, acima de 61 anos, em mais da metade do tempo de estudo, predominantemente em UTI. Além disso, o principal fenótipo para os não fermentadores foi MBL e Enterobacteriaceae KPC. Considerações finais: A resistência a múltiplas drogas reflete a combinação de mecanismos de resistência e o uso da vigilância epidemiológica para identificar os problemas e riscos de infecção aliados à prática de medidas de prevenção e controle podem auxiliar na redução das infecções.

Palavras-chave: *Acinetobacter baumannii*, enterobacteriaceae, infecção hospitalar, *Pseudomonas aeruginosa*, mecanismos de resistência.

Introduction

Infections caused by multidrug-resistant (MDR) are associated with increased costs, length of hospitalization, and, especially, morbidity and mortality rates, and Gram-negative bacilli are proportionally greater etiologic agents of infections in Latin America compared to the USA and Canada (Gales et al., 2012).

Non-fermenters Gram negative (NFGN) are resistant to most available antimicrobial drugs, mainly due to their ability to acquire resistance genes through horizontal transfer, but also due to their ability to persist in the open environment for long periods on nosocomial surfaces (Dias et al., 2016). Acquired carbapenem resistance in Gram-negative rods is often associated with acquired carbapenemase production (Metallo- β -lactamase – MBL, Carbapenemases other, such as *Klebsiella pneumoniae* carbapenemase – KPC or oxacillinase

and AmpC β -lactamase) (Chaudhary & Payasi, 2014). Extended-spectrum β -lactamases (ESBLs), the class A confer resistance to expanded-spectrum cephalosporins and have been extensively identified in members of the Enterobacteriaceae family but are also reported from non-fermenters (Potron et al., 2015).

Surveillance studies are performed to help target antimicrobial use, particularly empirical therapy, based on local patterns of organism resistance and implement infection control measures. (Edelsberg et al., 2014).

The aim of this study was to use a hospital database and phenotypic tests to determine the prevalence of clinically significant resistant bacterial pathogens in a Brazilian public hospital in five years.

Material and methods

Data for this study obtained from the laboratory database of a public hospital in Brazilian public hospital, in the years 2013 to 2017. Which was analyzed the blood, tracheal secretion, and catheter tip cultures, including microbiology orders, specimens, results of susceptibility testing and patient data. This study was approved by the ethics committee (CAAE: 16186213.8.0000.5152).

For the classification of resistance, they were defined as resistant to antibiotic therapy if designated as non-susceptible or intermediate based on the interpretation criteria of the Clinical and Laboratory Standards Institute (CLSI), as assessed by the participating clinical microbiology laboratory and phenotypic test BrCast (2017) and Georgios et al. (2014).

Carbapenem-resistant NFGN and Enterobacteriaceae rods were recovered and cultured on Trypticase Soy Agar (Kasvi, Italy) to perform all tests, followed by preparation of the suspension at 0.5 on the McFarland scale, was inoculated onto the surface of the Mueller-Hinton (Kasvi, Italy) agar plate. Incubations occurred at 37°C for 18-24hours.

The Double Disc Synergy Test (DDST) was used for the detection of Ambler class A β -lactamases and especially ESBL. In Mueller-Hinton agar plate, added amoxicillin/clavulanic acid disc at the center of the plate and ceftazidime, ceftriaxone and cefepime discs around. Considered positive when there was the formation of the of ghost zone.

On combined disk test, meropenem disc test with 10 μ L of the EDTA (0.1 M), cloxacillin-CLX (Sigma-Aldrich, USA) (75mg/mL) and the Phenylboronic acid-PBA (Sigma-Aldrich, USA) (40mg/mL). The interpretation was based on the 5mm increase in the phenylboronic acid discs for the production of KPC and the 5mm increase in the EDTA discs for the production of MBL. We do not consider other types of results.

Positive controls of specimens containing the target molecule known used in all protocols and statistical analyses were run using BioEstat version 5.0. All the analyses matched in order to correspond to the study design, using contingency table and ANOVA one way, considering the confidence interval 95%.

Results

Over the sampled period, NFGN bacteria were isolated from 355 hospital-admitted patients, especially in the intensive care unit (n = 247, 69.6%). Tracheal aspirate was the main clinical specimen associated with multidrug-resistant *Acinetobacter* and *Pseudomonas* (n= 224, 63.1%).

The sociodemographic data of the patients, infection, and resistance mechanism by carbapenem-resistant isolates presented in Table 1. Considering the *Acinetobacter*-associated patients, the average age of patients was 65.8 years old (ranging between 96 days old and 106 years old); 64 (43.5%) bacterial strains were isolated from female patients. *Pseudomonas*-associated patients had an average age of 61.9 years old (ranging from 16 to 96 years old); 78 (37.5%) bacterial strains were isolated from female patients.

Carbapenem-resistant NFGN most recovered in the years 2013 (63.1%) and 2014 (33.8%), with the production of enzymes of type MBL (24.5%) and KPC (7.0%).

Enterobacteriaceae bacteria were isolated from 23 hospital-admitted patients, mostly in bloodstream infection (BSI), 69.6%, from intensive care unit (n = 18, 78.3%). The mean patient age was 63 years old (ranging between 23 and 82 years old), seven (30.4%) bacterial strains were isolated from female patients. BSI or PNM were not detected in 2013 and 2014 and the highest prevalence was in 2017 (n= 16, 69.6%) and the phenotypic profile of KPC (n= 13, 56.5%).

Considering the characteristics of samples, including patients infected, by statistical analysis, the occurrence of *A. baumannii* was higher ($P < 0.05$) in patients older than 81 years old, in the ICU and the occurrence of serious infections (BSI and PNM). For *P. aeruginosa* the occurrence of PNM in 2013 and 2014 in relation to Enterobacteriaceae, what prevailed were BSI infections in 2017 and ESBL and KPC production.

Table 1. Characteristics of the samples, including patients infected by *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae family in a Brazilian public hospital.

	<i>A. baumannii</i>	<i>P. aeruginosa</i>	Enterobacteriaceae	P (CI 95%)
	N= 147 (%)	N= 208 (%)	N= 23 (%)	
Age (mean) ± SD	65.8 ± 18.6	61.9 ± 17.7	63.0 ± 19.0	0.12
0-20 years	4 (2.7)	1 (0.5)	-	0.16
21-40 years	7 (4.8)	18 (8.7)	2 (8.7)	0.35
41-60 years	41 (27.9)	68 (32.7)	8 (34.8)	0.57
61-80 years	57 (38.8)	88 (42.3)	12 (52.2)	0.45
≥81 years	38 (25.9)	28 (13.5)	1 (4.3)	0.002*
Female	64 (43.5)	78 (37.5)	7 (30.4)	0.34
UCI	119 (81.0)	128 (61.5)	18 (78.3)	0.0003*
BSI	69 (46.9)	62 (29.8)	16 (69.6)	<0.0001*
PNM	78 (53.1)	146 (70.2)	7 (30.4)	<0.0001*
2013	52 (35.4)	68 (32.7)	-	0.002*
2014	12 (8.2)	48 (23.1)	-	<0.0001*
2015	19 (12.9)	54 (26.0)	3 (13.0)	0.007*
2016	37 (25.2)	38 (18.3)	4 (17.4)	0.25
2017	27 (18.4)	-	16 (69.6)	<0.0001*
ESBL	-	2 (1.0)	9 (39.1)	<0.0001*
KPC	17 (11.6)	8 (3.8)	13 (56.5)	<0.0001*
MBL	23 (15.7)	64 (30.8)	4 (17.4)	0.003*

CI: confidence interval; SD: standard deviation; UCI: intensive care unit; BSI: bloodstream infection; PNM: pneumonia; ESBL: Extended-spectrum β -lactamase; KPC: *Klebsiella pneumoniae* carbapenemase; MBL: metallo β -lactamase; *: statistical significance

All the studied bacterial samples were resistant to imipenem or meropenem (Table 2). For the most common profile NFGN was the MBL and the resistance to piperacillin-tazobactam; ceftazidime; cefepime; ciprofloxacin; ampicillin-sulbactam; tigecycline; gentamicin; aztreonam; and two samples of *P. aeruginosa* showed resistance to polymyxin B (1.1%) and MBL positive. To Enterobacteriaceae, *Klebsiella pneumoniae* was the most frequent bacterial species (47.8%) resistance to cefepime; tigecycline; piperacillin-tazobactam; ceftazidime (Table 2). Others were *Enterobacter cloacae* (21.7%), *E. gergoviae* (4.3%), *Escherichia coli* (8.7%) and *Serratia marcescens* (17.4%).

Table 2. Main phenotypes of resistance of the samples of *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae family in a Brazilian public hospital.

Pathogen/antibiotic resistant	N	Main phenotypic
Carbapenem-resistant <i>A. baumannii</i>	147	Resistant to PIP-TAZ; CAZ; CEF; CIP (100%); AMP-SUT (93.4%) MBL
Carbapenem-resistant <i>P. aeruginosa</i>	208	Resistant to AMP-SUT; TIG (100%); CEF; GEN; ATM; CIP (≥70%); POL (1.1%) KPC
Carbapenem-resistant Enterobacteriaceae	23	Resistant to CEF; TIG (100%); PIP-TAZ; CAZ (≥95%)

KPC: *Klebsiella pneumoniae* carbapenemase; MBL: metallo β -lactamase; PIP-TAZ: piperacillin tazobactam; CAZ: ceftazidime; CEF: cefepime; CIP: ciprofloxacin; AMP-SUT: ampicillin sulbactam; TIG: tigecycline; GEN: gentamicin; ATM: aztreonam; POL: polymyxin.

Discussion

According to the literature, colonization and infection by multidrug-resistant bacteria, especially species of *Pseudomonas* and *Acinetobacter*, is an important and predictive factor for intra-hospital patient death,

mainly of immunocompromised individuals (Dias et al., 2016). In this present study, the data may reflect a real setting of patients admitted to a tertiary hospital, the high risk of colonization and infection by MDR bacteria.

Carbapenems are antimicrobials used worldwide as the last resort antibacterial agents for the treatment of infections caused by MDR gram-negative organisms (Saw et al., 2016). However, resistance to carbapenems has been emerging steadily and thus putting clinical application of these antibiotics under threat. This resistance is mainly mediated by carbapenemases such as Ambler class B metallo- β -lactamases (MBL), which have been increasingly reported in Enterobacteriaceae and *Pseudomonas* spp. (Chaudhary & Payase, 2014), and findings KPC positive *K. pneumoniae* and *Pseudomonas* spp. are endemic in Brazil (Munoz-Price et al., 2013), consistent with the data shown in this study.

Antibiotic selection pressure shown to have an impact on the spread of MDR. Antimicrobial susceptibility test results for pathogens implicated in hospital infectious are an important source of information about the scope and magnitude of emerging and endemic antimicrobial-resistant infections (Weiner et al., 2016).

It is important to use simple and reliable tests to the identification of carbapenemase-producing clinical isolates among gram-negative organisms, in order to select an appropriate antibiotic therapy. Carbapenemase-producing organisms remain the leading cause of therapy failure with commonly used drugs due to false susceptibility patterns (Chaudhary & Payase, 2014). This study showed that also highlighted an alarming rise in MBLs in Brazilian patient's population, including *A. baumannii*.

If genotypic confirmation is not immediately available, to the phenotypic confirmation testing should be performed to avoid delay in notifying potential carbapenemase producers in clinical practice (Stuart & Leverstein-Van Hall, 2010).

Resistance to antimicrobial agents in *A. baumannii* and *P. aeruginosa* is high for β -lactams, aminoglycosides and fluoroquinolones. Although not almost all class A ESBLs possesses any significant carbapenemase activity, the rare identification of the ESBLs in *A. baumannii* may be due to limited horizontal gene transfer occurring between these different bacterial genders (Potron et al., 2015). Although in Enterobacteriaceae are frequent (Gashaw et al., 2018). MBL enzymes are not the most commonly identified in *A. baumannii*, but are mainly in *P. aeruginosa* (Potron et al., 2015). This represents a new paradigm in antibiotic resistance since it highlights that NFGN may be a source of an important resistance trait for Enterobacteriaceae.

A. baumannii naturally produces a gene encoding an AmpC-type cephalosporinase. This gene usually expressed at a basal and low level; therefore, the amount of AmpC produced has no significant impact on the activity of expanded-spectrum cephalosporins (Potron et al., 2015). For this reason, the combination meropenem disc test results combined with cloxacillin were not considered.

Since resistance to carbapenem in the studied institution is high, in the last years colistin became the first-line drug for treating infections caused by Gram-negative pathogens. Colistin remains as the most active antimicrobial agent against *Acinetobacter* spp. and *P. aeruginosa* with stable susceptibility rates (Gales et al., 2012). However, two BSI samples detected by *P. aeruginosa* resistant to polymyxin B, generating concern about the evolution and outcome of antimicrobial resistance.

Conclusion

This study showed that: (i) most of the broad spectrum resistance patterns identified in Enterobacteriaceae can also be identified in *P. aeruginosa* and *A. baumannii*; (ii) multiple resistance mechanisms; and (iii) no single phenotypic test is sufficient to detect and differentiate carbapenem resistance among gram-negative bacilli, but detection of carbapenem resistance (Metallo- β -lactamase, KPC and others) is essential because it limits treatment options and may spread to susceptible bacteria by gene transfer.

Antibiotics according to the susceptibility standard and infection control measures are necessary in preventing the emergence of MDR bacteria in the study institution. However, increased resistance to β -lactams and fluoroquinolones is one of the alarming signs in the region.

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