Bilophila wadsworthia: a Unique Gram-negative Anaerobic Rod

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Although comprising less than 0.01% of the normal human gastrointestinal microbiota, Bilophila wadsworthia is the third most common anaerobe recovered from clinical material obtained from patients with perforated and gangrenous appendicitis. Since its discovery in 1988, B. wadsworthia has been recovered from clinical specimens associated with a variety of infections, including sepsis, liver abscesses, cholecystitis, Fournier’s gangrene, soft tissue abscesses, empyema, osteomyelitis, Bartholinitis, and hidradenitis suppurativa. In addition, it has been found in the saliva and vaginal fluids of asymptomatic adults and even in the periodontal pockets of dogs.

The organism is asaccharolytic, fastidious, and is easily recognized by its strong catalase reaction with 15% H₂O₂, production of hydrogen sulfide, and growth stimulation by bile (oxgall) and pyruvate. Approximately 75% of strains are urease positive. When grown on pyruvate-containing media, > 85% of strains demonstrate β-lactamase production. Ribosomal RNA-based phylogenetic studies show Bilophila to be a homogeneous species, most closely related to Desulfovibrio species.

Both adherence to human cells and endotoxin have been observed, and preliminary work suggests that environmental iron has a role in expression of outer membrane proteins. Penicillin-binding proteins appear to mediate the organism’s susceptibility to at least some β-lactam agents, which induce spheroplast formation that results in a haze of growth on agar dilution susceptibility test plates which is difficult to interpret. Bilophila strains are inhibited in vitro by most antibiotics.

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Introduction

Bilophila wadsworthia was first recognized in 1988 during the course of a study designed to describe the microbiota of gangrenous and perforated appendicitis and the effects of various antibiotic regimens on patient recovery. The organism was recovered from approximately one-half of all specimens of appendiceal tissue or peritoneal fluid collected from patients with appendicitis [1], although it had not been recognized previously [2,3]. Since its description, it has been the subject of or has been mentioned in approximately 20 publications. This review attempts to summarize the current information available with regard to this unique anaerobic organism.

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Characteristics of the Organism

Strains of *B. wadsworthia* are asaccharolytic and able to reduce nitrate to nitrite and occasionally to N\(_2\); approximately 75% of strains are urease positive ([2], M. McTeague, personal communication). There appears to be no genetic difference among urease-positive and urease-negative strains [4]. Growth is stimulated by 20% bile (oxgall) and 1% pyruvate [2]. In fact, demonstration of \(\beta\)-lactamase production requires growth on a pyruvate-containing medium [5]. Members of the genus produce a major acetic acid peak and minor to trace amounts of succinic acid as detected by gas-liquid chromatography performed on growth in pyruvate-supplemented pre-reduced, anaerobically sterilized (PRAS) peptone-yeast and peptone-yeast-glucose broths [2].

Two key distinguishing characteristics are its very rapid and strong catalase reaction with 15% \(\text{H}_2\text{O}_2\), the standard catalase reagent used for anaerobic bacteria, and production of hydrogen sulfide from sulfur-containing amino acids. The production of \(\text{H}_2\text{S}\) results in the presence of a small dark central spot on colonies grown for at least 48–72 h on Bacteroides bile esculin (BBE) agar. This characteristic feature on the otherwise normal human volunteers [2]. The same author was able to recover the species from periodontal pockets of three of 16 (19%) dogs with periodontitis that were sampled [8]. The rate of recovery from saliva and human vaginal secretions from 100 normal volunteers was only 4% and 3%, respectively [8].

At least five possible virulence mechanisms have been associated with *Bilophila* species. Similar to *Bacteroides fragilis* and unlike most other anaerobic species, *Bilophila* strains were shown to induce intra-abdominal abscess formation when injected as pure cultures into the peritoneal cavities of mice (Andrew Onderdonk, personal communication). Although abscess formation is usually associated with presence of capsular polysaccharide, *Bilophila* does not appear to have a capsule; thus the specific virulence factor responsible for abscess formation is not yet known. As do most Gram-negative bacteria, *Bilophila* species induces clotting of *Limulus* amoebocyte lysate and promotes procoagulant activity by human mononuclear cells, both characteristics associated with the presence of endotoxin [9]. However, the magnitude of the endotoxin-like activity of the *Bilophila* species tested was less than that observed for typical Gram-negative organisms [9]. Tissue culture assays for cytotoxicity demonstrated some cytotoxic activity in two cell lines, but this factor has not been further studied. Additional in vitro studies demonstrated great variability in the ability of strains of *Bilophila* to adhere to a human embryonic intestinal cell line (Sharon Gerardo, personal communication), although the adherence characteristics of individual strains were stable during multiple passages. No structural features were detected by electron microscopy to account for the adherence capabilities of some strains, although differences in outer membrane proteins between adherent and non-adherent strains were found (Gerardo). Finally, the presence of iron-regulated outer membrane proteins, which may have a role in virulence, has been demonstrated [10]. Under iron-depleted conditions, four new outer membrane proteins were expressed by all four separate strains of *Bilophila wadsworthia* tested [10]. The contribution of these proteins, if any, to the virulence of these organisms is unknown.

Clinical Associations

Several publications have catalogued the clinical syndromes and specimen types from which *Bilophila wadsworthia* has been isolated. In addition to isolates listed in previous reviews [8,11,12], the organism has been isolated from soft tissue infection in an intra-
venous drug user [13], and both cholesteatoma and brain abscess material from a patient with chronic otitis media [14]. Table 1 summarizes published data on the number and types of specimens from which *Bilophila* has been isolated. Although it is most commonly seen in specimens from patients with appendicitis, this species can clearly be expected as a component of anaerobic infections in any site. Interestingly, its association with appendicitis varies with the stage of disease, as determined by histopathology [15]. Investigation of 59 isolates from patients with appendicitis revealed that *Bilophila* was present in 25% of specimens from patients in the acute stage of disease, in 37% of specimens from patients with gangrenous appendicitis, and in 55% of specimens obtained from patients with perforated appendicitis [15]. On average, half of all properly collected tissues or peritoneal fluids from patients with appendicitis are found to harbor *Bilophila wadsworthia*, the third most common anaerobe isolated from such specimens [1].

### Antimicrobial Susceptibilities

Similar to several other genera of anaerobes, such as *Fusobacterium*, agar dilution susceptibilities of *Bilophila* spp. occasionally exhibited a trailing ‘haze’ of growth that made endpoint determination difficult [5,7,16]. The organisms in the haze were found to be spheroplasts, often as large as 30 µm across, or eight times the diameter of the unaffected cell [5,7]. Triphenyltetrazolium chloride, a dye that is reduced to a red color by enzymes of viable bacteria, was used to clarify the endpoint determination [5]. During the course of this study, it was determined that many enzymes of *Bilophila wadsworthia*, and presumably other anaerobic bacteria, are inhibited in the presence of oxygen ([5], Summanen, personal communication). It was also discovered that reliable susceptibility and β-lactamase results were dependent on the addition of 1% pyruvate to the testing growth medium [5]. Further investigation of the action of β-lactam antibiotics on cell wall structure was undertaken using imipenem. Inhibition of penicillin-binding proteins was found to be associated with spheroplast formation and ‘hazy’ growth on agar plates [7]. These distorted cells, however, were viable and able to return to normal morphology upon subculture. Time-kill kinetic studies of eight strains of *Bilophila* in pyruvate-containing medium illustrated the difficulty of interpreting susceptibility testing results. In these studies, breakpoint concentrations of ticarcillin/clavulanate, ampicillin/subactam, imipenem, and cefoxitin effected two-to-four-log decreases in CFU over 30 h of incubation, but only two of the strains were killed completely (by ticarcillin/clavulanate, ampicillin/subactam, or imipenem) in these trials [16]. A proportion of the initial inoculum of most strains was still viable at the end of the 30 h time period of the experiment, and CFUs did not decline at all for several strains growing in clindamycin and chloramphenicol [16]. Perhaps these remaining viable cells were the distorted forms and spheroplasts seen in the triphenyltetrazolium chloride studies cited. Only metronidazole displayed consistent bactericidal activity against all eight strains reported [16]. Clinical implications of these results, however, are not known.

With rare exceptions, *Bilophila* species are recovered in mixed cultures with other aerobic and anaerobic bacteria and are easy to overlook. Laboratory scientists should be encouraged to further characterize small, translucent colonies of Gram-negative bacilli on primary anaerobic blood agar plates or small, clear colonies with black centers on Bacteroides bile esculin agar. With just a rapid catalase test, many of these can be identified as *Bilophila wadsworthia*. By increasing the numbers of such strains recognized, we will increase our knowledge of the role of this organism in disease.

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References


