

## *Bacteroides gracilis*, an Important Anaerobic Bacterial Pathogen

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Clinical isolates of agar-pitting, formate-fumarate-requiring, anaerobic gram-negative bacilli were recharacterized in consideration of the species descriptions of *Bacteroides ureolyticus* and the newly described *B. gracilis*, *Campylobacter concisus*, and *Wolinella* sp. During an 11-year period, 7.5% (101 of 1,341) of all specimens yielding anaerobes were found to contain an organism in this group. Of the 71 isolates that were available for study, 43 were *B. ureolyticus*, 23 were *B. gracilis*, and 5 were in the *Wolinella-C. concisus* group. The role in infection and patterns of antimicrobial susceptibility for *B. ureolyticus* and *B. gracilis* were studied. Review of the clinical data indicated that 83% of *B. gracilis* strains were recovered from patients with serious visceral or head and neck infection, whereas 73% of *B. ureolyticus* isolates were recovered from superficial soft-tissue or bone infections. The strains of *B. ureolyticus* were uniformly susceptible to the tested antimicrobial agents. *B. gracilis*, however, showed some striking resistance, with penicillin active against only 67% and the cephalosporins active against 67 to 84% of the isolates tested. The association of *B. gracilis* with serious deep-tissue infection, coupled with the relatively high frequency of antibiotic resistance, indicates that it is an important, previously unrecognized, pathogen.

The role of anaerobic gram-negative bacteria in infection has been well documented (4). Pathogenic roles are best established for members of the *Bacteroides fragilis* and the pigmented *Bacteroides* groups; however, increasing information is available on the role of some of the less well known members of the family *Bacteroidaceae* in clinical infections (12). Recognition of the importance of these other organisms is related to improvement in laboratory recovery of these potential pathogens from clinical specimens and to new taxonomic information.

Among the less well known members of the family *Bacteroidaceae* is the group of bile-sensitive, formate-fumarate-requiring organisms that are usually recognized by their agar-pitting capabilities. Historically, the classification and nomenclature for this group of organisms has been confusing. Initially, *Bacteroides corrodens* was the name applied to both anaerobic and facultative gram-negative bacilli that formed "corroding" colonies on agar surfaces (3, 8). In 1972, however, Jackson and Goodman renamed the facultative strains *Eikenella corrodens* and reserved the name *Bacteroides corrodens* for the strictly anaerobic strains (10). Subsequently, Jackson and Goodman characterized and renamed this *B. corrodens* group as *Bacteroides ureolyticus* (11). In addition, other strains of agar-corroding anaerobic bacilli, which did not conform to the description of *B. ureolyticus*, have been reported. In 1981, four new species of these agar-pitting bacilli were described based on their guanine-plus-cytosine DNA contents, DNA homologies, and cluster analyses of their phenotypic features (15). Thus it is apparent that the species originally designated *B. corrodens* actually included several related, but different, species.

The new taxonomy for the anaerobic, agar-pitting, gram-negative bacilli places the nonmotile strains in the genus *Bacteroides* and the motile strains in the group of "anaerobic vibrios" (including *Wolinella recta*, *W. curva*, and *Campylo-*

*bacter concisus*) (15, 16). The nonmotile strains are separated into the species *B. ureolyticus* and *B. gracilis*, primarily on the basis of urease production. Previous reports on the clinical significance of *B. ureolyticus* are scanty, and those for *B. gracilis* are nonexistent. To assess and compare the clinical significance of these similar species, we have evaluated them in terms of the sites and types of infections with which they are associated, the bacteriology of these infections, and their antimicrobial susceptibility.

### MATERIALS AND METHODS

**Source of specimens and bacterial strains.** The Wadsworth Anaerobic Bacteriologic Research Laboratory performs complete bacteriologic evaluation of specimens obtained from patients suspected of having infections involving anaerobic bacteria. The laboratory also accepts anaerobic isolates for identification from the clinical microbiology laboratory of the medical center and from outside hospitals. All anaerobic isolates are stored in skim milk at  $-70^{\circ}\text{C}$ .

Records of the Wadsworth Anaerobic Bacteriologic Research Laboratory from January 1973 to May 1984 were reviewed. All clinical isolates originally identified as *B. corrodens*, "B. corrodens-like," *B. ureolyticus*, "B. ureolyticus-like," *B. gracilis*, or *Wolinella* were chosen for study. The source and complete bacteriology of each specimen yielding one of these isolates were noted. Duplicate or subsequent specimens from the same patients were not evaluated.

**Bacterial characterization.** Clinical isolates of agar-pitting, anaerobic gram-negative bacilli that were recovered before 1981 had been characterized according to the old nomenclature. To reidentify these strains in consideration of the new taxonomy, the stock strains were evaluated in terms of Gram stain reaction, colonial morphology, growth under anaerobic, aerobic, and  $\text{CO}_2$  atmospheric conditions, susceptibility to special-potency antibiotic disks, nitrate reduction, growth stimulation in broth by formate and fumarate, and biochemical tests including production of catalase,

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indole, and urease. All tests were carried out by methods described previously (14). Motility was determined by wet-mount examination and dark-field microscopy of young broth cultures. The clinical isolates recovered after 1981 were already correctly identified according to this schema and did not require further laboratory evaluation. The type strains of *B. ureolyticus* (strain ATCC 33387) and *B. gracilis* (strain ATCC 33236) were used as reference strains for all bacterial characterization tests.

The microbiologic characteristics shared by *B. ureolyticus*, *B. gracilis*, *C. concisus*, and the *Wolinella* sp. were as follows. They were anaerobic gram-negative bacilli, sensitive to colistin 10- $\mu$ g and kanamycin 1,000- $\mu$ g disks, but resistant to a vancomycin 5- $\mu$ g disk, catalase negative, indole negative, able to reduce nitrate, unable to grow in unsupplemented broth culture, and stimulated in broth culture by formate and fumarate. The *B. ureolyticus* isolates produced urease, whereas the other species did not. Only *C. concisus* and the *Wolinella* sp. were motile.

**Clinical data.** Clinical information on patients was obtained from medical charts, autopsy reports, discharge summaries, operative reports, Infectious Disease service records, and records of the referring physician.

**Antimicrobial susceptibility and beta-lactamase testing.** MICs were determined by a brucella-laked blood agar dilution method, as described previously (14). A qualitative assay for beta-lactamase was performed with the chromogenic cephalosporin nitrocefin (Cefinase; BBL Microbiology Systems).

## RESULTS

In the Wadsworth Anaerobic Bacteriologic Research Laboratory from January 1973 to May 1984, 101 isolates were recovered that had been called *B. corrodens*, "*B. corrodens*-like," *B. ureolyticus*, "*B. ureolyticus*-like," *B. gracilis*, or *Wolinella* (depending on the accepted nomenclature at the time of isolation). During this period, 7.5% (101 of 1,341) of all cultures positive for anaerobes yielded one of these organisms (discounting duplicate cultures). Of these 101 isolates there were 71 that had survived storage and on which clinical data were available. On recharacterization, 43 were *B. ureolyticus* and 23 were *B. gracilis*. An additional five urease-negative isolates belonged to the *Wolinella-C. concisus* group.

The complete bacteriology for each specimen yielding *B. ureolyticus* or *B. gracilis* was reviewed. *B. ureolyticus* was recovered in association with both aerobes and anaerobes in

TABLE 1. Sites of isolation of *B. ureolyticus* and *B. gracilis*

Specimen source	No. of patients with:	
	<i>B. ureolyticus</i>	<i>B. gracilis</i>
Central nervous system	1	2
Head and neck	0	2
TTA <sup>a</sup> or pleural fluid	3	8
Tracheostomy suction (sputum)	0	1 <sup>b</sup>
Intra-abdominal or pelvic sample	4	6
Perirectal sample	3	0
Soft tissue and bone below waist	20	0
Soft tissue and bone above waist	12	4

<sup>a</sup> TTA, Percutaneous transtracheal aspirate.

<sup>b</sup> This patient had an aspiration pneumonia, and the only specimen obtainable was sputum suctioned through a tracheostomy. The clinical significance of this culture is uncertain.

TABLE 2. Clinical diagnosis and outcome of patients from whom a specimen yielded *B. gracilis*

Diagnosis	No. of patients studied	No. of patients with clinical outcome of:	
		Cure	Death
Brain abscess	2	2	0
Peritonsillar abscess	1	1	0
Actinomycotic submandibular mass	1	1	0
Lung abscess or empyema or both	7	6 <sup>a</sup>	1
Aspiration pneumonia	2	1	1
Intra-abdominal abscess	4	3	1
Liver abscess, multiple	1	0	1
Peritonitis	1	1	0
Abdominal wound infection	1	1	0
Soft-tissue abscess	3	3	0

<sup>a</sup> Two patients with lung abscess or empyema or both required prolonged therapy (greater than 1 year) for definitive cure.

87% (33 of 38) of the cases. Typically, three or more different anaerobic isolates were present in these specimens. *B. gracilis* was recovered in association with both aerobes and anaerobes in 52% (12 of 23) of cases. Another 44% of cases yielded only other anaerobes in addition to *B. gracilis*. Generally, the specimens containing only anaerobes represented cases of pleuropulmonary or central nervous system infection, whereas those also containing aerobes or facultative bacilli were from intra-abdominal or perirectal infections. Neither *B. gracilis* nor *B. ureolyticus* was isolated in pure culture.

Clinical data were reviewed to determine the sources of the *B. ureolyticus* and *B. gracilis* isolates (Table 1). Seventy-four percent (32 of 43) of *B. ureolyticus* isolates were recovered from superficial soft-tissue or bone infections or both. Most of these specimens were swab cultures of a skin ulcer or abscess, although a few tissue or bone samples were also cultured. There were only eight cases in which *B. ureolyticus* was recovered from a significant deep-tissue infection (three intra-abdominal abscesses, one gynecologic pelvic abscess, three aspiration pneumonias, and one brain abscess). The remaining three cases were perirectal abscesses. In comparison, *B. gracilis* was primarily recovered from sites of deep-tissue infection and was recovered from superficial soft-tissue or bone sites only rarely. Eighty-three percent (19 of 23) of the specimens had been obtained from patients with a serious visceral or head and neck infection (nine pleuropulmonary infections, five intra-abdominal abscesses, two brain abscesses, one peritonitis due to a ruptured appendix, one retropharyngeal-peritonsillar abscess, and one large actinomycotic submandibular mass).

Table 2 summarizes the cases that yielded *B. gracilis* on culture. These patients ranged in age from 12 to 85. Of the 23 patients, 21 were male, but this was biased by the predominantly veteran population. Of the nine patients with pleuropulmonary infection, three were alcoholics, two had other underlying alterations of consciousness, and two had severe gingivitis. The two patients with brain abscess both had sinusitis as a contiguous source of infection. All cases of intra-abdominal abscess or peritonitis resulted from underlying biliary tree obstruction or appendiceal perforation. Overall, 19 of the 23 patients were cured with a variety of antimicrobial agents as well as appropriate surgical drainage procedures. There were four deaths among the patients, all of which were related to the infections involving *B. gracilis*.

Susceptibility testing revealed some notable differences

between *B. ureolyticus* and *B. gracilis* spp. The strains of *B. ureolyticus* were uniformly susceptible to the tested antimicrobial agents, including penicillins, cephalosporins, erythromycin, clindamycin, chloramphenicol, metronidazole, and aminoglycosides. The *B. gracilis* isolates, however, were much less susceptible to the antimicrobials agents. Only 67% of the *B. gracilis* strains were inhibited by penicillin G at a concentration of 16 U/ml or less. For the cephalosporins, susceptibilities at the breakpoint ranged from 67 to 84%, with the broad-spectrum drugs generally showing the least activity. The percent susceptible to clindamycin depended on whether a breakpoint of 4 or 8 µg/ml was chosen, since the MICs were exactly 8 µg/ml for five of the strains. With the lower breakpoint (the Food and Drug Administration-approved breakpoint is 4.8 µg/ml), only 68% of the *B. gracilis* isolates were susceptible to clindamycin. Beta-lactamase production was not detected in any of the *B. gracilis* or *B. ureolyticus* isolates. (Complete methodology and results of antimicrobial susceptibility testing has been submitted for publication elsewhere.)

### DISCUSSION

The recovery of *B. ureolyticus* (previously *B. corrodens*) has been reported in a variety of clinical settings, including cases of brain abscess (9), subdural empyema (17), bite wounds (7), pleuropulmonary infection (1), perirectal abscess (13), and necrotizing fasciitis (6), among others. However, clinical reports from before 1978, when criteria for the identification of *B. ureolyticus* were strictly defined, must be interpreted cautiously. More recently, Duerden et al. reported the isolation of 103 strains of *B. ureolyticus* that do conform to the present taxonomic criteria for this species (2). All of their isolates were recovered from fairly superficial necrotic or gangrenous lesions. Of the isolates, 42 were from perineal, genital or perianal sites, 16 were from gangrenous lesions of the lower extremities, 15 were from soft-tissue abscesses, and the remainder were from a variety of soft-tissue and dental sites. Similarly, the majority of *B. ureolyticus* isolates in our study were recovered from sites of soft-tissue infection. These were usually on the extremities and were mild.

*B. gracilis* was first described as a species in 1981. Previously, these bacteria would have been categorized under various other designations, including *B. corrodens*, "*B. corrodens*-like," "*B. ureolyticus*-like," or *Bacteroides* sp. The original isolation of *B. gracilis* was from a human gingival crevice; there have been no subsequent reports of isolation from other sources. Thus, although the closely related *B. ureolyticus* has been implicated as a pathogen in infections, *B. gracilis* has, to date, had no recognized pathogenic role. In this investigation, we identified 23 strains of *B. gracilis* and found 83% of them to be associated with serious visceral or head and neck infections. Anaerobic pleuropulmonary infections were the single largest disease category. Because all of the isolates of *B. gracilis* were recovered from polymicrobial infections, the role of *B. gracilis* itself in the pathogenesis of these serious infections remains somewhat uncertain. However, the frequency of association of this species with serious anaerobic infections implies virulence.

Antimicrobial susceptibility testing revealed a strikingly high percentage (33%) of *B. gracilis* isolates to be resistant to penicillin. The usual mechanism of penicillin resistance among anaerobes (e.g., *B. fragilis*) is through the production of beta-lactamases. Beta-lactamase was not detected for any of our penicillin-resistant isolates of *B. gracilis*, although the

use of a single assay method may not exclude this possibility (5). The list of penicillin-resistant anaerobes that have been recovered from infections continues to grow. Empiric antimicrobial regimens for the treatment of a seriously ill patient with an anaerobic infection, including pleuropulmonary disease, should take this into account. The presence of *B. gracilis* may explain the failure of some cases of anaerobic infections, such as pleuropulmonary infections, to respond to penicillin, despite the absence of a recognized penicillin-resistant pathogen. The significant resistance of *B. gracilis* to antimicrobial agents suggests that it may be more frequently encountered in the future, as it is selected out under the pressure of exposure to antimicrobial agents.

The taxonomy of agar-pitting, anaerobic, gram-negative bacilli has undergone recent revision, and several new species have been described. In our 11-year review of the clinical occurrence of these organisms, *B. ureolyticus* and *B. gracilis* accounted for 60 and 32% of such isolates, respectively. The association of *B. gracilis* with serious deep-tissue infection, coupled with the relatively high frequency of antibiotic resistance, indicates an important clinical role for this organism.

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