JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1983, p. 770–774
0095-1137/83/100770-05$02.00/0

Vol. 18, No. 4

Bacteriology of Dental Abscesses of Endodontic Origin

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Received 18 November 1982/Accepted 13 July 1983

Aspirates have been cultured from 10 dental abscesses of endodontic origin, all of which had penetrated beyond the bony alveolus to produce fluctuant swelling. Sampling was by syringe aspiration. Strict anaerobic techniques, including the use of an anaerobic chamber, were used for serial dilution and plating. Randomly selected colonies (100) from each culture were purified, characterized, and identified. Seventy percent of the bacterial isolates were either strict anaerobes or microaerophilic. One abscess yielded a pure culture of a viridans streptococcus, *Streptococcus milleri*. *Streptococcus intermedius* dominated the flora in a second abscess. The common oral streptococcus, *Streptococcus sanguis*, constituted only 2% of the isolates from one additional infection. *Fusobacterium nucleatum*, *Bacteroides melaninogenicus*, other *Bacteroides* including *B. oralis* and *B. ruminicola*, anaerobic diphtheroids, *Peptostreptococcus micros*, and *Staphylococcus epidermis* were other predominant isolates.

One of the more commonly encountered dental infections is the periapical abscess which originates at the apex of the root canal, usually of a nonvital tooth. In addition to the acute pain caused by this confined lesion, the presence of a periapical abscess is often associated with a relatively rapid destruction of the alveolar bone supporting the tooth. The presence of a fluctuant swelling indicates that the infection has progressed through the cortical bone. The periapical abscess is particularly significant for its potential to spread to sinuses and other fascial spaces of the head and neck, with possible lethal consequences. Therefore, early recognition of infection and appropriate therapy are essential.

Historically, bacteria of the viridans streptococcus group have been thought to be the major organisms in the periapical abscess. More recent studies incorporating anaerobic sampling and culture techniques have reported a greater number of mixed infections and larger proportions of gram-negative rods (1, 5, 7, 9, 13–15). *Fusobacterium nucleatum* and *Streptococcus mitis* were found frequently in aspirates of periapical abscesses examined by Oguntebi et al. (13). In a study of dental abscesses which included those of both endodontic and periodontic origin, Sabiston and Gold (14) found *F. nucleatum* in seven of eight infections, and as a major component in five of the eight. In a similar study, Sabiston et al. (15) reported that 66% of the species isolated were anaerobic gram-negative rods. However, none of the above studies has investigated the relative proportion of various anaerobic and facultative species in individual abscesses with regard to the etiology of mixed infection. The purpose of this endeavor, therefore, was to evaluate the complexity of the bacterial flora of acute abscesses of presumptive endodontic origin by identification and enumeration of species representing the predominant isolates.

MATERIALS AND METHODS

Selection of patients and specimen collection. The 10 patients included in this study presented at the University of Washington Health Sciences Center with fluctuant swelling of dental origin. These swellings were identified as periapical abscesses of endodontic origin on the basis of (i) radiographic evidence of a periapical lesion, (ii) a nonvital tooth, determined by thermal and electric pulp tests, and (iii) the lack of purulent exudate from periodontal sources when the subgingival areas surrounding the teeth in question were probed. None of the patients reported or showed signs of any concurrent systemic disease or were under antibiotic therapy.

The fluctuant area was swabbed three times with sterile cotton swabs to remove debris and saliva. This was accomplished by beginning over the intended sampling point and swabbing in increasingly larger concentric circles. Pus was then aspirated with a 1-ml tuberculin syringe that had been previously flushed with the prereduced anaerobic transport medium of Williams et al. (21) containing cysteine hydrochloride and dithiothreitol as reducing agents. The aspirate was transported within 15 min directly to an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) containing 80% nitrogen, 10% hydrogen, and 10% carbon dioxide.

Cultivation and isolation. All isolation and purification procedures were done in the anaerobic chamber.

Tenfold dilutions were made in prereduced anaerobically sterilized tubes containing the transport medium. Samples of each dilution were spread on blood agar plates made with Columbia base agar (BBL Microbiology Systems, Cockeysville, Md.) enriched with menadione (0.5 μg/ml), hemin (5.0 μg/ml), and 5% defibrinated sheep blood. Since the anaerobe chamber was at room temperature, plates were packed in anaerobe jars within the chamber environment and removed for incubation at 37°C. The isolation plates were examined after incubation at 37°C for 6 to 7 days, and 100 isolates were randomly selected. These were restreaked twice to obtain pure cultures.

Characterization and identification of isolates. To cope with the problem of the taxonomic identification of such a large number of isolates, a two-step procedure was used. First, isolates were identified to the genus level. This was accomplished through the following battery of eight tests or observations: Gram reaction; cell morphology as determined from Gram-stained smears and dark-field microscopy; colony morphology; catalase reaction; indole production; nitrate reduction; ability to grow anaerobically with 10% carbon dioxide; and gas chromatogrpahic analysis of metabolic end products. The above tests were applied as previously reported (20, 21).

The isolates were further characterized by another 37 fermentation and biochemical reactions as previously described (20). Prereduced anaerobically sterilized peptone-yeast extract (8) was the basal medium used for physiological characterization of most isolates. Inocula (2 ml) from 24- to 48-h cultures in basal medium were suspended in 100 ml of the same basal medium immediately after the latter had been autoclaved and cooled to room temperature. A 2-ml aliquot of the suspended inoculum was then aseptically dispensed into culture tubes previously autoclaved with 0.2 ml of a 10× concentrate of substrate in distilled water. A 0.5-ml amount of inoculum was added to 5 ml each of milk and gelatin medium. Culture tubes, with loose-fitting plastic caps, were incubated under an anaerobic atmosphere (10% hydrogen, 10% carbon dioxide, and 80% nitrogen) achieved by evacuation and replacement. Residual oxygen was removed by palladium catalyst. After incubation for 7 days at 37°C, acid production was measured with a pH meter. Assays for indole production, reduction of nitrate and nitrite, and deamination of urea, arginine, lysine, and ornithine were performed as described previously by Cowan (6). The hydrolysis of esculin, gelatin, and starch and the reactions in milk were assayed by the methods of Holdeman et al. (8).

From these tests, the identification of the anaerobic isolates was determined by using the keys in the *Anaerobe Laboratory Manual* (8). In instances in which the test results did not match well with those of described species, the identification of the isolates was left at the genus level.

RESULTS

Ten fluctuant abscesses of presumptive endodontic origin were sampled by syringe aspiration and cultured under anaerobic conditions. The number of colony-forming units per milliliter of pus was determined for six specimens and ranged from 3.3×10^6 to 1.2×10^9. Each abscess was characterized bacteriologically by the identification of 100 isolates representing the prominent cultivable flora (Table 1). Of the 1,000 isolates picked for identification, 83 lost viability during the initial purification steps.

TABLE 1. Bacteriology of individual abscess specimens

Specimen no.	Microorganisms isolated	Proportion (%)
1	*Streptococcus milleri*	98
2	Anaerobic gram-positive cocci	51
	Bacteroides species	11
	Fusobacterium nucleatum	11
	Bacteroides oralis	10
	Bacteroides melaninogenicus	6
3	*Fusobacterium nucleatum*	89
	Fusobacterium species	8
	Streptococcus sanguis	2
	Staphylococcus epidermidis	1
4	Anaerobic diphtheroides	30
	Bacteroides species (2)	41
	Fusobacterium nucleatum	26
5	*Streptococcus intermedius*	47
	Bacteroides melaninogenicus	21
	Bacteroides distasonis	12
	Bacteroides oralis	2
	Bacteroides corrodens	2
	Fusobacterium nucleatum	1
6	*Staphylococcus epidermidis*	66
	Peptostreptococcus micros	17
	Fusobacterium nucleatum	15
	Bacteroides species (2)	2
7	*Peptostreptococcus micros*	35
	Eubacterium lentum	14
	Bacteroides ruminicola subsp. *brevis*	13
	Bacteroides corrodens	9
8	*Peptostreptococcus micros*	34
	Bacteroides species (3)	35
	Bacteroides melaninogenicus	11
	Bacteroides asaccharolyticus	3
	Fusobacterium nucleatum	7
	Lactobacillus species	5
9	*Peptostreptococcus micros*	38
	Lactobacillus fermentum	22
	Bacteroides oralis	14
	Bacteroides ruminicola subsp. *brevis*	12
10	*Peptostreptococcus micros*	53
	Peptostreptococcus anaerobius	27
	Bacteroides corrodens	9
	Capnocytophaga ochracea	6

Anaerobic bacteria were cultured from 9 of the 10 abscesses; isolates were exclusively anaerobic in 6. Anaerobes accounted for 70% of the total isolates cultured. There was an average of 4.5 bacterial species per specimen. In one abscess, the infection was monobacterial, due to the viridans streptococcus *Streptococcus milleri*. No spirochetes were noted either by darkfield examination of pus or in Gram-stained smears.

Gram-negative anaerobic rods represented the largest group of isolates, accounting for 37% of the total; they were cultured from 9 of the 10 abscesses. The most numerous members of this group were *F. nucleatum* (15% of the total isolates) and *Bacteroides melaninogenicus* subsp. *melaninogenicus* (4%). *F. nucleatum* was the most frequently encountered organism cultured. It was isolated from 6 of the 10 abscesses and accounted for 89% of the flora cultured from specimen 3. This was the only specimen in which the predominant organism was a gram-negative rod. *B. melaninogenicus* subsp. *melaninogenicus* was cultured from three abscesses. Gliding rods, recently described as members of a new genus, *Capnocytophaga* (11), were isolated from a single specimen.

Anaerobic cocci accounted for 25.6% of all isolates. *Peptostreptococcus micros* was the predominant species in this group. It was encountered as the most numerous anaerobic isolate in 5 of the 10 specimens, constituting 17 to 53% of the flora of these abscesses; 17.7% of all isolates cultured (Table 2) belonged to this single species. *Peptostreptococcus anaerobius* was isolated as 27% of the flora from specimen 10, and an unidentified anaerobic gram-positive coccus was the dominant organism (52%) in specimen 2.

Facultative streptococci, including *S. sanguis*, *S. milleri*, and S. intermedius, were each cultured from one abscess. With the exception of *S. sanguis*, they dominated the flora in the abscess from which they were cultured, representing 2, 98, and 47% of the cultivable flora, respectively. *Staphylococcus epidermidis* was the major isolate (66%) from specimen 6, and a single isolate was cultured from specimen 2.

Gram-positive rods were not numerous. Only *Lactobacillus fermentum* and *Eubacterium lentum* could be identified. One additional species of *Lactobacillus* and an unknown anaerobic diphtheroid were also isolated.

DISCUSSION

The relative proportions of various anaerobic and facultative bacterial species in individual acute abscesses are seldom investigated with regard to the etiology of mixed infections. The present study was done after there had been marked advances in anaerobic oral microbiology. The prominence of anaerobic organisms in the mouth, particularly in the dense microbial plaque that accumulates on the teeth and in the subgingival sulcus, and the potential of these oral bacterial populations to serve as reservoirs of ectopic infections, are now generally appreciated. This study confirms in a quantitative manner the predominance of anaerobic bacteria in periapical abscesses, specifically those that have eroded through alveolar bone. Seventy percent of the isolates, chosen on a random basis, were either strict anaerobes or microaerophilic. Facultative gram-positive cocci accounted for only 21% of the isolates. Of the 10 abscesses studied, 9 yielded a mixed flora, with anaerobes predominating in most of them. Such observations are important for several reasons: the pathogenic potential of individual components of multimicrobial or mixed infections, particularly abscesses, needs to be further elucidated, and the presence of bacterial species in these abscesses

TABLE 2. Summary of relative proportions of bacterial isolates in 10 periapical abscesses

Bacterial group	% isolates (no. of abscesses)
Anaerobic gram-negative rods	
Bacteroides asaccharolyticus	0.3 (1)
Bacteroides melaninogenicus	3.8 (3)
Bacteroides ruminicola	2.5 (2)
Bacteroides oralis	2.4 (3)
Bacteroides corrodens	1.8 (3)
Bacteroides distasonis	1.2 (1)
Bacteroides species	8.7 (4)
Capnocytophaga ochracea	0.6 (1)
Fusobacterium nucleatum	14.8 (6)
Fusobacterium species	0.8 (1)
Anaerobic gram-positive cocci	
Peptostreptococcus micros	17.7 (5)
Peptostreptococcus anaerobius	2.7 (1)
Unidentified	5.2 (1)
Facultative gram-positive cocci	
Streptococcus milleri	9.8 (1)
Streptococcus intermedius	4.7 (1)
Staphylococcus epidermidis	6.6 (2)
Anaerobic gram-positive rods	
Lactobacillus fermentum	2.2 (1)
Lactobacillus species	0.5 (1)
Eubacterium lentum	1.4 (1)
Unidentified	3.0 (1)
Facultative gram-positive rods	0
Facultative gram-negative rods	0
Facultative gram-negative cocci	0
Anaerobic gram-negative cocci	0

known to be resistant to penicillin has therapeutic significance.

The general microbial composition of the periapical abscesses examined in this study is comparable with that described in previous reports. All studies report an average of less than five unique species identified per specimen. Sabiston et al. (15) found an average of 3.8 species in a study of 65 dental abscesses of both pulpal and periodontal origin. In a report limited to the bacteriology of periapical abscesses, Oguntebi et al. (13) recovered an average of 2.5 species. The latter investigators excluded the so-called "perio-endo" lesion by excluding teeth with associated periodontal pockets. Our result of 4.5 species per specimen agrees most closely with the report of Brook et al. (3) in which an average of 4.9 species was obtained from periapical abscesses in children. This consistency among studies is particularly noteworthy in light of the fact that "representative colony types" were isolated and characterized in the other three studies, whereas we characterized each of 100 randomly selected colonies per specimen.

Anaerobic bacteria were recovered in the absence of aerobes in 6 of 10 specimens (60%) examined in this study, 8 of 12 cases (67%) studied by Brook et al. (3), and only 1 of 10 cases (10%) reported by Oguntebi et al. (13). This information is not directly accessible in the report by Sabiston et al. (15). However, these authors did report facultative streptococci in 41 of 58 culturally positive specimens and estimated them to be greater than 20% of the total isolates in 18 specimens. Facultative streptococci were identified as 9 of 25 total strains recovered from 10 abscesses studied by Oguntebi et al. (13). Of their anaerobic strains, only four species were identified: *B. melaninogenicus* subsp. *intermedius*, *F. nucleatum*, *P. anaerobius*, and *P. micros*. It should be noted here that the Oguntebi group cultured aspirates of nonfluctuant lesions which had not yet penetrated through the alveolar bone by puncturing through that bone with the aspirating needle. It is thus possible that the Oguntebi group selected for lesions which do not "point" or fistularize. A far greater diversity of anaerobic species is characteristic of the abscess flora reported both in the present study and in that of Brook et al. (3), 17 and 19 species from 10 and 12 cases, respectively.

The two species most commonly encountered among the isolates from the periapical abscesses cultured in this study, *F. nucleatum* and *P. micros*, are prominent asaccharolytic organisms cultured from diseased periodontal pockets (16, 18, 19). They are also important constituents of acute periodontal abscesses (12). The latter type of abscesses originate beneath the gingiva if the coronal portion of the sulcus or periodontal pocket becomes occluded by food impaction or gingival shrinkage, such as that accompanying a reversal of the local inflammatory processes. *F. nucleatum* was similarly the most prominent anaerobe encountered in the study of Oguntebi et al. (13). However, the latter authors report *S. mitis*, rather than the anaerobic coccus *P. micros*, to occur frequently in association with the fusobacterium.

One of the goals of a quantitative bacteriological analysis such as this is to eventually be able to point out certain of the more common or successful combinations of bacteria responsible for oral abscess formation, as well as those responsible for those abscesses that develop from hematogenic spread of oral organisms. A comparison with the bacteriology of necrotic dental pulp is informative in this regard. In a study with a nonselective colony isolation procedure analogous to ours, Kantz and Henry (10) characterized the flora of 16 culture-positive pulp chambers of traumatized, nonvital teeth. A variety of anaerobes were cultured, with isolates of *F. nucleatum* being the most numerous. Facultative streptococci were prominent, but only one patient yielded anaerobic cocci (*Peptococcus morbillorum*). *Bacteroides*, *Corynebacterium*, *Peptostreptococcus*, and *Fusobacterium* species dominated the anaerobic isolates in a semiquantitative study of the microorganisms recovered from 54 culturally positive pulp chambers by Bergenholtz (2). In an attempt to determine the relative importance of the various organisms isolated from infected pulp in potential abscess formation, Sundqvist et al. (17) used combinations of bacteria isolated from the root canals of teeth with necrotic pulps and periapical bone destruction to test for the capacity to induce abscess formation and transmissible infections when inoculated subcutaneously into guinea pigs. All combinations which gave transmissible infections contained strains *B. melaninogenicus* or *B. asaccharolyticus*. *P. micros* also appeared to be essential for achieving pathogenicity. It would thus appear that a list of significant oral pathogens is emerging from studies such as these.

Although the primary therapeutic modality for most odontogenic infections is incision and drainage, appropriate antibiotic therapy is important in halting local as well as hematogenous spread of oral infection. The antibiotic most frequently used for treating orofacial infections is penicillin. However, there is no doubt that anaerobes can no longer be considered predictable in their susceptibility to antibiotics. *Bacteroides* species now commonly found to be resistant to penicillin are encountered in oral infections.

In a study of 61 pyogenic dental infections, Kannangara et al. (9) isolated organisms reported as *Bacteroides fragilis* from 18 infections (12 mandible fractures, 4 dental abscesses, and 2 postsurgical infections). Six of their *B. fragilis* isolates were resistant to penicillin at 16 μg/ml. Chow et al. (5) reported the isolation of *B. fragilis* from 3 of 13 cases of mandibular osteomyelitis. Organisms reported as *B. melaninogenicus* were isolated by Chow and co-workers from 1 of 4 periapical abscesses, 6 of 14 fascial-space abscesses, and 8 of 13 cases of mandibular osteomyelitis. In a study of the susceptibility of respiratory tract anaerobes, many of which are derived from the oral flora, Busch et al. (4) found 35 to 45% of all *Bacteroides* isolates to be resistant to penicillins G or V at concentrations approximating peak serum levels achieved after oral administration. Of the 64 strains of *Bacteroides* tested, 25 were *B. melaninogenicus*. Beta lactamase production was detected in isolates recovered from cultures of 4 of the 12 periapical abscesses in children studied by Brook et al. (3). These included 3 to 9 isolates of the *B. melaninogenicus* group and one of three *Bacteroides oralis* isolates. Careful speciation and in vitro susceptibility testing are therefore going to be necessary to provide better guidance for the therapy of oral infections, since increasing reports of beta lactamase production in anaerobes may soon make penicillin therapy without susceptibility testing a questionable practice in these situations.

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