Antimicrobial resistance of aerobes and facultative anaerobes isolated from the oral cavity

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ABSTRACT

Objectives: This study evaluated the resistance to antimicrobials of aerobes and facultative anaerobes isolated from patients wearing complete dentures, patients with gingivitis and periodontitis, and periodontally health subjects. Material and Methods: Three hundred and four isolates were tested. The minimal inhibitory concentrations of the drugs were evaluated through the agar dilution method using Mueller-Hinton agar. Results: The most active antimicrobial drugs were the carbapenems (meropenem and imipenem), and resistance to these drugs was restrict to 1.6-2.3% of the isolates, as well as ciprofloxacin and rifampin. Microbial resistance to ampicillin, amoxicillin/clavulanic acid, cefoxitin, cephalothin, amikacin, chloramphenicol and nalidixic acid was particularly high. In most cases, the resistance to β-lactams was mediated by the production of hydrolytic enzymes, especially in gram-negative enteric rods, while *enterococci* did not evidence production of these enzymes. The association amoxicillin/clavulanic acid was not effective in 28.3% of the tested isolates. Conclusions: The results of this investigation confirmed that the oral cavity of patients with periodontitis and gingivitis, and particularly edentulous patients wearing complete dentures could harbor microorganisms with several antimicrobial resistance markers, and these microorganisms are frequently implicated in multiresistant, systemic, oral or nosocomial infections.

Key words: Periodontitis. Gingivitis. Bacteria. Anti-bacterial agents.

INTRODUCTION

Oral cavity may act as a reservoir for superinfecting microorganisms commonly associated to systemic and opportunistic infections[12] especially in elderly wearing complete dentures[5]. In addition, either use or misuse of antimicrobial drugs associated with poor oral hygiene would facilitate the colonization of the oral cavity by these microorganisms, as well as the dissemination of their resistance genes amongst the members of oral microbiota[10,12].

The use of complete dentures[5] or the development of periodontitis[1,10] may create suitable nutritional conditions for superinfecting pathogens, such as Gram-negative enteric rods, pseudomonads, and *enterococci*, which may be commonly associated with refractory oral and nosocomial infections. Moreover, the loss of the balance between the host's immune response and the microbiota's virulence has resulted in several oral infections, such as denture stomatitis and endodontic, periodontal or periapical infections.

However, in case of history of previous use of antimicrobials or immune suppression, the clinician may suspect of the participation of facultative anaerobes and aerobes in the infectious process. These microorganisms have presented a very diverse antimicrobial susceptibility profile in comparison to strict anaerobes[4]. In addition, in spite of the role that facultative anaerobes and aerobes would play in head and neck infections, most of dentists have been instructed to prescribe antimicrobial drugs only directed against strict anaerobes[4].

Although local and surgical procedures have remained the basis of odontogenic infections treatment, antimicrobial drugs may act as adjuvants in this therapeutic, especially in anatomical sites

where surgical procedures could not be performed. However, the determination of resistance patterns to antimicrobial drugs of oral microorganisms has not constituted a routine procedure[9].

This study evaluated the frequency of antimicrobial resistance among isolates of aerobic and facultative anaerobic bacteria harvested from the oral cavity of patients wearing complete dentures, patients with gingivitis and chronic periodontitis, and periodontally healthy subjects.

MATERIAL AND METHODS

Study Population

A total of 250 patients (84 males and 166 females; mean age 43.03 years), followed up within an 8-year period at the Araçatuba Dental School, UNESP, Brazil, from February, 1998 to March, 2008 were enrolled in this study. Forty-one patients wore complete dentures, 89 exhibited gingivitis, 70 chronic periodontitis and 50 were periodontally healthy, following the criteria described in the literature[23]. Demographic and additional characteristics of the patients are presented in Table 1.

Thirteen patients had received amoxicillin or ampicillin due to medical prescription three months before sample collection, while two patients received azithromycin. Two patients had used trimethoprim/sulfamethoxazole for treatment of oral minor infections, respiratory or urinary infections. Exclusion criteria included: diabetes, systemic diseases and other chronic infections (except for periodontitis or gingivitis), prosthetic heart valves, previous endocarditis, transplants, pregnant or lactating women, and history of antimicrobial drug use within the period of three months before sample collection. Since it is not possible to determine with accuracy of antimicrobial drug use in individuals with history of self-medication, it was established that these patients should be excluded from the study.

A written informed consent form approved by the Institutional Review Board of Araçatuba Dental School, UNESP (Proc.27/2000 and 34/2006) was signed by all participants. After sample collection, the dentate patients were referred to restorative and periodontal treatment, while edentulous patients were directed to prosthetic dentistry.

Microorganisms

Clinical samples of resting saliva, oral mucosa, tongue, and both supragingival and subgingival biofilm were collected from periodontally healthy subjects and patients with gingivitis and periodontitis. Supragingival samples were obtained by scaling; subgingival samples were obtained by using 3 sterile paper points (Dentsply Ind. Co. Ltd., Petrópolis, RJ, Brazil), which were inserted into the apical region of periodontal pockets or gingival crevices for 60

Table 1- Demographic and additional characteristics of the patients

Characteristic	Dentate patients N (%)	Edentulous patients N (%)
Gender		
Male (N=84)	65 (31.1)	19 (46.3)
Female (N=166)	144 (68.9)	22 (53.7)
Education		
Illiterate (N=27)	16 (7.7)	11 (26.8)
Elementary school (N=179)	149 (71.3)	30 (73.2)
High school (N=44)	44 (21.0)	0 (0.0)
History of tobacco consumption		
Yes[1] (N=45)	82 (39.2)	15 (36.6)
No (N=5)	127 (60.8)	26 (63.4)
History of alcohol consumption		
Yes[2] (N=75)	61 (29.2)	14 (34.1)
No (N=175)	148 (70.8)	27 (65.9)
History of antimicrobial drugs use		
Yes (N=15)	13 (6.2)	2 (4.9)
No (N=235)	196 (93.7)	39 (95.1)
History of oral surgeries in the previous 5 years		
Yes (N=29)	22 (10.5)	7 (17.1)
No (N=221)	187 (89.5)	34 (82.9)

[1]At least 10 cigarettes per day over the last 5 years.[2]At least two daily doses of cachaça, a distilled alcoholic beverage product of distillation of fermented sugarcane juice with alcoholic contents of 38-48% v/v.

s. Oral mucosa samples were collected by a sterile swab, while saliva was collected by using Salivette devices (Cortisol-Salivette, Sarstedt AG & Co., Nümbrecht, Nordrhein-Westfalen, Germany). In the edentulous patients wearing complete dentures, clinical samples from palate, dorsum of tongue, and fornix were collected by using swabs[5]. Clinical samples were transferred to a VMGA III medium[18]. The clinical samples of subgingival biofilm were pooled before transportation.

Clinical specimens were inoculated in peptone water and ethyl violet azide broth (Difco Laboratories, Detroit, MI, USA), and incubated both at room temperature and 37°C, for 3-7 days. After that, from the bacterial growth observed in peptone water, aliquots of 0.1 ml were transferred to Eosin Methylene Blue agar, SS agar, MacConkey agar (Difco Laboratories) and Brilliant Green agar. From the tubes containing EVA broth, 0.1 mL was transferred to Bile Esculin agar (Difco Laboratories). Agar plates were incubated in aerobiosis, at 37°C, for 48 h[10].

Clinical specimens were also diluted in VMG I[18] and plated on tryptic soy agar (TSA), supplemented with both 0.5% yeast extract and 5% horse blood, and incubated in aerobiosis, at 37°C, for 48 h, for isolation of non-enteric aerobes and facultative anaerobes. The isolates were identified by Gram staining, colony morphology on agar plates, respiratory test, catalase assay, and biochemical identification kits (API Test System, BioMérieux, Marcelle l´Etoile, Provence-Alpes-Côte d'Azur, France).

A total of 304 isolates were subjected to susceptibility tests, as follows: *Bukholderia cepacia* complex (5 isolates), *Citrobacter freundii* (7 isolates), *Enterobacter cloacae* (18 isolates), *E. intermedius* (6 isolates), *E. sakazakii* (9 isolates), *Enterococcus* sp. (18 isolates), *E. faecalis* (31 isolates), *E. faecium* (8 isolates), *Escherichia coli* (6 isolates), *Klebsiella oxytoca* (11 isolates), *K. pneumoniae* (3 isolates), *Morganella morganii* (17 isolates), *Pantoea agglomerans* (7 isolates), *Proteus mirabilis* (5 isolates), *P. vulgaris* (7 isolates), *Providencia alcalifaciens* (6 isolates), *Pseudomonas aeruginosa* (15 isolates), *P. fluorescens* (4 isolates), *Serratia* sp. (9 isolates), *S. liquefaciens* (9 isolates), *Staphylococcus* sp. (9 isolates), *S. aureus* (10 isolates), *S. epidermidis* (17 isolates), *S. hominis* (8 isolates), *Streptococcus* sp. (9 isolates), *S. oralis* (7 isolates), *S. sanguinis* (9 isolates), *S. mitior* (4 isolates), *S. salivarius* (11 isolates), *S. mutans* (7 isolates), *S. pneumoniae* (6 isolates), and *S. pyogenes* (6 isolates).

Antimicrobial Susceptibility Tests

All isolates were examined for susceptibility to antimicrobial agents by agar dilution method[19]. When CLSI antimicrobial breakpoints were not established, the breakpoints adopted by the British Society for Antimicrobial Chemotherapy[3] were followed. Mueller-Hinton agar (MHA) was used for all isolates. In tests involving oral *streptococci*, 5% horse blood was added to MHA plates in order to support microbial growth.

Thus, five pure colonies of each bacterial strain were inoculated into 2 mL of sterile Mueller Hinton broth or brain heart infusion broth supplemented with yeast extract (oral *streptococci*) and incubated at 37°C for 12-24 h. The turbidity was adjusted to match a 0.5 McFarland turbidity standard. The bacterial inocula were standardized in 10^5 cells[9] and transferred to Mueller-Hinton agar plates containing the antimicrobial agent and control plates (without drugs), using a Steer's replicator (Cefar Diagnostica Ltda, São Paulo, SP, Brazil). The test and control agar plates were incubated aerobically or under CO_2 (10% CO_2 + conventional atmosphere, for oral *streptococci*) at 37ºC, for 48 h.

A total of 14 antibiotics or associations were tested. The antibiotics tested consisted of the following drugs: amikacin, ampicillin, amoxicillin/clavulanic acid, cefoxitin, cephalothin, chloramphenicol, ciprofloxacin, doxycycline, gentamicin, imepenem, meropenem, nalidixic acid, rifampin, and tetracycline. Antimicrobials were tested in twofold dilution series ranging from 0.06 to 256 µg/mL. After incubation, the organisms were classified as sensitive or resistant, according to CLSI[19] and BSAC[3] guidelines. *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *E. faecalis* ATCC 29212 were used in the assays involving facultative anaerobes.

Detection of β-lactamases

The isolates resistant to β-lactams were also tested for β-lactamase activity by both chromogenic cephalosporin and biological method[9]. These two methods were performed because nitrocefin-based β-lactamase assays have not proven useful in detecting β-lactamase production by some microorganisms. In all tests, *S. aureus* ATCC 29213 was used as the positive control of β-lactamase production.

The chromogenic cephalosporin β-lactamase assay using cefinase disks was performed according to the manufacturer's instructions (Calbiochem, San Diego, California, USA).This description was briefly the following: 6-mm-diameter filter paper disks impregnated with nitrocefin were moistened with 0.85% NaCl, and several fragments of the tested microorganisms' colonies were transferred to the disk. After 10-60 min, the disks were examined regarding the appearance of a pink-red coloration, which has been characteristic of the degradation of nitrocefin.

In the biological method, 20 µL of the resistant isolate cultures were plated on the surface of Mueller-Hinton agar containing 0.5 µg/mL of the tested β-lactam to which the tested microorganism showed to be resistant. These plates were then incubated in aerobiosis at 37°C, for 48 h. After this incubation period, the cultures were exposed to chloroform fumes for 20 min. and then covered with 5 mL of semi-solid brain heart infusion (BHI) agar (0.7% agar) previously inoculated with 10^6 cells of *S. pyogenes* FOA-94F14 sensitive to all tested β-lactams in a concentration of ≤0.06 µg/mL. The Petri dishes were then incubated under aerobiosis for 24 h at, 37°C. After incubation, presence or absence of streptococcal growth was checked. The presence of this growth halo was indicative of the β-lactam degradation.

Statistical analysis

Differences between clinical parameters and the frequency of pathogen detection or presence of microbial resistance for each subject were analyzed by the Chi-square, Mann-Whitney or Fisher's exact test. Inter-relationships among different microorganisms were evaluated using the Spearman's correlation coefficient test.

RESULTS

Significant levels of resistance were observed for all β-lactams, excepting for imepenem and meropenem, which respectively presented 2.3% and 1.6% of resistance. The most prominent resistance was observed for ampicillin, amoxicillin and cephalothin, which respectively reached 44.4%,

Table 2- Resistance to β-lactams of aerobes and facultative anaerobes isolated from oral cavity of patients wearing complete denture as well as gingivitis and periodontitis patients

Microorganisms (number of isolates)	Resistance Prevalence (%)							Production of β-lactamases (%)
	AM	AMX	AMC	CF	CP	IM	ME	
B. cepacia[1] (5)	100.0	100.0	60.0	20.0	40.0	0.0	0.0	40.0
C. freundii (7)	57.1	57.1	28.5	28.6	42.9	0.0	0.0	57.1
E. cloacae (18)	77.8	77.8	44.4	50.0	61.1	0.0	0.0	72.2
E. intermedius (6)	33.3	33.3	33.3	0.0	0.0	0.0	0.0	33.3
E. sakazakii (9)	44.4	44.4	11.1	22.2	100.0	0.0	0.0	44.4
Enterococcus sp. (18)	22.2	22.2	22.2	44.4	38.9	0.0	0.0	0.0
E. faecalis (31)	19.4	19.4	19.4	19.4	38.7	0.0	0.0	0.0
E. faecium (8)	50.0	50.0	50.0	62.5	50.0	25.0	12.5	0.0
E. coli (6)	66.7	66.7	16.7	0.0	0.0	0.0	0.0	66.7
K. oxytoca (11)	63.6	45.5	45.5	9.1	27.3	0.0	0.0	63.6
K. pneumoniae (3)	100.0	66.7	66.7	0.0	0.0	0.0	0.0	100.0
M. morganii (17)	70.6	70.6	29.4	23.5	52.9	5.9	5.9	70.6
P. agglomerans (7)	85.7	85.7	85.7	28.6	42.9	0.0	0.0	85.7
P. mirabilis (5)	60.0	60.0	0.0	0.0	40.0	0.0	0.0	80.0
P. vulgaris (7)	71.4	71.4	28.6	14.3	14.3	0.0	0.0	71.4
P. alcalifaciens (6)	66.7	66.7	66.7	16.7	33.3	0.0	0.0	66.7
P. aeruginosa (15)	86.7	86.7	66.7	40.0	60.0	13.3	13.3	40.0
P. fluorescens (4)	50.0	50.0	25.0	0.0	0.0	0.0	0.0	50.0
Serratia sp. (9)	77.8	77.8	44.4	33.3	55.6	0.0	0.0	77.8
S. liquefaciens (9)	66.7	66.7	66.7	22.2	33.3	0.0	0.0	66.7
Staphylococcus sp. (9)	44.4	44.4	11.1	44.4	44.4	11.1	11.1	66.7
S. aureus (10)	60.0	50.0	50.0	40.0	40.0	0.0	0.0	60.0
S. epidermidis (17)	23.5	23.5	11.8	23.5	29.4	12.5	0.0	35.3
S. hominis (8)	50.0	50.0	25.0	12.5	12.5	0.0	0.0	50.0
Streptococcus sp. (9)	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0
Streptococcus[2] *spp.* (50)	4.0	4.0	0.0	4.0	4.0	0.0	0.0	0.0
Total (304)	44.4	43.1	28.3	22.7	33.2	2.3	1.6	36.8

AM, ampicillin; AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; CF, cefoxitin; CP, cephalothin; IM, imepenem; ME, meropenem.

[1]*Burkholderia cepacia* complex

[2]*Streptococcus* species (N): *S. oralis* (7), *S. sanguinis* (9), *S. mitior* (4), *S. salivarius* (11), *S. mutans* (7), *S. pneumoniae* (6), *S. pyogenes* (6).

43.1% and 33.2% (Table 2). Enteric gram-negative rods and pseudomonads were the most resistant isolates. Out of 304 tested isolates, 178 were resistant to at least one β-lactam, representing 58.6% of all tested microorganisms, and 112 resistant isolates were β-lactamases producers, which represented 36.8% of all tested isolates and 62.9% of all β-lactam resistant bacteria. These hydrolyzing enzymes seemed to be the major mechanism of resistance to this class of antimicrobials, excepting for *enterococci*, which did not produce such compounds.

The association amoxicillin/clavulanic acid was active on less than half of ampicillin or amoxicillin resistant isolates. Resistance to this association was detected in 28.3% of the targeted microorganisms and it was particularly frequent in *E. cloacae*, genera *Klebsiella*, *Serratia* and *Pseudomonas,* as well as in *B. cepacia* complex, *E. faecium*, *P. agglomerans*, *P. alcalifaciens* and *S. aureus*.

In relation to cephalosporins, the resistance to both cefoxitin and cephalothin was disseminated in all tested microbial genera, particularly in pseudomonads, *E. cloacae*, *staphylococci*, and *enterococci*. Some isolates of *E. cloacae*, *K. oxytoca*, *P. agglomerans*, *P. alcalifaciens* and *Serratia* sp. presented a broad spectrum resistance to β-lactam antibiotics, and produced β-lactamases that were active on penicillins and cephalosporins. This would suggest that the oral cavity of dentate patients and, particularly, edentulous patients wearing complete dentures could harbor bacterial strains able to produce broad spectrum β-lactamases.

The results presented in Table 2 show that

Table 3- Susceptibility of the isolates to amikacin, chloramphenicol, ciprofloxacin, doxycycline, gentamicin, nalidixic acid, rifampin, and tetracycline

Microorganism (number of isolates)	Resistance Prevalence (%)							
	AK	CHR	CPR	DC	GE	NA	RF	TE
B. cepacia[1] (5)	0.0	20.0	0.0	20.0	0.0	20.0	40.0	40.0
C. freundii (7)	0.0	28.6	0.0	14.3	28.6	14.3	0.0	28.6
E. cloacae (18)	5.6	38.9	0.0	0.0	38.9	5.6	0.0	5.6
E. intermedius (6)	0.0	33.3	16.7	0.0	0.0	16.7	0.0	16.7
E. sakazakii (9)	0.0	33.3	0.0	0.0	0.0	11.1	0.0	11.1
Enterococcus sp. (18)	66.7	44.4	0.0	16.7	0.0	22.2	0.0	38.9
E. faecalis (31)	83.9	35.5	0.0	12.9	9.7	71.0	0.0	61.3
E. faecium (8)	87.5	62.5	0.0	25.0	0.0	37.5	0.0	37.5
E. coli (6)	0.0	16.7	0.0	33.3	0.0	0.0	0.0	16.7
K. oxytoca (11)	0.0	9.1	0.0	0.0	18.2	9.1	0.0	0.0
K. pneumoniae (3)	0.0	33.3	0.0	0.0	0.0	33.3	0.0	0.0
M. morganii (17)	5.9	29.4	0.0	5.9	5.9	11.8	5.9	41.2
P. agglomerans (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	28.5
P. mirabilis (5)	0.0	20.0	0.0	60.0	0.0	0.0	0.0	40.0
P. vulgaris (7)	0.0	28.6	0.0	28.6	14.3	0.0	0.0	14.3
P. alcalifaciens (6)	0.0	33.3	0.0	83.3	33.3	50.0	33.3	66.7
P. aeruginosa (15)	13.3	33.3	6.7	46.7	33.3	86.7	20.0	73.3
P. fluorescens (4)	0.0	25.0	0.0	0.0	0.0	0.0	0.0	25.0
Serratia sp. (9)	0.0	0.0	0.0	11.1	0.0	33.3	11.1	55.6
S. liquefaciens (9)	0.0	33.3	0.0	44.4	0.0	0.0	44.4	55.6
Staphylococcus sp. (9)	22.2	22.2	11.1	11.1	0.0	0.0	0.0	0.0
S. aureus (10)	30.0	20.0	40.0	20.0	10.0	20.0	10.0	60.0
S. epidermidis (17)	17.7	29.4	35.3	29.4	11.8	17.7	17.7	58.8
S. hominis (8)	0.0	12.5	37.5	12.5	0.0	0.0	0.0	50.0
Streptococcus sp. (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Streptococcus[2] *spp.* (50)	4.0	4.0	8.0	4.0	0.0	4.0	0.0	12.0
Total (304)	19.4	24.0	6.6	15.5	8.6	21.1	5.6	33.2

AK, amikacin; CHR, chloramphenicol; CPR, ciprofloxacin; DC, doxycycline; GE, gentamicin; NA, nalidixic acid; RF, rifampin; TE, tetracycline.

[1]*Burkholderia cepacia* complex

[2]*Streptococcus* species (N): *S. oralis* (7), *S. sanguinis* (9), *S. mitior* (4), *S. salivarius* (11), *S. mutans* (7), *S. pneumoniae* (6), *S. pyogenes* (6).

Table 4- Presence of antimicrobial resistant aerobes and facultative anaerobes in dentate patients with different periodontal health conditions and edentulous patients wearing complete dentures

Patients (N)	Presence of resistant isolates - number (%)					
	β-lactams	AMN	CHR	QNL	RF	TE
Periodontally healthy subjects (N=50)	5 (10.0)	0 (0.0)	2 (4.0)	3 (6.0)	0 (0.0)	11 (22.0)
Patients with gingivitis (N= 89)	29 (32.6)	21 (23.6)	10 (11.2)	9 (10.1)	2 (2.2)	20 (22.5)
Patients with periodontitis (N= 70)	55 (78.6)	31 (44.3)	9 (12.9)	12 (17.1)	1 (1.4)	28 (40.0)
Edentulous patients (N=41)	34 (82.9)	23 (56.1)	28 (68.3)	23 (56.1)	6 (14.6)	27 (65.9)

AMN, aminoglycosides; CHR, chloramphenicol; QNL, quinolones; RF, rifampin; TE, tetracycline.

carbapenems were the only β-lactams that had a significant antimicrobial activity on *enterococci*, *staphylococci*, pseudomonads and *Enterobacteriaceae*. The resistance to imepenem was similar to that observed to meropenem and restricted to a few isolates of *staphylococci*, *P. aeruginosa*, *E. faecium* and *M. morganii*.

Variable levels of resistance to aminoglycosides were also observed. Resistance to amikacin was more prevalent among gram-positive cocci of genera *Enterococcus,* and *Staphylococcus*, while gentamicin resistance was common in *P. aeruginosa*, *P. alcalifaciens*, *C. freundii,* and *E. cloacae* (Table 2). Resistance to chloramphenicol, doxycycline, nalidixic acid and, specially, tetracycline was frequent in most of the targeted microorganisms, while rifampin was effective against most of these isolates, excepting for some gram-negative enteric rods, *staphylococci*, and pseudomonads. The resistance to ciprofloxacin was almost restricted to *staphylococci* and *streptococci*, besides an isolate of *E. intermedius* and other isolate of *P. aeruginosa* (Table 3).

The main relationship between periodontal conditions and the presence of resistant microorganisms was linked to higher prevalence of enteric Gram-negative in patients with periodontitis or gingivitis, and these bacteria were less susceptible to antimicrobial agents. Antimicrobial use in the period prior to sample collection, and consumption of tobacco and alcohol did not significantly affect the occurrence of resistant microorganisms. The results suggest that factors that increase the presence of enteric bacteria in the oral cavity eventually collaborate with the increase in the prevalence of resistant microorganisms. Table 4 presents the prevalence of resistant microorganisms in edentulous patients wearing complete dentures, periodontally healthy subjects, patients with gingivitis and patients with periodontitis.

The results presented in Table 4 showed a close correlation between the presence of microorganisms resistant to β-lactams and the use of complete dentures (Chi-square test, $p<0.01$) and to a lesser extent, patients with periodontitis (Chi-square test, $p=0.012$) or gingivitis (Chi-square test, $p=0.02$). The same phenomenon was also detected in relation to resistance to aminoglycosides and chloramphenicol. However, the occurrence of tetracycline-, quinolone- and/or rifampin-resistant microorganisms was similar in periodontally healthy subjects and patients with gingivitis. The levels of resistance to rifampin were reduced and a low prevalence of such isolates was observed from all dentate patients.

DISCUSSION

The patterns of susceptibility to antimicrobial drugs amongst aerobes and facultative anaerobes have evidenced the presence of a multiresistance phenotype, both in isolates from human resident microbiota and from exogenous environment[8,16]. Accordingly, antibiotic resistance raised among commensal bacteria has been supposed to represent a major feature in the development of resistance within bacterial pathogens. In addition, the detection of resistant bacteria in commensal microbiota has pointed to the oral cavity as a possible source for transmission of genes associated to antimicrobial resistance in pathogenic bacteria[12].

β-Lactam agents, such as penicillins, cephalosporins and carbapenems, have been among the most frequently prescribed antibiotics worldwide. In this investigation, most of the tested microorganisms showed to be resistant to ampicillin. The levels of resistance to ampicillin in isolates of *Enterobacteriaceae* was similar to those described by literature[1,6,11], however, they were lower than those reported by Gonçalves, et al.[12] (2007).

The association between β-lactams and β-lactamase inhibitors has been frequently used in the treatment of odontogenic infections, particularly those infections where the patient had presented a history of previous use of β-lactams. However, in refractory mixed infections where aerobes and facultative anaerobes had been involved, this association has seemed to lead to a poor treatment outcome, since the expression of combined resistance to ampicillin and to amoxicillin/clavulanic

acid has been frequent in gram-negative enteric rods and *staphylococci*, as shown in Table 1 and also described in literature[6,12].

Enterococci have been often associated to refractory odontogenic infections, such as dental abscesses and both endodontic and periapical infections. The results presented here have evidenced that these *cocci* were just susceptible to carbapenems, ciprofloxacin, gentamicin and rifampin. On the other hand, *enterococci* resistant to ampicillin were also resistant to amoxicillin/clavulanic acid association, which was not in agreement with the findings of Ferrari, Cai and Bombana[7] (2005). These authors reported a high susceptibility to ampicillin and a variable susceptibility to ciprofloxacin for *enterococci* from endodontic infections, while Das, et al.[6] (2006) verified a high resistance of *E. faecalis* strains to gentamicin and ciprofloxacin.

The cephalosporins have greatly varied in susceptibility to β-lactamases. Cephalothin has been more resistant to hydrolysis by β-lactamases of *staphylococci*, whereas cefoxitin has been more resistant to β-lactamases produced by aerobic gram-negative rods[3]. Table 1 not only confirms these data, but also shows a disseminated resistance to both cefoxitin and cephalothin among aerobic gram-positive *cocci* and gram-negative *bacilli*, as also reported in literature[6,16].

It has been verified that carbapenems are the only β-lactams active against most of enteric microorganisms and other facultative anaerobes and aerobes[2], particularly *staphylococci*[20] and enteric gram-negative *bacilli*[20]. However, some resistance to carbapenems in aerobes and facultative anaerobes has been described, especially in gram-negative enteric rods, coagulase-positive *staphylococci* and several species of genera *Streptococcus* and *Enterococcus*[13,15]. The resistance to imepenem was similar to that observed to meropenem and restrict to a few isolates of *staphylococci*, *P. aeruginosa*, *E. faecium*, and *M. morganii*. This was also observed by Pillar, et al.[20] (2008).

In this present investigation, most of isolates resistant to β-lactam were β-lactamase producers. However, 37.1% of the β-lactam resistant microorganisms were not β-lactamase producers. Some of these "non-producers" could be producers of non-exportable β-lactamases, as it has been previously reported for some gram-negative bacteria[14]. Another explanations for this fact would be that these "non-producers" could harbor other mechanisms of resistance (e.g.: alteration of structure of penicillin-binding proteins), or that the method's sensitivity did not allow the detection of these enzymes[8].

Both the production of low affinity penicillin binding proteins[21] and the impermeability of the outer membrane to these drugs could also be involved in the resistance to β-lactams, even in β-lactamase producers. In this sense, *enterococci* in general and *E. faecium* in particular would be intrinsically more resistant to penicillin and ampicillin than the other *streptococci*. Ampicillin and amoxicillin resistance in *E. faecium* have occurred due to the expression of the low-affinity class B penicillin-binding protein 5 (PBP5). However, higher levels of resistance in clinical isolates have been only rarely associated with increased levels of PBP 5 expression[22]. More commonly, mutations that have been presumed to lower the affinity for β-lactam antibiotics have been identified within PBP5 genes of highly resistant clinical isolates[21].

Aminoglycoside antibiotics have not been usually recommended in the treatment of odontogenic infections. However, its use in association with other drugs, especially β-lactams, has been frequent in oral surgery. The susceptibility to gentamicin and amikacin was high among most of the tested microorganisms, but some *enterococci* were highly resistant to amikacin. On the other hand, resistance to gentamicin was more concentrated on *C. freundii*, *E. cloacae*, *P. aeruginosa*, and some *staphylococci*.

The activity of gentamicin and amikacin against most aerobes and facultative anaerobes has been well described in literature[11,12,16], and these drugs have been the most frequently used in nosocomial and opportunistic infections involving these microorganisms. However, resistance has been observed in *Enterobacteriaceae* and *Pseudomonadaceae*, ranging from 1.0% to 17.9% in gram-negative enteric rods for amikacin and from 2.8% to 38.5% for gentamicin[11,24]. An expressive resistance to these drugs has also been detected for *staphylococci* and *enterococci*, as well as genus *Klebsiella*[6,11,17].

Chloramphenicol has been a broad spectrum antimicrobial largely used in the treatment of nosocomial infections, particularly when *Enterobacteriaceae* species were involved and its use has been rare in dentistry. Table 3 shows that *enterococci* were the most resistant to chloramphenicol amongst the tested microorganisms. Moreover, some resistance was disseminated in most target microorganisms. On the other hand, this phenomenon has been described mainly in *enterococci* and *streptococci viridans*, although some enteric gram-negative rods resistant to this drug have been detected[11,12]. The results presented here, however, would not justify the use of this antimicrobial agent in the treatment of serious infections involving these superinfecting bacteria.

The resistance to ciprofloxacin was manly restricted to some isolates of genera *Staphylococcus* and *Streptococcus*, while just two isolates of gram-

negative rods (*E. intermedius* and *P. aeruginosa*) were resistants amongst the tested microorganisms. This was in accordance with most of available data in literature[1,11,12]. However, it contrasted with Huang, et al.[15] (2007), who showed high levels of resistance to ciprofloxacin in oxacillin-resistant *S. aureus* (100%), extended spectrum β-lactamase (ESBLs) producers *K. pneumoniae* (82%), *S. marcescens* (40%), *streptococci* (33%), and *P. aeruginosa* (9%). Since this drug has not been either frequently used by Brazilian dentists or as part of self-medication, it would be possible that the antimicrobial resistance profiles of isolates from hospitals would have been significantly different from those observed in commensal microbiota, as described by Pillar, et al.[20] (2008).

Resistance to tetracycline also has been often registered in facultative anaerobes and aerobes, which have seemed to be disseminated in the human and animal microbiota[11,12]. This phenomenon could be related to its extensive use in medicine, veterinary and dentistry. The distribution of the resistance to tetracycline, as observed in Table 2, was similar to that previously reported[11,16], and slightly higher than the results of Gonçalves, et al.[12] (2007). The resistance to tetracycline was similar between gram-positive *cocci* and gram-negative bacilli, as previously observed[16], while resistance to doxycycline was more prevalent among gram-negative enteric rods and pseudomonades, which represented 76.9% of all resistant isolates. The most commonly detected doxycycline resistant species were *C. freundii*, *E. cloacae*, *P. aeruginosa*, and *P. alcalifaciens.* The high resistance of pseudomonades from oral cavity to doxycycline was previously reported by Barbosa, Mayer and Saba-Chujfi[1] (2001). These results did not support the use of doxycycline or tetracycline in life threatening infections.

Rifampin has been widely used in the treatment of several life threatening infections as well as minor oral infections for many years[4]. In the clinical samples, the low frequency of rifampin resistance was relevant and confined to *staphylococci* and the genera *Providencia* and *Serratia*, as well as to pseudomonades. However, these authors showed that the rifampin resistance ranged from 17.2% to 30.0% among *enterococci*, while Ferrari, Cai and Bombana[7] (2005) detected this resistance in 58.3% of *enterococci*. On the other hand, the present investigation found that all *enterococci* were susceptible to rifampin, and these results seem to endorse rifampin as an important therapeutic alternative in mixed and nosocomial infections, particularly where clinical signs evidenced the participation of multi-drug resistant microorganisms.

Although patients with periodontitis and edentulous patients are considerably older than patients with gingivitis and periodontally healthy patients, the influence of age on the distribution of resistant microorganisms is reduced when data from patients with the same age and with different periodontal status are compared, showing that this factor alone does not seem relevant. Moreover, a great proportion of bacterial strains presented resistance to ampicillin, amoxicillin and amoxicillin/clavulanic acid and some isolates of *E. cloacae*, *K. oxytoca*, *P. agglomerans*, *P. alcalifaciens*, and *Serratia* sp. presented a broad spectrum resistance to β-lactam antibiotics, producing β-lactamases active on penicillins and cephalosporins.

The presence of the enterobacteria and pseudomonads and other superinfecting microorganisms may be relevant in gingivitis and periodontitis etiology, especially in immunosuppressed patients[1,10]. However, the role of enteric bacteria in the periodontal diseases etiology remains unclear, and it must be an alert to clinicians who use systemic antibiotics, such as ciprofloxacin, as an adjunct in the periodontitis treatment in these patients[9].

CONCLUSIONS

The results of this investigation confirmed that the oral cavity of patients with periodontitis and gingivitis, and particularly edentulous patients wearing complete dentures could harbor microorganisms with several antimicrobial resistance markers, and these microorganisms are frequently implicated in multiresistant, systemic, oral or nosocomial infections.

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