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# Bacteriologic and Clinical Study of *Bacteroides oris* and *Bacteroides buccae*

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**We characterized clinical isolates previously identified in our laboratory as *Bacteroides ruminicola*, the human strains of which are now classified as *Bacteroides oris* and *Bacteroides buccae*. A total of 72 isolates (55 *B. buccae* isolates and 17 *B. oris* isolates) recovered over a 10-year period were studied. They were differentiated from each other by special-potency antibiotic disks and the RapID-ANA system. The two organisms were associated with a variety of infections, the majority being pleuropulmonary (29.2%) and infections of the head and neck region (27.8%). The infections were always polymicrobial, usually with more than five organisms per specimen. A total of 44% of the *B. oris* strains and 27% of the *B. buccae* strains were resistant to penicillin G (breakpoint, 2 U/ml), and this correlated with the presence of beta-lactamase. Although *B. oris* and *B. buccae* are found with some frequency in human infections, they are present primarily as components of a mixed flora.**

In the 1950s, it was noted that several species of ruminal bacteria produce succinic acid as a major end product of carbohydrate fermentation. In 1958, Bryant et al. published their observations on two of these organisms, one of which they called *Bacteroides ruminicola* (3). This organism was a strictly anaerobic, nonmotile, nonsporeforming, gram-negative bacillus capable of fermenting a wide variety of carbohydrates. Two subspecies were recognized, *B. ruminicola* subsp. *brevis* and *B. ruminicola* subsp. *ruminicola*, which differed mainly in cell morphology and rumen fluid requirements. Subsequently, a number of strains resembling *B. ruminicola* were isolated as part of the normal flora of the upper respiratory and intestinal tracts of humans (2,5,6). The role of these strains as pathogens in human infections was reviewed in 1980 (10). However, it was later shown by DNA homology studies that these human strains are not related to *B. ruminicola*, and two new species, *Bacteroides oris* and *Bacteroides buccae*, were proposed by Holdeman et al. (9).

Like *B. ruminicola*, both *B. oris* and *B. buccae* are obligately anaerobic, nonsporeforming, gram-negative rods. They ferment a number of sugars, including the pentose sugars, arabinose, and xylose, which distinguishes them from *Bacteroides oralis*. The two species are similar morphologically, and, although they clearly differ from each other based on DNA homology studies and the polyacrylamide gel electrophoresis patterns of soluble proteins, no commonly used phenotypic test to differentiate them has been identified previously (9). Recently, the RapID-ANA system (Innovative Diagnostics Systems, Inc., Atlanta, Ga.) has been evaluated for its ability to correctly identify anaerobic bacteria on the basis of various enzyme activities. In *Bergey's Manual of Systematic Bacteriology*, Holdeman et al. state that *B. oris* can be differentiated physiologically from *B. buccae* by virtue of its having a negative β-glucosidase test (8). Subsequent testing could not verify this, but it has been noted that *B. oris* differs from *B. buccae* in the α-fucosidase and *N*-acetylglucosaminidase reactions (4). We evaluated these two species in terms of means of identification and of sites, types, and bacteriology of associated infections to assess their clinical significance.

## MATERIALS AND METHODS

**Source of specimens and bacterial strains.** The isolates examined were originally isolated from clinical specimens and were stored in skim milk at −70°C. Records of the Wadsworth Clinical Anaerobic Bacteriology Research Laboratory from January 1976 to July 1985 were reviewed. All clinical isolates identified as *B. ruminicola*, *B. oris*, and *B. buccae* were chosen for study. Duplicate or subsequent specimens from the same patient were not evaluated.

**Bacterial characterization.** All of the isolates used in this study were evaluated in terms of Gram stain reaction, colonial morphology, aerotolerance, susceptibility to special-potency antibiotic disks, and catalase, indole, and nitrate production (13). Fermentation of arabinose, cellobiose, glucose, salicin, sucrose, and xylose and bile susceptibility (2% oxgall) were assessed by using prereduced anaerobically sterilized media. Fluorescence (13) and pigment production were evaluated on sheep and rabbit blood agar media. The ability of the RapID-ANA system to differentiate our clinical isolates of *B. oris* and *B. buccae* was studied. The type strains of *B. oris* (ATCC 33573) and *B. buccae* (ATCC 33574) were used as reference strains.

**Bacteriologic and clinical data.** The source and complete bacteriology of each specimen yielding one of the isolates were noted. Clinical information on patients was obtained from medical records, discharge summaries, autopsy and surgical reports, Infectious Disease Section records, and records of referring physicians.

**Susceptibility and beta-lactamase testing.** Agar plate dilution testing with brucella-laked blood agar (13) was used with one modification (the inoculum was prepared from 24-h plate cultures) to determine susceptibility to the following 12

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TABLE 1. Sites of isolation of *B. buccae* and *B. oris*

| Site | No. of isolates | |
|---|---|---|
| | *B. buccae* | *B. oris* |
| Head and neck | 19 (34.5)[a] | 3 (17.6) |
| Pleuropulmonary[b] | 13 (23.6) | 7 (41.2) |
| Bone and soft tissue above the waist | 10 (18.2) | 2 (11.8) |
| Bone and soft tissue below the waist | 4 (7.3)[c] | 3 (17.6) |
| Intraabdominal | 7 (12.7)[d] | 2 (11.8) |
| Brain | 1 (1.8) | 0 |
| Blood | 1 (1.8) | 0 |

[a] The numbers in parentheses are percentages.
[b] One isolate of each species was from endotracheal suction.
[c] This includes one isolate from an abdominal wound infection.
[d] This includes one nasogastric specimen.

antimicrobial agents: penicillin G, ticarcillin, ticarcillin plus clavulanic acid (Timentin), imipenem, cefazolin, cefoxitin, ceftizoxime, clindamycin, erythromycin, doxycycline, chloramphenicol, and metronidazole. Broth disk susceptibility testing with bicarbonate-supplemented thioglycolate (13) was performed with metronidazole (16 μg/ml), clindamycin (1.6 μg/ml), and penicillin (2 U/ml). Beta-lactamase was assayed qualitatively with nitrocefin disks (Cefinase; BBL Microbiology Systems, Cockeysville, Md.).

## RESULTS

Our records revealed that between January 1976 and July 1985, 152 isolates from 136 patients were identified as *B. ruminicola*, *B. oris*, or *B. buccae*. Of these (excluding duplicate cultures), 72 strains were available for study; on characterization, 55 (76%) proved to be *B. buccae* strains, and 17 (24%) were *B. oris* strains.

As previously shown, Gram stain reactions and colonial morphology were indistinguishable for the two taxa. All strains fermented arabinose and xylose and were bile susceptible, and none produced indole or catalase or reduced nitrate. Both *B. oris* and *B. buccae* were resistant to vancomycin (5-μg disks) and kanamycin (1,000-μg disks). *B. buccae*, but not *B. oris*, was susceptible to colistin (10-μg disks). The majority of strains of both species fluoresced chartreuse on either sheep or rabbit blood agar at 72 h. Three strains of *B. oris* (18%) produced brown pigment on sheep blood agar after 2 to 6 weeks of incubation.

*B. buccae* was easily and correctly identified to the species level by RapID-ANA testing. *B. oris* was distinguishable from *B. buccae* on the basis of the α-fucosidase and *N*-acetylglucosaminidase reactions (both positive for *B. oris*). However, even with revised criteria (4), six strains of *B. oris* (35%) could not be differentiated from *B. oralis*, *Bacteroides loescheii*, *Bacteroides bivius*, and *Bacteroides denticola* by RapID-ANA testing. *B. oris* is readily distinguishable from these taxa on the basis of pentose sugar fermentation when prereduced anaerobically sterilized biochemicals are used.

Complete bacteriology was available for 68 of the 72 specimens studied. Three strains were referred to our laboratory for identification without further information. One specimen had been worked up anaerobically only. Neither *B. oris* nor *B. buccae* was isolated in pure culture. A total of 90% of the infections yielding *B. buccae* and 94% of the infections yielding *B. oris* involved five or more pathogens, with a combination of aerobes and anaerobes in all but one case.

A total of 52 patients had infections with *B. buccae*, 14 patients had infections with *B. oris*, and 3 patients had infections with both *B. oris* and *B. buccae*. The majority of isolates (58.3%) came from head and neck sources and pleuropulmonary sources (Table 1). There was no difference between the two species in terms of sites of isolation. Complete clinical data was available for 58 patients, 44 with infections involving *B. buccae*, 11 with infections involving *B. oris*, and 3 with infections involving both organisms. Outcome was generally good. Treatment in many instances involved both medical and surgical therapy, as in anaerobic infections generally. Three patients from whom *B. buccae* had been isolated died. In one, the role of infection as a cause of death was not clear. In another patient, the specimen was obtained by endotracheal suctioning, so that the significance of the isolate is questionable. The third patient who died had *Bacteroides fragilis* isolated from a blood culture premortem. Two patients in the *B. oris* group died. One of these had an isolate obtained by endotracheal tube suction, and the other patient clearly died of unrelated neurological complications. One patient infected with both *B. oris* and *B. buccae* died.

Agar plate dilution and broth disk susceptibility testing were performed with 52 *B. buccae* strains and 16 *B. oris* strains. All of the organisms were inhibited by ticarcillin, Timentin (ticarcillin plus clavulanic acid), imipenem, cefoxitin, ceftizoxime, clindamycin, chloramphenicol, doxycycline, erythromycin, and metronidazole at the breakpoint concentration or at a lower concentration. The breakpoints used were those listed in reference 10, except for ceftizoxime (32 μg/ml), imipenem (8 μg/ml), ticarcillin (128 μg/ml), and Timentin (128 μg of ticarcillin per ml). Penicillin G and cefazolin were less active against these two species (Table 2). Cefazolin inhibited 69% of the *B. oris* strains and 83% of the *B. buccae* strains at a breakpoint of 32 μg/ml; 56% of the *B. oris* isolates and 73% of the *B. buccae* isolates were inhibited by penicillin G at a breakpoint of 2 U/ml, and 75 and 83%, respectively, were inhibited at a breakpoint of 32 U/ml. The results of broth disk susceptibility testing correlated well with the results of the agar dilution method. All organisms with an MIC of penicillin G of >2 U/ml were beta-lactamase positive.

TABLE 2. Results of agar dilution susceptibility testing

| Antibiotic | Breakpoint[a] | *B. oris* | | | | *B. buccae* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | % Inhibited at breakpoint | MIC[b] | | | % Inhibited at breakpoint | MIC | | |
| | | | Range | 50% | 90% | | Range | 50% | 90% |
| Penicillin G | 32 | 75 | ≤0.5–64 | ≤0.5 | 64 | 83 | ≤0.5–128 | ≤0.5 | 64 |
| Penicillin G | 2 | 56 | | | | 73 | | | |
| Cefazolin | 32 | 69 | ≤0.5–128 | ≤0.5 | 128 | 83 | ≤0.5–128 | 2 | 64 |

[a] Expressed as units of drug per milliliter for penicillin G and micrograms of drug per milliliter for cefazolin.
[b] 50% and 90%, MIC for 50 and 90% of strains tested, respectively.

## DISCUSSION

In 1982, human isolates resembling *B. ruminicola* were described as two new species, *B. oris* and *B. buccae*, on the basis of DNA homology studies. Although these species are indistinguishable by many conventional tests, we found that they could be differentiated from each other by either special-potency antibiotic disks or the RapID-ANA system.

*B. oris* and *B. buccae* have been isolated from a number of human specimens under both their new taxonomic status (7, 9, 11) and their previous designation as *B. ruminicola* (1, 10, 12). In this study, we found both *B. oris* and *B. buccae* involved in a number of infections, *B. buccae* more commonly than *B. oris*. Most clinical specimens were from head and neck sources or pleuropulmonary sources. This is in keeping with their being found predominantly as part of the human oral flora (7, 9, 11). Also of note is the fact that the majority of bone and soft-tissue infections above the waist were bite wound infections. Strains were found in mixed infections, usually with a large number of other recognized pathogens.

Susceptibility testing revealed that 32% of the organisms were resistant to penicillin. This was found more commonly with *B. oris* than with *B. buccae* (44 compared with 27%) and correlated well with the presence of beta-lactamase. However, both species are susceptible to a large number of other antimicrobial agents and should not pose a therapeutic problem.

Although both of these organisms may be isolated from a variety of infections, they are found primarily as one element of a mixed flora. In such infections it is difficult to determine the role of individual isolates.

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