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## RESEARCH ARTICLE

### OCCURRENCE OF *PSEUDOMONAS AERUGINOSA* AND *KLEBSIELLA PNEUMONIAE* IN NASAL SECRETION OF UNDERGRADUATE STUDENTS OF HEALTH SCIENCES AREA WHO DO NOT ATTEND THE HOSPITAL ENVIRONMENT IN THE CITY OF BELFORD ROXO, PROVINCE OF RIO DE JANEIRO, BRAZIL

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#### ABSTRACT

Hospital infections are considered as a cause of the high mortality rate that occurs during the hospitalization period, and considered an important public health problem. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* are among the most frequent, easily transmitted and resilient microorganisms resistant to several antimicrobials. The aim of this research was to investigate the prevalence of *P. aeruginosa* and *K. pneumoniae* among undergraduate students of Health Sciences area who do not attend the hospital environment and test the sensitivity of these pathogens to antimicrobial agents. A total of 120 nasal material samples were analyzed. Isolation and identification of bacteria were performed by microbiological methods. The results showed that among the 120 examined students, 3 were colonized by *P. aeruginosa*, with a prevalence of 2.5%, and 2 colonized with *K. pneumoniae* with prevalence of 1.7%. No multidrug resistant strains were isolated. All strains showed variable degrees of susceptibility to the tested antimicrobials.

#### INTRODUCTION

The *Pseudomonas* genus in the past consisted of a large heterogeneous variety of non-fermenting carbohydrate bacteria that were grouped by their similarity of morphological characters. These bacteria were called “pseudomonads” because they generally appeared in pairs of cells resembling a single cell when viewed under an optical microscope. Studies carried out by several researchers split this in several new genera, including *Burkholderia* and *Stenotrophomonas*. Researchers believe that *P. aeruginosa* is the most important and most frequent as pathogen and is often the cause of several nosocomial infections.

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Species of this genus are found in soil, water, deteriorated organic matter, plants and are also found throughout the hospital environment and in humid places, including food, sinks, restrooms, cleaning clothes, respiratory physiotherapy equipment, hemodialysis devices, air conditioning systems and even disinfectant solutions (Murray *et al.*, 2004, Coura, 2014). *Pseudomonas aeruginosa* has several virulence factors, such as adhesins, toxins and enzymes, being the secretion system used by this bacterium the type III system. It is effective in injecting toxins into the host cell. Despite various factors of virulence, several factors act together in the genesis of the disease (Cimerman, 2013, Lewinson 2014). *Pseudomonas aeruginosa* infections produce an acute inflammatory response. The bacterium invades arteries and veins causing vascular thrombosis and hemorrhagic necrosis mainly in the skin and lungs. The invasion of blood vessels predisposes to dissemination and sepsis, leading to multiple nodular lesions in the lungs.

Sometimes disseminated infections cause typical skin lesions, called ecthymagangrenosum. These nodular necrotic lesions present sites where the bacterium has spread to the skin, invaded blood vessels and produced localized hemorrhagic infarcts (Rubin *et al.*, 2013). Secondary pneumonia caused by *Pseudomonas* can complicate infections caused by influenza virus or other respiratory tract infections (Kumar *et al.*, 2005; Brasileiro-Filho, 2011). *Klebsiella pneumoniae* and *Enterobacter* spp. are bacteria that account 10% of all hospital infections, including pneumonia, urinary tract infections, biliary tract and surgical wounds. Transmission among professionals working at the hospital environment represents a special risk in the transmission link of the professionals to hospitalized patients. Predisposing factors includes obstructive pulmonary disease, tracheal tube contamination, delayed catheters, debilitating diseases, and immunosuppressed or immunocompromised patients (Kumar *et al.*, 2005; Brasileiro-Filho, 2011).

Regarding the pathogenesis, *Klebsiella pneumoniae* and *Enterobacter* spp. are inhaled and multiply within the alveolar spaces. The pulmonary parenchyma becomes consolidated and the exudate consisting of mucus that fills the alveoli is dominated by macrophages, fibrin and edema fluid. As the exudate accumulates, the alveolar walls become compressed and then necrotic; several small abscesses can degenerate and cause cavitation (Lopes, 2016, Rubin *et al.*, 2013, Trabulsi&Alterthum, 2008). Considering the importance of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as important pathogens, the objective of this research was to investigate these microorganisms in the nasal secretion of undergraduate students of Health Sciences area who do not attend the hospital environment, to treat the possible carriers and avoid them to be sources of infection.

## METHODS

The research design has a cross-sectional, descriptive and prospective study, with a representative sample performed by 120 volunteer students of the first classes of undergraduate courses of Health Sciences area. The research was carried out from March to June 2016. The inclusion criteria to the research universe were: to be inscribed in a Health Sciences course, not to have attended a hospital environment and not to be a user of antibiotics. Each student was asked for authorization to collect material and use the results data for a scientific article and without personal identification of each participant, and the technique was informed of the importance of the study. The collection of nasal secretions was performed with sterile swabs. Each sample unit was immediately seeded into Petri dishes containing the culture media: blood agar, methylene blue agar (Teague), Cled-agar and *Pseudomonas*-selective agar. The dishes were incubated at 37°C for 24 hours. The developed colonies were identified by the morphotintorial characteristics for Gram method, cultural characteristics and biochemical tests. Antimicrobial susceptibility testing: the antibiograms were performed through agar diffusion technique according to the procedure standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). From cultures growth of *P. aeruginosa* and *K. pneumoniae*, a bacterial sample was transferred to 5mL of Brain Heart Infusion (BHI) medium and incubated for 5 hours. The suspension was prepared with the density adjusted to 0.5 of the McFarland

turbidityscale, which corresponds to the concentration of approximately 10<sup>8</sup> Colony-forming Units/mL. Using sterile swabs, the suspensions were seeded on the surface of Mueller-Hinton agar, in order to obtain confluent growth. Discs manufactured by Cefar industry impregnated with antibiotics were applied over the seeded surface. The plates were incubated at 37°C for 24 hours. The following antibiotics were tested: rifampicin, gentamicin, ciprofloxacin, norfloxacin, cephalothin, cefoclor, cephalixin, cefotaxime, nalidixic acid, carbemicillin, sulfazotrim and chloramphenicol. After this period, the diameter of growth inhibition halos was measured. For interpretation of the readings, samples with inhibition halos smaller than 10 mm were considered resistant, and the occurrence of any growth within the halo was interpreted as an evidence of a resistant subpopulation.

## RESULTS

The results shown that among 120 examined students, 3 were colonized by *Pseudomonas aeruginosa*, corresponding to a prevalence 2,5%, and 2 were colonized by *Klebsiella pneumoniae*, corresponding to a prevalence of 1,7%. No multidrug resistant strains were isolated. All the analyzed strains presented variable degrees of susceptibility to the tested antimicrobials (Tables 1 and 2).

**Table 1 - *Pseudomonas aeruginosa* strains isolated from nasal secretions of healthy carriers among students of Health Sciences and their sensibility to tested antibiotics**

Antibiotic	Positive sample1	Positive sample2	Positive sample3
Nalidixicacid	R	R	R
Carbenicilin	S	S	S
Cefalotin	R	R	R
Cefalexin	R	R	R
Cefoclor	R	R	R
Cefotaxime	R	R	R
Ciprofloxacin	S	S	R
Chloramphenicol	S	R	R
Gentamicin	S	S	S
Norfloxacin	R	R	R
Rifampicin	R	R	R
Sulfazotrim	R	R	R

**Tabela 2. *Klebsiella pneumoniae* strains isolated from nasal secretions of healthy carriers among students of Health Sciences and their sensibility to tested antibiotics**

Antibiotic	Positive sample1	Positive sample2
Nalidixicacid	R	R
Carbenicilin	S	S
Cefalotin	S	R
Cefalexin	S	R
Cefoclor	S	S
Cefotaxime	S	S
Ciprofloxacin	S	S
Chloramphenicol	R	R
Gentamicin	S	R
Norfloxacin	S	S
Rifampicin	R	R
Sulfazotrim	R	R

## DISCUSION

According to Gisket *al.* (2008), the isolation of bacteria resistant to multiple classes of antimicrobials is a reality in the clinical routine.

Among the factors that have promoted the increase of the frequency of infections by these microorganisms are the indiscriminate use of antimicrobials and the ease dissemination of mechanisms of resistance between the bacteria. We fully agree with the statements made by these authors when we contemplate the abusive or unnecessary use of antimicrobials daily in the medical practice, besides the cases of self-medication by the population. In relation to *Pseudomonas aeruginosa*, researchers Ossa-Giraldo *et al.* (2014) and Cortés *et al.* (2009) considered that this bacterium is the most frequent cause of infections associated to health care, mainly in immunosuppressed or immunocompromised patients, burned people, patients with cystic fibrosis and patients hospitalized in intensive care units. Generally bacteria isolated from lesions of these individuals are resistant to several classes of antibiotics, making the treatment difficult, and are associated with high mortality rates and high hospital admission costs. This fact is also observed in hospitals in cities of the Baixada Fluminense region, Province of Rio de Janeiro, Brazil, area of the city of our research.

Norberg *et al.* (2002) investigated the presence of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in nasopharyngeal secretion of 100 students of the basic nursing course not frequenters of the hospital environment. The results showed 3% positivity for *P. aeruginosa* and 2% for *K. pneumoniae*. These authors commented that the isolated strains showed resistance to several antibiotics among the tested ones and drew the attention of health professionals to the possibility of these asymptomatic carriers transmit these pathogens to hospitalized patients through direct or indirect contact. In our results, we found a higher degree of sensitivity of these bacteria to the antibiotics tested than those found by these authors. Pandey & Manna (2016) researched various organic materials from 300 patients hospitalized at the Medical College & Hospital of Jaipur, India. The results showed that all cultivated samples were positive for *Pseudomonas aeruginosa*, which 210 (70%) patients were men and 90 (30%) were women. They alerted health professionals about the importance of this bacterium as an important cause of hospital infection. We believe that the hygienic conditions and manipulation of patients contribute to the mechanisms of dissemination of the bacteria among patients, manipulators and vice versa. Silva-Junior & Brites (2014) considered that hospital infection is a major concern in health centers due to its high morbidity and mortality rate, especially among patients with long hospital stay. Among the several causes, there are important infections by *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, which besides the health aggressions acquire progressive changes in the profile of resistance to antibiotics. These authors studied cases of nosocomial infection in the period from 2011 to 2013, using the data registry of the Hospital Espanhol, Province of Bahia, Brazil. *Pseudomonas* was the most frequently identified bacteria in the registries with 65.7% while *A. baumannii* obtained a frequency of 34.3%. The study reported that the bacterium related to the highest mortality rate was *A. baumannii*, with 73.3% of the cases. The authors recommended that hospitals should maintain strict vigilance in infection control to minimize costs and hospital stay and consequent loss of life. We corroborate the ideas of these authors and also propose the control of the asymptomatic carriers responsible for the dissemination of these pathogens.

A research of nosocomial bacteremias caused by multidrug resistant *Pseudomonas aeruginosa* was performed by Grochelet *et al.* (2015) at the Edmonton Hospital, Alberta, Canada, between 2007 and 2014. The authors identified 210 cases of bacteremia and found that the main sources of primary infection were respiratory tract (6%), urinary tract (19.6%), gastrointestinal tract (6%) and skin (7%). Antibiotic sensitivity tests revealed that 21.6% of *P. aeruginosa* tested samples were considered multidrug resistant, a fact that makes treatment difficult and represents a high risk of mortality. The three bacterial samples isolated from the nostrils of the Health Science students and non-frequenters of hospital environment studied by us were resistant to several tested antibiotics but cannot be classified as multidrug resistant.

## Conclusion

From the analysis of the results, we concluded that the research of nosocomial tract of 120 undergraduate students of Health Sciences area who do not attend the hospital environment showed that 2.5% of the individuals surveyed were colonized with *Pseudomonas aeruginosa* and 1.7% were colonized by *Klebsiella pneumoniae*. No multidrug resistant strains were isolated. All strains showed variable degrees of susceptibility to the tested antimicrobials.

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