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## Epidemiologic analysis of clinical isolates of *Pseudomonas aeruginosa* from an university hospital

*Análise epidemiológica de isolados clínicos de Pseudomonas aeruginosa provenientes de hospital universitário*

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### ABSTRACT

**Objectives:** *Pseudomonas aeruginosa* is an increasingly prevalent opportunistic pathogen in hospital infection cases. Its high resistance rates to many antimicrobials has given this microorganism a relevant role among other highly prevalent bacteria involved in nosocomial infections. This study aimed to analyze epidemiologic characteristics of *P. aeruginosa* and to evaluate its susceptibility to antimicrobial agents at Hospital das Clínicas of the Universidade Federal de Pernambuco

**Methods:** A retrospective study was performed based on the registry book of miscellaneous secretions from the bacteriology laboratory of the Hospital das Clínicas involving the period between January and June 2008. Among the secretions registered, were identified the positives samples for *P. aeruginosa*, whose origin was analyzed, as well as its susceptibility profile to routinely used in our laboratory antimicrobials.

**Results:** The bacteria most frequently isolated from miscellaneous secretions bacteria were *P. aeruginosa* (26%) and *S. aureus* (25%). *P. aeruginosa* was mainly isolated from respiratory infections, with 33% of positive samples for this organism from tracheal secretions and 21% from nasal. The most effective antimicrobials against *P. aeruginosa* were: amikacin, imipenem, meropenem and aztreonam.

**Conclusions:** These results show a high prevalence of *P. aeruginosa* in the Hospital das Clínicas of the Universidade Federal de Pernambuco. Despite featuring high resistance rates to older antimicrobials, as cephalosporins first and second generations and chloramphenicol, this pathogen showed good susceptibility to agents routinely used in this hospital.

**Keywords:** *Pseudomonas aeruginosa*; *Pseudomonas* infections; Antimicrobial drugs resistance; Nosocomial infections

### INTRODUCTION

*Pseudomonas aeruginosa* has been outstanding through the years among the most frequently isolated infective organisms in hospital environments. Although the technological advance and the large number of antimicrobials available, thousands evolution years gave this organism natural and acquired resistance mechanisms which, sometimes, defeat modern therapeutics.

In a susceptible patient, *P. aeruginosa* may infect any body region. It can cause infections in skins of burned patients<sup>(1)</sup> and neonates,<sup>(2)</sup> eye,<sup>(3)</sup> wounds,<sup>(4)</sup> bones and joints,<sup>(5)</sup> urinary tract<sup>(6)</sup> and most frequently, respiratory tract infections,<sup>(7-12)</sup> or in any region where natural protection mecha-

nisms are weakened. Thus, intensive care unit (ICU) patients are particularly prone to *P. aeruginosa* infections. This organism may easily spread within hospital facilities, as additionally it is resistant to chemical disinfectants and antiseptics as ammonium quaternary compounds, phenol and hexachlorophene.<sup>(13)</sup>

It is currently quite impossible talking on hospital infections and not mentioning *P. aeruginosa*. In the last four decades, this organism was responsible for 10% of all reported nosocomial infection cases.<sup>(14-16)</sup> Started in 1997, the SENTRY (Antimicrobial Surveillance Program) is a surveillance study of antimicrobials resistance involving worldwide medical centers.<sup>(7)</sup> Nevertheless the several investigators involved in this program efforts, Brazilian data published then were not actually representative.<sup>(17)</sup> According to the Brazilian National Health Surveillance Agency, the multi-center trials included few Brazilian centers an a small sample size, beneath a continent-sized country reality.<sup>(17)</sup> In 1999 the MYSTIC (Meropenem Yearly Susceptibility Information Collection) was started in Brazil, as an yearly surveillance program comparing several broad spectrum antimicrobials' activity among carbapenem using centers.<sup>(18)</sup>

Thus, epidemiologic studies help monitoring high pathogenic potential germs as *P. aeruginosa*. Then, this study aim was to analyze the *P. aeruginosa* prevalence among other organisms isolated in patients at the Hospital das Clínicas of the Universidade Federal de Pernambuco between January and June 2008, as well as to evaluate their origin and susceptibility profile to routine antimicrobials used in this hospital's laboratory.

## METHODS

A retrospective study was performed examining the period between January and June 2008, based on microbiologic data from the Hospital das Clinicas (HC) of the Universidade Federal de Pernambuco's bacteriology laboratory registry book of miscellaneous secretions. Clinical samples from all wards were analyzed according to their origin, month and bacteriological data.

*P. aeruginosa* was identified by analysis of suspect microbial colonies in sheep blood agar (5%) and next replicated in selective and differential media, ammonium chloride acetyl-methyl agar (cetrimide), along with biochemic oxydase production test, motility and pyocyanin production tests, in order to isolate and

identify the infection-involved organism gender and species. Several records regarding each organism were counted aiming to estimate *P. aeruginosa* prevalence over other isolates. *P. aeruginosa* positive record clinical samples were also analyzed.

The *P. aeruginosa* resistance and sensitivity profile analysis was performed by the Kirby-Bauer diffusion method, in compliance with the National Committee for Clinical and Laboratory Standards (NCCLS) (2006) criteria<sup>(19)</sup> and according to the antimicrobial groups (cephalosporins, aminoglycosides, carbapenems, quinolones, sulphs, tetracyclins, in addition to aztreonam and chloramphenicol).

This study is part of the project approved by the Human Research Ethics Committee of the Universidade Federal de Pernambuco Health Sciences Center, registration number CEP/CCS/UFPE N° 015/08.

## RESULTS

*P. aeruginosa* was the most frequently isolated germ during the study period, and was identified in 182 (26%) of the 701 satisfactory bacterial growth samples. Ninety seven (53.3%) of these 182 secretions were from intensive care unit patients, and due to lack of complementary data in the samples registration book, we are not able to describe the patients' clinical profile (hospital stay length, previous antimicrobials use, previous admissions, structural lung damage, chemotherapy, etc). The second most frequent organism was *Staphylococcus aureus*, identified in 173 (25%) positive cultures. One hundred and thirty six (19%) cultures had no gender or species identified, and were recorded as gram-negative non-fermenting bacillus (GNNFB), thus changing a clear diagnosis and bringing a bias to organisms prevalence, as *P. aeruginosa* itself. Other organisms as *Klebsiella pneumoniae*, *Proteus mirabilis* and *Escherichia coli*, were less frequent in the several secretions (Table 1).

Among the 182 *P. aeruginosa* positive records, 60 (34%) were from tracheal materials and 39 (21%) from nasal ones. Other less frequent than respiratory secretions materials were catheter tip (8%), bony fragments (7%), surgical wounds (6%), pressure sores (6%), ulcers (6%), skin lesions (3%), sputum (2%), eye secretion (1%). In another 7% there was no record of the sample origin (Table 2).

The antimicrobial susceptibility profile analysis showed that *P. aeruginosa* had, overall, good sensitivity to routinely used in the HC bacteriology laboratory

**Table 1 - Frequency of several organisms isolated from secretions samples**

Organism	Number of isolates	% isolates*
<i>Pseudomonas aeruginosa</i>	182	26
<i>Staphylococcus aureus</i>	173	25
GNNFB	136	19
<i>Klebsiella pneumoniae</i>	97	14
<i>Proteus mirabilis</i>	80	11
<i>Escherichia coli</i>	30	4
Outros	132	19

GNNFB – gram-negative non-fermenting bacillus. \*The isolated percent (% isolates) regards the number of organisms isolated by total positive cultures (701).

**Table 2 – *P. aeruginosa* samples origin**

Sample origin	Amount	%*
Tracheal secretion	60	33
Nasal secretion	39	21
Catheter tip	14	8
Bony fragment	12	7
Surgery wound	11	6
Pressure sores	11	6
Ulcers	11	6
Skin lesions	6	3
Sputum	3	2
Eye secretion	2	1
Others	13	7

\*Percent regarding the rate between each sample type amount and total *P. aeruginosa* positive samples (182).

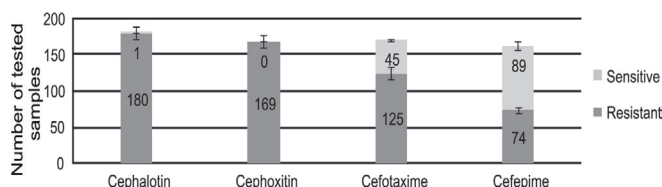
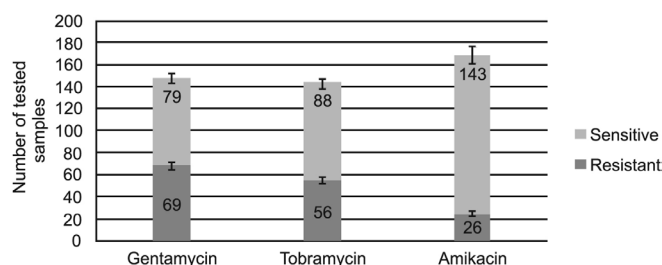
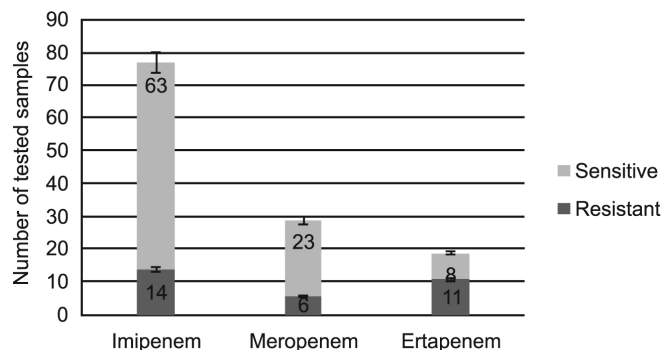
antimicrobials. Regarding the cephalosporin group, resistance was inversely proportional to their generation. Among the 182 *P. aeruginosa* isolates, 181 were tested for cefalotin (first generation cephalosporin – C1), and 180 of them were resistant. For cefoxitin (second generation cephalosporin – C2), the resistance ratio was even higher, and 100% of the 169 samples were not inhibited by this drug. A decrease in this high resistance started to be seen from cefotaxime (third generation cephalosporin – C3), where 73% (125) of the 170 tested samples were resistant, and for cefepime (fourth generation cephalosporin – C4) this ratio was 45% (74%) (Figure 1).

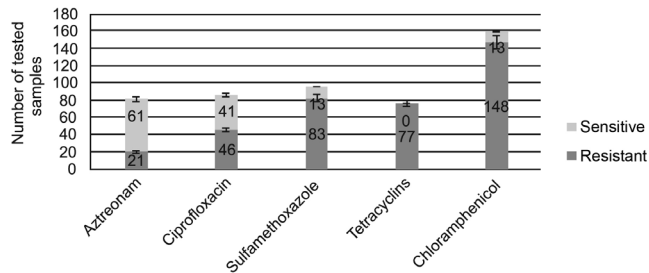
The aminoglycosides group resistance was lower than for cephalosporins. For gentamycin, 46.6% (69) of the 148 isolates tested were resistant. For tobramycin, the sensitivity was even higher, and 61.1% (88) of

the 144 tested samples were sensitive to the drug. For amikacin, only 26 (15.4%) of the 196 *P. aeruginosa* tested were not inhibited by this drug (Figure 2).

The HC patients' samples featured good sensitivity to carbapenem antimicrobials in the analyzed period. From the 77 samples evaluated for imipenem sensitivity, 63 (81.8%) were sensitive to the drug. Only 29 samples were tested for meropenem, however 23 (79.3%) were inhibited by this drug. For ertapenem, the number tested was even lower (19), and the sensitivity to this antimicrobial was 42.1% (Figure 3).

Other HC's bacteriology laboratory routinely used antibiogram antimicrobials are [antibiotic (% sensitive)]: aztreonam (74.4%); ciprofloxacin (47.1%); sulfamethoxazole (13.5%); tetracycline (0%); and chloramphenicol (8.0%) (Figure 4).

**Figure 1 - Cephalosporins susceptibility profile for *P. aeruginosa* tested samples.****Figure 2 – Aminoglycosides susceptibility profile for *P. aeruginosa* tested samples.****Figure 3 - Carbapenem susceptibility profile for *P. aeruginosa* tested samples.**



**Figure 4 - Other antimicrobials and their susceptibility profiles for *P. aeruginosa* tested samples.**

## DISCUSSION

The Hospital das Clínicas of the Universidade Federal de Pernambuco's most isolated germ was *P. aeruginosa*, followed by *S. aureus*. Lisboa et al.,<sup>20</sup> in a prevalence study in 16 intensive care units in the Rio Grande do Sul state [Brazil] found that 122 patients were infected and 51 (29%) of them acquired the infection at the ICU itself. Some years before, Sader et al.<sup>(7)</sup> surveyed data from 11 Brazilian hospitals between 1997 and 1998 as part of the SENTRY program, evaluating a total of 525 bacterial samples from these hospitals patients' lower respiratory tract. The five most frequent species were (n/%) : *Pseudomonas aeruginosa* (158/30.1%), *S. aureus* (103/19.6%), *Acinetobacter spp.* (68/13.0%), *Klebsiella spp.* (50/9.5%), and *Enterobacter spp.* (44/8.4%). In North America, Hoban et al.,<sup>(14)</sup> also studied SENTRY-related epidemiologic data. They studied 2,712 samples isolated from pneumonia patients in 30 different medical centers (25 in the United States and 5 in Canada). More than 30 microorganisms were identified in the samples, being the most prevalent *S. aureus* (28%) and *P. aeruginosa* (20%). More recently, Kiffer et al. performed a susceptibility study in Gram-negative bacteria involved in nosocomial infections as part of the fourth MYSTIC program in Brazil, 2003, and *P. aeruginosa* (30.3%) was the most prevalent organism among the 1,550 analyzed isolates.<sup>(18)</sup>

*P. aeruginosa* appears to have an increased respiratory tract tropism. In this study it was possible noticing that more than 50% samples were from respiratory origin (nasal and tracheal secretions). However, would this be due to this organism tropism? Or would it be just an infection favored by the patient's hospitalization? First, we should bear in mind that this is a facultative aerobic bacterium. As said, *P. aeruginosa* is an essentially opportunistic pathogen, and the hospitalized patient is weakened not only by the disease

condition, but also by hospital features as mechanic ventilation, drugs and the hospitalization emotional condition itself. The respiratory tract surface is protected by a mucus network rich in fibronectin, and to colonize it *P. aeruginosa* releases structure breaking proteases, exposing the receptors where the fimbria can connect. Virus-injured or irritated tissues, e.g., may ease the colonization process. This mechanism is called opportunistic adherence.<sup>(21)</sup>

Aiming to evaluate mechanic ventilation pneumonias cause, Guimarães and Rocco,<sup>(12)</sup> performed a study in 278 patients hospitalized at the Hospital Clementino Fraga Filho (of the Universidade Federal do Rio de Janeiro) intensive care unit, who were above 24 hours under mechanical support. Among other factors, 45.3% of the pneumonias were attributed to Gram-negative bacteria infections, and among them the most frequent was *P. aeruginosa*, representing 22% of the group. Another study at the Universidade Estadual de São Paulo by Villas Boas and Ruiz<sup>(22)</sup> aimed to evaluate the occurrence of hospital infections and associated risk-factors in their university hospital from September 1999 to February 2000. The highest infection prevalence was respiratory, mounting 27.6% of the infection cases, and the most present organism was *P. aeruginosa*, identified in 37.5% of the infected samples. In Fortaleza-CE [Brazil], the results found by Menezes et al.<sup>(23)</sup> were not very different from the above mentioned in a study performed at the Hospital Geral de Fortaleza's isolates from January to December 2002. The most frequent tracheal secretions bacteria were *P. aeruginosa* (16%) and *K. pneumoniae* (15%). During the SENTRY program first four years, Gales, Sader and Jones<sup>(24)</sup> surveyed data from hospitals all over Latin America aiming to evaluate the frequency of main pneumonia-associated pathogens. At the study end they found that the most frequent organisms were (n/%) : *Pseudomonas aeruginosa* (659/26.3%), *Staphylococcus aureus* (582/23.3%), *Klebsiella pneumoniae* (255/10.2%), *Acinetobacter spp.* (239/9.6%), and *Enterobacter spp.* (134/5.4%). Among 1,550 samples analyzed by Kiffer et al.,<sup>(18)</sup> 265 were from the respiratory tract, among which 121 (45.7%) were positive for *P. aeruginosa*. Thus, the hospitalized patient status, under mechanic ventilation, should be taken as a *P. aeruginosa* airways infection predisposing factor, as well as these organisms adaptability to these ways infection cannot be neglected. In other words, this high respiratory tract organism prevalence should not be explained only by immunologic deficiency in the hos-

pitalized subject, but also by the organism-associated infection ability.

In another trial performed between 2004 and 2006 in samples from the Hospital das Clínicas de Pernambuco and the Hospital Agamenon Magalhães (HAM), Recife-PE, Figueiredo et al. (2007) showed that cefepime was the most active cephalosporin against *P. aeruginosa*, with 58.6% sensitivity at the HC (in 162 tested), and 32% at the HAM (in 97 tested).<sup>(25)</sup> In the current trial, the cefepime sensitivity was lower, 45% in 163 analyzed samples.

In their studies, Leiser, Tognim and Bedendo,<sup>(11)</sup> found similar results regarding cephalosporin resistance in the ICU *P. aeruginosa* tested samples. Although the paper has no data on C1, no sensitivity was found at all for the samples tested against C2, as 100% of the tested samples were resistant. For C3, only 15% sensitivity was found and for C4 34.5% of the tested *P. aeruginosa* were sensitive. Sader et al.,<sup>(7)</sup> also reported high resistance to C1 and C2, with no records of sensitivity for these drugs at all. They also found sensitivity in only 5% for ceftriaxone (C3), however 57.6% of the samples tested sensitive to ceftazidime (C3) and 63.9% were sensitive to cefepime (C4).

Sader et al.<sup>(7)</sup> used the same aminoglycosides routinely used at HC in their trials, finding sensitivity to gentamycin in 56.3% of the tested *P. aeruginosa* samples; for tobramycin, 59.5% of the cases; for amikacin, in 63.9% of the isolates. In North America, Hoban et al.<sup>(14)</sup> found that the most effective antimicrobial for *P. aeruginosa* in their studies was amikacin, with 93.7% sensitivity. Following, tobramycin was this pathogen highest inhibitor, with 90.2% sensitivity.

The introduction of carbapenem antibiotics into the clinical practice represented an important advance in other  $\beta$ -lactam antibiotics-resistant bacteria.<sup>(26)</sup> Thus, carbapenems are the antimicrobial therapy of choice for severe hospital infections by gram-negative germs.<sup>(27)</sup> Sader et al.<sup>(7)</sup> reported 66.5% sensitivity to imipenem and 69% to meropenem. In North America, Hoban et al.<sup>(14)</sup> found that 89.1% of the tested samples were sensitive to meropenem and for imipenem this sensitivity was 85.6%. Gales, Sader and Jones<sup>(24)</sup> also recorded good action of the carbapenem meropenem, reporting sensitivity to this compound of 71.6%. Although promising, these data contrast with other reports of increased carbapenem resistance, mostly due to metallo- $\beta$ -lactamase production.<sup>(28,29)</sup>

## CONCLUSIONS

The above discussed results showed the high prevalence in the Hospital das Clínicas de Pernambuco during the evaluated period. It can also be observed that the respiratory tract is the most affected site by infections caused by this germ. Although very resistant to some antimicrobials, *P. aeruginosa* showed good sensitivity to carbapenems (except ertapenem) and amikacin. *P. aeruginosa* susceptibility to all other drugs was not relevant, at least not enough to allow these to be prescribed as empiric starting treatment in cases of suspected *P. aeruginosa* infection. Thus, appropriate antimicrobials use along with rigorous control of this and other pathogens dissemination may disrupt these organisms spread.

## RESUMO

**Objetivos:** A *Pseudomonas aeruginosa* é um patógeno oportunista que tem se destacado quanto à prevalência em casos de infecções hospitalares. Sua ampla resistência aos diversos grupos de antimicrobianos garante a este microrganismo um papel de destaque entre as bactérias mais prevalentes associadas à infecção nosocomial. O objetivo deste estudo foi realizar um levantamento epidemiológico da *P. aeruginosa*, bem como do seu perfil de susceptibilidade aos antimicrobianos no Hospital das Clínicas da Universidade Federal de Pernambuco.

**Métodos:** Foi realizado um estudo retrospectivo baseado no livro de registro de secreções diversas do laboratório de bacteriologia do Hospital das Clínicas no período compreendido entre janeiro a junho de 2008. Entre os registros, identificamos aqueles que foram positivos para a *P. aeruginosa*, analisando sua origem e perfil de susceptibilidade aos antimicrobianos utilizados na rotina daquele laboratório.

**Resultados:** As bactérias mais frequentes, isoladas das secreções diversas, foram *P. aeruginosa* (26%) e *S. aureus* (25%). Quanto à origem, a *P. aeruginosa* foi isolada principalmente de infecções respiratórias, pois 33% das amostras positivas para esta bactéria foram provenientes de secreções traqueais e 21% nasais. Os antimicrobianos mais eficazes contra a *P. aeruginosa* foram: amicacina, imipenem, meropenem e aztreonam.

**Conclusões:** Estes resultados mostram uma alta prevalência de *P. aeruginosa*, no Hospital das Clínicas da Universidade Federal de Pernambuco. Apesar de apresentar grande resistência a antimicrobianos mais antigos como as cefalosporinas de primeira e segunda geração, assim como cloranfenicol, em geral, este patógeno demonstrou boa sensibilidade às drogas utilizadas na rotina deste hospital.

**Descritores:** *Pseudomonas aeruginosa*; Infecções por pseudomonas; Resistência bacteriana a antimicrobianos; Infecção nosocomial

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