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High resistance against clindamycin, metronidazole and amoxicillin in *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* isolates of periodontal disease

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Abstract

Objectives: To test the antimicrobial sensitivity of two periodontal pathogens to a panel of five orally administrable antibiotics in periodontal disease.

Study design: A total of 69 isolates of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were processed using culture and biochemical tests. Selected colonies of *A. actinomycetemcomitans* and *P. gingivalis* were used to evaluate the antibacterial activity of clindamycin, metronidazole, amoxicillin, moxifloxacin and amoxicillin/clavulanic acid.

Results: Susceptibility testing revealed a sensitivity of 100% of *A. actinomycetemcomitans* and *P. gingivalis* to moxifloxacin and amoxicillin/clavulanic acid but moderate susceptibilities were found for the rest of antibiotics agents evaluated.

Conclusions: The widespread use of antibiotics is reflected in the level of resistance of *A. actinomycetemcomitans* and *P. gingivalis* in patients with periodontal infections. This suggests that antibiotic susceptibility testing is necessary to determine efficacy of antimicrobial agents. Clinical studies with antibiotics should take these differences into account.

Key words: Antibiotics, antimicrobial activity, resistance.

Introduction

Periodontitis, a chronic inflammatory disease, begins with a microbial infection, followed by a host-mediated destruction of soft tissue caused by hyperactivated or primed leukocytes and the generation of cytokines, eicosanoids, and matrix metalloproteinases that cause clinically significant connective tissue and bone de-

struction (1). *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are implicated in the pathogenesis of periodontitis (2,3). These bacteria are able to produce virulence factors that act locally within the sulcus, and result in tissue destruction (2,3). Examples of virulence factors include proteolytic enzymes produced by *P. gingivalis* and leukotoxins produced by *A. actinomycetemcomitans* (3). *P. gingivalis* has shown

the ability to invade human gingival fibroblasts in cell culture while *P. gingivalis* and *A. actinomycetemcomitans* have all demonstrated the ability to invade human oral epithelial cells in cell culture (4). By the way, it is noteworthy that 35% of nosocomial pneumonia has been demonstrated to be related to periodontopathogens (5). Invasiveness and ubiquitous intraoral distribution may be the main reasons for the reported observation of rather poor results after conventional, merely mechanical treatment of periodontal infections (6). Thus, adjunctive systemic antibiotics have been advocated in order to suppress the organism in subgingival plaque (7). The adjunctive use of systemically administered antibiotics has been shown to provide a better clinical outcome, particularly in terms of pocket depth reduction and attachment-level gain, than scaling and root planing in chronic periodontitis subjects (6,7). Commonly used antibiotics include clindamycin, metronidazole and amoxicillin (6,8). Besides, positive responses have been reported with amoxicillin/clavulanic (6,8) and moxifloxacin (7) in the treatment of periodontitis.

Most of the knowledge available on the microbial composition of subgingival plaque is based on studies from the United States and Europe (8-10), where therapeutic oral care programs are available to significant portions of the population. Temporal and geographic changes in antibiotic susceptibility among anaerobes have been reported in Europe (8,10). Besides, the development of resistance and significant variation in the local prevalence of resistance are well recognized in aerobic and anaerobic bacteria in Latin America (9). Determinations of MIC values permit early recognition of emerging resistance. For relevant comparative analysis distributions of the minimal inhibitory concentration (MIC) values are mandatory as the terms susceptible, intermediate and resistant are not universally applicable since breakpoints may vary in different countries (11).

To the best of our knowledge, there are no studies that determine the resistance of *A. actinomycetemcomitans* and *P. gingivalis* to a panel of five orally administrable antibiotics in periodontal disease. Thus, the present study aimed to test, in vitro, the susceptibility to different antibiotics of *A. actinomycetemcomitans* and *P. gingivalis* isolated from subgingival plaque in periodontal infections.

Study Design

Population studied, clinical evaluation, microbiological sampling and microbiological procedures.

A total of 76 adult patients in good general health and with no current medication usage were included in the study after giving informed consent. Medical history and clinical and radiographic examination were conducted for each patient. One of the authors (CA) carried out a complete periodontal examination. The following

clinical parameters were recorded: probing depth (PD), clinical attachment level (CAL), and percentages of sites with bleeding on probing (BOP) and plaque. A marked probe (UNC-15, Hu-Friedy, Chicago, IL) was used in all instances. Periodontal diagnosis was established based on the consensus report of the American Academy of Periodontology (AAP) (12).

Subgingival microbial samples were taken from the six deepest pockets. After removing supragingival plaque with curets and isolating the area with cotton pellets, the paper points were inserted into each periodontal pocket for 20 seconds. The paper points (Maillefer, Ballaigues, Switzerland) were pooled in screw cap vials containing Viability Medium Göttenborg Anaerobically (VMGA) III medium (13). All samples were labeled properly and processed within 4 hours after sampling. The samples were analyzed using microbial culture techniques for the presence of periodontopathic bacteria according to Slots (14). Briefly, most samples were processed at room temperature (25°C) and incubated in CO₂ and anaerobic culture systems. Brucella blood agar medium was incubated at 35°C in an anaerobic jar for 7 days. The Trypticase Soy Serum Bacitracin Vancomycin agar (TSBV) medium was incubated in 10% CO₂ at 37°C for 4 days. Presumptive identification was performed according to the methods described (14), and using a commercial identification micromethod system (RapID ANA II, Remel, Norcross, GA, USA) for *A. actinomycetemcomitans* and *P. gingivalis*. Total viable counts (TVC) were defined as the total number of colony-forming units obtained on non-selective media plates. Species found on selective media were enumerated and their percentage of TVC was calculated.

Antimicrobial susceptibility testing

The susceptibility/resistance of selected colonies of *A. actinomycetemcomitans* and *P. gingivalis* from pure cultures to five antibiotics were measured. The following antimicrobials were tested: clindamycin, metronidazole, amoxicillin, moxifloxacin and amoxicillin/clavulanic acid (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 x 10⁸ CFU/ml). Using a sterile glass rod, 0.1 ml of the inoculums was spread over Brucella blood agar plates (BD, Sparks, MD, USA) and dried for 15 minutes at room temperature. E-test strips were gently placed onto the agar surface and incubated under anaerobic conditions for 4 days. The elliptical zone of inhibition was examined after 96 hours of incubation. The reading at the intersection of the bacterial zone of inhibition and the E-strip represented the MIC of the organism. The MIC breakpoints were interpreted according to the Clinical Laboratory Standards Institute guidelines (15). In the case of *A. 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 (15).

Statistical Analysis

Descriptive analyses were carried out for clinical and microbiological parameters. A statistical program was used for all the statistical analyses (SPSS, Statistical Package for the Social Sciences, version 15, Chicago, IL).

Results

Table 1 depicts the clinical characteristics of study subjects. A total of 45 women (59.2%) and 31 men (40.8%) with chronic periodontitis were studied. From the 76 study patients a total of 69 bacterial colonies could be

clearly identified. Table 2 shows the in vitro susceptibility of *A. actinomycetemcomitans* (n=18) and *P. gingivalis* (n=51) to the five antibiotics tested. Susceptibility testing revealed a sensitivity of 100% of *A. actinomycetemcomitans* and *P. gingivalis* to moxifloxacin and amoxicillin/clavulanic acid but moderate susceptibilities were found for the rest of antibiotics agents evaluated.

Discussion

The overuse, misuse and widespread prophylactic application of antimicrobial drugs are some of the factors that have led to the emergence of drug resistant microorganisms (11,16). Microbial resistance has become a worldwide medical, economic and public health problem although microbial resistance rates are not equally distributed around the world (9-11,16). *A. actinomycetemcomitans* and *P. gingivalis* represent two important periodontal pathogens. In this study, we investigated the susceptibility of *P. gingivalis* and *A. actinomycetemcomitans* from patients with untreated chronic periodontitis; their invasiveness and ubiquitous intraoral distribution may be the main reasons for the reported observation of poor results after mechanical therapy (6). Additionally, bacterial resistance to penicillin has become a problem of great clinical significance in Latin America because of its widespread use for many years (11,16). In our study, *P. gingivalis* was highly susceptible to moxifloxacin and amoxicillin/clavulanic acid (Table 2). These findings are in agreement with previous studies which have found that *P. gingivalis* is highly susceptible to these antibiotics (7,8,17,18). Nevertheless, in our study 23.52%, 21.56% and 25.49% of the isolates were

Table 1. Clinical parameters of the periodontal sites being selected for subgingival plaque sampling.

Clinical Parameter	Mean SD
Clinical attachment level (mm±SD)	4.3±1.99
Probing depth (mm±SD)	3.4±1.48
% sites with plaque (mm±SD)	56±28
% of sites with bleeding on probing (mm±SD)	77±21

Table 2. Minimal inhibitory concentration (MIC, mg/ml) across 18 strains of *A. actinomycetemcomitans* and 51 strains of *P. gingivalis*.

Microorganism and antibiotic	Range	MIC (mg/L) ^a		R ^b (%)	IR ^c (%)	S ^d (%)
		50%	90%			
<i>A. actinomycetemcomitans</i>						
Clindamycin	0.016>256	0.125	>256	83.33	5.56	11.11
Metronidazole	1.5>256	6	>256	88.88	5.56	5.56
Amoxicillin	0.064-32	0.25	32	77.77	-	22.23
Moxifloxacin	0.19-0.5	0.38	0.5	-	-	100
Amoxicillin/clavulanic acid	0.02-0.75	0.25	0.5	-	-	100
<i>P. gingivalis</i>						
Clindamycin	0.08≥16	8	≥16	23.52	7.8	68.68
Metronidazole	0.08≥16	0.256	≥16	21.56	9.8	68.64
Amoxicillin	0.016>256	0.125	>256	25.49	-	74.51
Moxifloxacin	0.006-0.032	0.023	0.032	-	-	100
Amoxicillin/clavulanic acid	<0.016-0.064	<0.016	<0.016	-	-	100

a50% and 90% indicate the MIC values at which 50% and 90% of isolates were inhibited respectively. bResistance. cIntermediate resistance. dSensitive

non-susceptible to clindamycin, metronidazole and amoxicillin respectively. Similar results have been reported by other investigations that detected resistances, although lower (19). In this viewpoint, it is important to note that *P. gingivalis* has recently been shown to be able of transfer plasmid DNA, chromosomal DNA, or both which would supply a functional system to also transfer resistance determinants (2).

The present findings support that *A. actinomycetemcomitans* was the least vulnerable microorganisms. Amongst the drugs tested, moxifloxacin and amoxicillin/clavulanic were active against all *A. actinomycetemcomitans* isolates in vitro (Table 2). Previous studies of the susceptibility of *A. actinomycetemcomitans* have also shown high susceptibility to these two antibiotics (6-8, 17,18). In this study, clindamycin, metronidazole and amoxicillin resistance was found amongst *A. actinomycetemcomitans* isolates. Clindamycin, metronidazole and amoxicillin resistance observed in this study agrees with the studies of van Winkelhoff et al. (8), Madinier et al. (20), and Kulik et al. (17) where 82%, 72% and 33% of the *A. actinomycetemcomitans* isolates were non-susceptible. Once again resistance values found in our study were higher. On the other hand, metronidazole in combination with amoxicillin has been shown to be successful in the treatment of *A. actinomycetemcomitans* associated periodontal disease (6). In contrast, the MIC90 value of >256 µg/ml of the Spanish *A. actinomycetemcomitans* may indicate that the clinical value of this mixture therapy may not be equal in Spain as it is in other European countries (8). Analogous situations may possibly arise in Latin America.

Multidrug-resistance is of great concern in skin and soft tissue infections, especially in Latin America with a calculated occurrence rate of 24.7% compared with markedly lower rates in Europe (10.8%) and North America (3.2%) (16). Latin America also has the lowest susceptibilities of all regions for the following antimicrobials: aztreonam, cefepime, ceftazidime, gentamicin, ciprofloxacin, imipenem, polymyxin B, and piperacillin/tazobactam (16). Imipenem susceptibilities were lowest in Latin America at 65.3% compared with Europe (80.7%) and North America (88.7%) (16). Imipenem resistance is due in part to the appearance and dissemination of metallo-β-lactamase enzymes that have been described repeatedly in Latin America (16). Moreover, an increasing prevalence of penicillin resistant pneumococci has been observed throughout the world, and Latin America echoes this trend (11).

The results of the present study have shown that moxifloxacin and amoxicillin/clavulanic acid were the most active antibiotics against *A. actinomycetemcomitans* and *P. gingivalis* with all the isolates being susceptible. Less effective were clindamycin, metronidazole and amoxicillin. Owing to geographical differences as well

as differences over time, we suggest that the overuse and misuse of antibiotics could be influencing the manifestation of more highly resistant strains associated with periodontal infections in our population. Ongoing longitudinal surveillance studies have been crucial to the detection and monitoring of regional antimicrobial resistance patterns, and continue to provide important insights that may serve to modify local prescribing guidelines.

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Conflict of Interest and Sources of Funding Statement

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