Aerobic Bacterial Flora of Oral and Nasal Fluids of Canines with Reference to Bacteria Associated with Bites[†]

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Oral and nasal fluids of 50 dogs were examined to determine the prevalence of aerobic bacteria frequently associated with animal bite wounds. The most frequently isolated microorganisms included: IIj, EF-4, *Pasteurella multocida*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, group D streptococci, *Corynebacterium* sp., enterobacteria, *Neisseria* sp., *Moraxella* sp., and *Bacillus* sp. Other species and genera were infrequently recovered and may represent transient flora. The high incidence of IIj, EF-4, *P. multocida*, and *S. aureus*, all known human pathogens, suggests that they should be considered as probable contaminants in bite wounds.

The incidence of animal bites is of increasing concern. A surveillance program in 12 states showed attack rates of 215 to 809 per 100,000 population. A total of 84% of the bites were by dogs (28). *Pasteurella multocida* has been frequently incriminated in infections resulting from animal bites (7, 10, 11, 14, 27). Other species of bacteria less commonly associated with bite wounds are *P. pneumotropica* (31), an unidentified gram-negative rod (5), group EF-4 (EF-4), group M-5 (M-5), and IIj (26). Knowledge of the incidence of these species and others likely to contaminate dog bite wounds would be valuable.

Several studies have been conducted on oral, throat, and nasal flora of the canine (1-3, 6, 12, 17, 18, 21-23, 30). Only one reported isolating any of the newly recognized species. Saphir and Carter examined the gingival flora of 50 dogs in Michigan (21) and recovered several species previously unreported from the mouth of the dog.

A species of bacteria not previously recognized was recovered from a nasal swab of a dog admitted to the Kansas State University Veterinary Teaching Hospital in September, 1972, with a complaint of respiratory distress. Because of concern regarding its etiological significance, a subculture was submitted to the Center for Disease Control (CDC) for identification. It was an unnamed species that had been given the alphanumeric designation group IIj. Several of the CDC isolates had been recovered from humans with infected animal bite wounds. The cultural characteristics of this species have been reported (26). The realization in 1972 that IIj was present but largely unrecognized in the upper respiratory tract of the canine raised questions as to its incidence. Independent of Saphir and Carter, we began a survey to determine the incidence of IIj and other aerobic species of bacteria in the oral and nasal fluids of the dog. Such information would provide a basis to assess significance of future isolations of IIj and its role as a pathogen in the dog and potential pathogen of humans.

MATERIALS AND METHODS

Collection of specimens. Fluids were collected on sterile cotton-tipped applicators from oral and nasal vestibules of 50 dogs of assorted ages, sexes, and breeds. Subjects were routine admissions to the veterinary teaching hospital or animals maintained for teaching at the College of Veterinary Medicine, Kansas State University. No animals had apparent bacterial infections or were receiving antimicrobial therapy. In an attempt to sample a representative portion of the population, specimens were collected at irregular intervals.

Isolation and identification procedures. Within 30 min of collection, specimens were directly streaked for isolation onto tryptic soy blood agar, MacConkey agar, mannitol salt agar, and phenylethanol blood agar (Difco Laboratories). A 5% concentration of citrated bovine blood was added to tryptic soy blood agar and phenylethanol blood agar. All media were incubated at 35°C in an atmosphere of 5% CO₂. After 24, 48, and 72 h of incubation, the plates were examined and different colonial types were subcultured by streaking for isolation. After selection of isolated colonies from the tryptic soy blood agar, the area of initial inoculation was thoroughly searched with a loop for viscous growth. This type of growth, characteristic for many group IIj isolates, was subcultured by streaking for isolation. After subculture, colonial and cellular mor-

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phology were reported for each strain isolated. Each pure culture was identified, when possible, with the aid of generally accepted procedures and keys (4, 9, 15, 24, 29).

Antimicrobial sensitivity tests. Antimicrobial sensitivity was conducted on 10 isolates each of group II and EF-4. Tests were conducted by standardized single disk diffusion (16) with the following exceptions. Group II tests were conducted on tryptic soy blood agar, and EF-4 tests were conducted on Mueller-Hinton agar supplemented with 5% citrated bovine blood in 100-mm petri plates with a 4-mm medium depth. Two antimicrobial disks were placed on each plate. Parallel controls with *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were conducted on the alternative and standard media.

RESULTS

Aerobic bacteriological examination of oral and nasal fluids of 50 dogs resulted in isolating microorganisms belonging to 30 different genera. Additional isolates were placed into nine different CDC alphanumeric designations and five groups of unidentifiable species. Microorganism recovery frequencies are presented in Table 1.

Microorganisms identified as to the species recovered from 50% or more of the dogs included: IIj, EF-4, *P. multocida*, *S. aureus*, and *S. epidermidis*. Genera recognized at such a frequency were *Streptococcus*, *Corynebacterium*, *Neisseria*, *Moraxella*, and *Bacillus*. Many species within these genera were identified. Species in the family *Enterobacteriaceae* were also recovered in the above 50% frequency range.

The most frequently isolated species known to be associated with bite wounds or potentially pathogenic were IIj, EF-4, *P. multocida*, and *S. aureus*. Less frequently recovered species in this category were group M-5, *P. pneumotropica*, and unidentified no. 5. Other species potentially pathogenic to man were recovered at low frequencies.

CDC alphanumeric species. The most frequently isolated species was IIj. Two different colonial types were recognized. The most predominant type was very viscous, a characteristic useful in searching for isolates and as an aid to preliminary identification. A less frequently recovered colonial type was butyrous. Both types had identical biochemical characteristics. One animal had both types in its nasal fluids, and nine had both types in oral fluids. One or both types were recovered from the nasal fluids of 30% and from the oral fluids of 90% of the dogs. When the microorganism was recovered from nasal fluids, it was also recovered from oral fluids. More capsular material on the cells of the viscous type, as determined by capsular stains, appeared to be the primary difference between the two colony types.

Sufficient criteria to identify this species are available (26). The most useful single criterion that we found for tentative identification was its rapid production of alkalinity on Christensen urea agar. Gram-negative, oxidase-positive microorganisms with a compatible colonial morphology were urea positive within 5 min. In most instances, further biochemical examination confirmed an identification of IIj.

The second most frequently isolated microorganism, EF-4, was recovered from 82% of the dogs. Strains were typical of those described (26). Additional alphanumeric strains were recovered infrequently.

Unidentifiable species. Five species unidentifiable with available keys were recovered at low frequencies. Unidentifiable species no. 1, 2, 3, and 4 were extremely fastidious, gram-negative, strict aerobes that grew slightly better under 5% increased carbon dioxide tension. Unfortunately, the strains died before extensive biochemical characterization was completed. Reactions determined are presented in Table 2.

Unidentifiable species no. 5, recovered from the oral fluids of four dogs, was a particularly interesting microorganism. R. E. Weaver of CDC forwarded three strains of an unidentified microorganism (C3556, C7570, and C8936) to compare with our unidentified strains. His strains had been recovered from septicemias in humans with a history of dog bites (R. E. Weaver, personal communication). Our unidentified species no. 5 was identical to his strains. They were also identical to an unidentified gram-negative rod isolated from blood cultures of 17 humans, 10 of whom had histories of a recent dog bite (5). The next edition of The Identification of Unusual Gram-Negative Bacteria will refer to this microorganism as DF-2 (Weaver, personal communication).

This microorganism produced a greenish discolorization of bovine erythrocytes under areas of heavy growth and in stabs. Isolated colonies were 0.5 to 1.0 mm in diameter after 72 h of incubation at 35° C under 5% increased carbon dioxide tension. Colonies were smaller when incubated under aerobic conditions, and no growth was obtained anaerobically. The colonies were gray, semitranslucent, circular, convex, smooth, butyrous, and glistening, with an odor similar to that produced by certain *Alcaligenes* sp. and *Pseudomonas* sp.

Cells stained Gram negative were nonmotile, fusiform, long, thin, and moderately pleomorphic. Some cells were barred or vacuolated. Stains for polymetaphosphate, glycogen, and polybetahydroxybutyric acid failed to reveal the nature of this irregular staining. The microorganism was fastidious and failed to grow in some

	acteria from oral and nasal fluids of 50 dogs No. of dogs harboring					
Microorganisms	Nasal	Oral	Nasal and oral	Total		
CDC alphanumeric designations						
Group IIj	15	45	15	45		
Group EF-4	21	37	17	41		
Group M-5		6		6		
Group TM-1		2		2		
Group HB-5		2		2		
Group M-4f		1		1		
Group Ve-biotype 2		1		1		
Group IIK-biotype 1	1	1		2		
Group IVe	1	1		2		
Unidentifiable species		_				
No. 1		2		2		
No. 2	1	2		3		
No. 3	2	3		5		
No. 4	1	1		2		
No. 5		4		4		
Gram-negative aerobic rods and cocci	_					
Pseudomonas cepacia	3	1		4		
P. denitrificans		1		1		
P. aeruginosa	1	3		4		
P. putida	2	1		3		
P. fluorescens	1	2		3		
P. diminuta	1			1		
P. maltophila		1		1		
P. stutzeri		1		1		
Alcaligenes denitrificans	1	1	1	1		
Brucella canis	2			2		
Bordetella bronchiseptica	1	3	1	3		
Gram-negative cocci and coccobacilli						
Neisseria flavescens	14	10	3	21		
N. mucosa	4	1	1	4		
N. sicca	2	2		4		
Neisseria sp.	4	1		5		
Branhamella catarrhalis	5	1		6		
Moraxella osloensis	3	4	1	6		
M. phenylpyruvica		1		1		
M. nonliquefaciens	1			1		
Moraxella sp.	12	13	3	22		
Acinetobacter calcoaceticus						
subsp. anitratis	3	3	2	4		
subsp. lwoffi	1	3	-	4		
Gram-negative facultatively anaerobic rods						
Escherichia coli	8	11	5	14		
Klebsiella pneumoniae	2	5	1	6		
Enterobacter agglomerans	$\overline{2}$	1	1	2		
E. cloacae	1	-	-	1		
E. aerogenes	-	1		1		
Serratia marcescens		ī		1		
Proteus mirabilis		1		1		
Aeromonas hydrophila	1	2	1	2		
Flavobacterium sp.	2	-	*	$\frac{1}{2}$		
Haemophilus aphrophilus	3	3		6		
Pasteurella multocida	7	30	4	33		
P. gallinarum	2	4	1	5		
P. pneumotropica	-	2	-	2		

TABLE 1. Isolation frequency of bacteria from oral and nasal fluids of 50 dogs

	No. of dogs harboring					
Microorganisms	Nasal	Oral	Nasal and oral	Total		
P. ureae	1			1		
Pasteurella sp.	6	8		14		
Actinobacillus lignieresii		1		1		
Streptobacillus sp.	3	6	1	8		
Eikenella corrodens	3	8	2	9		
Gliding bacteria						
Simonsiella sp.	1	9		10		
Gram-positive cocci						
Micrococcus sp.	11	4	1	16		
Staphylococcus aureus	30	21	15	36		
S. epidermidis	23	20	11	32		
Streptococcus sp. group D	12	24	4	32		
Streptococcus sp. group G	1	7		8		
Streptococcus sp. group M		1		1		
Streptococcus sp. group F	1	1		2		
Streptococcus sp. group E		3		3		
Endospore-forming rods						
Bacillus cereus	7	5		12		
B. subtilis	5	6		11		
B. megaterium		2		2		
B. pumilus	2	$\overline{2}$		4		
B. circulans	3	1		4		
Bacillus sp.	2	2	2	2		
Gram-positive asporogenous rod-shaped bacteria						
Lactobacillus sp.	3	4		7		
Actinomycetes and related organisms						
Corynebacterium sp.	15	30	11	34		
Brevibacterium acetyliticum		1		1		
Actinomyces viscosus		1		1		
Streptomyces sp.	1			1		

TABLE	1-Continued	
I ADLD	1 - 0 - 0 - 0 - 0 - 0 - 0 - 0 - 0 - 0 -	

differential media even when supplemented with sterile bovine serum. It did not grow on Mac-Conkey agar and was nitrate, indole, urea, and citrate negative. Tests for cytochrome oxidase, catalase, and esculin hydrolysis were positive. Growth was insufficient and acid was not detected in triple sugar iron agar, oxidative-fermentative media, or phenol red carbohydrate broths. Acid was produced from glucose, lactose, and maltose but not from sucrose, xylose, or mannitol in heart infusion broth supplemented with 4% sterile bovine serum without an indicator. A few drops of 1% aqueous bromothymol blue were added after incubation to detect acid production.

Gram-negative facultatively anaerobic rods. Enterobacteria were recovered from 52% of the dogs. Of the seven species in this family recognized, *E. coli* was most frequently encountered.

Four recognizable Pasteurella species were

recovered. *P. multocida* was isolated from 66% of the dogs. Six nasal and eight oral isolates were classified as *Pasteurella* sp. They were similar to those previously reported from the oral cavity of the dog and described as *Pasteurella*-like (21).

Strains referred to as *Streptobacillus* sp. were similar to those described by Smith (23) and referred to as *S. canis*. Placing these strains into this genus was based primarily on microscopic morphology and the precedence cited. They were gram-negative pleomorphic rods to long filaments with one or more oval or pyriform bulbous swellings similar to those described for *S. moniliformis*. All strains were fastidious and required addition of serum to differential media for growth. The microorganism fermented glucose and was oxidase, catalase, and nitrate positive. No reactions were noted in other differential media, and reversion to L-phase variants was not observed. **Gliding bacteria.** We recovered 10 isolates of a gram-negative, multicellular, filamentous microorganism. Filaments were composed of irregular numbers of cells approximately 3 to 4 μ m in their long axis (width of filament) by 0.5 to 1 μ m in their short axis. Terminal cells were decreased in size, forming rounded ends (Fig. 1). These organisms, viewed as they tumbled in a

	Characteristic ^a of organism no.:							
Determination	1	2	3	4				
Colony morphology	Flat, transparent	Tiny, clear, rough, adher- ent	Flat, white to gray	Tiny, white-gray				
Organism morphology	Thin, filamen- tous	Coccobacilli Pleomorphic cocci to fila- ments		Pleomorphic plump rods				
Gram reaction	-	-	– to var.					
		Weak staining						
Motility	_	-	-	-				
Cytochrome oxidase	+	-	_	-				
Triple sugar iron agar	NC/NC	A/NC	K/K	K/NC				
OF-glucose	NC	NC	Oxidizer	NC				
Nitrate	+	Ins. Gr.	_	+				
Urea	-	-	-	-				
Indole	-	Ins. Gr.	_	_				
Citrate	ND	ND	+	ND				
Casein	ND	ND	+	ND				
10% Glucose	ND	ND	Α	ND				
10% Lactose ND		ND	K	ND				

 TABLE 2. Characteristics determined on unidentifiable species no. 1, 2, 3, and 4

a –, Negative; +, positive; NC, no change; ND, not done; A, acid; Ins. Gr., insufficient growth; var., variable; and K, alkaline.

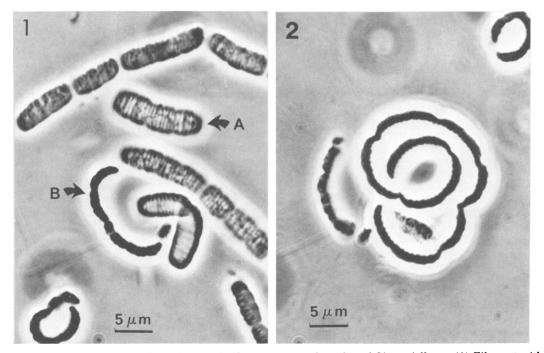


FIG. 1. Phase-contrast photomicrograph of a wet mount of a cultured Simonsiella sp. (A) Filament with the broad surface in the microscopic plane; (B) filament with the narrow surface in the microscopic plane. FIG. 2. Phase-contrast photomicrograph of a wet mount of a cultured Simonsiella sp., coiling of a filament.

flowing wet mount by phase microscopy, had a convex dorsal, concave ventral differentiation (19, 20; J. Pangborn, D. A. Kuhn, and J. R. Woods, First Int. Congr. Bacteriol., 1973, Abstracts II, p. 164). Cultured ribbon-like filaments tended to coil (Fig. 2). Neither motility nor flagella were demonstrated. Slime tracks trailed behind filaments growing with their ventral surface in contact with the substrate, indicating gliding motility. These isolates were identified as members of the genus *Simonsiella* on the basis of characteristic microscopic morphology (18, 20, 24, 25). The isolates were nonhemolytic on bovine, rabbit, and horse blood agar.

Gram-negative aerobic rods and cocci. Organisms of this part were infrequently recovered. The most likely primary pathogen isolated was *Brucella canis*.

Gram-negative cocci and coccobacilli. *Neisseria* sp. were recovered from 68% of the dogs. A total of 29 were identifiable as to species. Five isolates classified as *Neisseria* sp. were strongly oxidase-positive, very dysgonic gramnegative diplococci. They failed to grow in differential media and were placed in this genus on a morphological basis.

A total of 60% of the dogs harbored Moraxella sp. Of the 34 isolates, 9 were identifiable as to species. A total of 25 isolates, referred to as Moraxella sp., were similar to M. phenylpyruvica. They deaminated phenylalanine, hydrolyzed gelatin and casein, but failed to produce urease. They were similar or identical to those phenylalanine-positive strains which are not M. phenylpyruvica but have not been studied in detail and classified (13).

Gram-positive cocci. Micrococci and staphylococci were frequently isolated. *Micrococcus* sp., *S. aureus*, and *S. epidermidis* were frequently recovered from a single dog. *S. aureus*, a potential pathogen, was recovered from 72% of the dogs. Group D streptococci were recovered from 64% of the dogs. Streptococci were grouped on the basis of serological examination. Pathogenic streptococci in groups G, M, F, and E were infrequently isolated.

Endospore-forming rods. *Bacillus* sp. were recovered from 70% of the dogs. Five identifiable species were recognized. An unidentified species of *Bacillus* was isolated from the oral and nasal fluids of two dogs.

Gram-positive asporogenous rod-shaped bacteria. The cultural conditions that we used were not optimal for recovery of lactobacilli. However, members of this genus were recovered from the oral fluids of four and nasal fluids of three dogs.

Actinomycetes and related organisms.

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Bacteria classified as *Corynebacterium* sp. were recovered from 68% of the dogs. They were typical, small, gram-positive pleomorphic rods. They were not identifiable as any of the recognized species and are best classified as diphtheroids. Other microorganisms in this part were infrequently isolated.

Antimicrobial sensitivity test results are presented in Tables 3 and 4. Interpretations of results on group IIj strains must be considered as probable as this species did not grow on Mueller-Hinton agar and tests were conducted on an alternative medium. Zones for the standard S. aureus and E. coli strains fell within expected ranges on the alternative medium (16). The permitted millimeter difference (ATCC 25923 minus ATCC 25922) was not within specifications for two of the agents. The difference for streptomycin should be -1 to 5 and was -3. The difference for tetracycline should be 0 to 6 and was 7. All of the strains tested were susceptible to a variety of commonly used antimicrobial agents. Due to extremely large zone diameters (up to 60 mm) obtained with the IIj strains, it was necessary to limit the number of antimicrobial disks to two per plate. When more than two disks were placed on a plate, overlapping zones precluded accurate measurement or entirely inhibited growth.

DISCUSSION

Various microorganisms were identified in the oral and nasal fluids of dogs. A total of 11 specific species or groups of species were recovered from 50% or more of the dogs. The most frequently isolated microorganisms included: IIj, EF-4, *P. multocida*, *S. aureus*, *S. epidermidis*, group D streptococci, *Corynebacterium* sp., *Enterobacteria*, *Neisseria* sp., *Moraxella* sp., and *Bacillus* sp. Either other species and genera isolated infrequently likely represent transient flora, or conditions of growth were insufficient for their optimal recovery.

Only recently have organisms other than P. multocida been reported as causing infections after animal bite wounds. Of the cultures of IIj, EF-4, and M-5 examined by Tatum et al. (26), 46% were isolated from humans with infected animal bite or scratch wounds. An additional 35% were from wounds or from other extraintestinal sources such as spinal fluids, blood, and sputa for which a complete history of animal contact was unknown. A total of 14% of the cultures were recovered from the upper respiratory tract of dogs and cats (26).

Butler et al. (5) reported recovery of a fastidious, unidentified gram-negative rod from blood cultures of 17 patients with fever, 10 of whom

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Determination			Group II	i				' zone diam nm)	E. coli ^b zone diam (mm)	
	Zone diam (mm)			Interpretation ^c (no. of isolates)			Observed	Expected	Observed	Expected
	Observed range	Mean	\mathbf{SD}^d	s	I	R	Observed	range ^e	Observed	range
Ampicillin	47-65	56.7	6.4	10			32	24-35	17	15-20
Bacitracin	24-40	33.3	5.1	10			17	17-22	6	
Carbenicillin	50-65	55.1	5.1	10			31	_	17	-
Cephalothin	45-60	55.5	5.7	10			34	25-37	18	18-23
Chloramphenicol	38-50	43.5	4.0	10			23	19-26	23	21-27
Clindamycin	30-50	40.8	6.4	10			25	23-29	6	-
Erythromycin	40-52	44.6	4.7	10			23	22 - 30	11	8-14
Gentamicin	20-30	24.8	3.5	10			21	19-27	20	19-26
Kanamycin	12 - 25	18.1	4.1	6	2	2	20	19-26	20	17 - 25
Methicillin	32-40	38.2	3.2	10			21	17 - 22	6	
Nalidixic acid	28-38	34.1	3.5	10			18		24	
Neomycin	20-40	25.9	5.9	10			20	18-26	17	17 - 23
Nitrofurantoin	38-50	44.4	5.5	10			18		21	
Nitrofurazone	36-53	44.1	5.8	10			21		21	
Penicillin	45-60	52.8	5.9	10			30	26 - 37	6	
Polymyxin B	6-22	10.5	6.3	4		6	9	7-13	14	12-16
Streptomycin	24-50	32.3	7.5	10			17	14-22	20	12-20
Tetracycline	30-54	42.5	7.0	10			28	19–28	21	18-25
Trimethaprim- sulfamethoxi- zole	44-60	51.2	5.8	10			25		22	
Triple sulfa	6-34	25.1	8.5	9		1	16		17	

 TABLE 3. Antimicrobial sensitivity of 10 group IIj strains, S. aureus, and E. coli by disk diffusion on tryptic soy, bovine blood agar

^a ATCC 25923.

^b ATCC 25922.

^c This interpretation of sensitivity should be considered tentative since the test was not conducted on Mueller-Hinton agar. S, Susceptible; I, intermediate; R, resistant.

 d SD, Standard deviation.

^e Expected ranges in zone diameter on Mueller-Hinton agar (16).

had a recent dog bite. The syndromes included cellulitis, primary bacteremia, purulent meningitis, and endocarditis. Their microorganism appears identical to four isolates that we designated as unidentified no. 5.

We found all of the above-mentioned human pathogens as a portion of the normal oral or nasal flora of the dog. One or more of the following were isolated from the oral or nasal fluids of all dogs: IIj, EF-4, *P. multocida*, M-5, or unidentified no. 5. Two dogs had four, 27 had three, 17 had two, and 4 had one of the five organisms in their fluids. These five species represented 31% of the total number of isolates from oral fluids and 16.4% from nasal fluids. Most dogs probably carry one or more of the species (all potential human pathogens) as a portion of their normal oral and/or nasal flora. The combination of IIj, EF-4, and *P. multocida* was the most common, occurring in 23 dogs.

P. multocida has been the microorganism

most commonly associated with infected animal bite wounds (9, 10, 11, 14, 17). Other microorganisms are now being recognized in association with bite wounds.

Isolating IIj and EF-4 from oral and/or nasal secretions of 90 and 74%, respectively, of the dogs examined suggests that these organisms would commonly contaminate bite wounds, possibly even more often than *P. multocida*, yet they have gone largely unrecognized. Although little is known of *P. multocida* virulence factors, it may be relatively more virulent than other, more prevalent, pathogenic microorganisms.

Some clinicians and clinical microbiologists view with suspicion or ignore isolates of bacteria not previously recognized as pathogenic or that cannot be positively identified. Past isolates of some of these newly recognized pathogens may have been reported, "no pathogens isolated," for lack of identification or recognition. Considering the frequency with which we recovered these

otood agar									
Determination	Z	one diam (mm)	Interpretation ^a (no. of isolates)						
	Observed range	Mean	SD ^b	Susceptible	Intermediate	Resistant			
Ampicillin	29-43	36.9	5.0	10					
Bacitracin	9-30	19.4	5.8	9	1				
Carbenicillin	37-50	45.6	6.0	10					
Cephalothin	6-39	26.8	14.4	7		3			
Chloramphenicol	37-45	42.1	3.1	10					
Clindamycin	6	6				10			
Erythromycin	22-38	30.8	5.9	10					
Gentamicin	12-23	19.5	4.4	9		1			
Kanamycin	12-23	19.0	4.6	7		3			
Methicillin	6-25	10.6	7.0	3	1	6			
Nalidixic acid	36-46	42.0	4.3	10					
Neomycin	6-18	14.1	3.9	3	4	3			
Nitrofurantoin	25-50	38.2	8.0	10					
Nitrofurazone	24-40	33.3	6.5	10					
Penicillin	20-42	35.6	7.3	9	1				
Polymyxin B	17-24	21.3	3.0	10					
Streptomycin	6-26	15.1	7.8	6		4			
Tetracycline	33-45	39.6	3.8	10		-			
Trimethaprim-sul- famethoxizole	25–35	30.6	3.3	10					
Triple sulfa	30-45	39.8	4.8	10					

 TABLE 4. Antimicrobial sensitivity of 10 group EF-4 strains by disk diffusion on Mueller-Hinton bovine blood agar

^a This interpretation of antimicrobial sensitivity should be considered as tentative because there is a lack of interpretive standards for group EF-4 strains.

^b SD, Standard deviation.

microorganisms, we doubt that they are emerging species. More likely, they have been present for years and only recently recognized.

Of 17 patients with infections of the unidentified gram-negative rod, 15 had debilitating diseases or splenectomy that compromised normal host defense mechanisms (5). It is well known that such individuals are susceptible to agents that otherwise would not be pathogenic.

Many clearly separable species of microorganisms, which lack descriptions and/or a genus and species designation, were recovered. Attempts to place them into a genus now would be pure conjecture. However, we concur with Saphir and Carter (21) that, based on available characteristics, IIj most likely is a *Moraxella* or *Brucella*; EF-4, a *Pasteurella* or *Actinobacillus*; and M-5, a *Moraxella*. Taxonomic studies, including deoxyribonucleic acid base ratio and hybridization, should be completed on these and other unnamed species.

Saphir and Carter reported isolating Caryophanon from the gingival flora of the dog (21). Identification was made on the basis of microscopic morphology. They note discrepancies in regard to the Gram reaction of this microorganism; all of their isolates were gram negative. Members of the genus Caryophanon, as described, are gram-positive cells, which are "large rods or filaments up to 3 μ m in diameter." They are "divided by closely spaced cross walls into numerous disc-shaped cells which are less than 1 μ m long when growth is active." "Transverse fission may give rise to shorter, even spherical forms." Cells are motile by lateral flagella. This microorganism has most frequently been recovered from cow dung (8).

We recovered 10 isolates that clearly fit the description of the genus Simonsiella, a gliding bacterium in the order Cytophagales. It has been recovered from or demonstrated in the oral cavity of a variety of animals (18, 24, 25). Nyby et al. (18) found Simonsiella in the oral cavity of 66 of 67 (98%) of dogs they surveyed and considered it a portion of the normal bacterial flora. Stained smears of our isolates were similar to those presented by Saphir and Carter (21), which they classified as Caryophanon. Their isolates were gram negative, morphologically similar to Simonsiella, and found in the oral cavity of the dog. Caryophanon is gram positive and most commonly associated with cow dung (8). We concur with Nyby et al. (18) that the Saphir and Carter isolates probably were Simonsiella rather than Caryophanon.

Our Simonsiella isolates did not hemolyze bovine, horse, or rabbit erythrocytes. The previously reported species (S. muelleri and S. crassa) hemolyze horse and/or rabbit erythrocytes (24, 25). A Simonsiella sp. from the oral cavity of the dog that differs from the previously described species hemolyzes horse and rabbit erythrocytes (D. A. Kuhn, personal communication). Although not completely characterized, our isolates may represent an additional new species from the oral cavity of the dog.

Since our first recognition of IIj and EF-4, we have routinely isolated both from clinical specimens in dogs and cats, most often from the upper respiratory tract. Because of their high incidence in the oral and nasal cavities, we are not concerned about their pathogenicity there. Both organisms have been recovered in nearly pure or mixed culture from cases of male or female urogenital-tract infections, suppurative dermatitis, gastrointestinal disease, abscesses, and cellulitis. Because dogs and cats lick, these areas are likely to be contaminated with oral and nasal secretions. The true pathogenic significance of these species is yet to be determined; however, when nearly pure cultures are recovered from lesions, an etiological significance must be considered.

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