**Antimicrobial susceptibility of periodontal pathogens isolated from Slovenian patients**

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**INTRODUCTION and PURPOSE**

Periodontitis is an inflammatory destructive disorder of the tissues that support the teeth. Loss of alveolar bone is a major characteristic of the disease. Periodontitis is caused by a subgingival biofilm consisting of several different anaerobic and facultative anaerobic bacteria. The clinically most important cultivable periodontal bacterial species are *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia/Prevotella nigrescens*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*. Since antibiotic susceptibility of periodontal pathogens in Slovenia has never been tested before the aim of our study was to investigate the antimicrobial susceptibility of five periodontal pathogens isolated from Slovenian patients to six commonly used antibiotics in periodontics.

**MATERIALS and METHODS**

A total of 140 periodontal bacterial isolates were isolated from subgingival plaque samples in patients suffering from chronic periodontitis (50 *P. intermedia/P. nigrescens*, 45 *F. nucleatum*, 30 *P. gingivalis* and 15 *A. actinomycetemcomitans* isolates).

Samples were grown on 5% horse blood agar plates (no. 2; Oxoid Ltd., Basingstoke, UK) supplemented with 5 mg/L haemin and 1 mg/L menadione (BA plates) and incubated in 80% N₂, 10% H₂ and 10% CO₂ for 7 days at 37 °C. *A. actinomycetemcomitans* was isolated from Dental-d 1 agar plates (BHI agar, vacuum sealed Petri dishes furnished with vancomycin, sodium format with cymohexamide) incubated in air + 5% CO₂ at 35 °C for 5 days.

Identification of isolates was based on their colony morphology using a ring-light-equipped stereomicroscope Olympus SZX7 (Olympus, Japan) (Figure 1), Gram staining (Gram Stain Kit Recton Dickinson and Company, New Veney, USA), catalase production with ID Color catalogue (bioMérieux, Marcy l’Etoile, France) and confirmed with Matrix-Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry on MALDI Biotyper (Bruker Daltonik, Germany).

Antibiotic susceptibility of the test bacteria to amoxicillin, amoxicillin/clavulanic acid (AMC), clindamycin, azithromycin, metronidazole and tetracycline was determined by Etest (AB Biodisk bioMérieux, Marcy l’Etoile, France) (Figure 2).

For anaerobic bacteria, a suspension from 48h bacterial cultures of 2 McFarland was prepared and applied to a pre-reduced BA plate. Minimum inhibitory concentrations (MICs) were determined after 48h incubation at 37 °C in an anaerobic atmosphere. For *A. actinomycetemcomitans* a suspension of 2 McFarland was prepared and applied to a BA plate. MICs were determined after 72h of incubation at 37°C in air + 5% CO₂. MICs and MIC₅₀ values were determined and percentage of susceptible bacterial isolates was calculated using CLSI and EUCAST breakpoints.

**RESULTS**

The results of the susceptibility testing are presented in Table. All *A. actinomycetemcomitans* isolates were susceptible to all antibiotics tested except one (6.7%) resistant to azithromycin. MIC₅₀ values for all tested antibiotics with available EUCAST and/or CLSI breakpoints in *A. actinomycetemcomitans*, *P. intermedia/P. nigrescens* and *F. nucleatum* were well below breakpoint values. In contrast MIC₅₀ of clindamycin for *P. gingivalis* was 256 µg/ml, which is also significantly higher than reported in published studies from other European countries (Int J Antimicrob Ag 2012, 40: 450-4). *Clin Periodontol* 2005, 32, 893-8. Int J Antimicrob Ag 1999, 12, 41-6; Int Antimicrob Chemother 2006, 61: 1087-99).

**CONCLUSIONS**

The majority of periodontal pathogens in Slovenian patients were susceptible for antibiotics commonly used for the treatment of chronic periodontitis, yet resistant strains were found.

The highest (almost 5%) resistance among all tested bacterial isolates was observed for clarinamycin. More than 13% of *P. gingivalis* isolates were clindamycin resistant.

Significant differences in susceptibility exist in different countries. Thus periodic surveillance of antibacterial susceptibility of periodontal pathogens in a country/region is necessary to assure optimal antibiotic treatment of periodontal disease when this treatment approach is necessary.

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