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ORIGINAL ARTICLE

Determination and identification of antibiotic-resistant oral streptococci isolated from active dental infections in adults

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ABSTRACT

Objective: To determine and identify antibiotic-resistant bacteria (ARB) of oral streptococci from active dental infections in adults and its association with age and gender.
Material and methods: This cross-sectional study included 59 subjects from 18 to 62 years old. Ninety-eighth samples obtained from the subjects were cultivated in agar plates containing antibiotics amoxicillin/clavulanic acid (A-CA), clindamycin, and moxifloxacin (concentrations of 16, 32 or 64 µg/ml). PCR assay was performed to identify bacterial species.
Results: The bacterial species that showed more antibiotic-resistance (AR) was *S. mutans* (45.9%), followed by *S. gordonii* (21.6%), *S. oralis* (17.6%), *S. sanguinis* (9.5%), *S. salivarius* (5.4%) and *S. sobrinus* (0%). Moreover, clindamycin (59.4%) showed the highest frequency of AR. Moxifloxacin and A-CA showed an susceptibility >99.1%, while clindamycin showed the lowest efficacy (93.3%); there was a significant statistically difference ($p < .01$). The age group between 26 and 50 years old (32.2%) and females (28.8%) showed more multiresistance. Clindamycin showed a statistical difference ($p < .05$) when comparing groups by gender.
Conclusions: Clindamycin was the antibiotic with the highest frequency of ARB and lower bactericidal effect. Moxifloxacin and A-CA showed the highest efficacy and the lowest ARB frequency. *Streptococcus mutans* was the bacterial specie that showed an increased frequency of AR.

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Introduction

Oral cavity infections are a significant public health problem and a leading cause of pathology. Besides, infections are the third cause of antibiotic prescription, 10% of all antibiotics are used for oral infections treatment, and dentists represent 7% of all antimicrobial prescriptions [1]. The factors mentioned above together with self-medication are the primary factors to produce antibiotic-resistance bacteria (ARB) [2,3].

It is known that odontogenic infections are caused not only by a single organism but by polymicrobial biofilms [4,5]. The biofilm is a mixture of Gram-negative and Gram-positive bacteria (in some cases up to 6 different species have been isolated) capable of causing local infections. In fact, oral cavity is colonized by several microorganisms, which comprises between 300 and 500 species of bacteria, fungi and protozoa, and only 10% are regularly isolated using conventional culture techniques [2]. Besides, it has been reported that oral infections affect the metabolic control of systemic chronic diseases such as diabetes mellitus and rheumatoid arthritis [6–8].

On the other hand, the goal of the antimicrobial treatment is to avoid the continuity of the infection, reduce the bacterial inoculation and prevent the spread of damage to surrounding tissues and organs [9]. Some drugs used for the antibiotic therapy are penicillin and clindamycin, which are the first choice in the treatment of odontogenic infections [10] along with new synthetic antibiotics such as fluoroquinolones [11]. Also, the new antibacterial agent moxifloxacin has been used recently; its benefits are wide-spectrum, excellent bioavailability, long half-life and superior tissue penetration. Besides, it has 94% of clinical success and 95% bacterial eradication [12].

However, some bacteria are becoming resistant to these antibiotics, producing a global health problem due to it generates ineffective treatments and increases the risk of spreading refractory infections [6]. Dental active infections are biofilms that could create resistance to an antimicrobial drug to which it was initially vulnerable. One possible cause of ARB is that cells living within the biofilm where they exchange genetic information that produces physiological resistance [13]. The biofilm shows a greater resistance to antibiotics up to 1000 times compared to planktonic bacteria [14,15]. The aim of this study was to determine and identify ARB of oral streptococci from active dental infections in adults and its association with age and gender.

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Table 1. Specific primers used to determine oral streptococci.

Target	Name	Primer sequence	Annealing (°C)	Expected size (bp)
S. mutans gtfD	MKD-F	5′GGCACCACAACATTGGGAAGCTCAGTT3′	70	433
	MKD-R	5′GGAATGGCCGCTAAGTCAACAGGAT3′		
S. sobrinus gtfT	MKT-F	5′GATGATTTGGCTCAGGATCAATCCTC3′	70	328
	MKT-R	5′ACTGAGCCAGTAGTAGACTTGGCAACT3′		
S. salivarius gtfK	MKK-F	5′GTGTTGCCACATCTTCACTCGCTTCGG3′	66	544
	MKK-R	5′CGTTGATGTGCTTGAAAGGGCACCATT3′		
S. sanguinis gtfP	MKP-F	5′GGATAGTGGCTCAGGGCAGCCAGTT3′	70	313
	MKP-R	5′GAACAGTTGCTGGACTTGCTTCTC3′		
S. oralis gtfR	MKR-F	5′TCCCGGTCAGCAAACTCCAGCC3′	66	374
	MKR-R	5′GCAACCTTTGGGATTTGCAAC3′		
S. gordonii gtfG	MKG-R	5′CTATGCGGATGATGCTAATCAAGTG3′	70	440
	MKG-R	5′GGAGTCGCTATAATCTTGTCAGAAA3′		

Materials and methods

Subjects and clinical preparation

This cross-sectional study comprised 59 adults between 18 and 62 years old, who attended for dental treatment due to active infections with acute symptoms in the permanent dentition. The subjects were selected by non-probabilistic consecutive sampling from the northern-central region of San Luis Potosi, Mexico. All were recruited from the Clinic of Periodontics (Dental School of Potosina University, Mexico) and divided into three groups of age (18–25, 26–50 and >50 years old). Subjects completed a health and dental questionnaire that included information about systemic health, dental and periodontal status. Informed written consent was obtained before the clinical examination; it embodied the ethical principles of the Declaration of Helsinki (Version 2013). Also, the study was approved by the Clinical Studies Committee of the Master's Degree in Advanced Education General Dentistry Program at San Luis Potosi University (MCO2015-2).

Dental sample

The field and tooth disinfection procedure were carried out with 30% of H_2O_2, followed by 5% tincture of iodine and then 5% NaOCl. Dental caries was removed using a sterile bur, and the access was performed without water coolant to ensure minimal contamination. In the case of purulent infections, a sterile saline solution was introduced into the canals, and the bacterial biofilm was disrupted using a sterile endodontic file. Then, the adherent bacterial sample was aseptically transferred to an Eppendorf vial containing 1 ml of sterile phosphate-buffered saline (PBS). All collected samples were stored at −40 °C until the microbial and molecular assays were carried out.

Bacterial culture

Neat and serial ten-fold dilutions of the 59 subjects samples were cultivated on brain heart infusion (BHI) agar plates containing 16 µg/ml and 32 µg/ml of amoxicillin and clavulanic acid (Augmentin, Glaxo SmithKline, WestSussex, UK) and moxifloxacin (Avelox, Bayer HealthCare AGD-51368, Leverkusen, Germany). Clindamycin (Dalacin C, Pharmacia & Upjohn SA de CV, Mexico) agar plates were prepared containing 32 and 64 µg/ml. All agar plates were incubated under anaerobic conditions for 36–48 hours at 37 °C. For the control group, agar plates without any antibiotics were used. Also, anaerobic culture conditions (Hampshire, England) were established using BBL Gaspack System jars (BBL, Loveton Circle, Sparks, MD, USA) with an anaerobiosis generator system (Anaerogen, Oxoid, UK). Once bacteria grew on the agar plates, two to five colonies with different phenotypes were randomly selected from each patient sample for oral streptococci bacterial identification by polymerase chain reaction (PCR) assay.

The reference criteria of The Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standars) and colony-forming units (CFUs) values were considered for each of the antibiotics [16]. All bacterial samples were handled and tested under identical conditions to evaluate: (1) Bacterial resistant: number of CFUs that grew from each antibiotic agar plate (A-AC, clindamycin, moxifloxacin at different concentration): (2) Bacterial susceptibility: percentage of all bacteria killed in each antibiotic agar plate compared with the control group (bactericidal effect); (3) Multiresistance: bacterial resistance to at least one antibiotic.

DNA extraction and PCR assay

Ninety-eight colonies were collected from the agar plates of the 59 subjects. All clinical samples were processed under aseptic conditions to prevent contamination from the environment. Asepsis was also maintained during DNA extraction and PCR assay.

DNA extraction was performed from 500 µl of each sample from infected teeth; the protocol was cited in a previous study [6]. PCR assays were carried out in 25 µl of a reaction mixture containing 1 U Taq DNA Polymerase (Roche, Indianapolis, IN), 0.5 µM of oligonucleotides, 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$ and 10 ng of DNA template. Specific primers for each bacterial species used in the study are listed in Table 1 [17].

PCRs were performed in a thermal cycler (iCycler, BIO-RAD Laboratories, Hercules, CA) with the cycling parameters previously reported according to each set of primers. The PCR products were analysed by electrophoresis on a 2% agarose gel with Tris-acetate-EDTA buffer, using a 100-bp DNA ladder

Table 2. Bacterial resistance (CFU), bacterial susceptibility and antibiotic resistance of oral streptococci isolated from active dental infections.

	Control	A-AC-16	A-AC-32	Clin-32	Clin-64	Mox-16	Mox-32
Bacterial resistance* (CFU)							
Mean	1.0×10^4	5.9×10^1	2.5×10^1	4.5×10^2	1.2×10^2	4.3×10^2	6.1×10^1
SD	1.0×10^4	2.7×10^2	1.1×10^2	5.4×10^2	1.9×10^2	9.1×10^2	3.7×10^1
Bacterial susceptibility*							
Percentage	–	99.4	99.7	93.4	98.4	99.4	99.9
SD	–	2.3	1.3	13.1	2.6	1.2	0.5

A-AC: amoxicillin/clavulanic acid (16 and 32 μg/ml); CFU: colony-forming units; Clin: clindamycin (32 and 64 μg/ml); Mox: moxifloxacin (16 and 32 μg/ml); $n = 59$; SD: standard deviation.
*$p < .001$ (ANOVA).

marker (New England Biolab, Beverly, MA) to estimate the molecular size. Each gel was stained with ethidium bromide (0.5 μg/ml) and photographed under ultraviolet light (Chemi Doc, BIORAD Laboratories, Hercules, CA). Positive reactions were determined by the presence of bands of the expected sizes.

DNA of the following reference strains was used as positive control for PCR assay: *S. mutans* (ATCC35665; GS5, MT8148), *S. salivarius* (ATCC25975), *S. sanguinis* (ATCC10556), *S. gordonii* (ATCC33399), *S. oralis* (ATCC35037), and *S. sobrinus* (6715). All primers were tested with the different reference strains mentioned above to avoid false-positive results. A negative control was also included in each PCR assay.

Statistical analysis

The primary outcomes were bacterial resistance, bacterial susceptibility and multiresistance. All data are expressed as a mean and standard deviation. Qualitative data are expressed as frequency and proportion. Levene and Shapiro–Wilk tests were used to verify normality and homogeneity of all variables. The non-parametric Mann–Whitney *U*-test was used to detect the statistical differences among groups in quantitative variables, and the chi-square test was applied for qualitative samples. JMP program version 9.0 (SAS Institute, Cary, NC) and STATA version 12 (Stata Corp., College Station, TX) was used for statistical analysis. Statistical significance was set at a $p < .05$.

Results

Age, gender and sites of the active dental infections

This study included 59 subjects between 18 to 62 years old with a mean age of 38.2 ± 12.04. The sample included 31 females (53%) and 28 (47%) males. Regarding the site of the active infection, the most frequent location was vestibular with 50 samples (85%), followed by four (7%) intraconduct, three (5%) from pulp chamber and two (3%) from lingual sites. According to the maxillaries distribution of the endodontic infections, the upper maxilla was the most affected with 37 (63%) samples, and 22 (37%) samples were obtained from mandible sites. When both maxillaries were divided into posterior and anterior areas, the posterior area was the most affected with 33 samples (56%), and anterior area showed a frequency of 26 (44%) samples (data not shown in tables).

Bacterial resistance and bacterial susceptibility of oral streptococci isolated from active dental infections

Table 2 shows that all antibiotics produced bacterial resistance, measured by CFU. However, the antibiotic with more bacterial resistance was clindamycin at a concentration of 32 μg/ml and moxifloxacin at 16 μg/ml, while the antibiotic with the lowest ABR was A-AC at 32 μg/dl showed a statistically significant difference ($p < .01$). On the other hand, all antibiotic concentrations showed an excellent bacterial susceptibility above 93%. For example, moxifloxacin at a concentration of 32 μg/ml showed the highest bactericidal effect (99.9%), followed by A-AC (99.7%) at a concentration of 32 μg/ml. The antibiotic with the lowest bactericidal effect (93.4%) was clindamycin at a concentration of 32 μg/ml; there was a statistically significant difference ($p < .01$) (Table 2).

Patient parameters, bacterial resistance and susceptibility to antibiotics, and its association with gender

When comparing the sample by gender, males showed a higher frequency of participants in the group 26–50, followed by >50, and none between 18 and 25 years old. However, women reported more pain presence ($p < .05$) and more variety in the use of antibiotics (ciprofloxacin, ampicillin, and ampicillin/clavulanic acid) during the active dental infection ($p < .05$). Regarding bacterial resistance, women had a significantly increased amount of resistant bacteria to clindamycin at concentrations of 32 and 64 μg/ml. There was a statistical significance when comparing groups by age and gender ($p < .05$).

Most antibiotics used in this study had above 98% of bactericidal effect in both males and females ($p > .05$). Also, the bacterial susceptibility (bacteria killed by antibiotics) was similar for men and women in all antibiotic and concentrations used in this study. However, clindamycin at a concentration of 32 and 64 μg/ml showed the lowest bactericidal effect in both groups (93%). There was a significant statistical difference when comparing by gender (Table 3).

Oral streptococci isolated from active dental infections

Ninety-eight colonies were recollected and analysed from the study sample. However, only 74 were positive for oral streptococci resistant to antibiotics. Table 4 shows the ARB

Table 3. Bacterial resistance and susceptibility to antibiotics by gender.

	Women Mean ± SD	Men Mean ± SD	*p* value
Bacterial resistance (CFU)			
Control	$9.3 \times 10^3 \pm 4.7 \times 10^3$	$1.1 \times 10^4 \pm 1.4 \times 10^4$	.35
A-AC-16	$9.3 \times 10^1 \pm 3.4 \times 10^2$	$2.0 \times 10^1 \pm 1.1 \times 10^2$	.21
A-AC-32	$3.1 \times 10^1 \pm 1.2 \times 10^2$	$1.8 \times 10^1 \pm 9.8 \times 10^1$	.65
Clin-32	$5.6 \times 10^2 \pm 5.8 \times 10^2$	$3.2 \times 10^2 \pm 4.7 \times 10^2$	.049[a]
Clin-64	$1.5 \times 10^2 \pm 2.0 \times 10^2$	$8.2 \times 10^1 \pm 1.8 \times 10^2$	.040[a]
Mox-16	$4.0 \times 10^2 \pm 8.6 \times 10^2$	$4.7 \times 10^1 \pm 9.8 \times 10^1$	.93
Mox-32	$2.6 \pm 1.4 \times 10^1$	$10 \pm 5.2 \times 10^1$	.92
Bacterial susceptibility	% ± SD	% ± SD	
A-AC-16	99.1 ± 2.8	99.7 ± 1.4	.21
A-AC-32	99.7 ± 1.3	99.8 ± 1.2	.65
Clin-32	93.4 ± 6.7	93.3 ± 17.8	.06
Clin-64	98.2 ± 2.83	98.7 ± 2.9	.09
Mox-16	99.5 ± 1.03	99.3 ± 1.4	.82
Moxi-32	99.9 ± 0.2	99.9 ± 0.7	.92

A-AC: amoxicillin/clavulanic acid (16 and 32 µg/ml); Clin: clindamycin (32 and 64 µg/ml); $n = 59$; Mox: moxifloxacin (16 and 32 µg/ml); SD: standard deviation.
[a]Mann–Whitney *U* test.

Table 4. Bacterial resistance to antibiotics of oral streptococci isolated active dental infections.

Antibiotic	*S. mutans* *n* (%)	*S. oralis* *n* (%)	*S. salivarius* *n* (%)	*S. sanguinis* *n* (%)	*S. gordoni* *n* (%)	*S. sobrinus* *n* (%)	Total
A-AC	0	0	1 (1.4)	0	1 (1.4)	0	2 (2.8)
Clin	28 (37.8)	2 (2.7)	3 (4.0)	4 (5.5)	7 (9.4)	0	44 (59.4)
Mox	6 (8.1)	11 (14.9)	0	3 (4.0)	8 (10.8)	0	28 (37.8)
Total	34 (45.9)	13 (17.6)	4 (5.4)	7 (9.5)	16 (21.6)	0	74 (100)

A-AC: Amoxicillin/clavulanic acid; Clin: clindamycin; Mox: moxifloxacin.

Table 5. Multiresistance to antibiotics of oral streptococci isolated from active dental infections and its association with age and gender.

	No resistance *n* (%)	Resistance to 1 antibiotic *n* (%)	Resistance to 2 antibiotics *n* (%)	Resistance to 3 antibiotics *n* (%)	*p* value
Age					
18 a 25	2 (3.3)	3 (5.1)	1 (1.7)	0 (0)	.54
26 a 50	23 (39.0)	13 (22.0)	4 (6.8)	2 (3.4)	
>50	6 (10.2)	2 (3.4)	3 (5.1)	0 (0)	
Total	31 (52.5)	18 (30.5)	8 (13.6)	2 (3.4)	
Gender					
Women	14 (23.7)	11 (18.6)	5 (8.5)	1 (1.7)	.72
Men	17 (28.8)	7 (11.9)	3 (5.1)	1 (1.7)	
Total	31 (52.5)	18 (30.5)	8 (13.6)	2 (3.4)	

$n = 59$; χ^2 test was used for statistical analysis.

samples that were positive for each oral streptococci (*S. mutans*, *S. oralis, S. salivarius, S. sanguinis*, *S. gordoni, S. sobrinus)*. The strain with the most bacterial resistance to antibiotics was *S. mutans* (45.9%), followed by *S. gordonii* (21.6%), *S. oralis* (17.6%), *S. sanguinis* (9.5%) and *S. salivarius* with 5.4%; while no samples of *S. sobrinus* showed AR. Moreover, clindamycin (59.4%) showed the highest frequency of oral streptococci that showed AR, followed by moxifloxacin (37.8%) and A-AC (2.8%).

Multiresistance to antibiotics of clinical isolates of oral streptococci associated with age and gender

Table 5 indicates that 47.5% of the subjects presented multi-resistance to antibiotics. However, the majority of the sample was resistant to one antibiotic (30.5%). The group of age with more multi-resistance was the one between 26 and 50 years old (32.2%). About gender, females were more affected by ARB, 17 women (28.8%) were resistant to at least one antibiotic, while 11 men (18.7%) presented ARB. However, there was no significant difference on multi-resistance to antibiotics among the different groups of age, neither when comparing by gender.

Discussion

It is important to notice that in this study, 47.5% of the subjects presented oral streptococci (*S. mutans*, *S. oralis*, *S. salivarius*, *S. sanguinis* and *S. gordonii)* from active oral infections that were resistant to antibiotics. The bacterial resistance was mainly observed in clindamycin and moxifloxacin. Bacterial resistance probably depends on the complexity of the bacterial community (biofilm) involved. For example, a study that evaluated a dose–response effect noted that lower antibiotic concentrations showed more bacterial resistance in planktonic bacteria, but dental infections are organized as a biofilm [18]. This microbial biofilms are more tolerant to antibiotic treatment and cause problematic infections through different mechanisms [19,20] such as the transfer of genetic information among bacterial species that provides AR in active dental infections [6]. It has been suggested that this bacterial resistance could be promoted by gene coding, either chromosome or plasmid borne; the difference is that chromosomal DNA is relatively stable, whereas plasmid DNA is easily mobilized from one strain to another bacterial specie [6]. It is possible that ARB could be associated to plasmid-borne since our samples harbor multi-resistant antibiotic bacteria.

Nevertheless, there is little information about ARB related to gender and age. There are reports that active oral infection is more frequent in males. However, it has been observed that there is no difference concerning gender [21] as same as declared in this study. Regarding age, it has been reported a wide range of age, from children to adults [18,21]. In this study, we divided the population into three segments and found no statistical difference between groups of age and the presence of multi-resistant antibiotics oral streptococci. Another strength of the present study was that all patients were affected by active dental infections rather than pericoronitis which produces a different bacterial biofilm [21].

One of the bacterial specie that presented more AR was *Streptococcus mutans* (45.9%), probably due to its high genetic diversity that allowed it to produce different substances and contains different genes that contribute to spreading the infection through facilitating the escape from neutrophil killing through degradation [22]. Besides, it has been reported the presence of Quorum Sensing Inhibitor which is a cell-density-dependent communication process that responds to the inter/intra-species signals and elicits responses that show behavioural changes towards aggressive forms [23]. Furthermore, genetic analysis of *Streptococcus mutans* had shown a high diversity of virulence-related genes, metabolic pathways and transmission of AR genes [24,25]. All this together provides to the *Streptococcus mutans* a high resistance to antibiotics and other chemical agents such as fluor [26,27].

On the other hand, this study observed that moxifloxacin (32 μg/ml) showed the highest bactericidal effect, followed by A-AC (32 μg/ml), and clindamycin (32 μg/ml). Moxifloxacin had shown a bacterial susceptibility of 99.9%, which suggests an excellent antibiotic therapy for active dental infections that include abscesses/cellulitis, and bone infiltration. Still, moxifloxacin showed an AR of 37.8%, especially for *S. oralis* and *S. gordonii* [28,29]. However, these findings agree with other reports that suggested that moxifloxacin is an excellent alternative, especially in cases of dental extractions [30].

Traditionally, penicillin has been recommended as the first-line antibiotic because of its sensitivity, low incidence of side effects, and low cost of production [31]. However, there are several reports about the recovery of ARB from isolated of active infections from primary [6,32], permanent dentitions and orofacial infections [21,33]. Therefore, some researchers have considered the natural and semisynthetic penicillin (amoxicillin) as the first choice for infections. Nevertheless, it has been reported that amoxicillin at 8 μg/ml showed an ARB of 60% and 50% at a concentration of 16 μg/ml in active dental infections in primary dentition.

Another antibiotic used in oral infections is clavulanic acid (CA), which is a broad-spectrum sz-lactamase inhibitor produced from *Streptomyces clavuligerus*, with bactericidal activity against both Gram-positive and Gram-negative bacteria. Although CA itself has minimal antibacterial *in vitro* activity, when combined with beta-lactam antibiotics (amoxicillin) enhances its activity against none-sz-lactamase producing microorganisms. Besides, it has a promising activity against pathogens that cause bone infections [33]. However, some clinicians prefer the combination of A-CA due to the low level of bacterial resistance to this combination, broad-spectrum action, pharmacokinetic profile and tolerance [11]. It has been reported that A-CA had a small frequency of ARB compared with A-CA in active dental infections in primary and permanent dentitions [6,32]. These findings are in agreement with our results and reports from different countries, which suggest that A-CA is an excellent antibiotic alternative for refractory dental infections, especially in acute endodontic abscesses/cellulitis [33–35]. It has been reported that A-AC had a bactericidal activity of 98.8% and the presence of 20% of ARB (these studies were focused on anaerobic bacteria) [20,36]. In this study, A-CA was assessed against oral streptococci for bactericidal activity and ARB, and it showed a bactericidal effect of >99% and a small frequency of ARB (2.8%).

Furthermore, clindamycin is considered to be the gold standard for the treatment of anaerobic bacterial infections. However, ARB has increased steadily over the past years in different countries [1,6,29,37], as observed in this study where clindamycin showed the greatest frequency of ARB from dental infections.

Finally, the American Heart Association recommends antibiotic prophylaxis to prevent the development of infectious endocarditis in high-risk cardiac patients; being amoxicillin the first choice (2 g) and clindamycin the second (600 mg), both administered one hour before dental procedures [38]. However, all evidence mentioned in this report supports that those guidelines for antibiotic prophylaxis should be modified to the actual knowledge of science. Our results suggest that A-AC as first choice and moxifloxacin for penicillin-allergic subjects could be used to prevent bacterial endocarditis and probably another approach should be considered [39].

Conclusions

From all subjects involved in this study, 52.5% showed no antibiotic resistance, while 30.5% showed ABR to one antibiotic, 13.6% to two, and 3.4% to three antibiotics. The age group between 26–50 years old (32.2%) and females (28.8%) showed more multi-resistance.

Clindamycin was the antibiotic that showed both the higher ARB and the lowest bactericidal effect. There was a significant statistical difference when comparing groups by gender ($p < .05$). Moxifloxacin and A-AC displayed a strong bactericidal effect (>99%) and the lowest frequency of ARB, especially A-AC.

Streptococcus mutans exhibited the highest values of AR. This bacteria specie from clinical isolates was multi-resistant (AR to >1 antibiotic) possibly due to its genetic diversity.

Disclosure statement

The authors declare they have no conflict of interests and no financial support or relationships that may pose a conflict of interest.

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