Identity of *Bacteroides* isolates and previously named *Bacteroides* spp in clinical specimens of animal origin

Spencer S. Jang, BA, and Dwight C. Hirsh, DVM, PhD

SUMMARY

During the years 1984 through 1987, 2,574 isolates of obligately anaerobic bacteria were isolated from samples submitted for analysis. The most common anaerobic isolates were members of the genus *Bacteroides*, representing 44.6% of the isolates. Of these, the most commonly isolated identifiable microorganisms were bile-resistant and nonpigmented, belonging to the *B fragilis* group of *Bacteroides*. Importantly, obvious predilections for any one species or group of *Bacteroides* were not apparent for animal or site (condition), except that the proportion of isolates belonging to the nonpigmented, bile-resistant group obtained from the respiratory tract was significantly ($P < 0.005$) higher than that not belonging to this group. On the other hand, the proportion of isolates of the nonpigmented, bile-resistant group obtained from abscesses was significantly lower than that not belonging to this group.

Obligate anaerobic bacteria are a relevant component of infectious processes in human beings and other animals. In the latter group, 26% of samples obtained from pyonecrotic processes will contain, on average, 2 species of obligate anaerobes.[1]

Members of the genus *Bacteroides* represent the most-common obligate anaerobe isolated from animals.[2-5] However, little published information is available regarding the species of *Bacteroides* isolated, together with the kinds of animals and sites from which the isolates were obtained. The objective of the study reported here was to determine the species of *Bacteroides* most commonly isolated from animals, along with the sites affected.

Materials and Methods

Source of isolates—Isolates were from specimens obtained from animals brought to the teaching hospital, from Jan 1, 1984 through Dec 31, 1987. Specimens were processed as described.[1]

Samples excluded from anaerobic culture techniques were those obtained from sites with normal flora. Samples from the respiratory tract, when obtained from a live animal, were obtained by transtracheal aspiration.

Identity of isolates—Briefly, portions of each specimen were plated onto prereduced anaerobically sterilized (PRAS) blood agar plates containing *Brucella* agar base with hemin and vitamin K1.[a] Inoculated plates were stored under flowing oxygen-free CO_2 until placed in an incubator within an anaerobic chamber[a] containing a palladium catalyst, together with a mixture of 10% CO_2, 10% H_2, and 80% N_2. Colonies growing anaerobically were subcultured to a chocolate (bovine) agar plate or blood (bovine) agar plate, which was incubated at 37 C in an atmosphere of 10% CO_2 and air. Isolates not growing under these conditions were assumed to be obligate anaerobes. At this point, isolates were inoculated into PRAS chopped meat carbohydrate broth[b] (CMC) in the anaerobic chamber. The inoculated CMC was incubated at 37 C for 48 hours or until no further growth was observed after 5 days. Tubes containing slower-growing isolates were supplemented with PRAS horse serum[b] or formate-fumarate.[b] If growth was not enhanced by either supplement, the isolate was reinoculated into PRAS peptone yeast glucose[b] (PYG) or PRAS PYG with formate-fumarate.[b] Portions of the incubated broth were analyzed by gas liquid chromatography and were used as a source of inoculum for other PRAS media. Catalase (using 3% hydrogen peroxide) and spot indole (paradimethylaminocinnamaldehyde[a]) tests were performed, using colonies from a PRAS *Brucella* blood agar plate containing a pure culture of the isolate. Identity of the isolates was established by the use of gram staining, microscopic morphology, gas chromatographic results, and determination of utilization of various prereduced anaerobically sterilized substrates.[6-8,b] Stimulation of growth in bile was determined by inoculating the isolate into 2 tubes of PRAS PYG, one containing 20% bile,[b] the other without. After 48 hours' incubation, tubes were examined and the degree of growth or stimulation was determined. Isolates were defined as pigmented if they produced a black or fluorescent (brick red color when examined under UV light) colony when grown on PRAS *Brucella* agar plates containing hemin and vitamin K1 or subcultured to laked rabbit blood agar.

The isolates of *Bacteroides* were grouped into 1 of 4 groups, depending on whether the isolate was nonpigmented and grew in or was stimulated by bile (nonpig-

Received for publication Dec 8, 1990.
From the Microbiology Service, Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, CA 95616.

[a] Anaerobe Systems, San Jose, Calif.
[b] Carr-Scarborough Microbiologicals Inc, Stone Mountain, Ga.

Table 1—Obligate anaerobes isolated from animals at the teaching hospital during 1984–1987

Organism	No.	(%)
Bacteroides spp	1148	(44.6)
Fusobacterium spp	696	(27.1)
Clostridium spp	332	(12.9)
Peptostreptococcus spp	326	(12.7)
Nonspore-forming gram-positive rods	72	(2.8)

mented, bile-resistant group), produced pigment and did not grow in the presence of bile (pigmented, bile-sensitive group), or did not produce pigment and did not grow in the presence of bile (nonpigmented, bile-sensitive group). The fourth group was a miscellaneous group containing unspeciated members of the genus *Bacteroides*.

Results and Discussion

During the period of this study, 2,574 isolates of obligate anaerobes were obtained. Of these, 1,148 (44.6%), belonged to the genus *Bacteroides* (Table 1).

Approximately 35% of the isolates of *Bacteroides* could not be speciated (Table 2), a finding in keeping with other published surveys.[1,2,5,9] It is somewhat disconcerting that such a large number of undescribed species of obligate anaerobes from animals remains. Though nearly half the unspeciated *Bacteroides* was isolated from ruminants, this finding was not statistically significant by use of the χ^2 test.

The most commonly identifiable *Bacteroides* isolates belonged to the nonpigmented, bile-resistant group; of that group, those belonging to the *B fragilis* group were the most prevalent (Table 2). The next most prevalent isolates were those belonging to the pigmented, bile-sensitive group. These findings were in agreement with recent reports dealing with prevalence of various obligate anaerobes in clinical veterinary medicine. That is, pigmented species of *Bacteroides* are no longer the dominant species, as was reported some years ago.[2,5,9,10] Since those earlier reports, the proportion of pigmented isolates has gradually decreased, whereas the proportion of isolates belonging to the *B fragilis* group has steadily increased.[1] An explanation for this observation is not readily apparent. No doubt, techniques in sample collection and iso-

Table 2—*Bacteroides* spp isolated from animals, 1984 to 1987

Group/organism	Total	Animal species						
		Equine	Canine	Feline	Ruminant	Porcine	Exotic*	Avian
Nonpigmented, bile-resistant *B fragilis* group								
B fragilis	157	54	34	23	32	2	12	1
B uniformis	110	25	16	9	52	3	4	1
B thetaiotamicron	29	7	6	1	10	1	3	1
B ovatus	23	5	4	2	9	0	2	1
B distasonis	23	7	3	2	7	0	4	0
B vulgatus	17	3	5	2	4	0	2	1
Total	359	101	68	39	114	6	27	5
Non-*B fragilis* group								
B eggerthii	31	3	4	1	18	0	5	0
B splanchnicus	3	0	0	0	2	0	1	0
B tectum	2	0	1	0	0	0	1	0
Total	35	3	5	1	20	0	7	0
Pigmented, bile-sensitive group								
Porphyromonas asaccharolyticus	89	7	28	18	32	1	3	0
B levii	41	3	3	2	30	1	2	0
B melaninogenicus	20	3	2	1	11	0	3	0
B intermedius	19	1	1	0	12	1	4	0
B gingivalis	14	2	10	1	0	0	1	0
B macacae	12	1	1	2	7	0	0	1
B corporis	9	1	1	3	3	0	1	0
B loescheii	6	2	4	0	0	0	0	0
B denticola	1	1	0	0	0	0	0	0
Total	211	21	50	27	95	3	14	1
B bivius	53	9	10	7	21	0	5	1
Fibrobacter succinogenes	16	2	11	2	1	0	0	0
B oralis	12	4	3	2	3	0	0	0
B putridinus	9	2	1	2	4	0	0	0
B disiens	8	4	3	0	0	0	1	0
B capillosus	7	3	2	0	2	0	0	0
Ruminobacter amylophilus	6	2	1	0	3	0	0	0
B ruminicola								
ss brevis	5	1	2	0	2	0	0	0
ss ruminicola	3	1	1	0	1	0	0	0
B coagulans	3	0	0	1	2	0	0	0
B pneumosites	3	2	0	0	1	0	0	0
B gracilis	2	1	0	0	2	0	0	0
Tissierella praecutis	2	1	1	0	0	0	0	0
B buccae	2	0	2	0	0	0	0	0
B zoogleoformans	2	1	1	0	0	0	0	0
Mitsuokella multiacidus	2	1	0	0	1	0	0	0
B ureolyticus	1	0	0	0	1	0	0	0
Sabaldella termiditus	1	1	0	0	0	0	0	0
Total	137	35	35	14	45	0	6	1
Miscellaneous group								
Bacteroides spp	406	75	85	40	168	7	28	3
Total	1,148	235	243	121	442	16	82	10

* Includes non-human primates, llamas, snakes, mice, rats, rabbits.

Table 3—Anatomic site or condition* from which *Bacteroides* spp were isolated

Group/site	Total	Animal species: Equine	Canine	Feline	Ruminant	Porcine	Exotic	Avian
Nonpigmented, bile-resistant								
Respiratory tract	116	29	14	11	45	2	13	2
Body cavity	81	22	21	15	21	0	2	0
Abscess	52	16	12	4	11	1	6	2
Organ	30	12	1	0	11	1	5	0
Joint	22	3	1	0	16	0	2	0
Wound	19	3	5	3	6	0	2	0
Mass, tissue	21	5	8	1	5	1	0	1
Draining tract	10	2	0	0	7	0	1	0
Pigmented, bile-sensitive								
Respiratory tract	46	7	5	10	21	0	2	1
Body cavity	31	3	12	6	10	0	0	0
Abscess	48	7	8	4	22	3	4	0
Organ	12	0	5	0	7	0	0	0
Joint	12	0	1	0	10	0	1	0
Wound	13	1	3	3	4	0	2	0
Mass, tissue	12	0	6	1	5	0	0	0
Draining tract	12	1	5	6	0	0	0	0
Nonpigmented, bile-sensitive								
Respiratory tract	45	15	5	4	18	0	0	0
Body cavity	16	5	2	4	4	0	1	0
Abscess	36	14	7	3	12	0	0	0
Organ	7	1	3	1	2	0	0	0
Joint	2	0	1	0	1	0	0	0
Wound	3	1	1	0	1	0	0	0
Mass, tissue	10	0	7	0	2	0	1	0
Draining tract	6	2	4	0	0	0	0	0
Miscellaneous								
Respiratory tract	123	27	23	7	53	5	5	4
Body cavity	78	13	26	13	19	0	7	0
Abscess	89	12	16	7	43	1	15	1
Organ	21	2	1	1	17	0	2	0
Joint	10	1	0	0	8	1	0	0
Wound	10	0	5	0	4	0	1	0
Mass, tissue	19	6	7	1	3	0	2	0
Draining tract	27	8	7	6	6	0	1	0

* Representing 88 to 93% of isolates.

lation and identification of obligate anaerobes differed. However, we believe it is unlikely that improvement of anaerobic technique would account for a decrease in isolation of the pigmented *Bacteroides* species because most of the members of this group, in particular those belonging to the *B melaninogenicus-asaccharolyticus* group, are equally as susceptible to oxygen as is *B fragilis*.[11] Because most members of the *B fragilis* group isolated from animals are more resistant to antimicrobial agents, it is conceivable that the selective pressures resulting from antimicrobial usage (though data are not available to indicate increase in the usage of such drugs in animals) has resulted in an increase in the proportion of obligate anaerobes belonging to the *B fragilis* group comprising the normal flora of animals in general. An increase in the proportion of obligate anaerobes belonging to the *B fragilis* group isolated from clinical specimens might be the result.

Our data were at variance with recent reports of the contents of oral cavity-associated diseases of cats wherein *B tectum, B heparinolyticus, B salivosus,* and *B gingivalis* constituted approximately 91% of isolates of bite wound abscesses and 90% of cases of pyothorax.[12,13] It is possible that the newly described species, *B heparinolyticus* and *B salivosus*, constitute some of our isolates tabulated as *Bacteroides* spp. However, we isolated few *B tectum* or *B gingivalis*.

Bacteroides spp were isolated from a variety of animal species, sites, and conditions (Table 3; isolates from the most commonly encountered sites or conditions). Obvious predilections were not apparent, except that the proportion of isolates belonging to the bile-resistant, nonpigmented group obtained from the respiratory tract (29%) was significantly ($P < 0.005$; χ^2 test) higher than that of isolates not belonging to this group (23%). On the other hand, the proportion of isolates of the bile-resistant, nonpigmented group obtained from abscesses (13%) was significantly ($P < 0.001$; χ^2 test) lower than that of isolates not belonging to this group (23%). However, the seeming lack of predilection, with the aforementioned exceptions, of isolates belonging to the nonpigmented, bile-resistant group was an observation with clinical relevance; most of the isolates belonging to this group produce a cephalosporinase and are, thus, resistant to the penicillins (ampicillin) and first-generation cephalosporins.[1] This observation, taken together with the increased prevalence of this group in pyonecrotic processes in animals, makes choice of an antimicrobic prior to availability of susceptibility test results, more difficult (ie, no longer can penicillin (ampicillin) be used with impunity when treating an infectious process with a suspected anaerobic component).

References

1. Hirsh DC, Indiveri MC, Jang SS, et al. Changes in prevalence and susceptibility of obligate anaerobes in clinical veterinary practice. *J Am Vet Med Assoc* 1985;186:1086–1089.

2. Berg JN, Fales WH, Scanlan CM. Occurrence of anaerobic bacteria in diseases of the dog and cat. *Am J Vet Res* 1979;40:876–881.

3. Chirino-Trejo JM, Prescott JF. The identification and antimicro-

bial susceptibility of anaerobic bacteria from pneumonic cattle lungs. *Can J Comp Med* 1983;47:270–276.

4. Love DN, Jones RF, Bailey M, et al. Isolation and characterization of bacteria from pyothorax (empyaemia) in cats. *Vet Microbiol* 1982;7:455–461.

5. Prescott JF. Identification of some anaerobic bacteria in nonspecific anaerobic infections in animals. *Can J Comp Med* 1979;43:194–199.

6. Finegold SM, Baron EJ. *Bailey and Scott's diagnostic microbiology*. 7th ed. St Louis: CV Mosby Co, 1986;500–550.

7. Holdeman LV, Cato EP, Moore WEC. *Anaerobe laboratory manual*. 4th ed. Blacksburg, Va: Polytechnical Institute and State University, 1977.

8. Sutter VL, Citron DM, Edelstein MAC, et al. *Wadsworth anaerobic bacteriology manual*. 4th ed. Belmont, Calif: Star Publishing Co, 1985.

9. Hirsh DC, Biberstein EL, Jang SS. Obligate anaerobes in clinical veterinary practice. *J Clin Microbiol* 1979;10:188–191.

10. Biberstein EL, Knight HD, England K. *Bacteroides melaninogenicus* in diseases of domestic animals. *J Am Vet Med Assoc* 1968;153:1045–1049.

11. Loesche WJ. Oxygen sensitivity of various anaerobic bacteria. *Appl Microbiol* 1969;18:723–727.

12. Love DN, Johnson JL, Jones RF, et al. *Bacteroides salivosus* sp nov, an asaccharolytic, black-pigmented species from cats. *Int J Syst Bacteriol* 1987;37:307–309.

13. Love DN, Johnson JL, Moore LVH. *Bacteroides* species from the oral cavity and oral-associated diseases of cats. *Vet Microbiol* 1989;19:275–281.