

## PRESENCE AND ANTIMICROBIAL PROFILE OF GRAM-NEGATIVE FACULTATIVE ANAEROBE RODS IN PATIENTS WITH CHRONIC PERIODONTITIS AND GINGIVITIS

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### ABSTRACT

Chronic periodontitis is a multifactorial infectious disease associated with Gram-negative anaerobes which are part of the subgingival microflora. In recent years, studies have been conducted to assess the presence of Gram-negative facultative anaerobes (*Enterobacteriaceae*) and their participation in the development and progression of chronic periodontitis. The aim of this study was to determine the presence of *Enterobacteriaceae* in patients with chronic periodontitis and gingivitis and to assess antimicrobial susceptibility of clinical isolates. A descriptive, observational study was performed including 64 patients with chronic periodontitis and 22 patients with gingivitis. Microbiological samples were taken from the gingival sulcus using paper points, which then were placed in thioglycollate broth. Samples were incubated for 4 hours at 37°C and finally replated on MacConkey agar. Bacteria were identified using the API-20E system (Biomérieux, France) and antimicrobial susceptibility was determined using the disk diffusion method. The evaluation of samples showed presence of 29 enterobacterial species

distributed as follows: 7 in the group with gingivitis and 22 in the group with chronic periodontitis. In the chronic periodontitis group the most common species were: *K. oxytoca* n=5, *S. liquefaciens* n=4 and *K. pneumoniae* and *E. coli* with n=3. The gingivitis group had the highest frequency of *Erwinia* sp. (n= 2). Clinical isolates showed very low sensitivity levels to  $\beta$ -lactam ampicillin and amoxicillin/clavulanic acid, 17.2% and 27.6%, respectively, and higher sensitivity levels to ciprofloxacin (96.6%), amikacin (79.3%), gentamicin (68.9%) and ceftazidime, ceftriaxone, kanamycin and trimethoprim-sulfa (65.5%). In conclusion, the existence of a high frequency of enterobacteria in patients with chronic periodontitis and gingivitis shows that periodontologists should pay greater attention to prevention protocols, and develop mechanical and antimicrobial therapies in which antimicrobial susceptibility profile reports should be considered as part of periodontal treatment.

**Key words:** Gram-Negative Aerobic Rods and Cocci; *Enterobacteriaceae*; chronic periodontitis.

## PRESENCIA Y PERFIL ANTIMICROBIANO DE BACILOS GRAM-NEGATIVOS ANAEROBIOS FACULTATIVOS EN PACIENTES CON PERIODONTITIS CRÓNICA Y GINGIVITIS

### RESUMEN

La periodontitis crónica es una enfermedad infecciosa multifactorial asociada a bacilos Gram-negativos anaerobios estrictos que hacen parte de la microflora subgingival. En los últimos años se han realizado estudios para valorar la presencia de bacilos Gram-negativos anaerobios facultativos (*enterobacterias*) y su importancia en el desarrollo y progresión de la periodontitis crónica. El objetivo de este estudio fue determinar la presencia de enterobacterias en pacientes con periodontitis crónica y gingivitis y conocer la susceptibilidad antimicrobiana de los aislamientos clínicos. Se realizó un estudio observacional y descriptivo en el que se incluyeron 64 pacientes con periodontitis crónica y 22 pacientes con gingivitis. Las muestras tomadas en el surco gingival con conos de papel se depositaron en caldo tioglicolato, se incubaron durante 4 horas a 37 °C y se sembraron finalmente en Agar MacConkey. En la identificación de las bacterias se utilizó el sistema API-20E (Biomérieux, France) y la susceptibilidad antimicrobiana se realizó por el método de difusión en disco. En los dos grupos se identificaron 29 especies enterobacterianas, 7

en el grupo con gingivitis y 22 en el grupo con periodontitis crónica. En el grupo de periodontitis crónica las especies más frecuentes fueron: *K. oxytoca* n=5, *S. liquefaciens* n=4 y *K. pneumoniae* y *E. coli* con n=3. En el grupo con gingivitis, *Erwinia* sp tuvo la mayor frecuencia (n=2). Los aislamientos clínicos presentaron niveles muy bajos de sensibilidad a los B-lactámicos ampicilina y amoxicilina/ ac.clavulánico, 17.2% y 27.6%, respectivamente, y mayor sensibilidad a ciprofloxacina (96.6%), amikacina (79.3%), gentamicina (68.9%) y a ceftazidima, ceftriaxona, kanamicina y trimetoprim-sulfa (65.5%). En conclusión, la alta frecuencia de enterobacterias en pacientes con periodontitis crónica y gingivitis debe conducir a los profesionales en periodoncia a trabajar con mayor determinación en protocolos de prevención y a desarrollar terapias mecánicas y antimicrobianas en las cuales se tengan en cuenta, como parte del tratamiento periodontal, los perfiles antimicrobianos reportados.

**Palabras clave:** bacilos gram-negativos, enterobacterias, periodontitis crónica.

## INTRODUCTION

Chronic periodontitis is a multifactorial infectious disease with high prevalence worldwide, in which the leading role of Gram-negative subgingival microflora has been defined, with a group of fully identified bacteria including *P. gingivalis*, *T. denticola*, *T. forsythia*, *A. actinomycetemcomitans*, *P. intermedia*, *E. corrodens*, *C. rectus*, *F. nucleatum*, *Capnocytophaga* sp. and *P. micra*<sup>1,2</sup>.

Factors inherent to the host such as heredity, smoking and environmental factors are important and determinant in the progression and severity of the disease<sup>1</sup>.

Although strict anaerobe periodontal pathogenic microorganisms are directly involved in the onset and progression of chronic periodontitis, it is also important to mention that for several years, facultative anaerobe Gram-negative enteric rods (enterobacteria) have also been found in the gingival sulcus of patients with chronic periodontitis<sup>3-10</sup>.

In the human oral environment, in addition to enterobacteria being found in the gingival sulcus of patients with chronic periodontitis, they have been isolated from mucosa and teeth<sup>3,11,12</sup>. The presence of enterobacteria in the oral cavity is basically due to orofecal transmission, deficient oral hygiene, or contamination from grooming accessories, food or drink<sup>13,14</sup>.

Another outstanding feature of enterobacteria is their implication as key pathogens in some cases of refractory periodontitis<sup>4,5</sup>. Similarly, these opportunistic microorganisms may be major players as a result of the inadequate use of antibiotics, which may suppress normal oral microbiota, leading to persistent colonization by opportunists<sup>15</sup>.

It is important to mention that if antimicrobial therapy is needed in chronic periodontitis to eradicate enterobacteria as well as regular periodontal pathogenic microorganisms, their resistance or sensitivity to antibiotics needs to be known<sup>7</sup>. In this regard, a variety of patterns of antimicrobial resistance have been found for these Gram-negative microorganisms isolated from the oral cavity, which may also be implicated in hospital-acquired, systemic and multi-resistant infections<sup>7</sup>.

To sum up, many studies suggest that enteric bacteria can play a major part in periodontal disease<sup>3,6,10</sup>. New studies therefore aim to assess the importance of enterobacteria in the progression of periodontal disease, their frequency of appearance in patients

with chronic periodontitis and gingivitis, relationship with age and socio-demographics of study populations, and antimicrobial susceptibility or resistance profile<sup>3,16-18</sup>.

The working hypothesis for this study suggests that there is high frequency of enterobacteria in patients with gingivitis and chronic periodontitis and high antimicrobial resistance. Thus, the aim of this study was to determine presence of enterobacteria in patients with gingivitis and chronic periodontitis, and to assess the antimicrobial susceptibility of clinical isolates. Finding these microorganisms and knowing their antimicrobial susceptibility profile could enable dentists to take them into account in prevention and control processes and in designing and applying new therapies, which should include antimicrobial therapy.

## MATERIALS AND METHODS

### Study Features

This was an observational, descriptive study which finally included 22 patients diagnosed with gingivitis and 64 patients diagnosed with untreated chronic periodontitis, who visited the pre- and post-graduate Periodontology clinics at the School of Dentistry of the Pontificia Universidad Javeriana from January 2010 to October 2011. One of the researchers performed a complete periodontal evaluation (full mouth) on all patients, using a Williams probe (Williams color-coded probe PQW, Hu-Friedy, Chicago-Illinois, USA), including gingival margin, bleeding on probing, probing depth and clinical attachment level, and classified them according to the recommendations of the 1999 International Consensus of the American Academy of Periodontology<sup>19</sup>.

Inclusion criteria were: patients diagnosed with chronic periodontitis or gingivitis, with at least 10 teeth, without systemic compromise, over 20 years old, who had not received prior periodontal treatment (within the last 6 months). Exclusion criteria were: patients undergoing antibiotic or corticosteroid therapy within the last three months, pregnant or lactating women and smokers. The study was approved by the Ethics and Research Committee of the School of Dentistry of the Pontificia Universidad Javeriana. All patients signed an informed consent form which explained the nature of the project and its associated benefits. A survey was conducted to determine each patient's systemic condition and whether the patient met the inclusion and exclusion criteria.

## Sampling

The clinical director of the study performed calibration, after which periodontal probing was done, 6 surfaces of all teeth were measured in order to select which surface to include in the sample (mesial vestibular, mesial lingual / palatal and vestibular / palatal interproximal surfaces). To take the sample from the gingival sulcus, 5 sites with pocket probing depth  $\geq 4$ mm and clinical attachment level  $\geq 2$ mm were selected for the group with chronic periodontitis, and pocket probing depth  $\leq 3$ mm and absence of gingival inflammation for the group with gingivitis. Supragingival film was removed with sterile gauze, the zone was isolated with sterile cotton, and paper points (New Stetic<sup>®</sup>) were placed depending on the depth of the gingival sulcus. Paper points were removed after 1 minute in the gingival sulcus, and placed in Eppendorf tubes containing 900  $\mu$ l thioglycollate broth (BBL<sup>™</sup> Fluid, Becton, Dickinson and Company) supplemented with hemin and menadione.

## Isolation, identification and assessment of antimicrobial susceptibility

The Eppendorf tubes containing the samples were taken to the laboratory and incubated under anaerobic conditions at 37°C for 4 hours, after which they were centrifuged (Eppendorf<sup>®</sup> centrifuge) at 4000 rpm for 10 minutes. After centrifuging, 300 $\mu$ l were discarded and the remaining 600 $\mu$ l were vortexed (Maxi mix II Thermolyne<sup>®</sup>) to mix the sample homogeneously. Immediately 50 $\mu$ l were plated on a Petri dish with MacConkey agar (Oxoid<sup>®</sup>) and incu-

bated under aerobic conditions at 37°C for 48 hours. After microbial growth, the macroscopic characteristics of the colonies were observed and a Gram stain was performed. Oxidase test, nitrate test and OF test in glucose and lactose were performed on Gram-negative colonies. Enterobacteria are oxidase-negative, glucose-fermenting, nitrate-reducing Gram-negative rods. Species were identified using an API 20E (Biomérieux<sup>®</sup> S.A., Marcy-l'Etoile/France) identification system. Clinical isolate antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method following the recommendations of the Clinical and Laboratory Standards Institute (CLSI). The following antimicrobial agents were assessed: amikacin, ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, gentamicin, kanamycin, tobramycin, trimethoprim-sulfamethoxazole and ciprofloxacin.

## Statistical analysis

Univariate and bivariate descriptive statistical analyses were performed (frequency distribution of categorical variables, mean, median and standard deviation of continuous variables). The chi-square test was used to establish a bivariate analysis between presence or absence of enterobacteria with the demographic variables age and sex (male, female). Non-parametric Mann-Whitney-Wilcoxon test was used to establish differences in pocket probing depth, attachment level and severity of periodontitis and presence or absence of enterobacteria. Values of  $P < 0.05$  were interpreted as statistically significant.

**Table 1: Main demographic and clinical findings for patients with chronic periodontitis.**

Characteristics	Enterobacteria Present	Enterobacteria Absent	Statistics
Number	22 (34.4%)	42 (65.6%)	
Age (years) <sup>a</sup>	49.8 $\pm$ 6.36	50.9 $\pm$ 5.35	
Gender Female	7	21	$P > 0.05$
Gender Male	15	21	$P < 0.05$
Pocket probing depth (mm) <sup>a</sup>	5.60 $\pm$ 1.75	5.57 $\pm$ 1.78	$P > 0.05$
Attachment level (mm) <sup>a</sup>	5.71 $\pm$ 1.89	5.78 $\pm$ 1.78	$P > 0.05$
Severity of periodontitis (mm) <sup>a</sup>	5.36 $\pm$ 1.23	5.39 $\pm$ 1.12	$P > 0.05$

<sup>a</sup> Values show mean  $\pm$  standard deviation

## RESULTS

The study sample consisted of 86 patients, of whom 44 (51.2%) were female and 42 (48.8%) male. Out of the 86 patients, 22 (25.6%) had gingivitis and 64 (74.4%) had chronic periodontitis. In the group of patients with gingivitis there were 16 females and 6 males, while in the group with chronic periodontitis there were 28 females and 36 males.

Table 1 describes the clinical and demographic characteristics of patients with chronic periodontitis with or without enterobacteria. In this group, enterobacteria were present at a frequency of 34.4% (22/64), strongly associated to male patients ( $P < 0.05$ ). There was no statistically significant difference in age regarding the chronic periodontitis group without enterobacteria ( $P > 0.05$ ). There was no statistically

significant difference ( $P>0.05$ ) in clinical characteristics (pocket probing depth, attachment level and severity of chronic periodontitis) between chronic periodontitis patients with or without enterobacteria. The 29 enterobacteria isolated from the two study groups were distributed as follows: 7 (24.1%) in the group with gingivitis (6 females and 1 male) and 22 (75.9%) in the group with chronic periodontitis (15 males and 7 females). No patient with enterobacteria ( $n=29$ ) had more than one species. There was no statistically significant difference ( $P>0.05$ ) in enterobacteria frequency between the two groups of patients (34.4% vs. 31.8%).

Figure 1 shows that the greatest presence of enterobacteria in patients diagnosed with gingivitis was at ages 20 to 30 years ( $n=5$ ), while in patients diagnosed with chronic periodontitis the greatest frequency of enterobacteria was at ages 41 to 50 years ( $n=9$ ).

In chronic periodontitis patients with and without enterobacteria, mean pocket probing depth was  $5.60 \pm 1.75$  and  $5.57 \pm 1.78$  respectively ( $P>0.05$ ) (Table 1). Mean attachment level in chronic periodontitis patients with and without enterobacteria was  $5.71 \pm 1.89$  and  $5.75 \pm 1.76$ , respectively (Table 1).

Table 2 shows distribution and frequency of enterobacteria species in each group. In the chronic periodontitis group the most frequent species were *K. oxytoca*  $n=5$ , *S. liquefaciens*  $n=4$ , and *K. pneumoniae* and *E. coli*  $n=3$ , while in the gingivitis group, *Erwinia* sp. had the highest frequency ( $n=2$ ).

Table 3 summarizes the *in vitro* susceptibility profiles of the 29 enterobacteria to the 10 antimicrobial agents. Sensitivity to the  $\beta$ -lactams ampicillin and

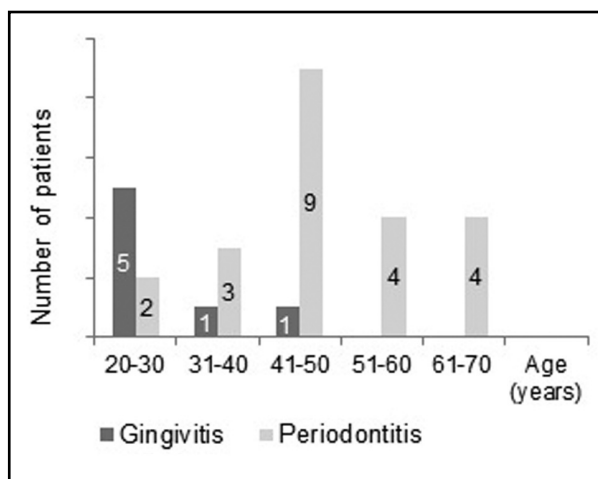


Fig. 1. Presence of enterobacteria according to age of patients with gingivitis and chronic periodontitis.

Table 2: Frequency of enterobacteria in the groups studied.

Species	Chronic periodontitis n= (%)	Gingivitis n= (%)
<i>Klebsiella pneumoniae</i>	3 (13.7)	1 (14.3)
<i>Klebsiella oxytoca</i>	5 (22.8)	0 (0.0)
<i>Escherichia coli</i>	3 (13.7)	0 (0.0)
<i>Hafnia alvei</i>	1 (4.5)	1 (14.3)
<i>Erwinia</i> sp	1 (4.5)	2 (28.6)
<i>Shigella</i> sp	1 (4.5)	1 (14.3)
<i>Serratia liquefaciens</i>	4 (18.2)	1 (14.3)
<i>Serratia marcescens</i>	1 (4.5)	1 (14.3)
<i>Serratia odorifera</i>	1 (4.5)	0 (0.0)
<i>Enterobacter cloacae</i>	2 (9.1)	0 (0.0)
<b>Total</b>	<b>22 (100)</b>	<b>7 (100)</b>

Table 3: Susceptibility to antimicrobial agents *in vitro* for the 29 enterobacteria strains isolated from patients with gingivitis and chronic periodontitis.

Antimicrobial agent	<i>K. pneumoniae</i> n= 4	<i>K. oxytoca</i> n=5	<i>E. coli</i> n= 3	<i>H. alvei</i> n= 2	<i>Erwinia</i> sp n=3	<i>Shigella</i> sp n=2	<i>S. liquefaciens</i> n=5	<i>S. marcescens</i> n=2	<i>S. odorifera</i> n= 1	<i>E. cloacae</i> n= 2	Total sensitive strains n= (%)
Amikacin	S (2)	S (3)	S (2)	S (2)	S (2)	S (2)	S (5)	S (2)	S (1)	S (2)	23 (79,3)
Ampicillin	S (0)	S (0)	S (1)	S (0)	S (1)	S (1)	S (2)	S (0)	S (0)	S (0)	5 (17,2)
Amox./Clav.	S (1)	S (2)	S (0)	S (0)	S (2)	S (1)	S (2)	S (0)	S (0)	S (0)	8 (27,6)
Cefotaxime	S (1)	S (2)	S (2)	S (1)	S (2)	S (2)	S (4)	S (2)	S (1)	S (1)	18 (62,1)
Ceftazidime	S (1)	S (2)	S (2)	S (2)	S (3)	S (2)	S (4)	S (2)	S (1)	S (0)	19 (65,5)
Ceftriaxone	S (2)	S (2)	S (2)	S (1)	S (2)	S (1)	S (4)	S (2)	S (1)	S (2)	19 (65,5)
Gentamicin	S (1)	S (2)	S (2)	S (1)	S (2)	S (2)	S (5)	S (2)	S (1)	S (2)	20 (68,9)
Kanamycin	S (1)	S (2)	S (1)	S (1)	S (2)	S (2)	S (5)	S (2)	S (1)	S (2)	19 (65,5)
Trim/Sulfa	S (1)	S (2)	S (2)	S (1)	S (3)	S (1)	S (4)	S (2)	S (1)	S (2)	19 (65,5)
Ciprofloxacin	S (4)	S (5)	S (3)	S (2)	S (2)	S (2)	S (5)	S (2)	S (1)	S (2)	28 (96,6)

amoxicillin/clavulanic acid was very low, at 17.2% y 27.6 %, respectively. The highest sensitivity was to ciprofloxacin (96.6%), followed in decreasing order by amikacin (79.3%), gentamicin (68.9%), ceftazidime, ceftriaxone, kanamycin and trimethoprim-sulfa (65.5%) and cefotaxime (62.1%).

*Serratia* species (*S. liquefaciens*, *S. marcescens*, *S. odorifera*) were highly sensitive (87.5% - 100%) to the antimicrobial agents, except ampicillin and clavulanic amoxicillin (25%). Similarly, the two *E. cloacae* strains were highly sensitive to 6 of the 10 antimicrobial agents (100%), with the exception of cefotaxime (50%) and ampicillin, amoxicillin/clavulanic acid and ceftazidime (0%). *Klebsiella* species (n=9) were less susceptible to antimicrobial agents; nevertheless, their susceptibility to ciprofloxacin and amikacin (100 and 55.6 %, respectively) is outstanding. *E. coli* strains (n=3) were sensitive to antimicrobial agents with values ranging from 33% to 100%.

## DISCUSSION

Enterobacteria are important not only because they may complicate the clinical picture of patients with chronic periodontitis, since they are key in cases of refractory periodontitis, but also because due to their pathogenic capacity they could produce systemic infections if they persisted after periodontal therapy and surgery<sup>4,5,7,20</sup>.

This study shows the frequency and antimicrobial susceptibility patterns of enterobacteria species isolated from patients with gingivitis and chronic periodontitis. It is noteworthy because it found enterobacteria in 35.3% and 31.8% of patients with chronic periodontitis and gingivitis, respectively. These frequencies were very similar to values reported for Sweden (34.9 %) and Brazil (31.2%), but differed from frequencies reported for Chile (17.6%), USA (28%), China (57%) and Rumania (61.1%)<sup>12,21-25</sup>.

Prior studies conducted in Colombia report frequencies of enterobacteria in patients with chronic periodontitis of 34.5, 27.9, 21.05, 16.7 and 13.1%<sup>6,9,16,17,26</sup>. It would appear that variations in frequency are due to specific socio-cultural and demographic features, as well as to differences in methods of sampling, transportation, processing and isolation by bacteriological culture<sup>9,16</sup>. The differences may also be due to situations created in the oral microbial ecosystem leading to overgrowth of these opportunistic microorganisms,

including deficient hygiene habits and attitudes, chronic baseline diseases, tobacco use, alcohol use, and prior antimicrobial therapies<sup>20</sup>.

In contrast to studies by Slots et al.<sup>20</sup> and Barbosa et al.<sup>3</sup>, who report greater presence of enterobacteria in females, this study found greater presence of enterobacteria in males (66.7%). The difference might be related to behavioral factors, primarily to the fact that male patients visit the periodontologist less frequently, with the consequent effect on eradication or control of these microorganisms<sup>6</sup>; however, this information was not available for this study. In recent years many efforts have been made to prove that enterobacteria worsen the prognosis and clinical picture in periodontitis<sup>4-6, 10</sup>. However, as there is no study on their presence in patients with gingivitis, their role in this pathology and subsequent implication in oral pathological processes is unknown. In this study on gingivitis patients, it is interesting to note the presence of enterobacteria in 31.8%. Further studies should be conducted to determine the composition of the subgingival microbiota and establish its significance to gingivitis patients.

In this study, the most frequent enterobacteria in patients with chronic periodontitis were *K. oxytoca* (n= 5, 22.8%), *S. liquefaciens* (n=4, 18.2%), *K.pneumoniae* (n=3, 13.7%) and *E. coli* (n=3, 13.7%), in contrast to the frequencies reported by Ardila et al.,<sup>6</sup> which were *K. pneumoniae* (n=12, 75%) and *S. marcescens* (n= 2, 12.5%) and the frequencies reported by Martínez-Pabón et al.<sup>9</sup>, which were *E. cloacae* (n=6, 37.5%), *S. marcescens* (n=4, 25%) and *K. pneumoniae* (n=3, 18.75%). All three studies establish that *K. pneumoniae*, *K. oxytoca*, *S. marcescens*, *S. liquefaciens*, *E. cloacae* and *E. coli* are the most frequently found enterobacteria, representing 75% of all strains found. The variability in species and frequencies may lead to identification of the source of contamination and transmission.

Regarding susceptibility to antimicrobial agents, this study found high sensitivity to ciprofloxacin (96.6%), amikacin (79.3%) and gentamicin (68.9%) and low sensitivity to ampicillin (17.2%) and amoxicillin/clavulanic acid (27.6%). These results in enterobacteria are very similar to those found by Ardila et al.<sup>6</sup>, who report high sensitivity to ciprofloxacin (100%) and low sensitivity to amoxicillin/clavulanic acid (25%); Botero et al.<sup>17</sup>, who report high sensitivity to ciprofloxacin (100%) and

low sensitivity to amoxicillin (12.5%) and Gaetti-Jardim et al.<sup>7</sup>, who report sensitivity to ciprofloxacin (93.4%) and amoxicillin/clavulanic acid (71.7%).

A study on 201 strains of enterobacteria and pseudomonas isolated from the oral cavity in different clinical situations (healthy, gingivitis and periodontitis) reported 42.6%, 65.4%, 97.6 %, 98.1 % and 63.5% sensitivity to ampicillin, amoxicillin/clavulanic acid, imipenem, meropenem and tetracycline, respectively, and  $\beta$ -lactamase production in 41.2% of the organisms assessed, with the highest frequency of the genetic determinant *bla*<sub>TEM</sub> as resistance indicator<sup>27</sup>. Although  $\beta$ -lactamases were not detected in our study, it is clear that the therapy options for infections caused by enteric Gram-negative rods that express these hydrolytic enzymes are limited, because they are usually resistant to all  $\beta$ -lactam antibiotics except carbapenems. It is also important to consider that bacteria in the oral cavity that are resistant to antimicrobial agents may be a source of transmission of antimicrobial resistance genes to other pathogenic bacteria<sup>18</sup>.

However, the strains assessed are highly sensitive to ciprofloxacin and the aminoglycosides evaluated (amikacin and gentamicin). Aminoglycosides, also known as amino sugars, are heterocyclic antibiotics that act on bacterial protein synthesis. They are not recommended individually in the treatment of infections of the oral cavity against Gram-negative microorganisms; nevertheless, they are used jointly with other antimicrobial agents, particularly  $\beta$ -lactams, in oral surgery<sup>7</sup>. Gaetti-Jardim et al. report high sensitivity to amikacin (80.6%) and gentamicin (91.4%)<sup>7</sup>. These studies leave an open window for considering the use of these antimicrobial agents against enterobacteria.

Ciprofloxacin is a synthetic antimicrobial agent of the fluorinated quinolone group, whose spectrum of action includes Gram-positive and Gram-negative bacteria. The results of this study are consistent with the high sensitivity found in other studies,

suggesting that ciprofloxacin has high potential for eradicating enterobacteria in patients with chronic periodontitis<sup>7, 18</sup>.

Because of their great pathogenic power, contaminating, colonizing and proliferating capacity, considerable frequency, facultative anaerobe characteristics and ability to use the nutrient richness of the gingival sulcus, enterobacteria could interact with periodontal pathogens when both are present in the micro-environment of the periodontal pocket, causing damage and complicating the clinical picture and prognosis of chronic periodontitis<sup>4,5,10,18</sup>. In this situation, it would be timely to consider the use of combined antimicrobial agents against the enterobacteria and classical periodontal pathogens implicated in periodontal disease.

One of the strengths of this study was to have determined the presence of enterobacteria not only in chronic periodontitis patients but also in gingivitis patients. The latter points to the need for implementing better strategies to reduce enterobacteria in gingivitis patients, so that they will not subsequently associate with periodontal pathogens in periodontal disease. Similarly, when antimicrobial therapy is used, the knowledge of susceptibility profiles to antimicrobial agents reported for the enterobacteria isolated from gingivitis and periodontitis patients should be considered. The main limitation of the study, due to its observational descriptive character, was that it did not determine the presence of enterobacteria longitudinally, with the aim of ascertaining whether colonization was persistent or transient.

## CONCLUSIONS

This study found high frequency of enterobacteria in chronic periodontitis and gingivitis patients. The clinical isolates identified had high sensitivity to ciprofloxacin and aminoglycosides and low sensitivity to the  $\beta$ -lactams ampicillin and amoxicillin/clavulanic acid.

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