Susceptibilities of *Eikenella corrodens*, *Prevotella intermedia*, and *Prevotella nigrescens* Clinical Isolates to Amoxicillin and Tetracycline

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The AB Biodisk Etest showed that 106 (100%) and 98 (92%) isolates of *Eikenella corrodens* were susceptible to amoxicillin and tetracycline, respectively. Twenty-three (68%) *Prevotella intermedia* isolates and 14 (67%) *Prevotella nigrescens* isolates were susceptible to amoxicillin. Seventy-nine percent of the *P. intermedia* isolates and 67% of the *P. nigrescens* isolates were susceptible to tetracycline. A higher percentage of β-lactamase-producing isolates of *P. intermedia* and *P. nigrescens* were identified with selective agar containing amoxicillin than with nonselective agar.

Periodontitis is a bacterial inflammatory disease characterized by the destruction of connective tissues, including alveolar bone, and it may eventually lead to tooth loss. Systemic or topical antibiotics have been employed as an adjunct in treating periodontal disease (10). A majority of the practicing periodontists in the United States prescribe antibiotics following periodontal surgery. Penicillin and tetracycline are among the most frequently prescribed antibiotics (10).

*Eikenella corrodens*, *Prevotella intermedia*, and *Prevotella nigrescens* have been implicated as the causative agents of periodontitis (4). *E. corrodens* is a gram-negative facultative anaerobic rod that is occasionally found in high numbers in people with periodontal disease (3). This organism has also been identified either as the sole pathogen or as part of mixed microflora in various extraoral sites (2, 3). *P. intermedia* and *P. nigrescens* are part of a heterogeneous group of gram-negative obligate anaerobic bacteria formerly designated as black-pigmented *Bacteroides* (7–9). The two species are phenotypically difficult to distinguish and are commonly identified as the *P. intermedia/nigrescens* group in primary culture. However, more recently developed 16S rRNA-based PCR methods can rapidly distinguish between these two microbial species (1, 11).

The aims of this study were to examine the susceptibilities to amoxicillin and tetracycline among *E. corrodens*, *P. intermedia*, and *P. nigrescens* clinical isolates. We also evaluated the use of agar containing either amoxicillin or tetracycline in primary culture to help identify resistant isolates of *P. intermedia* and *P. nigrescens*. The incidence of β-lactamase production was determined among *P. intermedia* and *P. nigrescens* isolates. The presence or absence of plasmid was also examined for *P. intermedia* and *P. nigrescens* isolates with resistance to antibiotics.

One hundred six *E. corrodens* clinical isolates were examined. The isolates were collected between 1985 and 1999. Ninety-one of these isolates originated from various intraoral sites (supra- and subgingival plaque samples, saliva, and mucosal surfaces) of periodontally healthy subjects (9 isolates), adult periodontitis patients (45 isolates), and localized juvenile periodontitis patients (37 isolates). The remaining *E. corrodens* isolates included 14 nonoral isolates (9 isolates from blood, 4 isolates from nonoral abscesses, and 1 isolate from a bite wound) and 1 isolate from sputum.

Sixty-five *P. intermedia* oral isolates and 33 *P. nigrescens* oral isolates were examined in this study. The isolates were recovered from the primary cultures of the subgingival plaque of periodontitis patients over 1 year from 1999 to 2000. The isolates can be divided into three categories based on the culture medium used in primary cultures; nonselective brucella blood agar (34 *P. intermedia* isolates and 21 *P. nigrescens* isolates), brucella blood agar containing 1 μg of amoxicillin per ml (21 *P. intermedia* isolates and 9 *P. nigrescens* isolates), and brucella blood agar containing 1 μg of tetracycline per ml (10 *P. intermedia* isolates and 3 *P. nigrescens* isolates). The species identities of the *P. intermedia* and *P. nigrescens* isolates were confirmed by 16S rRNA-based PCR detection methods as described previously (1).

The susceptibilities of *E. corrodens*, *P. intermedia*, and *P. nigrescens* to amoxicillin and tetracycline were determined by the Etest method (AB Biodisk, Piscataway, N.J.). *E. corrodens* was grown on brucella blood agar plates for 3 days in 5% CO₂ at 37°C and washed off the plates with Todd-Hewitt broth. *P. intermedia* and *P. nigrescens* were grown on brucella blood agar plates anaerobically at 37°C for 3 to 5 days and washed off the plates with dilution broth consisting of sodium chloride (5,000 μg/ml), Thiotone peptone (2,500 μg/ml), and tryptose (2,500 μg/ml). The *E. corrodens*, *P. intermedia*, and *P. nigrescens* bacterial suspensions were adjusted to a turbidity of a 1.0 McFarland standard and inoculated onto brucella blood agar plates with sterile cotton swabs. The Etest strips (AB Biodisk) were placed onto the agar surface. The plates were cultured for 3 days, and the MICs were determined according to the manufacturer’s guidelines. Selected *P. intermedia* and *P. nigrescens* isolates were further subjected to a β-lactamase test with Ce-fimase β-lactamase disc (Becton Dickenson Microbiology Sys-
The presence of plasmid in *P. intermedia* and *P. nigrescens* isolates was evaluated by plasmid extraction with the QIAprep Miniprep kit (Qiagen, Inc., Valencia, Calif.) according to the manufacturer’s recommendation.

Table 1 shows antimicrobial susceptibilities of *E. corrodens*, *P. intermedia*, and *P. nigrescens*. Because no interpretive criteria exist for amoxicillin or for *E. corrodens* isolates recovered from nonselective brucella blood agar with amoxicillin (21 isolates) and *P. intermedia* (21 isolates) and *P. nigrescens* (9 isolates) recovered from selective agars containing amoxicillin were significantly higher than those for the corresponding species recovered from nonselective plates (*P* < 0.01; Mann-Whitney test).

Of isolates recovered from nonselective agar, 27 of the 34 (79%) *P. intermedia* isolates and 14 of the 21 (67%) *P. nigrescens* isolates were susceptible to tetracycline (MIC of ≤4 μg/ml). Five (15%) *P. intermedia* and six (29%) *P. nigrescens* isolates were of intermediate susceptibility to tetracycline (MIC = 8 μg/ml). Resistance was found in the remaining two (6%) *P. intermedia* isolates and one (5%) *P. nigrescens* isolate (MIC of ≥16 μg/ml). The MICs of tetracycline for *P. intermedia* (10 isolates) recovered from selective agar containing tetracycline were significantly higher than those for nonselective *P. intermedia* isolates (*P* < 0.01; Mann-Whitney test).

The use of selective agar containing amoxicillin increased the detection rates of β-lactamase among *P. intermedia* and *P. nigrescens* isolates from approximately one-third with nonselective agar (11 of 34 [32%] *P. intermedia* and 7 of 21 [33%] *P. nigrescens* isolates) to 100% with selective agar (21 of 21 *P. intermedia* and 9 of 9 *P. nigrescens* isolates) containing 1 μg of amoxicillin per ml. Thirty-one *P. intermedia* isolates and 12 *P. nigrescens* isolates recovered from selective agar plates with amoxicillin or tetracycline were examined for the presence of plasmid. None of the isolates examined revealed a plasmid.

In summary, the present study showed that 100% and 92% of the *E. corrodens* isolates were susceptible to amoxicillin and tetracycline, respectively. Of *P. intermedia* and *P. nigrescens* isolates recovered from nonselective agar, 67 to 68% were susceptible to amoxicillin, and 79% of *P. intermedia* isolates and 67% of *P. nigrescens* isolates were susceptible to tetracycline. Approximately one-third of the *P. intermedia* and *P. nigrescens* isolates recovered from nonselective agar plates...
were β-lactamase positive. A higher percentage of β-lactamase-producing isolates of *P. intermedia* and *P. nigrescens* were identified with selective agar containing amoxicillin than with nonselective agar.

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