JOURNAL OF CLINICAL MICROBIOLOGY, July 1992, p. 1882–1884 Vol. 30, No. 7
0095-1137/92/071882-03$02.00/0

Bilophila wadsworthia Isolates from Clinical Specimens

E. J. BARON,[1,2]* M. CURREN,[3] G. HENDERSON,[4] H. JOUSIMIES-SOMER,[5] K. LEE,[6]† K. LECHOWITZ,[7] C. A. STRONG,[1,2] P. SUMMANEN,[1] K. TUNÉR,[8]‡ AND S. M. FINEGOLD[1,2,9,10]

Microbiology Laboratory, Barnes Hospital, St. Louis, Missouri 63110[3]; Microbiology Laboratory, Auckland Hospital, Auckland 1, New Zealand[4]; National Public Health Institute, Anaerobe Reference Unit, 00300 Helsinki, Finland[5]; Department of Clinical Pathology, Yonsei University Wonju College of Medicine, Wonju, Korea[6]; Clinical Microbiology, University of California at Los Angeles Medical Center, Los Angeles, California 90024[7]; Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden[8]; Research Service W151[1] and Medical Service,[9] Veterans Administration Medical Center, West Los Angeles, California 90073; and Department of Medicine[2] and Department of Microbiology and Immunology,[10] University of California at Los Angeles School of Medicine, Los Angeles, California 90024

Received 12 March 1992/Accepted 28 April 1992

***Bilophila wadsworthia* is an anaerobic, gram-negative, asaccharolytic, bile-resistant, catalase-positive bacillus that is usually urease positive and was originally recognized in specimens of peritoneal fluid and tissue from patients with appendicitis. Additional isolations from clinical specimens, including a scrotal abscess, mandibular osteomyelitis, axillary hidradenitis suppurativa, pleural fluid, joint fluid, and blood, are described here.**

Bilophila wadsworthia is a recently described asaccharolytic, anaerobic, gram-negative, urease-positive bacillus that is stimulated by bile and strongly catalase positive (2). Urease-negative strains have also been described (2). Because it was initially isolated from patients with appendicitis (1, 3–5), the natural habitat of *Bilophila* sp. was assumed to be the gastrointestinal tract. Interestingly, at the time of its description, neither the collection of the Virginia Polytechnic Institute Anaerobe Laboratory nor that of the Centers for Disease Control contained any organisms similar to *Bilophila* sp. (6). We present here additional types of clinical infections and other materials from which *Bilophila* sp. has been recovered.

Information from case presentations of 11 patients from whom *B. wadsworthia* was isolated is summarized in Table 1. The diversity of infectious foci is apparent.

Additional isolates. Workers in Helsinki, Finland (H. J.-S. et al.), have isolated 28 strains from patients with appendicitis (approximately half of the subjects studied), which corresponds to the experience of the group at the Wadsworth Anaerobe Laboratory (1, 3–5). The same group of microbiologists in Finland, studying periodontal disease in dogs, isolated *B. wadsworthia* from the periodontal pockets of 3 of 16 dogs. Colonies were recognized initially on *Bacteroides* bile esculin (BBE) agar. There is no information to indicate that these organisms were important in the disease process.

These workers and the group at the Wadsworth Anaerobe Laboratory have also reported on *Bilophila* isolates from feces of healthy subjects (2, 8). The Finnish workers recovered *B. wadsworthia* from stool samples of 34 (59.7%) of 57 subjects studied; counts ranged from 3×10^3 to 2×10^8/g (wet weight) of stool.

In Sweden, one of us (K. T.) cultured saliva (1.0 ml each) from 100 healthy volunteers and vaginal swabs from an additional 100 asymptomatic women attending a gynecological clinic for annual evaluation. All cultures were inoculated onto BBE agar and incubated for a minimum of 7 days. *B. wadsworthia* was isolated from 4% of the saliva samples and 3% of the vaginal samples.

Researchers at the Mayo Clinic Laboratory, Rochester, Minn., have recently reported identifying *B. wadsworthia* (in association with other bacteria) in the blood cultures of two patients with liver abscesses. Although gram-negative bacilli were isolated from the liver abscess aspirates, those strains were not characterized further. It is likely that the blood isolates originated from the liver abscess flora (7).

Laboratory identification. *B. wadsworthia* and related species have probably been overlooked or misidentified because they grow slowly on routine anaerobic media, producing small, translucent, nondescript colonies on anaerobic blood agar plates. A colony can easily be distinguished on BBE agar, however, on which it appears after 3 days or longer as a transparent colony with a black center, the result of precipitation of ferrous sulfide due to hydrogen sulfide production by the organism. *B. wadsworthia* is unable to hydrolyze esculin. In areas where the inoculum is heavy, the black precipitate is visible in the agar underlying the growth. Bacteroides bile esculin agar is usually used as a selective and differential medium for recovery and recognition of *Bacteroides fragilis* group organisms, which are resistant to the high bile concentration (20%) in the medium and usually hydrolyze esculin, forming dark brown to black colonies. Most protocols for its use suggest that BBE be inspected after 48 h of anaerobic incubation, which allows sufficient time for detection of *B. fragilis* group colonies, and then discarded to prevent misidentification of later growth as *Bacteroides* organisms. Since *Bilophila* often appears only after 72 h or longer, it is not detected unless BBE plates are incubated for an extended time (we recommend 7 days).

Another feature useful for early suspicion of *Bilophila* species is the strongly positive catalase reaction observed when a 15% hydrogen peroxide reagent (standard formula

* Corresponding author.
† Present address: Yonsei University College of Medicine, Seoul, Korea.
‡ Present address: Roussel Nordiska AB, 126 12 Stockholm, Sweden.

TABLE 1. *B. wadsworthia* isolations from infections other than appendicitis

Specimen	Type of infection	Patient sex and age (yr); underlying condition	Amt of *Bilophila* growth	Other anaerobe(s) isolated	Aerobe(s) isolated	Facility
Pus	Erosive gingivitis; ?mandibular osteomyelitis	Male, 29; AIDS	Moderate[a]	*Prevotella intermedia*	Mixed oropharyngeal flora	Barnes Hospital[b]
Pus; necrotic tissue	Hidradenitis suppurativa	Female, 59; none	Moderate[a]	*Prevotella bivia*, *Prevotella melaninogenica*	Mixed skin flora	Barnes Hospital
Pus	Scrotal abscess, Fournier's gangrene	Male, 68; none	Numerous[a]	*Bacteroides distasonis*, *Bacteroides ovatus*, *Bacteroides loescheii-Bacteroides oralis* group, *Bacteroides fragilis*, *Peptostreptococcus* sp.	*Escherichia coli*, *Proteus vulgaris*	Wonju Medical College, Yonsei University Hospital[c]
Pericardial fluid	Liver abscess; pleural and pericardial effusions	Male, 6; none	Few	*Bacteroides fragilis, Bacteroides splanchnicus,* pigmented gram-negative rod	None	UCLA[d] Hospital
Pus	Scalp infection	Male, 35; AIDS, lymphoma of the scalp	Few	*Peptostreptococcus* sp., *Bacteroides* sp., *Prevotella intermedia*, *Fusobacterium nucleatum*, *Bacteroides gracilis*	*Staphylococcus aureus*	UCLA Hospital
Blood	Possible transient bacteremia	Male, 66; abdominal distension, diarrhea, aortic aneurysm	Not determined	None	None	Auckland Hospital[e]
Resected tissue	Infected bilateral patellar fractures	Male, 37; automobile accident	Few[a]	*Bacteroides gracilis*, *Prevotella* sp., *Peptostreptococcus micros*, *Bacteroides distasonis*, *Eubacterium lentum*, *Actinomyces* sp.	None	Wadsworth VA[f] Hospital[g]
Resected tissue	Osteomyelitis of the knee, sinus tract	Male, 34; gunshot wound	Few	*Bacteroides uniformis*, *Prevotella disiens*	*Escherichia coli*	Wadsworth VA Hospital
Empyema fluid	Pleural empyema	Male, 61; lung cancer	Moderate[a]	*Prevotella intermedia*, *Prevotella corporis*, *Peptostreptococcus anaerobius*	*Escherichia coli*, diphtheroids	Wadsworth VA Hospital
Bloody vaginal discharge	Lower abdominal pain	Female; 88; ?bladder malignancy	Not determined	*Bacteroides fragilis* group, others not identified	*Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Proteus mirabilis*	National Public Health Institute[h]
Pus	Decubitus ulcer of the buttocks	Unknown; unknown	Not determined	Mixed flora, not identified	Mixed flora, not identified	National Public Health Institute

[a] *Bilophila* sp. was likely to be important in the pathogenesis of the infection.
[b] St. Louis, Mo.
[c] Wonju, Korea.
[d] UCLA, University of California at Los Angeles.
[e] Auckland, New Zealand.
[f] VA, Veterans Administration.
[g] Los Angeles, Calif.
[h] Helsinki, Finland.

for use with anaerobic bacteria) is applied to colony paste on a glass slide (11). Only a few other anaerobes, notably, some *B. fragilis* group species, *Propionibacterium acnes*, *Actinomyces viscosus*, and a few others, are catalase positive, and none show the rapid, almost explosive evolution of bubbles displayed by some strains of *Bilophila* sp. This feature was instrumental in the early recognition of these organisms as unique.

Other characteristics are the ability to reduce nitrate to nitrite, stimulation of growth by pyruvate, and inability to ferment carbohydrates. Most strains produce the enzyme acid phosphatase. Metabolic end products detected by gas-liquid chromatography include a major acetic acid and various amounts of lactic and succinic acids. Although a positive urease test distinguishes *B. wadsworthia* from similar organisms, isolates of similar organisms have not been characterized extensively enough to determine whether they should also be placed into the species *B. wadsworthia* or into one or more separate species.

The preferred ecological niche of *Bilophila* sp. is unknown but is presumably the lower gastrointestinal tract. However, the organism is occasionally found in the oral and vaginal floras. It is felt to be virulent, since it is the third most common anaerobic isolate in studies of gangrenous and perforated appendicitis, although it is present in the feces of about 50% of humans in mean counts of only 10^5 to 10^6/g (with total flora counts of 10^{11}/g). Studies are in progress in the Wadsworth Anaerobe Laboratory to characterize its virulence mechanisms. The genus was originally thought to be resistant to antimicrobial agents used to treat anaerobic infections because of difficult-to-interpret growth patterns on agar dilution susceptibility plates. *Bilophila* species are now felt to be susceptible to imipenem, cefoxitin, and ticarcillin on the basis of tests performed with the spiral gradient method with endpoint determination enhanced by a triphenyltetrazolium chloride overlay (9). Contrary to earlier reports, most *Bilophila* strains produce β-lactamase and are resistant to penicillins and other β-lactam antibiotics that are not resistant to this enzyme (10).

The cases reported here do not document a significant role for *Bilophila* organisms in all of these infections, but it appears that the organism was likely important, at least as part of a mixed flora, in several of the patients (Table 1). However, more adequate culture techniques might lead to a different conclusion. The slow growth of *Bilophila* may lead to its being overlooked entirely or judged to be less important than it actually is.

By incubating BBE plates for up to 7 days to facilitate recognition of *Bilophila* colonies and testing all small, translucent colonies on anaerobic blood agar for a strong catalase reaction, microbiologists can enhance their chances of recovering strains of this species. Use of optimum media (including BBE agar and pyruvate as a growth factor), use of an anaerobic chamber for initial specimen handling, practice of optimum anaerobic laboratory techniques, and better recognition and reporting of *Bilophila* sp. (with quantitation of growth and good clinical evaluation) from clinical specimens will help to define the role of this genus in the infectious processes with which it is associated.

REFERENCES

1. **Baron, E. J., R. Bennion, J. Thompson, et al.** 1992. A microbiological comparison between acute and complicated appendicitis. Clin. Infect. Dis. **14:**227–231.
2. **Baron, E. J., P. Summanen, J. Downes, M. C. Roberts, H. M. Wexler, and S. M. Finegold.** 1989. *Bilophila wadsworthia*, gen. nov. and sp. nov., a unique gram-negative anaerobic rod recovered from appendicitis specimens and human faeces. J. Gen. Microbiol. **135:**3405–3411.
3. **Bennion, R. S., E. J. Baron, J. E. Thompson, et al.** 1990. The bacteriology of gangrenous and perforated appendicitis—revisited. Ann. Surg. **211:**165–171.
4. **Bennion, R. S., J. E. Thompson, E. J. Baron, and S. M. Finegold.** 1990. Gangrenous and perforated appendicitis with peritonitis: treatment and bacteriology. Clin. Ther. **12**(Suppl. B)**:**1–14.
5. **Finegold, S. M., R. S. Bennion, J. E. Thompson, H. M. Wexler, and E. J. Baron.** 1990. Gangrenous and/or perforated appendix: clinical outcome and in vitro susceptibility testing. Hosp. Pract. **25:**(S4):3–12.
6. **Holdeman-Moore, L. V., and V. R. Dowell. (Virginia Polytechnic Institute Anaerobe Laboratory and Centers for Disease Control Anaerobe Laboratory).** Personal communication.
7. **Kasten, M. J., J. E. Rosenblatt, and D. R. Gustafson.** Submitted for publication.
8. **Summanen, P., J. Downes, M. Karl, et al.** 1989. Characteristics and ultrastructure of an unusual gram-negative bacillus isolated from inflamed and noninflamed appendices. Abstr. Eur. Soc. Clin. Microbiol., abstr. PA 12, p. 5.
9. **Summanen, P., E. Molitoris, H. M. Wexler, and S. M. Finegold.** 1991. Use of triphenyltetrazolium chloride color reaction as an aid in susceptibility testing of *Bilophila wadsworthia*. Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., abstr. 182, p. 129.
10. **Summanen, P., H. M. Wexler, and S. M. Finegold.** Submitted for publication.
11. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1985. Wadsworth anaerobic bacteriology manual, ed. 4, p. 34. Star Publishing Co., Belmont, Calif.