Resistance to tetracycline and β-lactams and distribution of resistance markers in enteric microorganisms and pseudomonads isolated from the oral cavity

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ABSTRACT

This study evaluated the occurrence of enteric bacteria and pseudomonads resistant to tetracycline and β-lactams in the oral cavity of patients exhibiting gingivitis (n=89), periodontitis (n=79), periodontally healthy (n=50) and wearing complete dentures (n=41). Microbial identification and presence of resistance markers associated with the production of β-lactamases and tetracycline resistance were performed by using biochemical tests and PCR. Susceptibility tests were carried out in 201 isolates of enteric cocci and rods. Resistance to ampicillin, amoxicillin/clavulanic acid, imipenem, meropenem and tetracycline was detected in 57.4%, 34.6%, 2.4%, 1.9% and 36.5% of the isolates, respectively. β-lactamase production was observed in 41.2% of tested microorganisms, while the most commonly found β-lactamase genetic determinant was gene bla TEM. Tetracycline resistance was disseminated and a wide scope of tet genes were detected in all studied microbial genus.

Key words: Oral cavity. Enteric bacteria. PCR.

INTRODUCTION

The oral microbiota is composed of more than 500 different microbial species, most of them associated with oral health. However, sometimes the balance between the host’s immune system and microbial virulence is lost and opportunistic infections may arise. Hence, oral infectious diseases have been frequently associated with alterations in the host’s immune system, poor oral hygiene, denutrition, and alcoholism.

Associations between the occurrence of opportunistic and superinfecting pathogens with patients exhibiting different periodontal status or wearing complete dentures have been established. However, the role enteric bacteria and pseudomonads play in the etiology of periodontal disease needs further studies. In edentulous patients wearing complete dentures, the presence of enteric microorganisms may be associated with development of mucositis and usually reflects poor hygiene standards.

Suppression of the oral microbiota by abusive or intensive use of antibiotics may facilitate a persistent colonization of the oral cavity by these opportunistic microorganisms. These microorganisms may spread to microbial populations in nosocomial infections or to the dental biofilm, acting as reservoirs for antibiotic resistance genes.

Tetracyclines were among the most widely used drugs in dentistry in the 80’s. Their
effects on anaerobes and *Aggregatibacter actinomycetemcomitans* made these drugs the first choice in the treatment of aggressive periodontitis and necrotizing periodontitis. ß-lactams, such as ampicillin, amoxicillin, cefoxitin and others constitute the basis of antimicrobial treatment of head and neck infections. However, microbial resistance to these drugs has compromised the efficacy of this therapy and the dissemination of resistance genes among oral microorganisms needs further investigation, as the oral cavity may harbor some multiresistant microorganisms, particularly enteric rods and cocci.

Thus, the aim of this study was to evaluate the presence of antimicrobial resistance genes (tetracycline and ß-lactams) in enteric microorganisms isolated from the oral cavity of patients with gingivitis, periodontitis, periodontally healthy patients and patients wearing complete dentures, determining the distribution of most common ß-lactamase markers and tetracycline resistance markers.

**MATERIAL AND METHODS**

**Microorganisms and microbial identification**

Enteric microorganisms were isolated from 250 patients (84 males and 166 females), mean age 43.03 years, within an 10-year follow-up period (1998-2008) at the School of Dentistry of Araçatuba, São Paulo State University (UNESP), Brazil. Forty-one patients wore complete dentures, determining the distribution of most common ß-lactamase markers and tetracycline resistance markers.

Antimicrobials were tested in two-fold dilution series ranging from 0.06 µg/mL to 256 µg/mL. After incubation, the organisms were classified as sensitive or resistant, according to CLSI and BSAC 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.
useful in detecting β-lactamase production by some microorganisms. In all tests, *S. aureus* ATCC 29213 was used as the positive control for β-lactamase production.

Distribution of antimicrobial resistance determinants

Bacterial DNA from each β-lactamase producers placed in sterile ultra-pure water was obtained by using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA concentrations were determined with a spectrophotometer at A260nm (Model DU-640, Beckman Instruments, Richmond, Wash, USA).

Tetracycline-resistant isolates were screened for tetracycline resistance genes1,16 tet(A), tet(B), tet(C), tet(D), tet(E), tet(G), tet(K), tet(L), tet(M), tet(O), tet(Q), tet(S), and tet(T), while β-lactam-resistant microorganisms were screened for *bla*TEM, *bla*CTX-M and *bla*SHV genes3,8 performed in a DNA thermal cycler (AmpliTherm Thermal Cycler, Madison, WI, USA). The DNA extension.

RESULTS

In relation to susceptibility to antimicrobial drugs, significant levels of resistance were observed for all β-lactams, except for imipenem and meropenem, which presented 2.4% and 1.9% of resistance, respectively. Resistance to ampicillin, and cephalothin were detected in 57.4%, and 41.7% of tested bacteria, especially Pseudomonadaceae and Enterobacteriaceae. Out

Table 1- Resistance to β-lactams and tetracycline in enteric bacteria and pseudomonads

<table>
<thead>
<tr>
<th>TAXON (N)</th>
<th>Resistance prevalence N (%)</th>
<th>β-lactamase production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>AMC</td>
</tr>
<tr>
<td>A. bamanii (10)</td>
<td>6 (60.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>B. cenocepacia (5)</td>
<td>4 (80.0)</td>
<td>2 (40.0)</td>
</tr>
<tr>
<td>C. freundii (7)</td>
<td>4 (57.1)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>E. cloacae (18)</td>
<td>14 (77.8)</td>
<td>9 (50.0)</td>
</tr>
<tr>
<td>E. intermedius (6)</td>
<td>2 (33.3)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>E. sakazakii (9)</td>
<td>4 (44.4)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Enterococcus sp. (18)</td>
<td>4 (22.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>E. faecalis (31)</td>
<td>6 (19.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>E. faecium (8)</td>
<td>4 (50.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>E. coli (6)</td>
<td>4 (66.7)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>K. oxytoca (11)</td>
<td>7 (63.6)</td>
<td>5 (45.5)</td>
</tr>
<tr>
<td>K. pneumoniae (3)</td>
<td>3 (100.0)</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>M. morganii (17)</td>
<td>12 (70.6)</td>
<td>9 (52.9)</td>
</tr>
<tr>
<td>P. agglomerans (7)</td>
<td>6 (85.7)</td>
<td>6 (85.7)</td>
</tr>
<tr>
<td>P. mirabilis (5)</td>
<td>3 (60.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>P. vulgaris (7)</td>
<td>5 (71.4)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>P. alcalifaciens (6)</td>
<td>4 (66.7)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>P. aeruginosa (15)</td>
<td>13 (86.7)</td>
<td>13 (86.7)</td>
</tr>
<tr>
<td>P. fluorescens (4)</td>
<td>3 (75.8)</td>
<td>3 (75.0)</td>
</tr>
<tr>
<td>S. liquefaciens (9)</td>
<td>6 (66.7)</td>
<td>6 (66.7)</td>
</tr>
<tr>
<td>Serratia sp. (9)</td>
<td>7 (77.8)</td>
<td>5 (55.6)</td>
</tr>
<tr>
<td>Total (211)</td>
<td>121 (57.4)</td>
<td>73 (34.6)</td>
</tr>
</tbody>
</table>

AM= ampicillin; AMC= amoxicillin/clavulanic acid; CF= cefoxitin; CP= cephalotin; IM= imipenem; ME= meropenem; TE= tetracycline
of 121 bacterial isolates resistant to ampicillin or amoxicillin, 87 were β-lactamase producers of (41.2% of the isolated bacteria and 72.9% of ampicillin-resistant isolates). The production of these hydrolytic enzymes seems to be the major mechanism of resistance to β-lactams, excluding most pseudomonads, and enterococci, where β-lactamases were not detected (Table 1).

Most of β-lactamase Gram-negative producers harbored β-lactamases. The detection of antimicrobial resistance determinants evidenced that 29.9% of Gram-negative isolates resistant to ampicillin harbored blaTEM genes, while blaSHV and blaCTX-M were detected in 23.4% and 2.8% of the resistant isolates, respectively (Table 2).

These genes were not detected in enterococci (Table 3).

Resistance to tetracycline was also widely disseminated in the microbial enteric strains and 36.5% of tested microorganisms were resistant. The presence of tetracycline resistance determinants was widely disseminated among resistant Gram-negative isolates and enterococci. Tet(A) and tet(B) were the most common in Gram-negative bacteria; while tet(K), tet(M) and tet(O) were predominant in resistant enterococci. Tet(G), tet(Q) and tet(T) were not detected.
DISCUSSION

Enteric bacteria and pseudomonads have been involved in many oral and extra-oral infections, and some studies have evidenced that the oral cavity may act as a reservoir of enteric microorganisms and their virulence genes. In spite of the small participation of enteric bacteria and pseudomonads in the total microbial load present in the gingival sulcus, supragingival biofilm, saliva and other sites of the oral cavity, the occurrence of these pathogens should not be neglected. Antimicrobial resistance surveillance programs have provided sufficient data about antimicrobial susceptibility of clinically relevant enteric bacteria and pseudomonads from nosocomial infections and environment, although few reports describe the antimicrobial susceptibility of these organisms isolated from the oral cavity. In addition, information about the genetic determinants associated with this resistance is not clarified yet and most available data regards nosocomial infections, as mentioned above.

β-Lactam agents such as penicillins, cephalosporins, monobactams and carbapenems are among the most frequently prescribed antibiotics worldwide. In Gram-negative pathogens, β-lactamases remain the most important factor to β-lactam resistance, and their increasing prevalence, as well as their alarming evolution seems to be directly linked to their clinical use.

In the present study, the genetic bases of β-lactamase production in enteric Gram-negative rods were mainly associated with blaTEM gene, which evidenced a noticeable dissemination among Gram-negative enteric bacteria. Presence of β-lactamase genetic markers was significantly more pronounced in our study than previously reported in literature, even though the distribution of particular determinants in β-lactamase-producer strains was similar.

However, the introduction of new β-lactams with different activity spectra has led to a selection of different genes and mutations that confer resistance to these drugs, especially β-lactamase-producers, mainly in members of family Enterobacteriaceae. In this family, most β-lactamase producers harbor blaTEM, blaSHV, and blaCTX-M resistance determinants. Thus, the distribution of these resistance markers in enteric microorganisms distributed in the dental biofilm and mucosal surfaces remains unclear.

Therapeutic options for infections caused by Gram-negative organisms expressing β-lactamases are limited because these organisms are usually resistant to all β-lactam antibiotics, except the carbapenems. Several families of β-lactamases from Gram-negative organisms were identified, but no phenotypic test can differentiate them, impairing surveillance and epidemiological studies.

The genes screened in the β-lactamase family represent only a small part of the cellular defense mechanisms that prokaryotes developed to avoid the action of β-lactams. Enterobacteriaceae isolates that exhibited uncertain identification by PCR were later classified as K. oxytoca, Enterobacter spp. and C. freundii due to detection markers of β-lactam resistance. Moreover, K. oxytoca strains are known to express specific class A β-lactamases that were not considered in this study; while the resistance to β-lactams in Enterobacter sp. and C. freundii is generally attributed to the expression of chromosomal AmpC β-lactamases, as also described to some pseudomonads. Possibly, these lactamases may be responsible for the β-lactam resistance phenotype, specifically to penicillins and narrow-spectrum cephalosporins, registered in some isolates affiliated to these genera.

Enterococci in general and E. faecium in particular, are intrinsically more resistant to penicillin and ampicillin than the other streptococci. Ampicillin resistance in E. faecium is due to expression of the low-affinity class B penicillin-binding protein 5 (PBP5). Early studies suggested that higher levels of ampicillin resistance in E. faecium were achieved by increasing levels of PBP 5 expression. More commonly, mutations that are presumed to lower the affinity for β-lactam antibiotics have been identified within pbp5 genes of highly resistant clinical isolates. The results of the present investigation also suggested that enterococcal
resistance to \(\beta\)-lactams, especially ampicillin, is not related to gene \(bla_{TEM}\), as this gene and \(\beta\)-lactamase activities were not detected.

Tetracycline resistance was also often observed. The most common genetic determinants of tetracycline resistance are represented by genes \(tet\), which can be separated into genes that encode efflux proteins, especially genes \(tet(A), tet(B), tet(C), tet(D), tet(E), tet(G), tet(I), tet(K),\) and \(tet(L);\) those that protect the ribosomes from the action of tetracycline, genes \(tet(M) tet(O) tet(Q);\) and gene \(tet(X)\) that encodes a protein able to inactivate the antibiotic drug\(^{16}\). In Gram-positive cocci, the concomitant presence of two or more genes \(tet\) is common but this peculiarity was not confirmed in the present study, since only 5 isolates (17.2\%) of enterococci expressed simultaneously \(tet(K)\) and \(tet(M)\) determinants.

In \textit{Enterobacteriaceae}, the most common tetracycline resistance markers were \(tet(A)\) and \(tet(B),\) which were present in 45.8\% of the tetracycline resistant isolates, according to previous studies\(^{1,11,12,16}\). In enterococci, genes \(tet(K)\) and \(tet(M)\) represented 58.6\% of the detected resistance markers.

Heterogeneity of tetracycline resistance genes in Gram-negative enteric rods and enterococci was significant, as also previously reported\(^{11},\) although these genes were not detected in 18 enteric resistant isolates. There are several possible explanations for the non-detection of \(tet\) genes in 23.4\% of our resistant isolates. The most probable possibility is that we screened only 12 of the 38 recognized \(tet\) genes and some isolates present an inherent resistance to tetracycline as opposed to acquired resistance.

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**REFERENCES**


