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Analysis of susceptibility profile of *Pseudomonas* spp. and prevalence of bacterial samples from the surfaces of dental consulting-rooms

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ABSTRACT

The aim of this research was to evaluate the susceptibility profile of *Pseudomonas* spp. and the prevalence of bacterial samples isolated from horizontal surfaces surrounding wash-basins used by dentists in several adjoined consulting-rooms, at points next to and at a distance from the basin, before and after surgical procedures. Our results showed a high percentage of Gram-positive cocci and Gram-negative bacilli; 34.66% were *Staphylococcus* spp. and 30.12% were non-fermentative Gram-negative bacilli among which *Pseudomonas* spp. (40.90%) was the commonest genus. Analysis of the susceptibility profile of *Pseudomonas* spp. isolates by determining the minimal inhibitory concentration (MIC) of 14 antibiotics showed a great variation among the strains and high rates of resistance to cefazolin, ceftazidime and aztreonan. Of the 14 antibiotics tested, 59.03% were found to be active against all the environmental isolates. Strains were resistant to aztreonan (62.82%), while susceptibility to third generation cephalosporins was variable.

Keywords: Pseudomonas, susceptibility, dental consulting-room, *P. aeruginosa*, *P. stutzeri*.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen that causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a great variety of systemic infections (Bodey et al., 1983; Agarwal et al., 2005). The typical *Pseudomonas* bacterium in nature might be found in a biofilm, and is one of the most vigorous, fast-swimming bacteria seen in infusions and water samples (Hardalo & Edberg, 1997; Costerton et al., 1999). This species is found all over the world, and may be present as part of the normal flora of humans, although the prevalence of colonization of healthy individuals outside the hospital is rather low (Moss, 1995). While colonization usually precedes infections by *P. aeruginosa*, the exact source and mode of transmission of the pathogen are often unclear because of its ubiquitous presence in the environment.

The objective of this study was to analyze the susceptibility profile of *P.aeruginosa*, one of the most prevalent bacteria involved in cross-contamination, and the incidence of microbial contamination on surfaces in dental consulting-rooms, near to and distant from the dentists' wash basins, with the purpose of revealing any high risks of infections that could be reduced with effective contamination control procedures.

MATERIAL AND METHODS

Sample Collection: Samples (1246 isolates) were taken from the surfaces of several adjoining dental consulting-rooms with cotton swabs. The samples were collected once before and once after clinical procedures, near to and distant from the wash basins used by the dentists.

Isolation and identification of microorganisms: By using conventional procedures to collect the samples, each sample, after bacterial enrichment in BHI broth (Brain Heart Infusion broth, Merck, Darmstadt, Germany), was streaked on 5% sheep blood agar, MacConkey agar and mannitol salt agar to allow differentiation of microorganisms. Bacterial strains were isolated and identified in accordance with the Manual of Basic Procedures in Medical Microbiology for the Control of Nosocomial Infection (Brasil, 1991), by streaking one loopful on selective and nonselective media. Microorganisms of medical importance were identified to genus, species and subspecies levels. Commercial kits (Probac do Brasil Ltda., São Paulo, SP, Brazil), Gram-negative Combo 20 panel (MicroScan System, Dade-Behring, USA) and/or traditional methods were used to identify bacterial samples (Balows et al., 1991). Testing was performed according to manufacturer's instructions.

Susceptibility Tests: The antimicrobial susceptibility profiles were determined by microdilution broth method. The Minimal Inhibitory Concentration (MIC) was determined using Negative MIC Plus Panel Type 3 (MicroScan system, Dade-Behring, USA) and the drugs/ MIC range (in μg/mL) tested were cefazolin/2-32 (CZ), ceftazidime/1-32 (CAZ), cefotaxime/2-64 (CFT),

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ceftriaxone/2-64 (CAX), cefepime/2-16 (CPE), piperacillin/8-64 plus tazobactam/4 (PTZ), netilmicin/2-16 (NT), meropenem/1-8 (MER), imipenem/0.5-16 (IMP), azlocillin/64 (AZ), aztreonam/1-32 (AZT), ciprofloxacin/0.25-4 (CIP), ticarcillin/16-128 plus clavulanic acid/2 (TIM) and mezlocillin/16-128 (MZ). The results were classified into susceptible, intermediate or resistant, as per CLSI (Clinical and Laboratory Standard Institute) guidelines (Balows et al. 1991; NCCLS, 2000).

RESULTS

In this study, 1246 isolates were taken from the surfaces around wash-basins, close to and distant from the basins, in dental surgeries, before and after surgical procedures. The most prevalent group of bacteria found were the staphylococci (34.66%), followed by non-fermentative Gram-negative bacilli, contributing 30.12%.

The incidence of *Pseudomonas* spp. was 40.09% of the total Gram-negative bacilli, 145 isolates (92.94%) being collected around the basins and 7.05% (11 isolates) distant from them. (Table 1).

Given the pathogenic potential of this non-fermentative Gram-negative bacillus, some *Pseudomonas* strains were tested for *in vitro* susceptibility. The samples tested were selected on the basis of their highly variable susceptibility. Fifty percent of all antibiotics tested inhibited all *Pseudomonas* spp. strains. Third and fourth generation cephalosporins possessed lower activity against the isolates, which showed between 17.30 and 79.48% of resistance. The general analysis of susceptibility profiles, carried out as recommended in M7-A5/M100-S10 (NCCLS, 2000) showed that 18.77% of strains were resistant to all tested drugs, and of 156 tests performed, 23.53% of antimicrobial agents were ineffective. To the monobactam, aztreonam, 62.82% of environmental isolates showed resistance and to cefotaxime and cefazolin the percentages were 39.10 and 100% of resistance, respectively. The drugs with high activity against all the environmental isolates of *Pseudomonas* spp. were meropenem, imipenem, ciprofloxacin, ticarcillin and mezlocillin. (Table 2).

Table 1 - Identification of *Pseudomonas* spp. isolated from the surfaces of wash basin around sink (PP), wash basin distant sink (PD) of dental offices before (A) and after dental surgical procedures (B).

Samples/Isolates	Microorganisms
PPA/20	*Pseudomonas aeruginosa*
PPA/7	*Pseudomonas aeruginosa*
PPA/32	*Pseudomonas aeruginosa*
PPA/33	*Pseudomonas aeruginosa*
PPA/23	*Pseudomonas stutzeri*
PPB/7	*Pseudomonas stutzeri*
PPB/22	*Pseudomonas aeruginosa*
PPB/1	*Pseudomonas aeruginosa*
PDA/5	*Pseudomonas aeruginosa*
PDA/6	*Pseudomonas stutzeri*

Table 2 - MIC values (µg/mL) of the 156 samples of *Pseudomonas* spp. isolated from the surfaces of wash basin around the sink (PP), wash basin distant from sink (PD) of dental offices before (A) and after dental surgical procedures (B) of dental offices.

Antibiotics[a]	Samples									
	PPA 20	PPA 7	PPA 32	PPA 33	PPA 23	PDA 5	PDA 6	PPB 7	PPB 22	PPB 1
CZ	> 32 (R)[b]	> 32 (R)	> 32 (R)	> 32 (R)	> 32 (R)	> 32 (R)	> 32 (R)	> 32 (R)	> 32 (R)	> 32 (R)
CAZ	> 32 (R)	16 (I)[c]	8 (S)[d]	8 (S)	8 (S)	2 (S)	8 (S)	4 (S)	8 (S)	8 (S)
CFT	> 64 (R)	> 64 (R)	32 (I)	8 (S)	> 64 (R)	> 64 (R)	> 64 (R)	32 (I)	32 (I)	32 (I)
CAX	> 64 (R)	32 (I)	16 (S)	8 (S)	> 64 (R)	32 (I)	8 (S)	8 (S)	8 (S)	16 (S)
CPE	2 (S)	< 2 (S)	> 16 (R)	2 (S)	2 (S)	< 2 (S)	< 2 (S)	2 (S)	< 2 (S)	< 2 (S)
PTZ	< 8 (S)	< 8 (S)	< 8 (S)	< 8 (S)	< 8 (S)	< 8 (S)	< 8 (S)	< 8 (S)	< 8 (S)	< 8 (S)
NT	8 (S)	4 (S)	8 (S)	4 (S)	16 (I)	4 (S)	4 (S)	8 (S)	4 (S)	4 (S)
MER	< 1 (S)	< 1 (S)	< 1 (S)	< 1 (S)	< 1 (S)	< 1 (S)	< 1 (S)	< 1 (S)	< 1 (S)	< 1 (S)
IMP	< 0.5 (S)	< 0.5 (S)	1 (S)	1 (S)	< 0.5 (S)	< 0.5 (S)	< 0.5 (S)	1 (S)	< 0.5 (S)	< 0.5 (S)
AZ	< 64 (S)	< 64 (S)	< 64 (S)	< 64 (S)	< 64 (S)	< 64 (S)	< 64 (S)	< 64 (S)	< 64 (S)	< 64 (S)
AZT	> 32 (R)	4 (S)	> 32 (R)	4 (S)	> 32 (R)	4 (S)	4 (S)	4 (S)	> 32 (R)	> 32 (R)
CIP	1 (S)	1 (S)	1 (S)	1 (S)	1 (S)	< 0.25 (S)	< 0.25 (S)	1 (S)	1 (S)	<0.25 (S)
TIM	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)
MZ	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)	< 16 (S)

[a]Antibiotics: cefazolin (CZ), ceftazidime (CAZ), cefotaxime (CFT), ceftriaxone (CAX), cefepime (CPE), piperacillin plus tazobactam (PTZ), netilmicin (NT), meropenem (MER), imipenem (IMP), azlocillin (AZ), aztreonam (AZT), ciprofloxacin (CIP), ticarcillin plus clavulanic acid (TIM) and mezlocillin (MZ). [b](R): resistant; [c](I): intermediate; [d](S): sensible.

DISCUSSION

The transmission of infection during dental procedures can occur by direct contact with tissues and secretions or blood, by aerosols containing infectious agents and on the cutting edge of contamined instruments. The goal of our study was to demonstrate the importance of the evaluation of the presence of microorganisms in the dental surgery environment. The results showed that the isolated microorganisms were Gram-positive cocci and Gram-negative bacilli, among which the most prevalent species in each group were *S. aureus* and *Pseudomonas* spp, respectively. Pathogens such as *P. aeruginosa* and *P. stutzeri* have been isolated from some areas of dental consulting-rooms, suggesting a risk of infection associated with oral dissemination (Barbeau et al., 1998; Barbeau, 2000; Jensen et al., 1997; O'Donnell et al., 2005). In a microbiological study of selected risk areas in dental technology laboratories, *Staphylococcus* spp. were most commonly isolated from curing water baths and from air (Verran et al., 1996). In another study, bacteria were isolated from pumice slurry, the major contaminants being *Pseudomonas* spp., *Staphylococcus* spp. and *Bacillus* spp (Verran et al., 1997), the same agents that were found in our study.

P. aeruginosa continues to be a major pathogen in the surgical environment because it is the most prevalent species in the water used (Paviani et al., 2004) and one of the most important bacterial pathogens in cystic fibrosis-associated lung disease (Chambers et al., 2005). In the environment of the dental surgery this agent can infect dental equipment. Patients with cystic fibrosis often suffer from *P. aeruginosa* lung infection and although the source of the organism is not known there is a risk of contamination from dental equipment, because strains have been found both in water taken from dental equipment and clinical isolates. Additionally one case of genotypically-identified *Pseudomonas* was acquired in dental sessions (Jensen et al., 1997).

P. aeruginosa is naturally resistant to many antibiotics (Tadeu et al., 2000) and only a few antibiotics are effective against *Pseudomonas*, including fluoroquinolone, gentamicin and imipenem, and even these antibiotics are not effective against all strains. In a study, Pitten et al. (2001) demonstrated resistance of *P. aeruginosa* to beta-lactam antibiotics including carbapenens, aztreonam, aminoglycosides and quinolones and *in vitro* susceptibility only to polymyxin B, in clinical and environmental samples isolated from hospital infection episode. The detection of clusters of beta-lactamases that hydrolyze broad-spectrum beta-lactams has received great attention in the combatting of cross-infection because the environmental dissemination of such agents can lead to the transference of the metallo-beta-lactamase gene among *P. aeruginosa* and other Gram-negative bacilli (Panzig et al., 1999; Tsuji et al., 2005). Our results for the environmental isolates showed similar percentages to clinical isolates in the study of Cavallo et al. (2000) supporting the hypothesis that part of isolates of the *Pseudomonas* spp contamination could occur during the surgical procedures, when aerosols would be disseminated to places nearby, carrying mainly the resistant strains. Similarly, a high percentage of resistance to aztreonam was detected, and this is an optional drug alternative in therapy that involves cephalosporins (Somekh & Cordova, 2000). Furthermore, *P. aeruginosa* has shown resistance to other antibiotics, such as third generation cephalosporins (CAZ and CFT), as demonstrated in a study of clinical isolates (Panzig et al., 1999; Pitten et al., 2001; Blandino et al., 2004). In the present study, the results suggests that the resistance cannot be associated with areas of collection because the samples were from several dental surgeries located in different places and the same considerations apply to the surgical procedures. Interestingly, *P. stutzeri* was isolated near to and distant from the wash basin, with different susceptibility profile and resistance to CAX and AZT, suggesting the possibility that the strains were from surgical aerosols. The knowledge of the susceptibility profile of given bacterial groups will be important for an understanding not only of the dissemination of these agents as sources of infection but also of the ecology of this organism in the dental surgery environment. This study suggests that special attention should be paid to these areas, using, for example, hospital disinfection methods, to weaken the chain of cross-infection.

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RESUMO

Análise do perfil de susceptibilidade de ***Pseudomonas*** *spp. e da prevalência de bactérias isoladas de superfícies de consultórios odontológicos*

O objetivo deste trabalho foi avaliar o perfil de susceptibilidade de *Pseudomonas* spp. e a prevalência de amostras bacterianas isoladas de áreas ao redor e distante da cuba de pias de consultórios odontológicos, antes e após vários procedimentos cirúrgicos. Nossos resultados mostraram uma porcentagem elevada de cocos Gram-positivos e de bacilos Gram-negativos; sendo 34,66% pertencentes ao gênero *Staphylococcus* spp. e 30,12% de bacilos Gram-negativos não-fermentadores, dos quais *Pseudomonas* spp. (40,90%) eram os mais freqüentes. A análise do perfil de susceptibilidade com determinação da concentração inibitória mínima (CIM) das amostras de *Pseudomonas* spp. apresentou uma grande variação entre as linhagens com taxas elevadas de resistência ao cefazolina, ao ceftazidima e ao aztreonam. Foram testados 14 antibióticos frente aos isolados ambientais e observou-se que 59,03% eram ativos. As linhagens foram resistentes a aztreonam (62,82%) enquanto que a sensibilidade frente 'a

cefalosporinas de terceira geração foi variável.

Palavras-chave: *Pseudomonas*, susceptibilidade, consultórios odontológicos, *P. aeruginosa, P. stutzeri.*

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