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# Gingival Flora of the Dog with Special Reference to Bacteria Associated with Bites[1]

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Gingival scrapings from dogs were examined to determine their aerobic bacterial flora. Of particular interest was the frequent recovery of three unclassified groups of aerobic gram-negative bacteria, IIj, EF-4, and M-5, previously associated with human dog-bite infections. Although no set pattern was found between the variability and consistency of gingival microbiota as related to age, sex, or breed of dog, a certain characteristic flora can be predicted in the healthy canine gingiva. Members of the following genera were found: *Streptococcus, Staphylococcus, Actinomyces, Escherichia, Corynebacterium, Pasteurella, Caryophanon, Mycoplasma, Acinetobacter, Moraxella, Neisseria, Enterobacter,* and *Bacillus.*

Studies of the gingival flora of the dog are of special interest as this animal has a gingival crevicular epithelium similar to that found in humans (8). No reports were found dealing specifically with the bacterial flora of the canine gingiva.

The organisms found within the oral cavity of domestic animals consist of many commensals and potential pathogens (12, 14, 15). Studies of the oral bacterial flora of man and animals reveal the occurrence of many of the same major groups or genera of bacteria. Most of these organisms are nonpathogenic but several species can cause disease most frequently involving the oropharyngeal mucosa as a result of irritation, trauma, penetration of foreign bodies such as bones or sticks, carious teeth, and neoplasms (6). It is now well known that certain organisms presumably of the canine and feline oral cavity infect wounds resulting from animal bites. Animal bite wounds in humans are frequently infected with *Pasteurella multocida* (3, 5, 10).

The purpose of this investigation was to isolate and identify the aerobic bacterial flora found in gingival scrapings of dogs. Particular attention was directed to those organisms previously reported to be associated with human infections resulting from dog bites.

## MATERIALS AND METHODS

**Collection of material.** Gingival scrapings were taken, using sterilized gauze pads (Steri-Pad, 2 by 2 12 ply, Johnson & Johnson, New Brunswick, N.J.) and forceps, from 50 dogs in the East Lansing, Mich., area. The gauze was applied vigorously along the junction of the teeth and gums of both the upper and lower jaws. Only healthy dogs were included.

**Cultural and identification procedures.** Immediately after collection, the gauze pad was immersed in 10 ml of nutrient broth (Difco) in a small sterile flask. All samples were thoroughly agitated and then streaked 15 min after collection on blood agar plates (6% bovine blood), MacConkey plates, and PPLO agar (Difco). Blood agar plates containing 4% agar to prevent swarming of *Proteus* species were routinely inoculated. Plates were incubated at 37 C in the presence of 4% $CO_2$ for 24 h, examined, and then reincubated for an additional 24 to 48 h. Each different colonial type was subcultured. The colonial and cellular morphology of all strains was recorded and the biochemical characteristics of the organisms were determined by standard procedures. Each organism was identified, when possible, with the aid of generally accepted criteria (2, 16).

**Antibiotic susceptibility tests.** Antibiotic susceptibility tests were conducted on seven isolates of group IIj and five strains of group EF-4. The standard Kirby-Bauer procedure (1) was used on the IIj strains and the same procedure was used on the EF-4 isolates except that tryptose agar (Difco) plus 0.3% yeast extract base (Difco) was used instead of Mueller-Hinton agar because of poor growth on the latter.

## RESULTS

Scrapings from 50 dogs were examined. The organisms isolated included group IIj, group EF-4, *Moraxella* (including *M. phenylpyruvica, M. nonliquefaciens,* M-4, and M-5), *Enterobacter aerogenes, Escherichia coli, Acinetobacter calcoaceticus* (var. *lwoffi* and *anitratus*), *Pasteurella* (including *P. multocida* and *Pasteurella*-like organisms), *Neisseria, Staphylococ-*

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cus aureus, Staphylococcus epidermidis, and other Micrococcaceae, and hemolytic streptococci, Corynebacterium, Actinomyces (including A. viscosus), Bacillus, Caryophanon, and Mycoplasma.

**Group EF-4.** Bacteria of this type consisted of small gram-negative, short, rod- to coccoid-shaped organisms. Colonies averaged 1 to 2 mm in diameter and were circular, entire, opaque, convex, and mucoid and sometimes produced a yellow pigment. They were nonhemolytic, although an occasional greening of blood agar was observed. Typical biochemical reactions are listed in Table 1. Thirty percent of the dogs examined harbored this organism (Table 2).

**Group IIj.** Colonies of this group were circular, entire, translucent, smooth, glossy, and butyrous, and very sticky, making them difficult to remove from solid media. Growth in broth was poor. No hemolysis was seen on blood agar plates, although greening was occasionally observed around colonies. Organisms were gram-negative, medium-length rods. Most of the organisms in cultures died within 1 week. Table 1 lists the differential reactions of group IIj. A relatively large inoculum on Christensen urea agar showed rapid hydrolysis. Group IIj was seen in 38% of the dogs examined.

***Moraxella.*** Members of this genus were rod shaped or coccoid, usually occurring in pairs or chains. Of the 40% of the canine samples harboring bacteria of this type (Table 2), M-5 was the most frequent isolate (Table 3). M-5 resembled other bacteria of this genus and was identified by the biochemical reactions listed in Table 1. It was nonhemolytic and produced a soluble yellow or tan pigment.

***Pasteurella* and *Pasteurella*-like organisms.** These gram-negative coccoid to small rod-shaped organisms were found in 22% of the dogs (Table 2). Half of these isolates, identified from the reactions shown in Table 1, consisted of organisms typical of *P. multocida*. However, organisms similar in colonial and cellular morphology, but differing in key biochemical reactions from *P. multocida*, were also found and grouped as *Pasteurella*-like organisms (Table 1). These organisms, which appeared to belong to the *Pasteurella* genus, did not conform to any recognized species by accepted criteria.

***Caryophanon.*** This motile, filamentous, multicellular eubacterium (see Fig. 1) was found in 10 out of 50 dog samples (Table 2). It was identified as in other studies on the basis of its characteristic microscopic morphology. Some discrepancies exist in the literature relating to the gram reaction of *Caryophanon*, but the organisms recovered in this survey were distinctly gram negative.

***Neisseria.*** Isolates of *Neisseria catarrhalis*, more recently reclassified as *Branhamella catarrhalis* (12), were found in 5 dogs (Table 3). The other five isolates identified as *Neisseria* were gram-negative, oxidase-positive cocci that produced a yellow pigment. They could not be identified with known species.

***Actinomyces.*** Five isolates of *A. viscosus* were found in gingival scrapings (Table 3). The organism was identified on the basis of delayed growth pattern (48 to 72 h), a crumbly, off-white, and dry colonial morphology, gram-positiveness, nonacid-fastness, filamentous to "Y-shaped" cellular configuration, and the biochemical reactions shown in Table 1. Three isolates with similar colonial and cellular morphology but different biochemical characteristics were also recovered.

***Corynebacterium.*** Twenty-six percent of the canine samples yielded diphtheroids. Their colonies were all nonhemolytic, 0.5 to 1.0 mm in diameter, and white. The different strains varied in their biochemical reactions. Glucose and maltose were fermented in all cases, but lactose and sucrose fermentation varied (Table 1).

***Mycoplasma.*** These organisms, isolated on PPLO agar (Difco), had a characteristic "fried egg" appearance. Colonies were pleomorphic, extremely small on the agar medium, and opaque, with a yellowish central area. These organisms were recovered from 35 of the 41 dogs sampled. No attempt was made to speciate them.

**Miscellaneous unidentified bacteria.** A number of organisms isolated from gingival scrapings of dogs produced a yellow pigment, were nonmotile, weakly or nonfermentative, and catalase positive (Table 3). These bacteria were gram-negative, small, thin, rod- to coccoid-shaped cells and resembled species of *Flavobacterium*.

Organisms yielding granular, dry, nonhemolytic, rhizoid colonies were frequently recovered from dogs. Colonies were pinpoint in 24 h, but increased in size following further incubation. These filamentous organisms stained gram variable, were inactive biochemically, and could not be identified with known genera.

**Streptococci and staphylococci.** Alpha-hemolytic streptococci were isolated more frequently than other organisms, with the exception of *Mycoplasma*, and made up a large part of the flora of gingival scrapings from these dogs (Table 2). Isolates of beta- and gamma-hemolytic streptococci were much less frequent in comparison (Table 3). Of the 35 isolates of *Micrococcaceae*, over 45% consisted of *S. epidermidis* and 25% were yellow to white, pigmented, $\beta$-hemolytic *S. aureus* (Table 3).

TABLE 1. *Criteria used in the identification of some unusual aerobic microorganisms found in gingival scrapings from dogs*[a]

| Organisms | Catalase | Oxidase | O/F test | Glucose | Lactose | Sucrose | Maltose | Mannitol | TSI sl/butt | TSI gas/$H_2S$ | Urease | Simmon citrate | MacConkey agar | Gelatinase | Motility | Indole | Nitrate reduction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group IIj[b] | + | + | NG | − | − | − | − | − | $N^v$/N | −/− | + | − | − | + | − | + | − |
| Group EF-4[b] | + | + | F | A | − | − | − | − | K/$A^v$ | −/− | − | − | $+^w$ | $-^v$ | − | − | $+^v$ |
| M-5[b] | + | + | I | − | − | − | − | − | K/$K^v$ | −/− | − | − | $+^w$ | − | − | − | − |
| *Corynebacterium* species | + | | F | A | V | $A^v$ | A | | | | − | | − | | − | | − |
| *Actinomyces viscosus* | + | | F | (A) | $A^v$ | A | A | − | | | − | | | − | | − | + |
| *Acinetobacter calcoaceticus* | | | | | | | | | | | | | | | | | |
| var. *lwoffi* | + | − | I | − | − | | − | − | K/N | −/− | V | V | + | $-^v$ | − | − | − |
| var. *anitratus* | + | − | O | + | (+) | | $-^v$ | − | K/N | −/− | V | $+^v$ | + | V | − | − | − |
| *Pasteurella multocida* | + | + | F | A | $(-)^v$ | A | $(-)^v$ | V | A/A | −/− | − | − | − | − | − | + | + |
| *Pasteurella*-like | + | + | F | A | − | A | $A^v$ | | A/A | −/− | − | − | − | − | − | − | + |
| Unknown | + | + | OF | w | w | w | w | w | N/N | ***[c] | − | − | − | $+^d$ | − | − | − |

[a] TSI, triple sugar iron; *** $H_2S$, 4+ reaction on lead acetate paper, no gas in TSI; NG, neutral; F, fermentative; O, oxidative; v, occasionally variable; K, alkaline; A, acid; w, weak positive; V, variable; (+), most strains positive; (−), most strains negative; d, delayed; I, inactive; sl, slant.

[b] Reactions from Tatum et al. (16) with modifications.

[c] An organism frequently observed but not identifiable at present.

**Antibiotic susceptibility tests.** The results of the antibiotic susceptibility tests on groups IIj and EF-4 are presented in Table 4. The group IIj organisms showed wide and rather consistent susceptibility to widely used drugs with the exception of polymyxin B and sulfadimethoxine. Group EF-4 strains were more variable in their susceptibility, displaying considerable resistance to lincomycin, neomycin, and penicillin.

**Comparison on the basis of sex and breed.** Twenty-three female and 27 male dogs were examined. No differences were found in gingival scrapings between males and females. Also, no differences in aerobic flora were observed when dogs were grouped according to the American Kennel Club classification of breeds.

TABLE 2. *Frequency of isolation of aerobic bacteria recovered in gingival scrapings from 50 dogs*

| Bacteria | No. of positive samples | Incidence (%) |
|---|---|---|
| Gram negative | | |
| *Moraxella* | 21 | 40 |
| Group IIj | 20 | 38 |
| Group EF-4 | 15 | 30 |
| *Escherichia coli* | 11 | 22 |
| *Pasteurella*[a] | 11 | 22 |
| *Caryophanon* | 10 | 20 |
| *Neisseria*[b] | 10 | 20 |
| *Acinetobacter calcoaceticus* | 5 | 10 |
| *Enterobacter* | 1 | 2 |
| Gram positive | | |
| Streptococci | 41 | 82 |
| *Micrococcaceae* | 30 | 60 |
| *Corynebacterium* | 13 | 26 |
| Actinomycetes | 7 | 14 |
| *Bacillus* | 6 | 12 |

[a] Includes *Pasteurella*-like organisms.
[b] Includes *Branhamella catarrhalis*.

## DISCUSSION

The Center for Disease Control, Atlanta, Ga. (CDC) reported on 36 cultures of group IIj, 17 from human lesions resulting from bites or scratches of dogs and cats. The remainder were mostly recovered from other human specimens (16). There is as yet no evidence that group IIj causes disease in the dog. Without guanine-cytosine percentages and base homologies one can only speculate on generic classification at this time. Considering the characteristics described in Table 1 and its nonfermentative nature, group IIj appears to resemble the genera *Moraxella* (specifically *M. phenylpyruvica*) and *Brucella* (specifically *B. canis*) most closely.

CDC reported on 85 strains of group EF-4, 66 of which were recovered from humans, and of these 66, 34 had a human origin and 32 were from humans who had been bitten by dogs or cats (16). Group EF-4 strains have not been incriminated as a cause of disease in the dog. On the basis of the characteristics listed in Table 1, group EF-4 appears to resemble most closely the genera *Pasteurella* and *Actinobacillus*.

On the basis of biochemical reactions (Table 1) and cellular and colonial characteristics group M-5 bacteria resemble the genus *Moraxella* (16). Of 41 cultures studied at CDC, 25 were recovered from infected wounds caused by dog bites. There is yet no evidence that suggest M-5 causes disease in the dog.

TABLE 3. *Frequency of isolation of aerobic bacterial species recovered in gingival scrapings from 50 dogs*

| Gram-negative | No. of positive samples | Incidence (%) | Gram-positive | No. of positive samples | Incidence (%) |
|---|---|---|---|---|---|
| *Moraxella* | | | Streptococci | | |
| *M. phenylpyruvica* | 3 | 14.3 | Alpha-hemolytic streptococci | 36 | 80.0 |
| *M. nonliquefaciens* | 2 | 9.5 | Beta-hemolytic streptococci | 5 | 11.1 |
| M-5 | 9 | 42.8 | Gamma-hemolytic streptococci | 4 | 8.9 |
| M-4 | 1 | 4.8 | | | |
| *Moraxella* species | 6 | 28.6 | | | |
| *Pasteurella* | | | *Micrococcaceae* | | |
| *P. multocida* | 6 | 50.0 | *Staphylococcus aureus* | 9 | 25.7 |
| *Pasteurella*-like | 6 | 50.0 | *Staphylococcus epidermidis* | 16 | 45.7 |
| | | | Other species | 10 | 28.6 |
| *Neisseria* | | | Actinomycetes | | |
| *B. catarrhalis*[a] | 5 | 50.0 | *A. viscosus* | 5 | 62.5 |
| *Neisseria* species | 5 | 50.0 | *Actinomyces* sp. | 3 | 37.5 |
| *Acinetobacter calcoaceticus* | | | | | |
| var. *lwoffi* | 4 | 80.0 | | | |
| var. *anitratus* | 1 | 20.0 | | | |

[a] Present classification: *Branhamella catarrhalis*, formerly *Neisseria catarrhalis*.

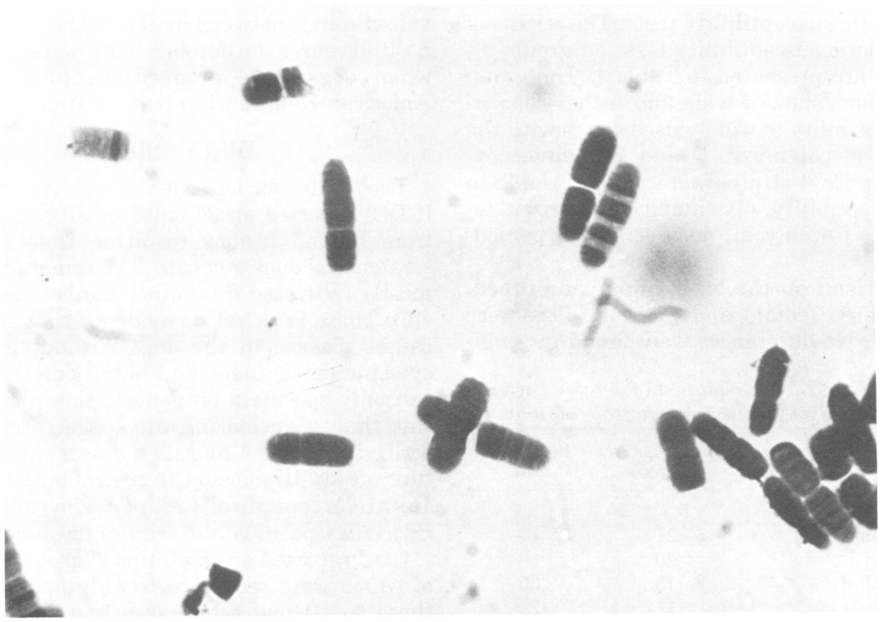

FIG. 1. *Gram-stained smear of Caryophanon cultures from the canine gingiva (×1600 approximately).*

TABLE 4. *Results of antibiotic susceptibility tests on 7 group IIj and 5 group EF-4 strains*[a]

| Antimicrobial agents | IIj strains | | | | | | | EF-4 strains | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 |
| Ampicillin | S[b] | S | S | S | S | S | S | S | S | S | S | S |
| Cephalothin | | | | | | | | S | R[c] | S | S | R |
| Chloramphenicol | S | S | S | S | S | S | S | S | S | S | S | S |
| Gentamicin | S | S | S | S | S | S | S | S | S | S | S | S |
| Lincomycin | S | S | S | S | S | S | S | R | R | S | S | R |
| Neomycin | S | S | E[d] | E | R | S | S | R | R | R | R | R |
| Nitrofurantoin | S | S | S | S | S | S | S | S | S | S | S | S |
| Novobiocin | | | | | | | | S | S | S | S | S |
| Penicillin | S | S | E | E | S | S | S | R | R | S | R | R |
| Polymyxin B | R | R | R | R | S | S | R | S | S | S | S | S |
| Sulfadimethoxine | R | R | R | R | S | S | S | S | S | S | S | S |
| Tetracycline | S | S | S | S | S | S | S | S | S | S | S | S |

[a] As interpretive standards have not been determined for IIj and EF-4 organisms, and because tryptose agar plus 0.3% yeast extract was used instead of Mueller-Hinton agar for the latter group, the results of antibiotic susceptibility tests should be considered tentative.
[b] S, Sensitive.
[c] R, Resistant.
[d] E, Equivocal.

*Caryophanon* was described as a gram-positive, motile, large rod or filament and was originally isolated from cow dung (8). *Caryophanon* has been seen not infrequently in Wright-stained smears from oral mucosa of dogs. Attempts were made without success to recover *Caryophanon* from several dogs that had yielded it earlier. It may be that *Caryophanon* is a transient and is unable to establish permanent residence in the gingiva.

The organisms classified as *Pasteurella*-like differed from *P. multocida* in their negative indole and occasional acid from maltose reaction. Smith (13) reported that dog strains of *P. multocida (P. septica)* frequently possessed such special characteristics as acid production from maltose, but not xylose and mannitol, and low pathogenicity for mice, saline and acid sensitivity, and absence of capsules. Other *Pasteurella*-like organisms recovered from humans bitten by dogs or cats were described as producing some gas from glucose (R. E. Wever, 1970, Seminar on Current Topics in Microbiology, 70th Meeting, American Society for Microbiology, Boston, Mass.).

*A. viscosus* was implicated as the causative agent of periodontal disease with subgingival plaque in hamsters (9). Although pathogenicity for man has not been observed, *A. viscosus* has been isolated from the human oral cavity (2). In this study, *A. viscosus* was found in the gingiva of five dogs with clean teeth and healthy gums. This organism has been reported as the cause of six cases of actinomycosis in dogs (4).

Although there was considerable variation in the oral flora among individuals, a number of organisms were recovered with fair consistency. These included streptococci, staphylococci, *Mycoplasma*, three gram-negative bacteria associated with dog bites in humans, namely groups IIj, EF-4, and M-5, and occasionally such potential pathogens as *Pasteurella* and *Actinomyces*. *Caryophanon*, *Neisseria*, *Acinetobacter*, *Corynebacterium*, and *Bacillus* were sporadically isolated.

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