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Odontogenic bacteria in periodontal disease and resistance patterns to common antibiotics used as treatment and prophylaxis in odontology in Spain

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SUMMARY

Resistance in streptococci or Gram-negative bacteria is associated with antibiotic consumption. Scarce information exists on the antibiotic susceptibility of bacterial isolates from patients with periodontitis in countries with high antibiotic consumption, as this is an area in which microbiological testing is not performed in daily practice. The present study was undertaken to explore the susceptibility of bacterial isolates in periodontitis to antibiotics prescribed in odontology in Spain as treatment for local infections or prophylaxis for distant focal infections. Periodontal samples were prospectively collected in 48 patients classified by pocket depth of <4 mm and ≥4 mm. Species were identified by culture, selecting the five most frequent morphotypes per sample, and polymerase chain reaction (PCR). Susceptibility was determined by E-test®. A total of 261 isolates were identified: 72.9% patients had Streptococcus oralis*; 70.8%* Streptococcus mitis*; 60.4%* Prevotella buccae*; 39.6%* Prevotella denticola*; 37.5%* Fusobacterium nucleatum*; 35.4%* Prevotella intermedia*; 25%* Capnocytophaga *spp.; 23%* Veillonella *spp.; 22.9%* Prevotella melaninogenica *and* Streptococcus sanguis*; and <20% other species.* Streptococcus viridans *resistance rates were 0% for amoxicillin, ≈10% for clindamycin, 9-22% for tetracycline, and for azithromycin ranged from 18.2% for* S. sanguis *to 47.7% for* S. mitis. Prevotella *isolates were susceptible to amoxicillin-clavulanic acid, with amoxicillin resistance ranging from 17.1% in* P. buccae *to 26.3% in* P. denticola*. Metronidazole resistance was <6% in all* Prevotella *species, while clindamycin resistance ranged from 0 to 21.1%. β-Lactamase production was positive in 54.1%* Prevotella *spp., 38.9%* F. nucleatum*, 30%* Capnocytophaga *spp., and 10%* Veillonella *spp. In this study, amoxicillin-clavulanic acid was the most active antibiotic against all species tested, followed by metronidazole in the case of anaerobes.*

Key words: Periodontopathogens - Antibiotics - Susceptibility - Periodontitis

Enfermedad periodontal, odontopatógenos y perfil de resistencia a los antibióticos habitualmente utilizados como tratamiento o profilaxis en odontología en España

RESUMEN

La resistencia de los estreptococos o de las bacterias gramnegativas se asocia al consumo antibiótico, pero existe escasa información sobre la sensibilidad de los aislamientos de pacientes con periodontitis en los países con alto consumo de antibióticos, como es España; datos que pueden ser importantes cuando en la práctica diaria no se realizan determinaciones microbiológicas. En este estudio se analiza la sensibilidad de aislamientos de periodontitis a los antibióticos prescritos habitualmente en España en odontología para el tratamiento de infecciones

locales o la profilaxis de infecciones a distancia. Se tomaron de forma prospectiva muestras periodontales de 48 pacientes clasificados, según la profundidad de la bolsa, en dos grupos: <4 mm y ≥4 mm. La identificación de las especies se realizó por PCR y por cultivo, seleccionando los cinco morfotipos más frecuentes en cada muestra. La sensibilidad antibiótica se determinó por E-test®. *Se identificaron 261 cepas. El 72,9% de los pacientes presentaron* Streptococcus oralis*, el 70,8%* Streptococcus mitis*, el 60,4%* Prevotella buccae*, el 39,6%* Prevotella denticola*, el 37,5%* Fusobacterium nucleatum*, el 35,4%* Prevotella intermedia*, el 25%* Capnocytophaga *spp., el 23%* Veillonella *spp., el 22,9%* Prevotella melaninogenica *y* Streptococcus sanguis*, y <20% otras especies. Las tasas de resistencia de* S. viridans *fueron 0% a la amoxicilina, ≈10% a la clindamicina y 9% a 22% a la tetraciclina; se halló resistencia a la azitromicina entre el 18,2% de* S. sanguis *y el 47,7% de* S. mitis*. Los aislamientos de* Prevotella *fueron sensibles a la amoxicilina-ácido clavulánico. La resistencia a la amoxicilina osciló entre el 17,1% de* P. buccae *y el 26,3% de* P. denticola*. La resistencia al metronidazol fue <6% en las especies de* Prevotella*, mientras que a la clindamicina osciló entre un 0% y un 21,1%. El 54,1% de* Prevotella *spp., el 38,9% de* F. nucleatum*, el 30% de* Capnocytophaga *spp. y el 10% de* Veillonella *spp. eran productores de betalactamasas. Amoxicilina-ácido clavulánico fue el antibiótico más activo frente a todas las especies aisladas, seguido del metronidazol en el caso de los anaerobios.*

Palabras clave: Periodontopatógenos - Antibióticos - Sensibilidad - Periodontitis

INTRODUCTION

The pathogenic potential of odontopathogens in odontogenic infections is determined by initial factors in the dentine lesions (1). In dental caries, the dominant bacterial group depends on the invasion of dental surfaces by bacterial species present in saliva and/or the supragingival plaque, and the progression of the lesion depends on the prevalence of acidogenic bacteria. The need for new nutritional resources leads to the migration of proteolytic bacteria to dentine tubules, from which radicular surfaces and subgingival plaque can be recolonized in the case of periodontitis (2). Debridement can select periodontopathogens, and this, together with the difficulty in controlling bacterial plaque biofilms, can explain the 20% failure rate of mechanical treatment (3). In this particular field a clinical efficacy rate of 80% is well accepted (4), and therefore a shift from a mechanical to a pharmacological, or to a mechanical plus pharmacological therapeutic approach is difficult, despite the demonstrated effectiveness of antibiotics that decrease anaerobic bacterial levels (5). Nonspecific bacterial growth and subsequent inflammatory disease have been postulated as one possible origin of periodontitis. In daily practice, antibiotic treatment is used in 20% of failures obtained after mechanical treatment, *i.e.,* refractory periodontitis (5).

Monitoring susceptibility of periodontal isolates may be important not only for treatment selection, but also for prevention of local or systemic infections after dental procedures and oral surgery when prophylaxis is indicated according to patient and procedure characteristics. Since 30% of the adult population has periodontitis with a gingival pocket depth of >4 mm (5), these periodontal isolates may be present in 30% of dental procedures and oral surgery. Local infectious complications are polymicrobial, with an aerobic/anaerobic component (6), while systemic infections occur after monomicrobial bacteremia in patients with underlying diseases. *Streptococcus viridans* bacteremia can be detected after dental manipulation in 75% and 30% patients with and without periodontal disease, respectively (7). Four to seven per cent of these bacteremias are due to periodontopathogens of the *Haemophilus* spp., *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella* spp. (HACEK) group (8), and anaerobic bacteremias can be detected when using adequate microbiological procedures (9).

This study explores bacterial isolates in patients with periodontitis and the antibiotic susceptibility to different antibiotics commonly prescribed as treatment or prophylaxis in odontology in Spain, a country with high antibiotic consumption, which is the main driving force for resistance in some species of the genus streptococci (10).

MATERIALS AND METHODS

Periodontal samples were collected prospectively in 48 patients treated at the Stomatology Department of the School of Dentistry at the Complutense University, Madrid, Spain, from January to May 2005. Adult patients aged at least 18 years and with a diagnosis of periodontitis were included. Patients who had received antimicrobial therapy in the previous 30 days were excluded. Samples were collected using four sterile paper points inserted gently into the subgingival sulcus after cleaning of the supragingival plaque; contamination by saliva was avoided. The paper points were left *in situ* for 10 sec. Two paper points were introduced into a sealed sterile tube and sent to the laboratory (Sanilab Molecular, Madrid, Spain) for polymerase chain reaction (PCR) testing (*A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tanne-*

rella forsythensis, *Treponema denticola*) using *micro-IDent®* test (Hain Lifescience GmbH., Nehren, Germany). The other two paper points were placed in a sealed sterile vial containing transport medium (anaerobic thioglycolate broth enriched with vitamin K and hemine; bioMedics, Madrid, Spain). The vials were maintained in a prereduced environment obtained using an anaerobic atmosphere generator (*Anaerogen™*; Oxoid Ltd., Basingstoke, UK) and sent to the laboratory (Department of Microbiology, Hospital Central de la Defensa, Madrid, Spain). Bacteriological procedures were performed no later than 60 min after sample collection. Inoculated thioglycolate tubes were agitated for 30 sec, and the homogenized sample was seeded using 0.02 ml per plate onto Columbia blood agar, enriched VX chocolate agar, colistin-nalidixic agar, McConkey agar, Schaedler agar and BBE amikacin agar (bioMedics). Additionally, 0.05 ml was seeded in a new enriched vitamin K and hemine anaerobic thioglycolate broth (bioMedics). Incubation was performed at 35 °C in an aerobic 5% CO_2-enriched atmosphere and in anaerobic conditions for culture of strict anaerobes using an anaerobic atmosphere generator. Plates were read on day 2 and day 7 after the initial processing, and after the plating from the enriched thioglycolate broth. Five of the most frequent morphotypes per sample were selected, isolated and frozen until identification and determination of susceptibility processes began.

Identification was performed using the following systems for aerobic and facultative bacteria: rapid ID 32 Strep (bioMérieux, Marcy-l'Etoile, France), ID GPC ViteK2 (bioMérieux), and ID GP Wider (Soria Melguizo, S.A., Madrid, Spain). Anaerobic bacteria were identified using rapid ID 32 A (bioMérieux). β-Lactamase production was determined using cefinase discs with nitrocefin (BBL Cefinase Discs, Becton Dickinson, Sparks, MD, USA).

The minimum inhibitory concentration (MIC) was determined for amoxicillin/clavulanic acid, amoxicillin, clindamycin, metronidazole, tetracycline and azithromycin, with antibiotic concentrations ranging from 0.02 to 256 mg/l, by *E-test®* (AB Biodisk, Solna, Sweden) in blood agar Columbia (bioMedics), and in enriched vitamin K, hemine, and 5% sheep blood *Brucella* agar (Reactivos para Diagnóstico S.L., Barcelona, Spain). *Bacteroides fragilis* ATCC 25285 was used as control strain. NCCLS/CLSI susceptibility and resistance breakpoints (mg/l) were considered (11, 12): amoxicillin/clavulanic acid ≤4/2 mg/l and ≥16/8 mg/l; amoxicillin ≤4 mg/l and ≥16 mg/l; clindamycin ≤2 mg/l and ≥8 mg/l; metronidazole ≤8 mg/l and ≥32 mg/l; tetracycline ≤4 mg/l and ≥16 mg/l for anaerobic bacteria, respectively; and amoxicillin/clavulanic acid ≤0.25/0.12 mg/l and ≥8/4 mg/l; amoxicillin ≤0.25 mg/l and ≥8 mg/l; clindamycin ≤0.25 mg/l and ≥1 mg/l; tetracycline ≤2 mg/l and ≥8 mg/l; and azithromycin ≤0.5 mg/l and ≥2 mg/l for streptococci, respectively.

Statistical methods

Analysis was performed using SPSS version 9.0 statistical package (SPSS Inc., Chicago, IL, USA). Comparisons between proportions were performed by Chi-square test or Fisher's exact test when necessary. Quantitative variables not following normality distribution were compared using the Mann-Whitney test.

RESULTS

Samples were collected in patients diagnosed with periodontitis with the following characteristics: age (mean ± SD) 50.0 ± 10.6 years (range 26-75); gender: 31 females and 17 males; and 14 smokers and 34 nonsmokers. Fourteen patients presented pocket depths of <4 mm and 34 patients depths of ≥4 mm.

PCR yielded positive results for *T. forsythensis* in 70.8% patients (34 of 48), for *T. denticola* in 62.5% (30 of 48), for *P. gingivalis* in 58.3% (28 of 48), for *P. intermedia* in 37.5% (18 of 48), and *A. actinomycetemcomitans* in 10.4% (five of 48). There was no relationship with gender or smoking status, because differences between smokers and nonsmokers did not reach statistical significance, *e.g., T. forsythensis* (92.3% *vs.* 65.7%; $p = 0.08$) and *T. denticola* (84.6% *vs.* 57.1%; $p = 0.09$). However, PCR detection of *T. denticola* was significantly higher ($p = 0.01$) in patients with pocket depth of ≥4 mm versus those with pocket depth of <4 mm (76.5% *vs.* 35.7%). In the case of *P. gingivalis* and *T. forsythensis,* differences between patients with pocket depths of ≥4 mm and <4 mm did not reach statistical significance: 67.6% *vs.* 35.7% ($p = 0.057$) for *P. gingivalis* and 79.4% *vs.* 57.1% ($p = 0.1$) for *T. forsythensis*, respectively.

A total of 261 isolates were obtained (representing a mean 5.4 isolates per patient): 47.9% of them were streptococci from the viridans group, 32.6% of them belonged to the genus *Prevotella*, 6.9% to the genus *Fusobacterium*, 4.6% to the genus *Capnocytophaga*, 3.8% to the genus *Veillonella*, and <1.5% to other genera such as *Gemella*, *Actinomyces*, and others.

The isolation rates (frequency per 100 patients) for the different species were 72.9% for *S. oralis;* 70.8% for *S. mitis;* 60.4% for *P. buccae;* 39.6% for *P. denticola;* 37.5% for *Fusobacterium nucleatum;* 35.4% for *P. intermedia;* 25%

for *Capnocytophaga* spp.*;* 23% for *Veillonella* spp.; 22.9% for *P. melaninogenica* and *S. sanguis;* and <20% for other species. Isolation rates were not related in any case to pocket depth, gender or age of patients. Isolation of *P. buccae* was significantly higher in smokers versus nonsmokers (84.6% *vs.* 51.4%; $p = 0.049$) while isolation of *P. denticola* was significantly higher in nonsmokers (48.6% *vs.* 15.4%; $p = 0.049$).

β-Lactamase production was positive in 54.1% of *Prevotella*, in 38.9% of *F. nucleatum*, in 30% of *Capnocytophaga*, and in 10% of *Veillonella*.

The antimicrobial susceptibility (range, MIC_{50} and MIC_{90}) and percentage of susceptible, intermediate and resistant strains for the different species are shown in Table I. Resistance rates of streptococci from the viridans group was 0% to amoxicillin/clavulanic acid or amoxicillin, around 10% for clindamycin (except for *S. sanguis* which had a resistance rate of 0%), from 9-22% for tetracycline, and the highest resistance rates corresponded to the macrolide azithromycin which ranged from 18.2% for *S. sanguis* to 47.7% for the most frequently isolated *S. mitis*.

All *Prevotella* isolates were susceptible to amoxicillin-clavulanic acid, while amoxicillin exhibited resistance rates ranging from 17.1% in *P. buccae* to 26.3% in *P. denticola*. Metronidazole resistance rates were below 6% in all *Prevotella* species, while clindamycin resistance ranged from 0% to 21.1%. All *Veillonella* spp. exhibited susceptibility to all antibiotics tested, while all *F. nucleatum* were susceptible to amoxicillin-clavulanic acid, clindamycin and metronidazole.

Against *Capnocytophaga*, amoxicillin-clavulanic acid and clindamycin exhibited the highest *in vitro* activity, with MIC_{90} values of 0.25 and 0.38 mg/l, respectively.

DISCUSSION

Antibiotic treatment of periodontal disease is empirical, as with most infectious diseases in the community, since symptoms provide no clues to help select an antibiotic. Antibiotics are used in the treatment of periodontitis when mechanical procedures have failed. If overgrowth of bacteria in plaque and the subsequent inflammatory disease are considered, a wide-spectrum antibiotic to kill as many species as possible could help in countering bacterial load. The antibiotic potency is important because the antibiotic should overcome the difficulty to control the bacterial plaque biofilm due to poor diffusion to the subgingival layer. The right choice of antimicrobial therapy, if needed, is based on the type of bacterial isolates responsible for the periodontal disease (13).

However, efficacy of antimicrobial therapy in periodontitis is based only on anecdotal reports and a few retrospective and prospective controlled and blinded studies (7). The general consensus is that appropriate antibiotic use is beneficial in the short-term and in some cases may be required to interfere with progression of periodontal attachment loss (7).

T. forsythensis and *P. gingivalis*, but not *A. actinomycetemcomitans,* have been significantly associated with attachment loss and alveolar bone loss (5). In this study, PCR detection of *T. denticola*, *P. gingivalis* and *T. forsythensis* was higher in patients with greater pocket depths (≥4 mm *vs.* <4 mm), but only reached statistical significance in the case of *T. denticola*. Other studies have shown that these three species are the only ones that can be statistically associated to increased pocket depth and bleeding (5). *A. actinomycetemcomitans* has been reported not to be associated with adult periodontitis (5). In our study, *A. actinomycetemcomitans* was only detected by PCR in 10.4% patients.

With respect to isolates, strains of the genera *Streptococcus* and *Prevotella* were the most frequently isolated microorganisms (47.9% and 32.6%, respectively). Susceptibility is important for prophylaxis of monomicrobial systemic infections after dental procedures in a population with a periodontitis prevalence of 30% (5), since *S. viridans* bacteremia occurs in 75% of subjects with periodontal disease after odontological invasive procedures (7). In this study, all *S. viridans* isolated were susceptible to aminopenicillins, but with a moderate percentage of strains exhibiting intermediate susceptibility. High doses of amoxicillin/clavulanic acid formulations (14) provide a complete pharmacodynamic coverage of these intermediate strains. A recent report showed the efficacy of amoxicillin, but not clindamycin, in preventing bacteremia following dental extractions (15), which can be associated with adequate efficacy indices (pharmacodynamic parameters) in the serum of amoxicillin against *S. viridans* and amoxicillin-clavulanic acid against all common isolates in odontogenic infections (16).

With regard to local concentrations, mean amoxicillin concentrations (after an oral 500-mg dose) in gingival crevicular fluid of 14 mg/ml (17) are above the maximum streptococcal MIC found in this study (2 mg/ml). This is not the case for clindamycin, with around 10% of *S. viridans* resistant strains exhibiting MICs that could not be covered by the mean gingival crevicular fluid concentrations reported (2.0 mg/ml) (18). In the case of tetracycline, serum and gingival crevicular fluid (0.61 mg/ml) concentrations (19) do not cover the high percentage of intermediate and resistant strains with MICs >2 mg/ml. As for azithromycin, the results obtained in this study suggest that

Table 1. Antimicrobial susceptibility of the 261 periodontal isolates.

	MIC (mg/l)			%		
Microorganism and antibiotics	Range	50%	90%	S	I	R
Streptococcus mitis (n = 44)						
Amoxicillin-clavulanic acid	≤0.02-0.75	0.05	0.25	90.9	9.1	0
Amoxicillin	≤0.02-1	0.06	0.38	86.4	13.6	0
Clindamycin	≤0.02->256	0.12	32	88.6	0	11.4
Metronidazole	>256->256	>256	>256	–	–	–
Tetracycline	0.12-98	0.39	48	70.5	6.8	22.7
Azithromycin	0.03->256	0.75	64	47.7	4.5	47.7
Streptococcus oralis (n = 37)						
Amoxicillin-clavulanic acid	≤0.02-0.64	0.06	0.25	91.9	8.1	0
Amoxicillin	0.02-0.64	0.06	0.38	86.5	13.5	0
Clindamycin	0.03->256	0.09	0.75	86.5	5.4	8.1
Metronidazole	3->256	>256	>256	–	–	–
Tetracycline	0.03-48	0.50	16	78.4	5.4	16.2
Azithromycin	≤0.02->256	3	24	67.6	2.7	29.7
Streptococcus sanguis (n = 11)						
Amoxicillin-clavulanic acid	0.06-2	0.38	1.50	45.5	54.5	0
Amoxicillin	0.06-2	0.25	1.50	54.5	45.5	0
Clindamycin	0.12-0.64	0.19	0.25	90.9	9.1	0
Metronidazole	>256->256	>256	>256	–	–	–
Tetracycline	0.19-12	0.38	0.50	90.9	0	9.1
Azithromycin	0.05-32	2	16	81.8	0	18.2
Streptococcus spp. (n = 33)[a]						
Amoxicillin-clavulanic acid	0.02-0.5	0.12	0.25	90.9	9.1	0
Amoxicillin	0.02-0.75	0.12	0.50	81.8	18.2	0
Clindamycin	≤0.02-128	0.12	0.94	78.8	12.1	9.1
Metronidazole	3->256	>256	>256	–	–	–
Tetracycline	0.02-48	0.50	6	78.8	12.1	9.1
Azithromycin	0.06->256	0.50	32	51.6	15.1	33.3
Prevotella buccae (n = 29)						
Amoxicillin-clavulanic acid	≤0.02-0.75	0.05	0.50	100	0	0
Amoxicillin	≤0.02->256	0.06	96	82.2	0	17.1
Clindamycin	≤0.02->256	0.02	0.23	89.7	0	10.3
Metronidazole	0.02->256	0.19	2	96.6	0	3.4
Tetracycline	≤0.02-96	0.35	32	71.4	10.7	17.9
Azithromycin	≤0.02->256	1	>256	–	–	–
Prevotella denticola (n = 19)						
Amoxicillin-clavulanic acid	≤0.02-1	0.05	1	100	0	0
Amoxicillin	≤0.02->256	1	128	63.2	10.5	26.3
Clindamycin	≤0.02->256	0.02	>256	78.9	0	21.1
Metronidazole	≤0.02-2	0.12	0.50	100	0	0
Tetracycline	0.03-96	0.50	48	61.1	16.7	22.2
Azithromycin	0.12->256	1.50	>256	–	–	–
Prevotella intermedia (n = 17)						
Amoxicillin-clavulanic acid	≤0.02-1	0.023	0.5	100	0	0
Amoxicillin	≤0.02-192	1	128	76.5	5.9	17.6
Clindamycin	≤0.02->256	0.02	>256	88.2	0	11.8
Metronidazole	0.02->256	0.25	6	94.1	0	5.9
Tetracycline	0.02-16	1.50	12	76.5	17.6	5.9
Azithromycin	0.02->256	0.12	32	–	–	–
Prevotella melaninogenica (n = 11)						
Amoxicillin-clavulanic acid	≤0.02-1.50	0.09	0.75	100	0	0
Amoxicillin	0.02->256	0.50	128	81.8	0	18.2
Clindamycin	≤0.02->256	0.02	0.23	90.9	0	9.1
Metronidazole	≤0.02-2	0.19	0.50	100	0	0
Tetracycline	≤0.02-32	0.50	32	63.6	0	36.4
Azithromycin	≤0.02->256	2	12	–	–	–

(Continued)

Table 1. Antimicrobial susceptibility of the 261 periodontal isolates. ***(Continued)***

Microorganism and antibiotics	MIC (mg/l)			%		
	Range	50%	90%	S	I	R
Prevotella spp. (n = 9)[b]						
Amoxicillin-clavulanic acid	≤0.02-0.19	0.02	0.19	100	0	0
Amoxicillin	≤0.02-8	0.25	8	88.9	11.1	0
Clindamycin	≤0.02-0.03	0.02	0.03	100	0	0
Metronidazole	≤0.02-0.50	0.09	0.50	100	0	0
Tetracycline	0.09-0.39	0.19	0.39	100	0	0
Azithromycin	0.06-6	0.75	6	–	–	–
Fusobacterium nucleatum (n = 18)						
Amoxicillin-clavulanic acid	≤0.02-0.38	0.32	0.38	100	0	0
Amoxicillin	≤0.02-48	0.12	32	83.3	0	16.7
Clindamycin	≤0.02-0.19	0.02	0.12	100	0	0
Metronidazole	≤0.02-6	≤0.016	0.94	100	0	0
Tetracycline	≤0.02-16	0.09	1.50	94.4	0	5.6
Azithromycin	≤0.02-4	0.25	2	–	–	–
Capnocytophaga spp. (n = 12)						
Amoxicillin-clavulanic acid	≤0.02-0.75	0.05	0.25	–	–	–
Amoxicillin	≤0.02->256	0.12	24	–	–	–
Clindamycin	≤0.02->256	≤0.016	0.38	–	–	–
Metronidazole	0.09->256	0.75	>256	–	–	–
Tetracycline	0.03-6	0.38	1	–	–	–
Azithromycin	0.25->256	1	32	–	–	–
Veillonella spp. (n = 10)						
Amoxicillin-clavulanic acid	0.03-4	0.50	2	100	0	0
Amoxicillin	0.03-8	0.75	3	90	10	0
Clindamycin	0.02-1.5	0.12	0.50	100	0	0
Metronidazole	0.25-12	3	8	90	10	0
Tetracycline	0.03-1	0.39	0.75	100	0	0
Azithromycin	1-64	4	24	–	–	–
Others (n = 11)[c]						
Amoxicillin-clavulanic acid	≤0.02-0.38	0.06	0.25	–	–	–
Amoxicillin	≤0.02-0.50	0.12	0.38	–	–	–
Clindamycin	≤0.02->256	0.250	>256	–	–	–
Metronidazole	≤0.02->256	>256	>256	–	–	–
Tetracycline	0.19-256	1	24	–	–	–
Azithromycin	≤0.02->256	0.19	>256	–	–	–

S: susceptible; I: intermediate; R: resistant. [a]Includes *S. gordonii* (n = 8); *S. salivaris* (n = 7); *S. constellatus* (n = 6); *S. anginosus* (n = 5); *S. parasanguis* (n = 3); *S. intermedium* (n = 2); and *S. vestibularis* (n = 2). [b]Includes *P. oralis (*n = 8) and *P. loescheii* (n = 1). [c]Includes *Gemella morbillorum* (n = 4); *Actinomyces* spp. (n = 2); *Actinomyces megei* (n = 1); *Bacteroides ovatus* (n = 1); *Bacteroides stercoris* (n = 1); *Cardiobacterium hominis* (n = 1); and *Eikenella corrodens* (n = 1).

it is inadequate due to high rates of resistance (MIC_{90} ≥16 mg/l). In Spain, high resistance to macrolides (20) is commonly associated with high resistance to tetracycline (21), clindamycin and azalides such as azithromycin in *S. viridans* (22).

With respect to *Prevotella*, 100% isolates were susceptible to amoxicillin-clavulanic acid, with resistance rates of the prevalent species *(P. buccae, P. denticola* and *P. intermedia)* ranging from 9.1% to 21% for clindamycin, from 0% to 5.9% for metronidazole, and from 5.9% to 36.4% for tetracycline. Mean gingival crevicular fluid concentrations of amoxicillin-clavulanic acid (14 mg/l for amoxicillin and 0.40 mg/l for clavulanic acid) (17) exceed the MIC determined against all isolates, while mean gingival crevicular fluid concentrations of other compounds do not cover the resistant strains, nor, in the case of clindamycin, a number of susceptible strains. As for metronidazole, the drug showing the lowest resistance rates excluding amoxicillin-clavulanic acid, mean concentrations (36.7 mg/l) (23) do not cover the MIC determined in two cases.

This study shows that amoxicillin-clavulanic acid is the most active antibiotic against all species tested, followed

by metronidazole in the case of anaerobes. No resistant strains to amoxicillin-clavulanic acid were found among all the isolates from this cohort of patients with periodontitis.

When considering isolates from patients with periodontal disease, three types of bacteria should be considered: i) periodontopathogens, such as *T. denticola* and other noncultivable spirochete that are penicillin-susceptible, the prevalence of which increase in line with severity (greater gingival pocket depth), as a basis for considering periodontitis an infectious disease; ii) bacteria responsible for the nonspecific bacterial growth and the subsequent inflammatory disease, with *Prevotella* spp. being the most prevalent anaerobe isolate; and iii) isolates of oral microbiota that are also responsible for increased bacteremia after dental manipulation in patients with periodontitis: group viridans streptococci, mainly *S. mitis* and *S. oralis*. When an antibiotic is needed it would be desirable to obtain coverage of these three types of direct or indirect pathogens.

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