Antimicrobial Susceptibilities of Porphyromonas gingivalis,
Prevotella intermedia, and Prevotella nigrescens spp.
Isolated in Spain

MARÍA T. ANDRÉS,1 WHASUN O. CHUNG,2 MARILYN C. ROBERTS,2 AND JOSÉ F. FIERRO1,3,*

Laboratory of Oral Microbiology, School of Stomatology,1 and Department of Functional Biology (Microbiology), Faculty of Medicine,3 University of Oviedo, Oviedo, Spain, and Department of Pathobiology, University of Washington, Seattle, Washington2

Received 27 April 1998/Returned for modification 16 July 1998/Accepted 26 August 1998

The susceptibilities of 143 Porphyromonas gingivalis, Prevotella intermedia, and Prevotella nigrescens isolates to 18 antimicrobial agents were tested. All P. gingivalis isolates were susceptible. In contrast, some Prevotella spp. (17%) were resistant to β-lactams, erythromycin, clindamycin, or tetracycline and carried resistance genes, ermF or tetQ, or β-lactamases.

Black-pigmented, gram-negative oral anaerobes such as Porphyromonas gingivalis and the Prevotella intermedia-Prevotella nigrescens group are thought to be pathogens in adult periodontitis (11). In addition, the P. intermedia-P. nigrescens group may be involved in both oral and nonoral infections (7, 8). Patients who do not respond to common surgical or mechanical periodontal therapy are often administered antibiotics as a complement to conventional treatment (12). However, limited susceptibility data has been available for these bacteria and no separate break points have been established by the National Committee for Clinical Laboratory Standards (NCCLS) (9).

The purpose of this study was to examine the susceptibilities of oral isolates of P. gingivalis and P. intermedia-P. nigrescens to 18 antimicrobial agents and to determine if there is a correlation between the MIC and the presence of known antibiotic resistance genes.

A total of 143 clinical isolates were obtained from different patients diagnosed with adult periodontitis at the School of Stomatology of the University of Oviedo, Oviedo, Spain, between 1995 and 1997. None of the patients had been given antibiotic therapy within the previous 6 months. The isolates were the most prevalent anaerobic species from oral samples collected from the supragingival plaque by means of sterile cotton pledgets and subgingival samples collected by using two sterile paper points, which were inserted at each study site. The API Rapid ID32 A system (bioMérieux, Marcy-l’Etoile, France) was used for initial identification. The P. intermedia-P. nigrescens isolates were verified with a 16S rRNA-based PCR assay, as described by Conrads et al. (4). P. gingivalis was also verified by PCR, as previously described (1).

The antibiotics used for susceptibility testing were as follows: benzylpenicillin and tetracycline (Antibióticos, S.A., Madrid, Spain); amoxicillin, ampicillin, and ticarcillin (Smith Kline & French, S.A., Madrid, Spain); piperacillin (Lederle Laboratorios, Puerto Rico); metronidazole and spiramycin (Rhone-Poulenc Rorer, S.A., Madrid, Spain); and clindamycin (Upjohn Farmacéutica, Madrid, Spain). MICs were determined under anaerobic conditions at 37°C by the agar dilution method described by the NCCLS using Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% sterile defibrinated sheep blood (9). Serial dilutions of the antibiotics ranging from 128 to 0.125 µg/ml were prepared and used on the same day. The final inoculum contained approximately 10⁵ CFU per spot. Quality control strains (Bacteroides fragilis ATCC 25285 and Bacteroides thetaiotaomicron ATCC 29741) were included with each run. Plates were read at 48 h. The MIC was defined as the lowest antimicrobial concentration which prevented visible growth of bacteria.

All 31 P. gingivalis isolates were susceptible to all of the antibiotics tested (Table 1), which is similar to previous findings (10). In contrast, some of the P. intermedia and P. nigrescens isolates were resistant to various antibiotics. We found that 14% (14 P. intermedia and 2 P. nigrescens isolates) were resistant to β-lactam antibiotics (penicillins and/or some cephalosporins). Cefinase disks (BBL Microbiology Systems, Cockeysville, Md.) were used to examine β-lactamase production. All 16 isolates had β-lactamase activities and had high MICs to penicillins and/or cephalosporins (Table 1). The percentage of β-lactamase-positive isolates observed in the P. intermedia-P. nigrescens group (14%) was lower than the 26% that has been reported previously (2, 14). The MICs for the cephalosporin-resistant strains of this study were similar to those exhibited for oral isolates of P. intermedia encoding a 2e cephalosporinase that we characterized recently (13).

Ninety percent of P. intermedia isolates were susceptible to erythromycin (MIC₉₀ ≤0.06 µg/ml), oleandomycin (MIC₉₀ ≤0.06 µg/ml), spiramycin (MIC₉₀ ≤0.125 µg/ml), and clindamycin (MIC₉₀ ≤0.125 µg/ml). All isolates of the P. intermedia-P. nigrescens group were susceptible to spiramycin (MIC₉₀ ≤0.125 µg/ml). Although no macrolide breakpoints are currently available for these anaerobes, we found high erythromycin, oleandomycin, and clindamycin MICs for two strains of P. nigrescens (15.4%). PCR assays for the ermF gene were performed with primers F₁ (5’ CGGGTCAAGCTTTAATGTTG 3’) and F₂ (GGACTACCTCATAGACAAG 3’), and the PCR products were amplified with Taq polymerase (Perkin-Elmer). The PCR products were purified using a Qiamp Tissue Kit (Qiagen). The purified DNA was sequenced with the fluorescent dye terminator method (Amersham) and an automated sequencer (Applied Biosystems). The sequencing reactions were performed with the forward primer (primF) and with the reverse primer (primR) using a sequencing machine (ABI-PRISM 310). The resulting sequences were analyzed with the Biosequence Analysis Software Package (version 1.0, 1992; Perkin-Elmer). The PCR products were amplified with Taq polymerase (Perkin-Elmer). The PCR products were purified using a Qiamp Tissue Kit (Qiagen). The purified DNA was sequenced with the fluorescent dye terminator method (Amersham) and an automated sequencer (Applied Biosystems). The sequencing reactions were performed with the forward primer (primF) and with the reverse primer (primR) using a sequencing machine (ABI-PRISM 310). The resulting sequences were analyzed with the Biosequence Analysis Software Package (version 1.0, 1992; Perkin-Elmer). The PCR products were amplified with Taq polymerase (Perkin-Elmer). The PCR products were purified using a Qiamp Tissue Kit (Qiagen). The purified DNA was sequenced with the fluorescent dye terminator method (Amersham) and an automated sequencer (Applied Biosystems). The sequencing reactions were performed with the forward primer (primF) and with the reverse primer (primR) using a sequencing machine (ABI-PRISM 310). The resulting sequences were analyzed with the Biosequence Analysis Software Package (version 1.0, 1992; Perkin-Elmer).
were confirmed by hybridization. Both resistant isolates were positive for the ermF gene.

Tetracycline inhibited 90% of the P. intermedia isolates (MIC<sub>90</sub>, 1 μg/ml) and was less effective against the isolates of P. nigrescens (MIC<sub>90</sub>, 16 μg/ml). By a previously described PCR assay for tetQ (7), two tetracycline-resistant P. nigrescens isolates were shown to carry the tetQ gene; one isolate also carried the ermF gene. The incidence of tetracycline resistance observed in this study for the P. intermedia-P. nigrescens group (3.6%) was much lower than the 26% reported previously for Prevotella spp. from other areas (6).

Metronidazole was highly active with the P. intermedia-P. nigrescens group (MIC<sub>90</sub>, 0.125 μg/ml). Resistance (32 μg/ml) was found in three isolates of P. intermedia. All isolates were susceptible to metronidazole-spiramycin (1:1.48), which is a combination used commonly in Spain for treatment of a variety of dental infections. The MIC<sub>90</sub> of this combination (≤0.032 μg/ml) was much lower than the MIC<sub>90</sub> of each antimicrobial agent tested separately, suggesting a synergistic effect that has been reported previously (3).

We have been able to link three classes (β-lactam, macro-lide-lincosamide, and tetracycline) of antibiotic resistance with known mechanisms of resistance (β-lactamases, tRNA methy- lases encoded by the ermF gene, or a ribosomal protection protein encoded by the tetQ gene) in the P. intermedia-P. nigrescens group. All three types of genes have been associated with conjugative elements in oral Prevotella spp. (5, 15), and gene transfer between different species has been demonstrated in the laboratory (5, 15). Thus, the P. intermedia-P. nigrescens group may function as an antibiotic resistance gene reservoir and may influence the success of antibiotic therapy in the oral cavity.

We thank M. C. Martín for technical support.

This work was supported in part by funds from the Sterilization Monitoring Service (CN-96-133-B1), School of Stomatology, University of Oviedo.

**REFERENCES**


