Predominant Microflora Associated with Human Dental Periapical Abscesses

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The microflora of periapical abscesses of teeth free of periodontal disease was studied. Per mucosal aspiration of abscess contents and anaerobic cultural, microscopic, and biochemical techniques indicated a mixed but somewhat specific and relatively limited facultative and obligate anaerobic flora with *Fusobacterium nucleatum* and *Streptococcus mitis* as a frequent pair.

Many cases of dental periapical abscess present annually worldwide. Such abscesses have been associated with either frank dental carious lesions with concomitant pulp tissue exposure or with advanced periodontal lesions; however, some are without obvious antecedent pathology (20, 23). The lesions can, if left untreated, lead to cellulitis, bacteremia, or abscesses at distant sites (15, 20, 21).

There is controversy regarding the specific bacterial etiology of periapical abscesses. Histological studies have reported the presence of bacteria (1, 3, 4, 13, 27). Although extensive characterizations of microflora associated with necrotic dental pulps (2, 12, 18, 25) and periodontal lesions (19, 26) have been reported, such has not been the case for periapical abscesses. Few of the early studies of periapical abscesses took precautions to assure the recovery and survival of fastidious microorganisms, particularly anaerobes, which may potentially occupy periapical alveolar bone. It should be recognized that culture of these usually painful lesions within clinical settings does present problems for anaerobic sample handling and transport to laboratories.

There have been studies of the bacteriological status of such abscesses from teeth subsequent to extraction (10, 23), but these data should be viewed with caution because of the probability of sample contamination from adjacent periodontal sources attendant to the extraction procedure (18). Other studies, attempting to sample the periapical flora after mechanical removal of dental root canal tissue, are also suspect because of the possibility that recovered bacteria either originated from the walls of infected root canals or were displaced into periapical lesions during canal instrumentation (7). Some studies of periapical abscess sites in adults indicate the

presence of a complex flora, predominantly of obligate anaerobes and facultative anaerobic cocci (21, 22), but unfortunately, no differentiation between abscesses of periodontal and of pulpal origin has been made. Such information now appears crucial because the microflora associated with periodontal abscess is heterogeneous (19, 26) and rich in *Bacteroides* sp. In addition, other investigators have demonstrated bacterial plaque surrounding root apices and entering root canals from the apical foramina of teeth in cases of advanced periodontal disease (14), a finding that supports the need for distinguishing between abscesses that involve or are adjacent to periapical regions and those discrete from periodontal lesions.

During the preparation of this paper, a report (5) appeared which indicates that periapical abscesses of 12 teeth of children who had been treated, presumably for dental carious exposure of the pulps, had an average of 4.9 isolate types in each of the per mucosally sampled lesions. The recovered species were facultative and obligate anaerobes.

Our study attempted to recover and identify the possibly fastidious and anaerobic microflora of dental periapical abscesses of adults with techniques designed to avoid contamination from contiguous, bacterially colonized periodontal and root canal regions. For the albeit small number of abscesses at the apices of teeth free of periodontal disease, a limited, mixed, and relatively specific microbial flora was detected.

Ten patients, 20 to 65 years old, were chosen for study from those treated for dental periapical abscesses at the University of Connecticut Health Center Dental Clinics. Excluded from study were patients with lesions associated with teeth having periodontal pockets or fistulous drainage to the oral mucosa or teeth with roots close to the maxillary sinus. Also excluded were patients with a history of rheumatic fever, heart disease, diabetes, immune or other systemic

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TABLE 1. Microflora associated with dental periapical abscesses

Subject	Sex/ age	Tooth designation ^a and status	No. of bacterial strains isolated	Identification
1	M/65	46, Temporary crown	2	<i>Streptococcus mitis</i> <i>Peptostreptococcus anaerobius</i>
2	F/38	25, Carious exposure	4	<i>Streptococcus mitis</i> <i>Fusobacterium nucleatum</i> <i>Bacteroides melaninogenicus</i> subsp. <i>intermedius</i> <i>Peptostreptococcus anaerobius</i>
3	F/29	46, Defective amalgam filling	3	<i>Streptococcus mitis</i> <i>Actinomyces viscosus</i> <i>Fusobacterium nucleatum</i>
4	M/35	46, Partial root filling	2	<i>Fusobacterium nucleatum</i> <i>Peptostreptococcus micros</i>
5	M/26	26, Defective amalgam filling	3	<i>Streptococcus mitis</i> <i>Fusobacterium nucleatum</i> <i>Bacteroides melaninogenicus</i> subsp. <i>intermedius</i>
6	M/29	37, Carious exposure of the pulp	2	<i>Streptococcus faecalis</i> <i>Actinomyces viscosus</i>
7	F/24	24, Defective amalgam filling	2	<i>Streptococcus faecalis</i> <i>Fusobacterium nucleatum</i>
8	M/24	36, Carious exposure of the pulp	2	<i>Streptococcus faecalis</i> <i>Actinomyces viscosus</i>
9	F/20	11, History of trauma, intact crown	3	<i>Streptococcus mitis</i> <i>Staphylococcus epidermidis</i> <i>Fusobacterium nucleatum</i>
10	M/32	11 and 21, History of trauma, intact crowns	2	<i>Streptococcus mitis</i> <i>Fusobacterium nucleatum</i>

^a Federation Dentaire International tooth designation.

compromise, or antibiotic therapy within 2 months of presentation.

For each patient, the oral mucosa overlying the abscess was scrubbed with tincture of iodine, and the area was anesthetized by distant injection of Lidocaine (2% wt/vol). A sterile 18-gauge needle fitted to a 5-ml disposable syringe was passed through the alveolar mucosa and bone into the abscess, from which the contents were withdrawn. The needle was immediately sealed by insertion into a sterile rubber block (9). The whole assembly was placed into an anaerobic jar (BBL Microbiology Systems, Cockeysville, Md.), immediately transported to and placed in an anerobic chamber (Coy Manufacturing, Ann Arbor, Mich.) containing an atmosphere of 85% N₂, 10% H₂, and 5% CO₂, and maintained at 37°C during sample handling.

Within 10 min of sampling, the contents of the syringe were expelled into 10 ml of prereduced Ringer solution supplemented with cysteine (4.5 mg/ml) and sodium metaphosphate (5 mg/ml; 17), and the suspension was agitated on a Vortex mixer to effect disruption of bacterial clumps. Samples were removed, and the morphology and motility of the cell types were determined with phase-contrast, Nomarski interference, darkfield optics, and brightfield microscopy after Gram staining. The remainder of the sample

was serially diluted in modified Ringer solution, and 0.02 ml of several dilutions was plated onto each of the following media: Trypticase (BBL) soy agar containing 5% (vol/vol) sheep blood, mitis salivarius agar (17), *Actinomyces* selective agar (CNAC-20; 6), a *Fusobacterium* selective medium (16), and an *Eikenella corrodens* selective medium (25). Replicate plates were incubated under anaerobic, microaerophilic (candle extinction jar), and aerobic atmospheres for up to 7 days at 37°C. Usually plates with >30 but <80 colony-forming units were studied; generally, 5 to 10 representative colonies of each morphology were selected from all media supporting their growth, and pure cultures were obtained for species characterization and identification. All cultures were grown and subsequently maintained in either Todd-Hewitt or Trypticase soy broth supplemented with potassium nitrate (2 mg/ml) and hemin (5 µg/ml; 16). The microorganisms were identified by examination of colonial morphologies and motility, growth on selective or differential media or both, Gram reaction, nutritional requirements, carbohydrate fermentation abilities, and a variety of biochemical tests including catalase activity, nitrate reduction, indole production, esculin, starch and gelatin hydrolysis, amino acid decarboxylase activities, and urease activity (8, 9). Acid end product

analyses were done when appropriate subsequent to the growth of the organisms on P-Y glucose (Scott Laboratories, Inc., Fiskeville, R.I.) by using methods previously described (11).

A total of 25 bacterial strains were isolated: 10 gram-positive facultative anaerobic cocci, 3 gram-positive anaerobic cocci, 9 gram-negative anaerobic rods, and 3 gram-positive facultative anaerobic pleomorphic rods (Table 1). Bright-field examination failed to reveal the presence of gram-negative cocci, and in addition, phase-contrast, Nomarski interference, and darkfield microscopy failed to reveal the presence of spirochetes, motile forms, or morphological forms not detected by culture.

The abscesses did not harbor a plethora of microbial species, in contrast to published observations of periodontal abscesses (19, 26) or necrotic pulp tissue (2, 12, 18, 25). No periapical abscess harbored more than four bacterial species, six abscesses harbored only two, and none harbored only a single species. In seven of the lesions, both facultative and obligate anaerobes were found, but in two cases only facultative anaerobes were found. Only one abscess contained an obligately anaerobic microflora. There was no discernible correlation between the apparent history of teeth associated with abscesses and the nature of the recoverable microflora. The most frequent microorganisms isolated from dental periapical abscesses in this study were *Fusobacterium nucleatum* and *Streptococcus mitis*, and the data suggested that these bacteria may exist as a frequent pair, being present in 6 of 10 cases. Thus, such lesions have a somewhat specific and relatively limited microflora.

The spectrum of isolated species is common to oral cavities, consistent with the probable oral origin of abscess isolates, and is somewhat similar to that described as recovered from periapical abscesses of the presumably carious teeth of children (5).

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