



Article/Artigo

Occurrence and the susceptibility to antimicrobial agents in *Pseudomonas aeruginosa* and *Acinetobacter* sp. at a tertiary hospital in southern Brazil

Ocorrência e perfil de sensibilidade a antimicrobianos em *Pseudomonas aeruginosa* e *Acinetobacter* sp. em um hospital terciário, no sul do Brasil

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ABSTRACT

Introduction: The main mechanism of emerging resistance in *Pseudomonas aeruginosa* and *Acinetobacter* sp. isolates is the production of metallo- β -lactamases (M β Ls). M β Ls are enzymes capable of hydrolyzing cephalosporins, penicillins and carbapenems, but not monobactams (aztreonam), which are often used as antimicrobial therapy to treat nosocomial infections. **Methods:** An observational descriptive and retrospective study was designed to assess the frequency of M β Ls among strains of *P. aeruginosa* and *Acinetobacter* sp. obtained from a tertiary hospital in southern Brazil. **Results:** M β L production was observed in 77.6% (n = 173/223) for *P. aeruginosa* isolates and 22.4% (n = 50/223) of *Acinetobacter* sp. isolates. The *Acinetobacter* sp. isolates showed 92.8% sensitivity to amikacin and *P. aeruginosa* isolates showed 58.9% sensitivity to aztreonam. **Conclusions:** The M β L indices determined confirm the global concern with this mechanism of resistance.

Keywords: M β L. Bacterial resistance. *Pseudomonas aeruginosa*. *Acinetobacter*.

RESUMO

Introdução: O principal mecanismo de resistência entre isolados de *Pseudomonas aeruginosa* e *Acinetobacter* sp. é a produção de metalo- β -lactamases (M β Ls). As M β Ls são enzimas capazes de hidrolisar cefalosporinas, penicilinas e carbapenêmicos, mas não monobactâmicos (aztreonam) antibióticos que se encontram entre as principais opções terapêuticas para o tratamento de infecções causadas por bactérias não fermentadoras de glicose. **Métodos:** Um estudo observacional, transversal, descritivo e retrospectivo foi desenvolvido para avaliar a frequência e o perfil de susceptibilidade cepas de *P. aeruginosa* e *Acinetobacter* sp. produtoras de M β Ls isoladas no Hospital São Vicente de Paulo, Passo Fundo, Brasil. **Resultados:** A produção de M β Ls foi observada em 77,6% (n = 173/223) dos isolados de *P. aeruginosa* e em 22,4% (n = 50/223) dos isolados de *Acinetobacter* sp. Dentre as cepas produtoras de M β L, a maioria apresentou mais de 90% de resistência a seis antimicrobianos dos 12 testados, enfatizando a resistência a ceftazidima, gentamicina, aztreonam, piperaciclina/tazobactam, cefepime, ciprofloxacina, meropenem e tobramicina. **Conclusões:** Os índices de M β L encontrados confirmam a preocupação mundial com a disseminação desse mecanismo de resistência.

Palavras-chaves: M β L. Resistência bacteriana. *Pseudomonas aeruginosa*. *Acinetobacter*.

INTRODUCTION

Hospital infections are a major concern for public health agencies and the internal committees of hospital infection control. Coco-bacilli and Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter* sp. respectively, are extremely important pathogens that cause serious infections in different parts of the human body and may lead to patient death¹⁻³.

In the last decade, particularly in *P. aeruginosa*, enzymes were identified that promote hydrolysis of cephalosporins, penicillins and carbapenems but not monobactams, such as aztreonam, which are called carbapenemases or metallo- β -lactamases (M β Ls) and confer resistance to these antimicrobial^{4,5}.

The first reports of *P. aeruginosa* isolates producing M β Ls in Japan occurred in 1991, since then Europe, Asia and America have also recorded such events⁶. In Brazil, the gene is prevalent *bla*_{SPM-1} originally isolated in São Paulo⁷. Currently, the increase in the number of isolates of *P. aeruginosa* with *bla* genes in intensive care units (ICUs) is a cause of serious concern⁸. Risk factors for nosocomial infections in this environment include exposure to highly invasive practices, such as mechanical ventilation, urinary catheters, intra-arterial and intravenous catheters and the use of antibiotics in large quantities⁹.

All metallo- β -lactamases (M β Ls) hydrolyze imipenem, but this ability varies considerably and the rate of hydrolysis may or may not be correlated with the level of bacterial resistance to carbapenems. These enzymes are inhibited by EDTA (ethylenediaminetetraacetic acid) and other chelators of divalent cations and is thus correlated with its mechanism of action^{10,11}.

This study was conducted in order to extend current knowledge regarding the incidence and susceptibility profiles of M β L-producing *P. aeruginosa* and *Acinetobacter* in a tertiary hospital in Passo Fundo, RS, Brazil.

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Received in 12/09/2010

Accepted in 08/11/2010

METHODS

Place of the study

This study was conducted at the Laboratory of Clinical Analysis of São Vicente de Paulo Hospital (*Hospital São Vicente de Paulo*, HSVP) in Passo Fundo, State of Rio Grande do Sul, Brazil. The hospital has approximately 590 beds and 60 beds for support in a constructed area of 50,000m², with about 28,000 admissions per year.

Study design and data collection

A retrospective cross-sectional observational study was conducted that included patients at the HSVP and bacterial cultures tests performed from June to December 2008. The data collected were compiled on a spreadsheet where the following information was recorded: name, age, sex, hospital ward, number of laboratory records for the patient, type of biological material, underlying disease, culture results, susceptibility to antimicrobials and use of antibiotics before and after treatment. The data of patients with positive cultures for MβL-producing or nonproducing *P. aeruginosa* and *Acinetobacter* sp. were compared. For ethical reasons, the name of the patient was noted to only to prevent replication and was not disclosed.

Data collection was performed by the internal system of HSVP computers in the Laboratory of Clinical Analysis using Mylab for Windows; this system is used to disclose the results of patients to medical assistants and in the Program Requirements and Medical Patients, where the outcome of hospitalized patients is registered.

Definitions

The bacterial isolates were identified by classic morphological and biochemical tests, such as Gram stain, cytochrome oxidase, motility, TSI and pyoverdine production. The profile of antimicrobial susceptibility was determined by the disk-diffusion method, in accordance with the standards of the Clinical Laboratory Standards Institute (CLSI), using the following agents: 30μg of ceftazidime (CAZ), 10mg of gentamicin (GEN), 110μg of piperacillin/tazobactam (PIT), 30μg of amikacin (AMI), 30μg of aztreonam (ATM), 30μg of cefepime (CEP), 5μg of ciprofloxacin (CIP), 10mg of meropenem (MER), 10mg of tobramycin (TOB), 20μg of ampicillin/sulbactam (SBA), 30μg of ceftriaxone (CRO) and 15μg of tigecycline (TGC). The phenotypic screening for the MβL production was performed by combined disk using imipenem (10ug) as the substrate and EDTA (750mcg) as an inhibitor¹².

Statistical analysis

The percentage of strains producing MβL was calculated among cultures positive for pathogens. The data of the positive cultures and MβL-producing strains were compared with the type of biological sample, the profile of antimicrobial susceptibility and demographics, such as sex, hospital and age. Comparative analysis between the presence of MβLs and the different traits was performed by the Fisher exact test and Chi square test (χ^2 test).

Qualitative categorical variables, such as the presence or absence of MβLs, type of biological sample, the hospital and patient sex were compared using univariate (*Mantel-Haenszel common odds ratio estimate*) and calculation of odds ratios (ORs) with 95% confidence interval (95%CI), using SPSS (version 11.0), where $p < 0.05$ was considered statistically significant.

RESULTS

A total of 7,849 cultures were analyzed during the study period. The percentage of positive cultures for pathogens was 2.8% ($n = 223/7849$) for *P. aeruginosa* and *Acinetobacter* sp. Among these, 77.6% ($n = 173/223$) were identified as *P. aeruginosa* and 22.4% ($n = 50/223$) were *Acinetobacter* sp. The screening test for MβLs was positive in 17.4% of isolates of *P. aeruginosa* and 6.3% of isolates of *Acinetobacter* sp.

The MβL-producing isolates were recovered mainly from samples of tracheal aspirate ($n = 19/223$, 8.5%), urine ($n = 10/2,234$, 48%), tracheal secretion ($n = 7/223$, 3.1%) and sputum ($n = 6/223$, 2.6%); however, the type of sample was not statistically significant for either of the pathogens studied.

Other types of biological samples, such as cultures of catheters, drains, bronchial washings, secretions and cerebrospinal fluid samples, occurred less frequently. Among the 53 MβL-producing isolates, 11 patients were female and 42 were male, although differences between sexes was only significant for isolates of *P. aeruginosa* ($p = 0.034$), with an odds ratio of 2.361 (95%CI, 0.987 to 5,649) for males.

A variation in rates of positive cultures for MβL-producing *P. aeruginosa* and *Acinetobacter* sp. was observed for different hospital sectors, with the highest rate of production of MβLs occurring in the central intensive care unit (ICU-C) with 21 isolates ($n = 21/53$, 39.6%), followed by the centre for critical care nursing (CCCN) with 17 infected patients ($n = 17/53$, 31.1%) and station 7 (S07) with seven cases ($n = 7/53$, 13.2%). A significant difference in the presence of MβL-producing *Acinetobacter* sp. ($p = 0.13$) was only observed for the ICU. For this sector, the odds ratio was 4.051 (95%CI, 1.343 to 13.213). The age group with the highest rate of MβL production was over 60 years-old ($n = 28/53$, 52.8%), followed by the range from 25 to 60 years-old ($n = 15/53$, 28.3%), from 12 to 25 years-old ($n = 9/53$, 16.9%) and up to 5 years-old ($n = 1/53$, 1.8%). Not one MβL producer was recovered from patients aged 5 to 12 years-old (**Table 1**), although age showed no significant difference ($p = 0.564$). The underlying diseases that manifested most often in relation to the presence of MβLs were the respiratory system, including pneumonia and bronchopneumonia, respiratory failure, chronic obstructive pulmonary disease and pulmonary embolism, and liver diseases, such as cirrhosis, renal and neurological complications.

Comparing the isolates of MβL-producing *Acinetobacter* sp. with the nonproducing strains, the former present much greater resistance to the antimicrobial agents evaluated. Among the MβL-producing strains, most isolates showed over 90% of antimicrobial resistance to six of the 12 drugs tested, particularly to ceftazidime, gentamicin, aztreonam, piperacillin/tazobactam, cefepime, ciprofloxacin, meropenem and tobramycin (**Figure 1**). Only 7.1% of the MβL-producing isolates presented resistance to amikacin and tigecycline, while 28.5% of these isolates were resistant to ampicillin/sulbactam, providing the only therapeutic options for these cases. Meropenem, which has a high resistance rate and is used as standard treatment in cases of *Acinetobacter* sp. cannot be used in cases of MβL production, because resistance is superior to 90%, but in cases of MβL-nonproducing strains, it is the antibiotic of choice.

In MβL-nonproducing strains of *Acinetobacter* sp. the levels of antibiotic resistance were lower, with 34% of isolates resistant to ceftazidime, 42% to gentamicin, 26% to piperacillin/tazobactam,

TABLE 1 - Comparison between the frequency of isolates of *Pseudomonas aeruginosa* and *Acinetobacter sp.* producers and nonproducers of metallo- β -lactamases according to the characteristics evaluated.

Characteristics observed	<i>Pseudomonas aeruginosa</i>		<i>Acinetobacter sp</i>	
	number isolated (total 173)		number isolated (total 50)	
	No-M β L	M β L	No-M β L	M β L
Biological sample				
tracheal aspirate	37	11	40	8
tracheal secretion	22	6	27	1
sputum	30	6	36	0
urine	33	10	44	0
Inpatient unit				
UCI-C	47	13	52	8
CCCN	16	13	25	4
Post 7	19	7	26	0
Gender				
male	112	32	144	10
female	62	7	66	4
Age (years)				
0-5	13	1	14	0
>5-12	4	0	4	0
>12-25	23	5	24	4
>25-60	60	14	73	1
>60	85	19	95	9

No-M β L: no-metallo- β -lactamases, M β L: metallo- β -lactamases, UCI-C: intensive care unit central, CCCN: centre for critical care nursing.

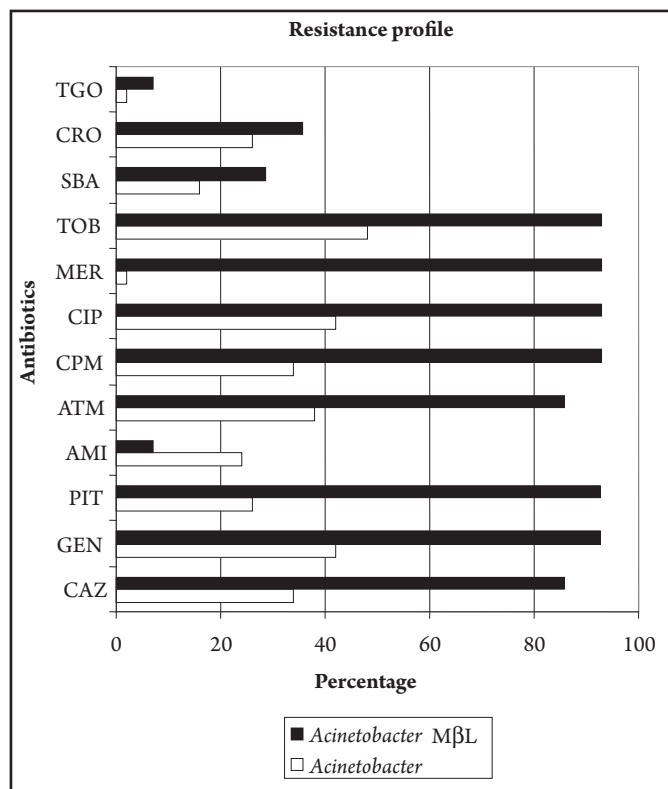


FIGURE 1 - Comparison between the resistance profiles of isolates of *Acinetobacter sp.* producers and not producing metallo- β -lactamases.

CAZ: ceftazidime, GEN: gentamicin, PIT: piperacilina/tazobactam, AMI: amikacin, ATM: aztreonam, CPM: cefepime, CIP: ciprofloxacin, MER: meropenem, TOB: tobramycin, SBA: ampicillin/sulbactam, CRO: ceftriaxone, TGO: tigecycline. M β L: metallo- β -lactamase.

24% to amikacin, 38% to aztreonam, 34% to cefepime, 42% to ciprofloxacin, 48% to tobramycin, 16% to ampicillin/sulbactam, 26% to ceftriaxone and only 2% to tigecycline and meropenem (Figure 1).

The M β L-producing strains of *P. aeruginosa* show greater resistance to the antibiotics tested than the nonproducing strains. However, M β L-producing strains present strength of more than 80% to only two antibiotics, ampicillin/sulbactam and meropenem, and resistance above 60% for six of the 10 antibiotics tested (Figure 2).

Among the M β L-nonproducing isolates of *P. aeruginosa*, resistance levels were much lower. The highest rates of resistance were observed against ampicillin/sulbactam (75.3%), followed by tobramycin (28.3%), gentamicin and ciprofloxacin (both 27.6%), amikacin (20.8%), aztreonam (17.9%), cefepime (16.4%), ceftazidime (12.6%), piperacillin/tazobactam (10.4%) and meropenem (3.7%).

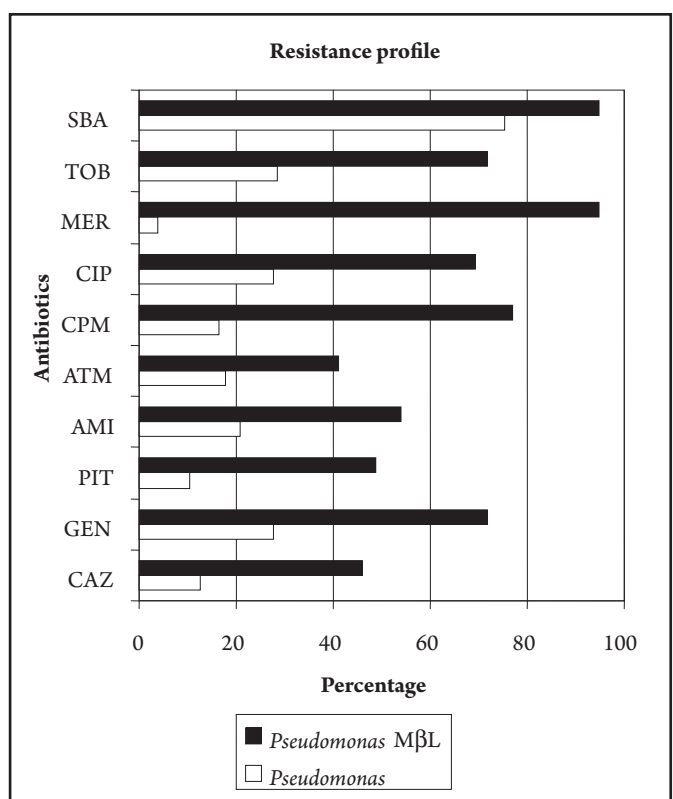


FIGURE 2 - Comparison between the resistance profiles of isolates of *Pseudomonas aeruginosa* producing and not producing metallo- β -lactamases.

CAZ: ceftazidime, GEN: gentamicin, PIT: piperacilina/tazobactam, AMI: amikacin, ATM: aztreonam, CPM: cefepime, CIP: ciprofloxacin, MER: meropenem, TOB: tobramycin, SBA: ampicillin/sulbactam, CRO: ceftriaxone, M β L: metallo- β -lactamase.

DISCUSSION

The frequency of M β Ls among isolates of *P. aeruginosa* in this study was 17.4%. In a study conducted at the same institution, Graf et al⁹ determined a rate of 10.8% of producers with phenotypes of resistance genes among isolates of *P. aeruginosa* in a period of three months. Sader et al⁵ verified a frequency of 19.7% for M β L-producing isolates at a hospital in São Paulo. Rates ranging from 70% of isolates resistant to carbapenems were reported by Gaspareto et al¹³ in Porto Alegre, in a two-year study. The high frequency of M β Ls verified emphasizes

the importance of phenotypically investigating the presence of MβLs in routine laboratory tests, since the enzymes are located in mobile genetic structures that can spread between bacterial strains and lead to epidemic outbreaks.

In this work, MβL-producing *Acinetobacter* sp. represented 6.3% of the samples identified. Similar rates are reported by the Mystic Program Brazil 2003, where 8.8% of bacterial isolates causing infections in ICUs were multidrug-resistant *Acinetobacter* sp.¹⁴.

Bacterial resistance to the antibiotics tested was consistent with several previous studies involving both pathogens. In MβL-producing *P. aeruginosa*, resistance exceeded 60% in six of the 11 antibiotics tested. The lowest resistance was observed against aztreonam, which can be used as a treatment option. For *Acinetobacter* sp. the lowest rates of resistance were observed against amikacin, tigecycline and sulbactam/ampicillin, making these the therapeutic options. The sensitivity profile of MβL-producing isolates of *Acinetobacter* sp. indicates the possible presence of other associated β-lactamase type oxacillinase (*bla*_{OXA23}), similar to the epidemic clone disseminated in Brazil¹⁵.

A significant increase in resistance to antibiotics was observed for these pathogens compared to MβL-nonproducing isolates. For *Acinetobacter* sp. even aztreonam shows diminished treatment effectiveness in pathogens producing this enzyme, probably due to the presence of other resistance genes, including those capable of hydrolyzing monobactams, such as extended spectrum β-lactamases (ESBLs). Reis et al¹⁶ reported that in some Brazilian hospitals, rates of carbapenem resistance increased approximately 12% for isolates of *Acinetobacter* sp. The profile of the *Acinetobacter* sp. strains could be the type of enzymes associated with *bla*_{OXA-23}. Dalla-Costa et al¹⁵ observed this strain in two hospitals in Curitiba, together with *P. aeruginosa*, and observed their similarity to MβL-producers due to carbapenem resistance and high sensitivity to aztreonam.

The high rate of resistance to most antibiotics tested indicates that the selection of resistant strains may be occurring and that the use of potent antibiotics may be contributing to this selection. Reis et al¹⁷ identified a strain of *Acinetobacter* sp. resistant even to polymyxin B, a worrying fact, since this is an extremely powerful drug and is often used as a last choice for the treatment of pan-resistant strains of *Acinetobacter* sp. According to Reis et al¹⁷, use of polymyxin B may have played a role in the selection of resistant strains, arguing against the theory of clonal spread. Furthermore, two isolates showed an identical genetic pattern, which raises the possibility of transmission of strains occurring between patients and the ability to create epidemics.

High rates of resistance observed in inpatient units, such as ICU-C, CCCN and Post 7, is probably related to the severity of cases among these patients, since these units are for hospitalized patients with more severe illnesses and greater susceptibility. MβL production occurred mainly in samples from the respiratory tract, stressing that both pathogens are important colonizers of artificial respiration and in patients with nosocomial pneumonia; another important risk factor is diabetes mellitus, which causes insulin resistance. Lautenbach et al¹⁸ reported that 44.6% of *P. aeruginosa* isolates were from respiratory tract samples. In nosocomial samples from Porto Alegre, Pulcinelli et al¹⁶ verified a rate of 70.7% in lower respiratory tract isolates of *P. aeruginosa*. In 2008, Carneiro et al¹⁹ reported that in a hospital in Londrina, Parana, the incidence of patients with ventilator-associated pneumonia was 25.2% for *Acinetobacter* sp.¹⁹.

According to the Manual of the Department of Infection Control, from 2009, of the HVSP, in-hospital precautions must be taken to prevent the spread of multidrug-resistant strains, which included contact precautions for patients colonized and/or infected, the use of aprons with long sleeves and appropriate use of procedure gloves and hand hygiene with antiseptic and water or alcohol gel before and after attending such patients, individual use of thermometers, stethoscopes and sphygmomanometers, individual, or preferably fourth cohort control within the hospital²⁰.

MβL rates (17.4% of *P. aeruginosa* isolates and 6.3% of *Acinetobacter* sp. isolates) verified among the HVSP isolates were similar to those of other Brazilian institutions. Detection of MβL production in routine laboratory exams provides the foundation and assists in the selection of the appropriate antimicrobial therapy. With the spread of MβL-producing strains in hospitals around the world, knowledge regarding the prevalence of MβL production is essential to develop policies for empirical therapy in high risk units, where rates of infection by these microorganisms are high. Surveillance, combined with monitoring and daily review of cultures in the microbiology laboratory and the use of antimicrobials, are effective measures to identify outbreaks in time to carry out measures to optimize and intervene in the conduct adopted in the various hospital sectors²¹. Thus, the rates of MβLs verified confirm the concern of the global emergency of this resistance mechanism.

ACKNOWLEDGMENTS

The authors would like to thank the Clinical Laboratory and the Committee on Infection Control at the São Vicente de Paulo Hospital for allowing them to develop this study. The authors are also grateful to Dr. Gilberto Barbosa da Luz for his review of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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