Journal of
PERIODONTAL RESEARCH

J Periodont Res 2010; 45: 557–563

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2010.01274.x

Antibiotic resistance of subgingival species in chronic periodontitis patients

C. M. Ardila[1,2], M. I. Granada[2], I. C. Guzmán[2]

[1]Epidemiology Group, University of Antioquia, Medellín, Colombia and [2]Department of Periodontology, School of Dentistry, University of Antioquia, Medellín, Colombia

Ardila CM, Granada MI, Guzmán IC. Antibiotic resistance of subgingival species in chronic periodontitis patients. J Periodont Res 2010; 45: 557–563.

Background and Objective: The increasing rate of resistance of microorganisms to penicillin and other antibiotics has generated concern among health authorities in Latin America. The present investigation determined the *in vitro* susceptibility of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, black-pigmented *Prevotella* spp. and *Aggregatibacter actinomycetemcomitans* to metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin and moxifloxacin in patients with chronic periodontitis.

Material and Methods: Subgingival plaque samples from patients with periodontitis were collected and cultured on selective and nonselective culture media. The antimicrobial susceptibility of periodontopathogenic isolates was studied in chronic periodontitis patients in Colombia. Metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin and moxifloxacin were tested on all bacterial isolates and the percentage of resistant strains was calculated.

Results: Of the 150 bacteria identified, 51 were *P. gingivalis*, 45 were black-pigmented *Prevotella* spp., 36 were *F. nucleatum* and 18 were *A. actinomycetemcomitans*. All the isolates were sensitive to amoxicillin/clavulanic acid and to moxifloxacin, but exhibited variable susceptibility patterns to the other antimicrobial agents tested.

Conclusion: The results of the present study suggest that periodontal microorganisms in patients with chronic periodontitis can be resistant to the antimicrobial agents commonly used in anti-infective periodontal therapy. We suggest that the indiscriminate use of antimicrobials could result in the appearance of more highly antibiotic-resistant strains of bacteria associated with periodontal diseases in our population compared with the populations of other countries.

Carlos M. Ardila, Epidemiology Group, University of Antioquia, Calle, 64 No 52–59 Medellín, Colombia
Tel: +57 4 2196772
Fax: +57 4 2195332
e-mail: martinardila@gmail.com

Key words: antibiotic resistance; antimicrobial(s); microbiology; periodontitis

Accepted for publication December 21, 2009

Microorganisms stoutly considered as etiologic agents of periodontitis include *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, black-pigmented *Prevotella* spp., *Aggregatibacter actinomycetemcomitans*, *Treponema denticola* and *Tannerella forsythia* (1). These bacteria activate many host immunoinflammatory processes and disrupt the host mechanisms responsible for bacterial clearance. For example, in *P. gingivalis*, high levels of proteolytic activity (2) are considered to be the most important virulence factors. *A. actinomycetemcomitans* synthesizes many toxins, such as leukotoxin and cytotoxin (3). Both *P. gingivalis* and *A. actinomycetemcomitans* can grow intracellularly *in vivo* as shown by the fact that a high percentage of human buccal epithelial

cells were found to be infected with these bacteria (4). Besides, the lipopolysaccharide molecule present in the cell wall of both black-pigmented *Prevotella* spp. and *F. nucleatum* may contribute to alveolar bone loss either by stimulating bone resorption or by inhibiting bone formation (5).

Several studies have examined the effect of different periodontal therapies on the clinical and microbiological parameters of periodontal diseases (6,7). The adjunctive application of systemically administered antibiotics has been shown to provide a better clinical and microbiological result in subjects with chronic periodontitis than no antibiotics (7–9). Metronidazole is one of the most commonly used agents to treat periodontal infections (10); however, because of the appearance of tetracycline-resistant *A. actinomycetemcomitan*s, the mixture of metronidazole and amoxicillin may be preferable to metronidazole alone (11). Moreover, good results have been reported with amoxicillin/clavulanic acid (12,13), clindamycin (12) and moxifloxacin (14) in periodontal therapy.

Sequential and geographic changes in antibiotic susceptibility among anaerobes have been reported in Europe (15–18). At present, concern about the correct empirical therapy arises because resistance is also observed among anaerobes previously considered as susceptible (17). Regrettably, only limited records are available on the subgingival microbial composition of periodontitis subjects in Latin America (19). The growing rate of resistance of microorganisms to penicillin and other antibiotics has generated concern among health authorities in Latin America (20). Increased resistance to antimicrobials is caused by a number of factors, including high antibiotic usage in this region (20).

To our knowledge, there are no investigations that study the resistance of periodontopathogens to adjunctive antibiotics used in periodontal treatment in Latin America. Geographical differences in the resistance of these microbes could impact periodontal treatment protocols, which may enable the establishment of specific therapeutic strategies.

The aim of this study was to investigate the *in vitro* susceptibility of *P. gingivalis*, *F. nucleatum*, black-pigmented *Prevotella* spp. and *A. actinomycetemcomitans* to metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin and moxifloxacin in Colombian patients with chronic periodontitis.

Material and methods

Subjects

Seventy-six systemically healthy Colombian subjects (45 women and 31 men; 27 to 66 years of age), who attended the dental clinics of the University of Antioquia, were invited to participate in this study between October 2008 and March 2009. Informed and written consent was obtained from each participant. The study design was approved by the Ethics Committee on Human Research of the University Investigation Department of the University of Antioquia, according to the Declaration of Helsinki on experimentation involving human subjects. Patients with a diagnosis of chronic periodontitis were considered to be candidates for the study. Exclusion criteria included diabetes, cardiovascular disease, or any other systemic disease that could alter the course of periodontal disease. Pregnant women, consumption of systemic antimicrobials or anti-inflammatory drugs in the previous (6 mo) and periodontal therapy during the last 6 mo also served as exclusion criteria.

Clinical evaluation

Medical history and clinical and radiographic examinations were conducted for each patient. One of the authors (C.A.) performed all clinical examinations. The presence or absence of bleeding on probing, plaque and suppuration were recorded. Probing depth and clinical attachment level were measured at all approximal, buccal and lingual surfaces, to the nearest millimeter, using a calibrated standard probe (UNC-15; Hu-Friedy, Chicago, IL, USA). The diagnosis of chronic periodontitis was made based on criteria defined at the workshop sponsored by the American Academy of Periodontology (21).

Microbial sampling

Microbial sampling of periodontitis patients was performed on periodontal pockets of ≥ 5 mm. The deepest six pockets were selected for sampling. After removing supragingival plaque using curets and isolating the area with cotton pellets, paper points (Maillefer, Ballaigues, Switzerland[)] were inserted into each periodontal pocket for 20 s. The paper points were transferred to a tube containing viability medium Göteborg anaerobic (VMGA) III (22). All samples were processed within 4 h after sampling. The samples were analyzed, using microbial culture techniques, to determine the presence of periodontopathic bacteria, according to Slots (23). Briefly, most samples were processed at room temperature (25°C) and incubated in CO_2 and anaerobic culture systems. Brucella blood agar medium was incubated at 35°C in an anaerobic jar for 7 d. The Trypticicase Soy Serum Bacitracin Vancomycin agar (TSBV) medium was incubated in 10% CO_2 at 37°C for 4 d. Presumptive identification was performed according to the methods described (23,24) and using a commercial identification micromethod system (RapID ANA II; Remel, Norcross, GA, USA) for *A. actinomycetemcomitans*, *P. gingivalis*, black-pigmented *Prevotella* spp. and *Fusobacterium* spp. The total viable counts were defined as the total number of colony-forming units obtained on culture plates containing nonselective media. The bacterial species that grew on selective media were enumerated and their percentage relative to the total viable counts was calculated. Each patient provided a pooled subgingival plaque sample. Equal numbers of isolates from each subject were used.

Antimicrobial susceptibility testing

Selected colonies of *P. gingivalis*, black-pigmented *Prevotella* spp., *A. actinomycetemcomitans* and *Fusobacterium* spp. from pure cultures were

used to test susceptibility to metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin and moxifloxacin (E-test®; AB Biodisk, Solna, Sweden). Briefly, viable colonies were homogenized in 0.85% saline, and the turbidity was adjusted to MacFarland 1.0 standard (3×10^8 colony-forming units/mL). Using a sterile glass rod, 0.1 mL of the inoculum was spread over Brucella blood agar plates (BD, Sparks, MD, USA) and dried for 15 min at room temperature. E-test strips were gently placed on the agar surface and incubated under anaerobic conditions for 4 d. The elliptic zone of inhibition was examined after 96 h of incubation. The reading at the intersection of the bacterial zone of inhibition and the E-strip represented the minimum inhibitory concentration (MIC) of the organism. The MIC breakpoints were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (25).

Statistical analysis

Descriptive analyses were carried out (mean, standard deviation, frequency of detection) for clinical and microbiological parameters. A statistical program was used for all the statistical analyses (Statistical Package for the Social Sciences, version 15; SPSS, Chicago, IL, USA).

Results

A total of 45 women (59.2%) and 31 men (40.8%) with chronic periodontitis were studied (age: 46 ± 8.08 years), of whom 21.05% (16 subjects) were current smokers. Table 1 describes the clinical characteristics of the patients.

From the 76 study patients a total of 150 bacterial colonies could be clearly identified (Table 2): *P. gingivalis* (51/150), black-pigmented *Prevotella* spp. (45/150), *F. nucleatum* (36/150) and *A. actinomycetemcomitans* (18/150). Table 3 lists the results of the susceptibility testing to metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin and moxifloxacin. All the isolates were sensitive to amoxicillin/clavulanic acid and to moxifloxacin but exhibited variable susceptibility patterns to the other antimicrobial agents tested. In total, 25.49%, 23.52% and 21.56% of the *P. gingivalis* isolates were resistant to amoxicillin, clindamycin and metronidazole, respectively. By contrast, 16.66% of the strains of *F. nucleatum* were amoxicillin-resistant, and 36.11% and 25% of the isolates were resistant to clindamycin and metronidazole, respectively. Black-pigmented *Prevotella* spp. were resistant to amoxicillin, clindamycin and metronidazole (35.55%, 22.22% and 26.66%, respectively). The antibiotics least effective against *A. actinomycetemcomitans* were amoxicillin, metronidazole and clindamycin (77.77%, 88.88% and 83.33% of resistance respectively). The MIC values (range, and the MIC_{50} and MIC_{90} values, namely the MIC values at which 50% and 90% of isolates were inhibited, respectively) of metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin and moxifloxacin are given in Table 3.

Table 1. Clinical data at sampled sites: probing depth (PD), clinical attachment level (CAL), percentage of sites with bleeding on probing (BOP), percentage of sites with plaque (Pl) and percentage of sites with suppuration (SUP)

Clinical parameter	Mean ± SD
PD (mm ± SD)	3.4 ± 1.48
CAL (mm ± SD)	4.3 ± 1.99
% BOP (mm ± SD)	77 ± 21
% Pl (mm ± SD)	56 ± 28
% SUP (mm ± SD)	4.3 ± 3.1

mm, millimeter; SD, standard deviation.

Discussion

In this study, we investigated the antibiotic susceptibility of the subgingival microflora from patients with untreated chronic periodontitis living in Colombia. To our knowledge there are no studies in the dental literature investigating the problem of antibiotic resistance in the periodontal microflora in Latin America. The rationale for studying patient populations from Colombia is that recent evidence shows a much higher use of antibiotics in Latin America (20) in comparison with other countries (26,27). Information from the present study may have therapeutic implications for the treatment of nonoral infections caused by oral pathogens. Dissemination of periodontal pathogens to other body sites frequently occurs and may cause serious diseases such as brain abscesses, lung infections, endocarditis and soft tissue infections (28). For these reasons, the study of the susceptibility of the subgingival microbiota in a particular country becomes pertinent to identify its possible impact on outcomes after treatment (28).

In the present study, the black-pigmented anaerobe, *P. gingivalis*, was highly susceptible to amoxicillin/clavulanic acid and moxifloxacin (Table 3). This is in accordance with other studies which show that this bacterium is highly susceptible to these antibiotics (29–33). However, in our study, 25.49%, 23.52% and 21.56% of the isolates were resistant to amoxicillin, clindamycin and metronidazole, respectively. This is in agreement with other investigations that detected antibiotic resistance, albeit lower, in subgingival *P. gingivalis* isolates and in isolates from odontogenic and periodontal abscesses (34,35). With this perspective, it is significant that *P. gingivalis* has recently been shown

Table 2. Frequency distribution (%) of a total of 150 identified bacterial colonies being sampled from 76 subjects

Microorganisms	Strains	Percentage
Porphyromonas gingivalis	51	34
Black-pigmented *Prevotella* spp. (*Prevotella intermedia*/ *Prevotella nigrescens* = 34; *Prevotella melaninogenica* = 11)	45	30
Fusobacterium nucleatum	36	24
Aggregatibacter actinomycetemcomitans	18	12

Table 3. In vitro susceptibility and percentage of resistant isolates to five antimicrobial agents[a]

Microorganism (no. of strains)	Range	MIC (μg/mL)[b]		Percentage resistant
		MIC_{50}	MIC_{90}	
Porphyromonas gingivalis (n = 51)				
Amoxicillin	0.016 > 256	0.125	> 256	25.49
Amoxicillin/clavulanic acid	< 0.016–0.064	< 0.016	< 0.016	0
Clindamycin	0.08 ≥ 16	8	≥ 16	23.52
Metronidazole	0.08 ≥ 16	0.256	≥ 16	21.56
Moxifloxacin	0.006–0.032	0.023	0.032	0
Prevotella intermedia/nigrescens (n = 34)				
Amoxicillin	0.016–32	0.064	32	35.55
Amoxicillin/clavulanic acid	0.016–1	0.023	0.094	0
Clindamycin	0.06–32	0.12	≥ 16	22.22
Metronidazole	0.08 ≥ 16	0.256	≥ 16	26.66
Moxifloxacin	0.002–0.5	0.064	0.25	0
Prevotella melaninogenica (n = 11)				
Amoxicillin	0.016–32	0.064	32	35.55
Amoxicillin/clavulanic acid	0.016–1	0.023	0.094	0
Clindamycin	0.06–32	0.12	≥ 16	22.22
Metronidazole	0.08 ≥ 16	0.256	≥ 16	26.66
Moxifloxacin	0.064–0.38	0.125	0.25	0
Fusobacterium nucleatum (n = 36)				
Amoxicillin	0.016 > 256	0.125	> 256	16.66
Amoxicillin/clavulanic acid	0.016–1	0.023	0.094	0
Clindamycin	0.08 ≥ 16	8	≥ 16	36.11
Metronidazole	0.08 > 16	0.256	≥ 16	25
Moxifloxacin	0.002–0.5	0.064	0.25	0
A. actinomycetemcomitans (n = 18)				
Amoxicillin	0.064–32	0.25	32	77.77
Amoxicillin/clavulanic acid	0.02–0.75	0.25	0.5	0
Clindamycin	0.016 > 256	0.125	> 256	83.33
Metronidazole	1.5 > 256	6	> 256	88.88
Moxifloxacin	0.19–0.5	0.38	0.5	0

[a]For anaerobic bacteria, the susceptibility and resistance breakpoint concentrations were as follows (25). Amoxicillin/clavulanic acid, ≤ 4 mg/L (amoxicillin) and ≤ 2 mg/L (clavulanic acid) and ≥ 16 mg/L (amoxicillin) and ≥ 8 mg/L (clavulanic acid), respectively; metronidazole, ≤ 2 and ≥ 8 mg/L, respectively; clindamycin, ≤ 2 and ≥ 8 mg/L, respectively; moxifloxacin, ≤ 2 and ≥ 8 mg/L, respectively. In the case of *Aggregatibacter actinomycetemcomitans*, the interpretive criteria for the HACEK group were applied for amoxicillin/clavulanic acid, whereas for metronidazole those for anaerobes were used. As no interpretive criteria exist for clindamycin, the interpretive criteria for anaerobes were applied (25).

[b] MIC_{50} and MIC_{90} indicate the MIC values at which 50% and 90% of isolates were inhibited, respectively.

to be capable of the conjugal transfer of chromosomal and plasmid DNA, which would provide a useful way to transfer resistance determinants (36).

In our study, *F. nucleatum* was 100% susceptible to amoxicillin/clavulanic acid and moxifloxacin (Table 3). This is in accordance with previous reports (33,37). Although penicillin resistance remains uncommon among *Fusobacterium* (38), in our hands, 16.66% of strains of *F. nucleatum* were amoxicillin resistant. This is in agreement with the results of Mosca *et al.* (17) who found resistance in 12.5% of *F. nucleatum* strains. According to King *et al.* (39), β-lactamases were the readily identified mechanism of resistance. By contrast, 36.11% and 25% of the isolates were not susceptible to clindamycin and metronidazole, respectively. Lakhssassi *et al.* (37) and van Winkelhoff *et al.* (40) reported resistances 33% and 20%, respectively of F. nucleatum to clindamicy and metronidazole, which was lower than reported in our study. However, other researchers (17), investigating periodontal samples from patients living in a selected area of southern Italy, showed that all the strains of *F. nucleatum* analyzed were susceptible to metronidazole.

Similarly to *F. nucleatum*, black-pigmented *Prevotella* spp. were also very susceptible to amoxicillin/clavulanic acid and moxifloxacin, and 35.55%, 22.22% and 26.66% of black-pigmented *Prevotella* spp. were resistant to amoxicillin, clindamycin and metronidazole, respectively (Table 3). Amoxicillin/clavulanic acid, moxifloxacin, metronidazole and clindamycin are generally regarded as highly effective against black-pigmented *Prevotella* spp. (29,34,40,41). However, similarly to the present study, resistance to amoxicillin, metronidazole and clindamycin were reported in previous studies (35,37). Interestingly, we detected a relatively high resistance to these antibiotics compared with findings from other parts of the world (37,42). Black-pigmented *Prevotella* spp. are known to produce β-lactamases (43). This may explain, in part, the resistance to amoxicillin of the *Prevotella* spp. in

this study, as all isolates were susceptible to amoxicillin/clavulanic acid. By contrast, as reported by other researchers (34), the proportion of isolates with antibiotic resistance could be mainly attributed to the varying degree of antibiotic use in different countries.

A. actinomycetemcomitans was the least antibiotic-susceptible species, with amoxicillin/clavulanic acid and moxifloxacin being the most effective antibiotics (Table 3). The high susceptibility of *A. actinomycetemcomitans* to these two antibiotics is corroborated by the results of other studies (29,33,34,44). We also found that the least-effective antibiotics were amoxicillin, metronidazole and clindamycin. This is in line with the studies of van Winkelhoff *et al.* (40) and Kulik *et al.* (29), where 33.3%, 72% and 82% of the *A. actinomycetemcomitans* isolates were resistant to amoxicillin, metronidazole and clindamycin, respectively. As noted, once again the antibiotic-resistance values found in our study were higher. It is important to note that even if we did not study tetracycline, 100% of subgingival *A. actinomycetemcomitans* isolated from Spanish patients grew on tetracycline-containing agar plates vs. 0% isolated from Dutch patients (40), probably because of the higher antibiotic use in Spain (45). It is known that the hydroxy metabolite of metronidazole is three to four times more active against *A. actinomycetemcomitans* and that it acts synergistically with amoxicillin against this pathogen (46). For this reason, metronidazole, in combination with amoxicillin, has been shown to be effective in the treatment of *A. actinomycetemcomitans*-associated periodontal disease (47). However, the MIC_{90} value (of > 256 μg/mL) of the Spanish *A. actinomycetemcomitans*, reported by van Winkelhoff *et al.* (40) indicates that the clinical efficacy of this combination therapy may not be as effective in Spain as it is in other European countries. Similar situations could occur in Latin America.

Earlier studies, comparing different European populations with different levels of antibiotic consumption, provide evidence that supports the view that standard guidelines for antimicrobial use in the treatment of periodontal infections should not be recommended, as major differences in the antimicrobial profile of major periodontal pathogens were found (34,40). The level of resistance varies between countries, which can be attributed to the different use of antibiotics (12,20). Among European countries, Switzerland is the country with the lowest antibiotic consumption per capita (48), which may partly explain why antibiotic resistance did not increase among isolates from the Basel area.

TOur results suggest that moxifloxacin has potent antibacterial activity against periodontal pathogens, which is comparable with that of amoxycillin/clavulanic acid and higher than that of clindamycin, metronidazole and amoxicillin. This could be explained by the fact that moxifloxacin and amoxycillin/clavulanic acid are not frequently used in the treatment of dental and medical infections because they are not included in the National Health Plan in Colombia. Additionally, moxifloxacin is new in our market and it has a reduced propensity to promote the development of resistance (49). Moreover, the high resistance of periodontopathogens to clindamycin, metronidazole and amoxicillin, observed in the present study, deserves special attention, because these antibiotics are frequently used as adjunctive antibiotics in periodontal treatment (9). One concern for the researchers in this study was that Sedlacek & Walker (50) demonstrated that a significantly higher amount of drug is required to have an inhibitory effect in the biofilm than in planktonically grown bacteria of the same strain. This must be taken into account when antibiotics are used as adjunctive to periodontal therapy, mainly in populations with a high resistance to antibiotics.

The prevalence of multidrug resistance continues to increase among many pathogens, largely because of the overuse and misuse of antimicrobial agents (20). Such use not only adds to the cost of medical care, but also needlessly exposes the patient to potential toxicity and risks that promote the development and spread of antimicrobial resistance in healthcare facilities (51). Surveillance of antimicrobial resistance is crucial for providing information on the degree of, and trends in, resistance, and for monitoring the result of interventions (52). Local observation data are decisive and should be utilized to direct clinical supervision, modernize treatment procedures, instruct prescribers and conduct infection-control policies (52).

Conclusions

The present investigation confirms that periodontal microorganisms in patients with chronic periodontitis can be resistant to antimicrobial agents commonly used in anti-infective periodontal therapy. The indiscriminate use of antimicrobials could result in the appearance of more highly antibiotic-resistant strains associated with periodontal diseases in our population compared with populations of other countries. Our results also support the notion that the use of antibiotics must be based on susceptibility testing, instead of a unique adjunctive antimicrobial regimen. These results could impact periodontal treatment, mainly with regard to the selection of adjunctive systemic antibiotics in Latin American populations. Further studies are needed to investigate these geographical variations in the antimicrobial drug resistance of the periodontal microflora in Latin America.

Acknowledgements

This study was supported, in part, by a grant from the National Public Health School and Epidemiology Group of the University of Antioquia. The authors report no conflicts of interest related to this study.

References

1. Genco RJ, Kornman KS, William R. Consensus report periodontal diseases: pathogenesis and microbiological factors. *Ann Periodontol* 1996;**1:**926–932.
2. Imamura T. The role of gingipains in the pathogenesis of periodontal disease. *J Periodontol* 2003;**74:**111–118.

3. Fives-Taylor PM, Meyer DH, Mintz KP, Brissette C. Virulence factors of *Actinobacillus actinomycetemcomitans*. *Periodontol 2000* 1999;**20:**136–167.
4. Rudney JD, Chen R, Sedgewick GJ. Intracellular *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in buccal epithelial cells collected from human subjects. *Infect Immun* 2001;**69:** 2700–2707.
5. Wilson M. Biological activities of lipopolysaccharides from oral bacteria and their relevance to the pathogenesis of chronic periodontitis. *Sci Prog* 1995; **78:**19–34.
6. Sanz M, Teughels W; Group A of European Workshop on Periodontology. Innovations in non-surgical periodontal therapy: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* 2008;**35:**3–7.
7. Herrera D, Alonso B, León R, Roldán S, Sanz M. Antimicrobial therapy in periodontitis: the use of systemic antimicrobials against the subgingival biofilm. *J Clin Periodontol* 2008;**35**(Suppl 8)**:** 45–66.
8. Haffajee AD, Torresyap G, Socransky SS. Clinical changes following four different periodontal therapies for the treatment of chronic periodontitis: 1 year results. *J Clin Periodontol* 2007;**34:**243–253.
9. Haffajee AD, Patel M, Socransky SS. Microbiological changes associated with four different periodontal therapies for the treatment of chronic periodontitis. *Oral Microbiol Immunol* 2008;**23:** 48–57.
10. Xajigeorgiou C, Sakellari D, Slini T, Baka A, Konstantinidis A. Clinical and microbiological effects of different antimicrobials on generalized aggressive periodontitis. *J Clin Periodontol* 2006;**33:** 254–264.
11. Ribeiro Edel P, Bittencourt S, Zanin IC *et al*. Full-mouth ultrasonic debridement associated with amoxicillin and metronidazole in the treatment of severe chronic periodontitis. *J Periodontol* 2009;**80:**1254–1264.
12. Walker C, Karpinia K. Rationale for use of antibiotics in periodontics. *J Periodontol* 2002;**73:**1188–1196.
13. Purucker P, Mertes H, Goodson JM, Bernimoulin JP. Local versus systemic adjunctive antibiotic therapy in 28 patients with generalized aggressive periodontitis. *J Periodontol* 2001;**72:**1241–1245.
14. Guentsch A, Jentsch H, Pfister W, Hoffmann T, Eick S. Moxifloxacin as an adjunctive antibiotic in the treatment of severe chronic periodontitis. *J Periodontol* 2008;**79:**1894–1903.
15. Albandar JM. Periodontal diseases in North America. *Periodontol 2000* 2002;**29:** 31–69.
16. Sheiham A, Netuveli GS. Periodontal diseases in Europe. *Periodontol 2000* 2002; **29:**104–121.
17. Mosca A, Miragliotta L, Iodice MA, Abbinante A, Miragliotta G. Antimicrobial profiles of *Prevotella* spp. and *Fusobacterium nucleatum* isolated from periodontal infections in a selected area of southern Italy. *Int J Antimicrob Agents* 2007; **30:**521–524.
18. Herrera D, van Winkelhoff AJ, Dellemijn-Kippuw N, Winkel EG, Sanz M. Beta-lactamase producing bacteria in the subgingival microflora of adult patients with periodontitis. A comparison between Spain and The Netherlands. *J Clin Periodontol* 2000;**27:**520–525.
19. Gjermo P, Rosing CK, Susin C, Oppermann R. Periodontal diseases in Central and South America. *Periodontol 2000* 2002;**29:**70–78.
20. Valenzuela MT, de Quadros C. Antibiotic resistance in Latin America: a cause for alarm. *Vaccine* 2009;**27**(suppl 3)**:**C25–C28.
21. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;**4:** 1–6.
22. Möller AJ. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. *Odontol Tidskr* 1966;**74**(suppl)**:**1–380.
23. Slots J. Rapid identification of important periodontal microorganisms by cultivation. *Oral Microbiol Immunol* 1986;**1:**48–57.
24. Slots J, Reynolds HS. Long-wave UV light fluorescence for identification of black-pigmented Bacteroides spp. *J Clin Microbiol* 1982;**16:**1148–1151.
25. Ambler J, Rennie R, Poupard J *et al*. Determination of moxifloxacin anaerobic susceptibility breakpoints according to the Clinical and Laboratory Standards Institute guidelines. *Diagn Microbiol Infect Dis* 2008;**61:**49–57.
26. Moet GJ, Jones RN, Biedenbach DJ, Stilwell MG, Fritsche TR. Contemporary causes of skin and soft tissue infections in North America, Latin America, and Europe: report from the SENTRY Antimicrobial Surveillance Program (1998–2004). *Diagn Microbiol Infect Dis* 2007;**57:**7–13.
27. van Winkelhoff AJ, Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in nonoral infections. *Periodontol 2000* 1999;**20:**122–135.
28. Herrera D, Contreras A, Gamonal J *et al*. Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain. *J Clin Periodontol* 2008; **35:**106–113.
29. Kulik EM, Lenkeit K, Chenaux S, Meyer J. Antimicrobial susceptibility of periodontopathogenic bacteria. *J Antimicrob Chemother* 2008;**61:**1087–1091.
30. Kuriyama T, Williams DW, Yanagisawa M *et al*. Antimicrobial susceptibility of 800 anaerobic isolates from patients with dentoalveolar infection to 13 oral antibiotics. *Oral Microbiol Immunol* 2007;**22:** 285–288.
31. Eick S, Pfister W. Efficacy of antibiotics against periodontopathogenic bacteria within epithelial cells: an in vitro study. *J Periodontol* 2004;**75:**1327–1334.
32. Eick S, Schmitt A, Sachse S, Schmidt KH, Pfister W. In vitro antibacterial activity of fluoroquinolones against *Porphyromonas gingivalis* strains. *J Antimicrob Chemother* 2004;**54:**553–556.
33. Milazzo I, Blandino G, Musumeci R, Nicoletti G, Lo Bue AM, Speciale A. Antibacterial activity of moxifloxacin against periodontal anaerobic pathogens involved in systemic infections. *Int J Antimicrob Agents* 2002;**20:**451–456.
34. van Winkelhoff AJ, Herrera D, Winkel EG, Dellemijn-Kippuw N, Vandenbroucke-Grauls CM, Sanz M. Antimicrobial resistance in the subgingival microflora in patients with adult periodontitis. A comparison between The Netherlands and Spain. *J Clin Periodontol* 2000;**27:**79–86.
35. Jaramillo A, Arce RM, Herrera D, Betancourth M, Botero JE, Contreras A. Clinical and microbiological characterization of periodontal abscesses. *J Clin Periodontol* 2005;**32:**1213–1218.
36. Tribble GD, Lamont GJ, Progulske-Fox A, Lamont RJ. Conjugal transfer of chromosomal DNA contributes to genetic variation in the oral pathogen *Porphyromonas gingivalis*. *J Bacteriol* 2007;**189:** 6382–6388.
37. Lakhssassi N, Elhajoui N, Lodter JP, Pineill JL, Sixou M. Antimicrobial susceptibility variation of 50 anaerobic periopathogens in aggressive periodontitis: an interindividual variability study. *Oral Microbiol Immunol* 2005;**20:**244–252.
38. Johnson CC. Susceptibility of anaerobic bacteria to beta-lactam antibiotics in the United States. *Clin Infect Dis* 1993;**16:**S371–S376.
39. King A, Downes J, Nord CE, Phillips I. Antimicrobial susceptibility of non-Bacteroides fragilis group anaerobic Gram-negative bacilli in Europe. *Clin Microbiol Infect* 1999;**5:**404–416.
40. van Winkelhoff AJ, Herrera D, Oteo A, Sanz M. Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in The Netherlands and Spain. *J Clin Periodontol* 2005;**32:** 893–898.
41. Sobottka I, Cachovan G, Sturenburg EM *et al*. In vitro activity of moxifloxacin against bacteria isolated from odontogenic abscesses. *Antimicrob Agents Chemother* 2002;**46:**4019–4021.

42. Luong N, Tsai J, Chen C. Susceptibilities of *Eikenella corrodens*, *Prevotella intermedia*, and *Prevotella nigrescens* clinical isolates to amoxicillin and tetracycline. *Antimicrob Agents Chemother* 2001;**45:** 3253–3255.
43. Handal T, Olsen I, Walker CB, Caugant DA. Detection and characterization of b-lactamase genes in subgingival bacteria from patients with refractory periodontitis. *FEMS Microbiol Lett* 2005;**242:**319–324.
44. Müller HP, Holderrieth S, Burkhardt U, Höffler U. In vitro antimicrobial susceptibility of oral strains of *Actinobacillus actinomycetemcomitans* to seven antibiotics. *J Clin Periodontol* 2002;**29:**736–742.
45. Cars O, Molstad S, Melander A. Variation in antibiotic use in the European Union. *Lancet* 2001;**357:**1851–1853.
46. Pavicic MJ, van Winkelhoff AJ, De Graaff J. In vitro susceptibility of *Actinobacillus actinomycetemcomitans* to a number of antimicrobial combinations. *Antimicrob Agents Chemother* 1992;**36:**2634–2638.
47. Winkel EG, Van der Weijden GA, Van Winkelhoff AJ, Timmerman MF, Van der Velden U. Metronidazole plus amoxicillin in the treatment of adult periodontitis patients. A double-blind placebo controlled study. *J Clin Periodontol* 2001; **28:**296–305.
48. Filippini M, Masiero G, Moschetti K. Socioeconomic determinants of regional differences in outpatient antibiotic consumption: evidence from Switzerland. *Health Policy* 2006;**22:**77–92.
49. Callegan MC, Ramirez R, Kane ST, Cochran DC, Jensen H. Antibacterial activity of the fourth-generation fluoroquinolones gatifloxacin and moxifloxacin against ocular pathogens. *Adv Ther* 2003;**20:**246–252.
50. Sedlacek MC, Walker C. Antibiotic resistance in an in vitro subgingival biofilm model. *Oral Microbiol Immunol* 2007; **22:**333–339.
51. Martone WJ, Nichols RL. Recognition, prevention, surveillance, and management of surgical site infections: introduction to the problem and symposium overview. *Clin Infect Dis* 2001;**33:**S67–S68.
52. Simonsen GS, Tapsall JW, Allegranzi B, Talbot EA, Lazzari S. The antimicrobial resistance containment and surveillance approach–a public health tool. *Bull World Health Organ* 2004;**82:**928–934.