Prevalence of carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in high complexity hospital

**ABSTRACT**

*Pseudomonas aeruginosa* and *Acinetobacter baumannii* are Gram-negative bacilli that in the last decades have become prevalent agents of hospital infection due to high antimicrobial resistance developed by these microorganisms. The present study is a retrospective analysis of all positive cultures for these microorganisms in the period of January 2004 to December 2008. Resistance levels of *A. baumannii* and *P. aeruginosa* to carbapenems was high and showed a trend to increase during the period of study. In recent years the increasing incidence and resistance levels of *A. baumannii* and *P. aeruginosa* to the antimicrobials used for their treatment in the hospital setting underscores the relevance of infections caused by these bacteria. The selective pressure caused by indiscriminated use of broad-spectrum antibiotics in empirical hospital infections is probably the main reason for such an increase with the consequent impact upon patient morbidity and mortality.

Keywords: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, drug resistance, carbapenems, hospital infection control program.


**INTRODUCTION**

Hospital infections are caused by several microorganisms, being of great relevance the ones caused by bacteria. Some of these represent higher risk for the patient due to a reduced sensitivity profile to antimicrobial agents, as observed in glucose non-fermenting Gram-negative bacilli. In this group, *Pseudomonas aeruginosa*, followed by *Acinetobacter baumannii* are bacteria largely isolated in hospitals worldwide, being associated to high morbidity and mortality rates in seriously ill patients.

*P. aeruginosa* and *A. baumannii* have become increasingly resistant to broad-spectrum cephalosporins used in the hospital setting leading to the use of more powerful β-lactam antibiotics, as the carbapenems. Currently, these agents are important options to treat nosocomial infections due to their high affinity for type 2 (PBP2) penicillin-binding proteins with, stability to many β-lactamases, including broad-spectrum (ESBL) and chromosomal (AmpC), besides showing excellent permeability through bacterial outer membrane.

Wide use of carbapenems in the hospital environment can cause more selective pressure on hospital microbiota, thus enhancing the subpopulation of microorganism with decreased sensibility or resistance to these antibiotics. Currently, bacterial samples of *P. aeruginosa* and *A. baumannii* resistant to most antimicrobial agents and sensitive only to polymyxin B have been isolated in most of the Brazilian hospitals.

Resistance to carbapenems are thought to result from the production of Ambler class D and B β-lactamases, also referred to as metallo-beta-lactamases (MBL). Additionally, the production of these enzymes has commonly been responsible for the resistance phenotype to these β-lactams.

Due to the potential relevance of the evolution of microbial resistance, the objective of this study was to investigate the prevalence of *P. aeruginosa* and *A. baumannii* resistant to carbapenems at the Santa Isabel Hospital in Blumenau, Santa Catarina state, Brazil.
METHODS

All positive cultures for *P. aeruginosa* and *A. baumannii* resistant to carbapenems during the study period of January 2004 to December 2008 were included. Blood, urine, tracheal aspirate, washed bronchi alveolar, sputum, purulent wound, skin ulcers, and catheter tip samples collected from patients admitted to all units (ICU and other yards) were eligible. Data for bacterial identification and sensitivity tests were obtained from the records of the Hospital Infection Control Commission (HICC). Samples were processed at the local microbiology laboratory.

Strains of *P. aeruginosa* were isolated on chocolate agar, blood agar and MacConkey agar based on colony morphology, oxidase positive test, and glucose non-fermenting kit (Probac).

Strains of *A. baumannii* were isolated on chocolate agar, blood agar and MacConkey agar based on colony morphology, negative oxidase test, and glucose non-fermenting kit (Probac).

For the sensitivity tests before placing the discs, Mueller-Hinton plates were inoculated with swabs immersed in the inoculation final solution and smeared on the entire plate surface. Afterwards, the plates were inverted and incubated at 35 ± 2°C for 20 to 24 hours. The sensitivity was investigated using the Kirby-Bauer method for reading the disc diffusion, according to the Clinical and Laboratory Standards Institute criteria.3

Annual resistance rates of each microorganism during the period of study were tested by the Fischer’s exact test.18 The p-value for significance was 0.05. Data analysis was performed with STATISTICA software, version 6.19

RESULTS

During the studied period 228 strains of *P. aeruginosa* and 140 strains of *A. baumannii* were isolated.

Figures 1 and 2 depict the sensitivity results of 140 strains of *A. baumannii*. In 2004 there was no report of resistance to meropenem, whereas 5.13% showed resistance to imipenem. In 2005, resistance rates significantly increased for both antibiotics: for imipenem it rose to 55% (p = 0.00003), and for meropenem the rate was 60% (p < 0.0001). From 2006 the resistance rates kept gradually increasing from 2006 and on, reaching rates of 77.27% to imipenem and 80% to meropenem in 2008.

Figures 3 and 4 show the sensitivity results for the 228 strains of *P. aeruginosa*. In 2004 there was only 6.06% of resistance to imipenem, whereas 5.13% showed resistance. In 2005, resistance rates significantly increased for both antibiotics: for imipenem it rose to 55% (p = 0.00003), and for meropenem the rate was 60% (p < 0.0001). From 2006 the resistance rates kept gradually increasing from 2006 and on, reaching rates of 77.27% to imipenem and 80% to meropenem in 2008.
DISCUSSION

There are a large number and variety of new resistance mechanisms that have emerged and their preliminary detection is important for infection control and adequate therapeutic guidance.

Among the resistance mechanisms to carbapenems described for *P. aeruginosa* and *A. baumannii* can be highlighted β-lactamases production, efflux pump and loss of porins can be highlighted.

β-lactamases have been grouped into four molecular classes A, B, C and D, based on the amino acids sequence homology according to Ambler classification (1980). The ones that belong to A, C and D classes are called serine-β-lactamases, and the others in B class are called metallo-β-lactamases (MBL). These enzymes have the common property of hydrolyzing; at least partially, imipenem or meropenem, besides they hydrolyze other penicillins and cephalosporins.

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In Brazil, the first report of acquired MBL was in 1997, in a sample with *P. aeruginosa*, reported as SPM-1 variant at the São Paulo Hospital/UNIFESP. In 1998, in the same hospital, samples of *Acinetobacter* spp with resistance or reduced sensitivity to carbapenems were isolated. Out of those, 54% (40/73) were IMP-1 producers. In 2003, a sample of *A. baumannii* presenting IMP variant was isolated which was resistant to imipenem, meropenem and broad-spectrum cephalosporins.

Resistance of *P. aeruginosa* and *A. baumannii* to Carbapenems may result from changes in the penicillin-binding proteins and porins. Since carbapenems enter the bacterium through the porins, one could postulate that porins changes could be involved in the resistance to these antibiotics.

Efflux pumps are a unique resistance mechanism against different classes of antimicrobials. AdeABC efflux pump, characterized in *A. baumannii*, together with the over-expression oxacilinases can result in resistance to carbapenems. In *P. aeruginosa*, the MexA-MexB-OprM efflux pump is responsible for antimicrobial transport to the external side of the bacterial cell.

At Saint Joseph Martin Hospital in Buenos Aires from 1998 to 2001, Rodriguez et al., reported 60% of *Acinetobacter* spp resistance to imipenem. Furthermore, *P. aeruginosa* showed an increasing resistance to imipenem from 15.4% to 68%.

A Latin America multicenter study, the SENTRY - Antimicrobial Surveillance Program – reported in 2001 *Acinetobacter* spp. resistance rates to imipenem of 16.7% and to meropenem of 18.17%. *P. aeruginosa* presented resistance of 37.8% to imipenem and 35.6% to meropenem.

The data obtained showed a significant increase of *A. baumannii* carbapenems resistance from 2005, whereas *P. aeruginosa* did not present statistically significant difference over the years. Molecular studies are essential to unfold resistance mechanisms, mainly associated to *A. baumannii*. Likewise, they will be useful to evaluate a possible cloned dissemination in the hospital environment.

CONCLUSION

New bacterial resistance patterns have emerged, and the gathering of different enzymes in different bacterial species shows the real potential for dissemination of these genetic elements and causes great concern.

The data presented showed high rates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* resistant to carbapenems, reducing the availability of effective agents. The fight against these microorganisms in hospitals can be accomplished through the constant presence of the hospital infection control committee, recognizing the local microbiota and having protocols for a rational use of antimicrobials.

REFERENCES