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# Recovery of Anaerobic Bacteria from Clinical Specimens in 12 Years at Two Military Hospitals

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**Examination of 15,844 clinical specimens submitted over 12 years (1973 to 1985) to the anaerobic microbiology laboratories in two military hospitals demonstrated the recovery of anaerobic bacteria in 4,458 (28.1%) specimens. The specimens yielded 6,557 anaerobic isolates (1.47 isolates per specimen). *Bacteroides* spp. accounted for 43% of all isolates; anaerobic gram-positive cocci, 26%; *Clostridium* spp., 7%; and *Fusobacterium* spp., 4%. *Bacteroides* spp. predominated in abscesses, obstetrical and gynecological (OBG) infections, abdominal infections, cysts, wounds, and tumors. Members of the *Bacteroides fragilis* group accounted for 44% of all *Bacteroides* spp., and of them, *B. fragilis* was mostly isolated in abscesses, wounds, abdomen, and blood. Pigmented *Bacteroides* spp. accounted for 21% of all *Bacteroides* sp. isolates and were mostly isolated in sinus, eye, chest, bone, and ear infections. *Bacteroides melaninogenicus* accounted for 42% of this group's isolates. *Bacteroides bivius* accounted for 9% of *Bacteroides* spp., and most isolates were found in OBG infections. Anaerobic gram-positive cocci were mostly isolated in OBG infections, abscesses, and wounds. The predominant anaerobic gram-positive cocci were *Peptostreptococcus magnus* (18%), *Peptostreptococcus asaccharolyticus* (17%), *Peptostreptococcus anaerobius* (16%), and *Peptostreptococcus prevotii* (13%). *Clostridium* spp. were mostly isolated from wounds, abscesses, abdominal infections, and blood. The predominant strain was *Clostridium perfringens* (48%). *Fusobacterium* spp. were recovered in abscesses and abdominal and OBG infections. The predominant isolate was *Fusobacterium nucleatum* (47%). These data illustrate the relative frequency of the different anaerobic bacteria in a variety of infections and demonstrate the predominance of certain isolates at different sites.**

Anaerobic bacteria are important pathogens that can cause a variety of infections in humans (7, 14). With the recently improved techniques for their isolation and identification, increasing numbers of reports have documented the importance of anaerobic bacteria in infectious processes. This knowledge has provided clinicians with new awareness of anaerobic bacteria and the need to direct diagnostic efforts toward their isolation and therapeutic intervention toward their eradication.

Most of the data in the literature regarding the incidence of recovery of anaerobes from clinical specimens are not entirely reliable. Finegold (14) summarized data from 10 studies that evaluated the frequency of recovery of anaerobes in clinical specimens. Most of these studies were completed before 1966, and three presented only general data. Although several other reports were published (2, 17), only one report (22) summarized data from 1973 to 1983. Since the publication of most of these reports, much improvement has occurred in the methods of collection, transportation, cultivation, and identification of anaerobic bacteria. Furthermore, many of the anaerobes have been reclassified and renamed according to newer criteria for their identification. Therefore, a more current evaluation of the incidence of recovery of anaerobic bacteria from clinical specimens is desirable. Such a study may better show the frequency of the recovery of anaerobes from clinical specimens today and may present the effect of these improvements on the recovery of these organisms.

The purpose of this report was to summarize the experience of the microbiology laboratories of two military hospitals, Walter Reed Army Medical Center and the Naval Medical Center, over 12 years (1973 to 1985) in the recovery of anaerobic bacteria from clinical specimens. The data provide details on the recovery of all anaerobic bacteria, including some of the newly recognized species and those that were recently reclassified.

## MATERIALS AND METHODS

Between June 1973 and June 1985, 15,844 clinical specimens were processed by the clinical anaerobic microbiology laboratories at Walter Reed Army Medical Center in Washington, D.C., and the Naval Medical Center in Bethesda, Md. Bacterial growth was observed in 4,458 (28.1%) specimens. These included 359 specimens from abdominal infections, 820 specimens from subcutaneous and soft tissue abscesses, 66 biliary tract infections, 9 human bites, 587 specimens of blood, 37 of bone, 220 central nervous system (CNS), 191 chest infections, 206 inclusion cysts, 25 ear, 55 eye, 13 vascular grafts, 63 joints, 70 lymph glands, 871 obstetrical and gynecological (OBG), 30 genitourinary, 102 sinuses, 61 necrotic tumors, 622 wounds, and 51 miscellaneous infections.

The microbiology laboratories accepted only specimens that were properly collected and submitted in transport media appropriate for anaerobic bacteria. Specimens accepted for processing were those collected in a fashion that avoided contamination by the normal skin or mucus surface flora (7, 22). These were specimens obtained during surgery, aseptic aspiration of body fluids, or needle or biopsy aspiration of abscesses or body cavities. Aspirates of lungs were obtained through transtracheal aspiration or biopsy, obstetrical specimens were collected through culdocentesis or during surgery, and urine was collected through suprapubic aspiration. When possible, pus and fluids were collected and transported in syringes. Tissues were transported in oxygen-free gassed-out tubes or in small volumes of thioglycolate broth. When possible, specimens were hand delivered. Some specimens were processed at the bedside or in the operating area by inoculating them in appropriate media and immediately placing the media in anaerobic jars. Swab cultures were placed in transport media (Port-a-Cul; BBL Microbiology Systems, Cockeysville, Md.; or Vacutainer, Becton Dickinson Vacutainer Systems, Rutherford, N.J.). However, no exact record is available regarding that information. Five milliliters of blood was collected aseptically from patients suspected of having bacteremia and was inoculated (10%, vol/vol) into one bottle each of aerobic and anaerobic BACTEC media. Anaerobic bottles were examined visually on days 1 through 7, read on a BACTEC 640 reader on days 2 and 4 through 7, and blindly subcultured on day 3.

The specimen material was plated onto prereduced vitamin $K_1$-enriched brucella blood agar, an anaerobic blood agar plate containing kanamycin and vancomycin, and a Columbia CNA anaerobic blood plate; inoculated into an enriched thioglycolate broth (containing hemin, sodium bicarbonate, and vitamin $K_1$ [22]); and incubated in GasPak jars (BBL). Cultures were examined at 48 and 96 h. Plates that showed growth were held until the organisms were processed and identified. All cultures that showed no growth were held for at least 5 days. Anaerobes were identified by the API anaerobic system (Analytab Products, Plainview, N.Y.) at Walter Reed and by the Minitek system (BBL) at the Naval Medical Center. In addition to these tests, when complete identification was not possible by the above methods, other carbohydrate tests (Scott Laboratories, Fiskeville, R.I.) and gas-liquid chromatography (22) were performed as needed to identify the organisms. The criteria for identification were according to previously published guidelines (16, 22).

## RESULTS

A total of 6,557 bacterial isolates were recovered from the 4,458 specimens that yielded growth (1.47 isolates per specimen) (Table 1). The frequency of recovery of the different anaerobic strains differs in various infection sites. The highest numbers of isolates per specimen were in ear infections (1.88), abscesses and genitourinary infections (1.73 each), cysts (1.69), wounds (1.59), and abdominal infections (1.53).

*Bacteroides* spp. accounted for 43% of anaerobic isolates (Table 1). They predominated in abdominal infections, abscesses, OBG infections, infected cysts, wounds, and tumors. They were less often recovered in infections of the lymph glands, joint, eye, and CNS.

A total of 2,835 isolates of *Bacteroides* spp. were recovered from the 4,458 specimens (0.64 isolate per specimen). The highest recovery rate of *Bacteroides* spp. per sample was observed with genitourinary specimens (Table 2), abscesses, abdominal and OBG infections, cysts, and wound infection. The lowest recovery rate was with lymph gland, eye, joint, and CNS infections.

The recovery of *Bacteroides* spp. varied among the different infections. Members of the *Bacteroides fragilis* group accounted for 44% of all *Bacteroides* isolates and were most frequently recovered (compared with other *Bacteroides* spp.) from abdominal infections (79% of all *Bacteroides* isolates) (Table 2), bile (76%), and blood cultures (74%). They were found least often in chest infections (34%), OBG infections (28%), cysts (27%), bone (12%), and sinuses (8%). These calculations disregarded infections with very few (<12) isolates of *Bacteroides* spp.

TABLE 1. Recovery of anaerobes from each infection site

| Specimen source | Total no. of specimens | Total no. of anaerobic isolates | No. of anaerobic isolates per specimen | % Recovery | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Bacteroides* spp. | *Fusobacterium* spp. | *Clostridium* spp. | *Lactobacillus* spp. | *Eubacterium* spp. | *Propionibacterium* spp. | *Bifidobacterium* spp. | *Actinomyces* spp. | *Veillonella* spp. | AGPC |
| Abdomen | 359 | 550 | 1.53 | 299 (55)[a] | 43 (8) | 71 (13) | 4 (1) | 31 (6) | 23 (4) | | | 8 (1) | 71 (13) |
| Abscess | 820 | 1,416 | 1.73 | 725 (51) | 97 (7) | 71 (5) | 7 (0.5) | 44 (3) | 54 (4) | 5 (0.5) | 2 (0.2) | 28 (2) | 383 (27) |
| Bile | 66 | 75 | 1.14 | 29 (39) | 1 (1) | 27 (36) | | | 9 (12) | | | | 9 (12) |
| Bites | 9 | 12 | 1.33 | 5 (42) | 1 (8) | | | | 2 (17) | | | | 4 (33) |
| Blood | 587 | 634 | 1.08 | 222 (35) | 24 (4) | 70 (11) | 1 (0.2) | 13 (2) | 229 (36) | 1 (0.2) | | 7 (1) | 67 (11) |
| Bone | 37 | 69 | 1.86 | 24 (35) | 4 (6) | 2 (3) | | 1 (1) | | | | 2 (3) | 27 (39) |
| CNS | 220 | 225 | 1.02 | 16 (7) | 2 (1) | 4 (2) | | | 163 (72) | | 1 (0.5) | | 39 (17) |
| Chest | 191 | 283 | 1.48 | 101 (37) | 31 (11) | 18 (6) | 1 (0.4) | 9 (3) | 51 (18) | 4 (1) | | 9 (3) | 59 (21) |
| Cysts | 206 | 348 | 1.69 | 153 (44) | 5 (1) | 6 (2) | 4 (1) | 6 (2) | 24 (7) | 1 (0.3) | | 10 (3) | 139 (40) |
| Ear | 25 | 47 | 1.88 | 12 (26) | 1 (2) | 1 (2) | | | 7 (15) | | | 1 (2) | 25 (53) |
| Eye | 55 | 66 | 1.20 | 8 (12) | 3 (5) | 11 (17) | | | 36 (55) | | | 4 (6) | 4 (6) |
| Genitourinary tract | 30 | 52 | 1.73 | 29 (56) | 2 (4) | 1 (2) | 1 (2) | 3 (6) | 3 (6) | | | 2 (4) | 11 (21) |
| Grafts | 13 | 15 | 1.15 | 4 (27) | | 1 (7) | | | 5 (33) | | | | 5 (33) |
| Joints | 63 | 69 | 1.10 | 9 (13) | | 8 (12) | | | 39 (57) | | | | 13 (19) |
| Lymph glands | 70 | 76 | 1.09 | 11 (15) | 3 (4) | | 1 (1) | | 48 (63) | 1 (1) | | 2 (3) | 10 (13) |
| OBG infections | 871 | 1,328 | 1.52 | 654 (49) | 42 (3) | 50 (4) | 15 (1) | 28 (2) | 28 (2) | 12 (1) | 1 (1) | 28 (2) | 470 (35) |
| Sinuses | 102 | 159 | 1.56 | 53 (33) | 11 (7) | 2 (1) | | 2 (1) | 36 (23) | | 1 (1) | 7 (4) | 47 (30) |
| Tumors | 61 | 79 | 1.30 | 33 (42) | 1 (1) | 1 (1) | | 1 (1) | 22 (28) | 1 (1) | | 1 (1) | 19 (24) |
| Wounds | 622 | 987 | 1.59 | 425 (43) | 20 (2) | 124 (13) | 6 (1) | 18 (2) | 66 (7) | 1 (0.1) | | 14 (1) | 313 (31) |
| Miscellaneous | 51 | 67 | 1.31 | 23 (34) | 3 (4) | 3 (4) | | 2 (3) | 20 (30) | 1 (1) | | 2 (3) | 13 (19) |
| Total | 4,458 | 6,557 | 1.47 | 2,835 (43) | 294 (4) | 471 (7) | 40 (1) | 158 (2) | 874 (13) | 27 (0.4) | 5 (0.1) | 125 (2) | 1,728 (26) |

[a] Number in parentheses is percentage of all anaerobic bacteria isolated from source indicated.

TABLE 2. Relative incidence of specific *Bacteroides* sp. in various infections (Walter Reed Army Medical and Naval Medical Centers, 1973 to 1985)

| Specimen source | Total no. of specimens | Avg no. of *Bacteroides* spp. per specimen | Total no. of *Bacteroides* spp. | Relative incidence | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *B. oris*, *B. buccae*[a] | *B. disiens* | *B. bivius* | *B. oralis* | *B. ureolyticus* | Pigmented *Bacteroides* spp. | *B. fragilis* group | *Bacteroides* spp. |
| Abdomen | 359 | 0.83 | 299 | 4 (1)[b] | 2 (1) | 2 (1) | | 3 (1) | 22 (7) | 236 (79) | 30 (10) |
| Abscess | 820 | 0.88 | 725 | 6 (1) | 3 (4) | 28 (4) | 19 (3) | 80 (11) | 209 (29) | 283 (39) | 97 (13) |
| Bile | 66 | 0.44 | 29 | 1 (3) | | | | | 1 (3) | 22 (76) | 5 (17) |
| Bites | 9 | 0.56 | 5 | | | | | | 1 (20) | 3 (60) | 1 (20) |
| Blood | 587 | 0.38 | 222 | | 2 (1) | 6 (3) | 3 (1) | 3 (1) | 14 (6) | 165 (74) | 29 (13) |
| Bone | 37 | 0.65 | 24 | | | 2 (8) | 2 (8) | 1 (4) | 11 (46) | 3 (12) | 5 (21) |
| CNS | 220 | 0.07 | 16 | | | | | | 2 (12) | 11 (69) | 3 (19) |
| Chest | 191 | 0.53 | 101 | | | 2 (2) | 3 (3) | | 48 (48) | 34 (34) | 12 (12) |
| Cysts | 206 | 0.74 | 153 | 3 (1) | 1 (1) | 9 (6) | 3 (2) | 25 (16) | 36 (24) | 41 (27) | 35 (23) |
| Ear | 25 | 0.48 | 12 | | | | | 5 (42) | 5 (42) | 1 (8) | 1 (8) |
| Eye | 55 | 0.15 | 8 | | | 1 (12) | | 1 (12) | 4 (50) | | 2 (25) |
| Genitourinary tract | 30 | 0.96 | 29 | | | 6 (21) | | 1 (4) | 5 (17) | 16 (55) | 1 (4) |
| Grafts | 13 | 0.31 | 4 | | | | | | | 2 (50) | 2 (50) |
| Joints | 63 | 0.14 | 9 | | | 1 (11) | | | 1 (11) | 6 (67) | 1 (11) |
| Lymph glands | 70 | 0.16 | 11 | | | | 1 (9) | 1 (9) | 3 (27) | 3 (27) | 3 (27) |
| OBG infections | 871 | 0.68 | 654 | 2 (0.5) | 15 (2) | 168 (26) | 11 (2) | 44 (7) | 117 (18) | 183 (28) | 114 (17) |
| Sinuses | 102 | 0.52 | 53 | 2 (4) | | | 2 (4) | 1 (2) | 29 (55) | 4 (8) | 15 (28) |
| Tumors | 61 | 0.54 | 33 | 1 (3) | | | 1 (3) | 1 (3) | 6 (18) | 16 (48) | 8 (24) |
| Wounds | 622 | 0.68 | 425 | 1 (0.5) | 2 (5) | 24 (6) | 5 (1) | 21 (5) | 86 (20) | 223 (52) | 63 (15) |
| Miscellaneous | 51 | 0.45 | 23 | | | 1 (4) | 1 (4) | | 8 (35) | 9 (39) | 4 (17) |
| Total | 4,458 | 0.64 | 2,835 | 20 (1) | 25 (1) | 250 (9) | 51 (2) | 189 (7) | 608 (21) | 1,261 (44) | 431 (15) |

[a] Characterization data separating *B. oris* and *B. buccae* were not available.
[b] Number in parentheses is percentage of all *Bacteroides* isolates from source indicated.

The recovery rates of the different members of the *B. fragilis* group also varied (Table 3). *B. fragilis* accounted for 63% of all *B. fragilis* group isolates; *Bacteroides thetaiotaomicron*, for 14%; *Bacteroides vulgatus* and *Bacteroides ovatus*, for 7% each; *Bacteroides distasonis*, for 6%; and *Bacteroides uniformis*, for 2%. The highest frequency of recovery of *B. fragilis* compared with other members of the *B. fragilis* group was in blood cultures (78% of all *B. fragilis* group isolates), tumors (75%), wounds (69%), abscesses (65%), and abdominal infection (59%). *B. thetaiotaomicron* was most frequently isolated from chest infections (35%), cysts (22%), and tumors (19%). *B. vulgatus* was mostly recovered from OBG infections (20%); *B. ovatus*, from bile infections (18%); *B. distasonis*, from OBG (10%) and abdominal (9%) infections; and *B. uniformis*, from wounds (3%) and abscesses (2%).

The incidence of recovery of the different members of the *B. fragilis* group varied. Of the 800 isolates of *B. fragilis*, 183 (23%) were found in abscesses; 153 (19%), in wounds; 139 (17%), in abdominal infections; 129 (16%), in blood; and 103 (13%), in OBG infections. Of 181 isolates of *B. thetaiotaomicron*, 42 (23%) were found in abscesses; 34 (19%) each, in abdomen and wounds; 23 (13%), in blood; 13 (7%), in OBG; and 12 (7%), in chest infections. Of 92 isolates of *B. vulgatus*, 36 (39%) were recovered from OBG infections, 18 (20%) were recovered from abdomen infections, and 13 (14%) were recovered from abscesses. Of 86 *B. ovatus* isolates, 26 (30%) were found in abscesses, 22 (26%) were found in abdomen infections, and 9 each (10%) were found in OBG infections and wounds. Of 81 *B. distasonis* isolates, 21 (26%) were isolated from abdomen infections, 19 (23%) were from OBG infections, and 13 (16%) each were found in abscesses and wounds. Of the 21 *B. uniformis* isolates, 7 (33%) were isolated from wounds and 6 (29%) were found in abscesses.

Pigmented *Bacteroides* spp. accounted for 21% of *Bacteroides* isolates (Table 4). They were found in (in descending order of frequency) sinuses, eye, chest, bone, ear, miscellaneous, abscesses, lymph glands, bites, cysts, wounds, tumors, OBG and genitourinary infections, CNS, joint, abdomen, blood, and bile infections.

The recovery rates of the pigmented *Bacteroides* spp. were different (Table 4). *Bacteroides melaninogenicus* accounted for 42% of all pigmented *Bacteroides* spp.; *Bacteroides asaccharolyticus*, for 28%; *Bacteroides intermedius*, for 22%; *Bacteroides corporis*, for 4%; *Bacteroides denticola*, for 3%; and *Bacteroides loescheii*, for 1%. The highest frequency of recovery of *B. melaninogenicus* was in bones, sinuses, blood, and chest. *B. asaccharolyticus* was most frequently recovered from abscesses, abdomen infections, and wounds. *B. intermedius* was most frequently recovered from cysts. *B. corporis* and *B. denticola* were mostly isolated from chest infections and abscesses.

The incidence of recovery of pigmented *Bacteroides* spp. and other *Bacteroides* spp. varied. Of 256 *B. melaninogenicus* isolates, 67 (26%) were found in abscesses; 58 (23%), in OBG infections; 38 (15%), in wounds; 21 (8%), in chest infections; 17 (7%), in cysts; and 15 (6%), in sinuses. Of 170 *B. asaccharolyticus* isolates, 71 (42%) were found in abscesses, 35 (21%) were found in OBG infections, and 24 (14%) were found in wounds. Of 134 *B. intermedius* isolates, 46 (34%) were found in abscesses, 21 (16%) were found in

TABLE 3. Relative incidence of *B. fragilis* group in various infections (Walter Reed Army and Naval Medical Centers, 1973 to 1985)

| Specimen source | Total no. of specimens | Total *B. fragilis* group (% all *Bacteroides* spp.) | Relative incidence | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *B. uniformis* | *B. thetaiotaomicron* | *B. vulgatus* | *B. ovatus* | *B. distasonis* | *B. fragilis* |
| Abdomen | 359 | 236 (79) | 2 (1)[a] | 34 (14) | 18 (8) | 22 (9) | 21 (9) | 139 (59) |
| Abscess | 820 | 283 (39) | 6 (2) | 42 (15) | 13 (5) | 26 (9) | 13 (5) | 183 (65) |
| Bile | 66 | 22 (76) | 1 (5) | 4 (18) | 2 (9) | 4 (18) | 2 (9) | 9 (41) |
| Bites | 9 | 3 (75) | | 1 (33) | | | | 2 (67) |
| Blood | 587 | 165 (76) | | 23 (14) | 5 (3) | 6 (4) | 2 (1) | 129 (78) |
| Bone | 37 | 3 (12) | | | | | | 3 (100) |
| CNS | 220 | 11 (69) | 1 (9) | 1 (9) | | | 1 (9) | 8 (73) |
| Chest | 191 | 34 (34) | | 12 (35) | 2 (6) | 3 (9) | 3 (9) | 14 (41) |
| Cysts | 206 | 41 (27) | | 9 (22) | 4 (10) | 5 (12) | 3 (7) | 20 (49) |
| Ear | 25 | 1 (8) | | | 1 (100) | | | |
| Eye | 55 | | | | | | | |
| Genitourinary tract | 30 | 16 (55) | 1 (6) | 2 (13) | 2 (13) | 1 (6) | 2 (13) | 8 (50) |
| Grafts | 13 | 2 (50) | | 1 (50) | 1 (50) | | | |
| Joints | 63 | 6 (67) | | | | | | 6 (100) |
| Lymph glands | 70 | 3 (27) | | 1 (33) | | | 1 (33) | 1 (33) |
| OBG infections | 871 | 183 (28) | 3 (2) | 13 (7) | 36 (20) | 9 (5) | 19 (10) | 103 (56) |
| Sinuses | 102 | 4 (8) | | 1 (25) | 1 (25) | | | 2 (50) |
| Tumors | 61 | 16 (47) | | 3 (19) | | 1 (6) | | 12 (75) |
| Wounds | 622 | 223 (52) | 7 (3) | 34 (15) | 7 (3) | 9 (4) | 13 (6) | 153 (69) |
| Miscellaneous | 51 | 9 (39) | | | | | 1 (11) | 8 (89) |
| Total | 445 | 1,261 (44) | 21 (2) | 181 (14) | 92 (7) | 86 (7) | 81 (6) | 800 (63) |

[a] Number in parentheses is percentage of all *B. fragilis* group from source indicated.

OBG infections, and 17 (13%) were found in wounds. Of 22 *B. corporis* isolates, 8 (36%) were found in abscesses (mostly in and adjacent to the oral area) and 5 (23%) were found in wounds. Of 17 *B. denticola* isolates, 10 (59%) were isolated from abscesses (all in or related to the oral cavity).

Of the 250 isolates of *B. bivius* (Table 2), 168 (67%) were recovered from OBG infections, 28 (11%) were recovered from abscesses, and 24 (10%) were found in wounds (mostly in and around the rectal or vulvovaginal area). Of 189 *B. ureolyticus* isolates, 80 (42%) were isolated from abscesses and 44 (23%) were found in OBG infections. Of 51 isolates of *B. oralis*, 19 (37%) were recovered from abscesses and 11 (22%) were recovered from OBG infections. Of 25 isolates of *B. disiens*, 15 (60%) were from OBG infections.

Anaerobic gram-positive cocci (AGPC) accounted for 26% of all isolates (Table 1). The infected sites where they predominated were ears, cysts, bones, and OBG. They were uncommonly found in CNS, abdomen, lymph gland, bile, and eye. Most isolates were found in OBG infections, abscesses, and wounds. The recovery rates differed for the 10 different AGPC and microaerophilic streptococci (Table 5). These were (in descending order of frequency) *Peptostreptococcus magnus*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevotii*, *Peptostreptococcus micros*, *Peptostreptococcus saccharolyticus*, and *Streptococcus intermedius*.

The highest frequency of recovery of *P. magnus* was with bone and chest infections. The highest recovery rate of *P. asaccharolyticus* and *P. anaerobius* was with OBG infections and wounds. Isolates of each of the most frequently recovered AGPC were recovered from abscesses, wounds, and OBG infections (Table 5).

*Clostridium* spp. made up 7% of all anaerobic isolates (Table 1). They predominated in infections of the bile, eye, abdomen, wounds, joint, and blood. They were less commonly found in ears, cysts, tumors, and sinuses. Most isolates were recovered from wounds, abscesses, abdominal infections, and blood. The most frequently recovered *Clostridium* sp. was *Clostridium perfringens* (48% of all clostridial isolates) (Table 6). Other species of *Clostridium* were found less frequently. Of 227 *C. perfringens* isolates, 59 (26%) were found in wounds and 37 (16%) were found in blood.

*Fusobacterium* spp. accounted for 4% of all isolates (Table 1). They were often found in infections of the chest, abdomen, and sinuses and in abscesses. They were infrequently recovered from OBG infections, ears, wounds, tumors, cysts, CNS, and bile. Most fusobacteria were recovered from abscesses and abdominal and OBG infections. The most common *Fusobacterium* sp. was *Fusobacterium nucleatum* (47% of all *Fusobacterium* spp.) (Table 7). Of 137 *F. nucleatum* isolates, 43 (31%) were recovered from abscesses and 24 (18%) were found in OBG infections.

Anaerobic, gram-positive, nonsporeforming rods accounted for about 20% of all isolates (Table 1). Strains of *Propionibacterium* spp. (97% identified as *Propionibacterium acnes*) were isolated in 13% of all specimens. They were often found in CNS, lymph glands, joints, and blood. They were infrequently recovered from wounds and OBG infections.

Strains of *Eubacterium* accounted for 2% of all anaerobic isolates (Table 1) and were most often isolated in abscesses (28% of all *Eubacterium* spp.) and OBG infections (18%). *Lactobacillus* spp. accounted for 1% of all anaerobic iso-

TABLE 4. Relative incidence of members of the pigmented *Bacteroides* spp. in various infections (Walter Reed Army and Naval Medical Centers, 1973 to 1985)

| Specimen source | Total no. of specimens | Total pigmented *Bacteroides* spp. (% all *Bacteroides* spp.) | Relative incidence | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *B. asaccharolyticus*[a] | *B. intermedius* | *B. melaninogenicus* | *B. denticola* | *B. loescheii* | *B. corporis* |
| Abdomen | 359 | 22 (7) | 7 (32)[b] | 5 (23) | 10 (45) | | | |
| Abscess | 820 | 209 (29) | 71 (34) | 46 (22) | 67 (32) | 10 (5) | 7 (3) | 8 (4) |
| Bile | 66 | 1 (3) | | | 1 (100) | | | |
| Bites | 9 | 1 (25) | | | 1 (100) | | | |
| Blood | 587 | 14 (6) | 3 (21) | 3 (21) | 7 (50) | 1 (7) | | |
| Bone | 37 | 11 (46) | 2 (18) | 3 (27) | 6 (55) | | | |
| CNS | 220 | 2 (12) | 1 (50) | | 1 (50) | | | |
| Chest | 191 | 48 (48) | 9 (19) | 10 (21) | 21 (44) | 3 (6) | 2 (4) | 3 (6) |
| Cysts | 206 | 36 (24) | 5 (14) | 12 (33) | 17 (47) | | | 2 (6) |
| Ear | 25 | 5 (42) | 1 (20) | 2 (40) | 2 (40) | | | |
| Eye | 55 | 4 (50) | | 2 (50) | 2 (50) | | | |
| Genitourinary tract | 30 | 5 (17) | 2 (40) | 1 (20) | 2 (40) | | | |
| Grafts | 13 | | | | | | | |
| Joints | 63 | 1 (11) | 1 (100) | | | | | |
| Lymph glands | 70 | 3 (27) | | 2 (67) | 1 (33) | | | |
| OBG infections | 871 | 117 (18) | 35 (30) | 21 (18) | 58 (50) | | | 3 (3) |
| Sinuses | 102 | 29 (55) | 6 (21) | 7 (24) | 15 (52) | 1 (3) | | |
| Tumors | 61 | 6 (19) | 2 (33) | 1 (17) | 3 (50) | | | |
| Wounds | 622 | 86 (20) | 24 (28) | 17 (20) | 38 (44) | 2 (2) | | 5 (6) |
| Miscellaneous | 51 | 8 (35) | 1 (12) | 2 (25) | 4 (50) | | | 1 (12) |
| Total | 4,458 | 608 (21) | 170 (28) | 134 (22) | 256 (42) | 17 (3) | 9 (1) | 22 (4) |

[a] Characterization data separating *B. asaccharolyticus*, *B. gingivalis*, and *B. endodontalis* were not available.
[b] Number in parentheses is percentage of all pigmented *Bacteroides* spp. from source indicated.

lates. Most isolates were recovered from OBG infections (37% of all *Lactobacillus* isolates). *Bifidobacterium* spp. accounted for 0.4% of all isolates and was mostly isolated in OBG infections (44% of all *Bifidobacterium* isolates).

## DISCUSSION

The data presented illustrate the relative frequencies of species of *Bacteroides*, AGPC, *Clostridium*, and *Fusobacterium* in various infections. About 81% of the anaerobic bacteria recovered from the clinical infections examined are members of these genera. The polymicrobial nature of anaerobic infections also was highlighted from the above data; the average number of isolates per specimen was 1.47. However, the number of isolates varied between one and seven. Although the pathogenic roles of all bacterial isolates have not been established, their synergistic characteristics in polymicrobial infections have been well established (8, 9, 11), and the virulence of species in genera such as *Bacteroides*, *Clostridium*, *Peptostreptococcus*, and *Fusobacterium* is well documented in animal studies (9) and for clinical infections (7, 14).

Species of *Bacteroides* are the most frequently recovered anaerobes in past studies (7) as well as in this report. Bacteroides are important anaerobic pathogens that can be recovered from a variety of infections (7, 14). Members of the *B. fragilis* group are primarily isolated from abdominal infections (5), pigmenting *Bacteroides* group members are isolated from oral and respiratory tract infections (6), and *B. disiens* and *B. bivius* are isolated from female genital tract infections (15, 20).

The relative distribution of the different *Bacteroides* species has important clinical implications for the management of infections involving anaerobic bacteria because of the differing antimicrobial susceptibilities of various *Bacteroides* species. Although most members of the *B. fragilis* group produce β-lactamase and resist penicillin, their susceptibilities to cephalosporins are variable (1) but predictable (23).

It is evident that members of the *B. fragilis* group are the most prevalent of the *Bacteroides* spp. isolated. *B. fragilis* is the most prevalent organism in the *B. fragilis* group, accounting for 41% (bile infections) to 78% (in bacteremia) of the isolates of the group. However, it should be remembered that the other members of the group account for the rest of the *B. fragilis* group isolates. This is of particular importance since these members are more resistant than *B. fragilis* to the newer cephalosporins. The growing resistance of *Bacteroides* species previously susceptible to penicillins has been noticed in the last decade (10, 19). These are members of the *B. melaninogenicus* group, *B. oralis*, *B. disiens*, *B. bivius*, *B. oris*, and *B. buccae*. The main mechanism of resistance is through the production of the enzyme β-lactamase. When choices are made between antimicrobial agents for the therapy of infections involving *Bacteroides* spp., their complete identification and testing for antimicrobial susceptibility and β-lactamase production are of practical importance.

The distribution of the various *Bacteroides* isolates in this report is similar, although not identical, to the recovery rate at the anaerobic laboratory at Wadsworth VA Medical Center between 1973 and 1983 (22), at the Mayo Clinic between 1971 and 1972 (18), at the Indiana University

TABLE 5. Relative incidence of AGPC in various infections (Walter Reed Army Medical and Naval Medical Centers, 1973 to 1985)

| Specimen source | Total no. of specimens | Total no. of AGPC isolates | No. of AGPC per specimen | Relative incidence | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Microaerophilic streptococci | *S. constellatus* | *S. intermedius* | *P. morbillorum* | *P. saccharolyticus* | *P. micros* | *P. prevotii* | *P. anaerobius* | *P. asaccharolyticus* | *P. magnus* | *Peptostreptococcus* spp. |
| Abdomen | 359 | 71 | 0.2 | 2 (2)[a] | | 2 (2) | 2 (2) | 1 (1) | 9 (13) | 8 (11) | 9 (13) | 9 (13) | 8 (11) | 21 (30) |
| Abscess | 820 | 383 | 0.47 | 5 (1) | 4 (1) | 7 (2) | 2 (1) | 7 (2) | 19 (5) | 42 (11) | 68 (18) | 49 (13) | 79 (21) | 101 (26) |
| Bile | 66 | 9 | 0.14 | | | | 1 (11) | 1 (11) | | | | 1 (11) | 1 (11) | 5 (55) |
| Bites | 9 | 4 | 0.44 | 1 | | | | | | | | | 2 (50) | 2 (50) |
| Blood | 587 | 67 | 0.11 | 1 (1) | | 2 (3) | | 7 (10) | 2 (3) | 16 (24) | 2 (3) | 12 (18) | 9 (13) | 16 (24) |
| Bone | 37 | 27 | 0.73 | | | 2 (5) | | | | 5 (19) | 2 (5) | 3 (11) | 11 (41) | 4 (15) |
| CNS | 220 | 39 | 0.17 | 2 (7) | | | 1 (3) | 7 (24) | 1 (3) | 9 (23) | 1 (3) | 5 (13) | 1 (3) | 12 (31) |
| Chest | 191 | 59 | 0.31 | | 2 (3) | 4 (7) | | 3 (5) | 3 (5) | 6 (10) | 6 (10) | 3 (5) | 18 (31) | 14 (24) |
| Cysts | 206 | 139 | 0.67 | 3 (2) | 2 (1) | 3 (2) | 2 (1) | 2 (1) | 1 (1) | 20 (14) | 22 (16) | 20 (14) | 25 (18) | 30 (28) |
| Ear | 25 | 25 | 1.0 | | | | | | 3 (12) | 3 (12) | 5 (20) | 4 (16) | 7 (28) | 3 (12) |
| Eye | 55 | 4 | 0.16 | | | | | | | | 1 (25) | 2 (50) | | 1 (25) |
| Genitourinary tract | 30 | 11 | 0.37 | | | | | | | 3 (27) | 3 (27) | 3 (27) | 2 (18) | |
| Grafts | 13 | 5 | 0.38 | | | | | | 1 (20) | | | 1 (20) | 2 (40) | 1 (20) |
| Joints | 63 | 13 | 0.21 | | | | | 2 (15) | | 2 (15) | | 2 (15) | 5 (38) | 2 (15) |
| Lymph glands | 70 | 10 | 0.14 | | | | | 1 (10) | | 2 (20) | | 1 (10) | 1 (10) | 5 (50) |
| OBG infections | 871 | 470 | 0.54 | 6 (1) | 1 (0.2) | 2 (0.5) | 2 (0.5) | 4 (1) | 9 (2) | 51 (11) | 125 (27) | 119 (25) | 52 (11) | 99 (21) |
| Sinuses | 102 | 47 | 0.46 | 4 (9) | 1 (2) | 2 (4) | 2 (4) | 1 (2) | 10 (21) | 5 (11) | 1 (2) | 3 (6) | 8 (17) | 10 (21) |
| Tumors | 61 | 19 | 0.31 | 1 (5) | | | | 1 (5) | | 3 (16) | 2 (11) | 1 (5) | 6 (32) | 5 (26) |
| Wounds | 622 | 313 | 0.5 | | 1 (0.3) | 4 (1) | 3 (1) | 10 (3) | 15 (5) | 57 (18) | 38 (12) | 53 (17) | 78 (25) | 54 (17) |
| Miscellaneous | 51 | 13 | 0.25 | 2 (15) | | | 1 (8) | 1 (8) | 1 (8) | | 2 (15) | 3 (23) | 3 (23) | |
| Total | 4,458 | 1,728 | 0.39 | 24 (1) | 13 (1) | 28 (2) | 15 (1) | 48 (3) | 74 (4) | 233 (13) | 285 (16) | 293 (17) | 318 (18) | 397 (23) |

[a] Number in parentheses is percentage of all anaerobic cocci in source indicated.

TABLE 6. Relative incidence of recovery of *Clostridium* spp. in various infections (Walter Reed Army Medical and Naval Medical Centers, 1973 to 1985)

| Specimen source | Total no. of specimens | Total no. of clostridial isolates | No. of clostridial isolates per specimen | Relative incidence | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *C. clostridioforme* | *C. difficile* | *C. bifermentans* | *C. cadaveris* | *C. innocuum* | *C. butyricum* | *C. septicum* | *C. perfringens* | *Clostridium* spp. |
| Abdomen | 359 | 71 | 0.20 | | | | | | 3 (4)[a] | 3 (4) | 31 (48) | 34 (48) |
| Abscess | 820 | 71 | 0.09 | | | | | | 2 (3) | 1 (1) | 16 (23) | 52 (73) |
| Bile | 66 | 27 | 0.41 | | | | | | 1 (4) | | 23 (85) | 3 (11) |
| Bites | 9 | | | | | | | | | | | |
| Blood | 587 | 70 | 0.12 | 2 (3) | | 2 (3) | 3 (4) | 2 (3) | 2 (3) | 10 (14) | 37 (53) | 12 (17) |
| Bone | 37 | 2 | 0.05 | | | | | | | | | 2 (100) |
| CNS | 220 | 4 | 0.02 | | 1 (25) | | | | | | 3 (75) | |
| Chest | 191 | 18 | 0.09 | | | 1 (6) | | | | | 13 (72) | 4 (22) |
| Cysts | 206 | 6 | 0.03 | | | | | 2 (33) | | | 1 (17) | 3 (50) |
| Ear | 25 | 1 | 0.04 | | | | | | | | | 1 (100) |
| Eye | 55 | 11 | 0.20 | | | | | | | | 8 (73) | 3 (27) |
| Genitourinary tract | 30 | 1 | 0.03 | | | | | | | | | 1 (100) |
| Grafts | 13 | 1 | 0.08 | | | | | | | | | 1 (100) |
| Joints | 63 | 8 | 0.15 | | | | 1 (13) | 1 (13) | | | 3 (37) | 3 (37) |
| Lymph glands | 70 | | | | | | | | | | | |
| OBG infections | 871 | 50 | 0.06 | | | | | | | | 30 (60) | 20 (40) |
| Sinuses | 102 | 2 | 0.02 | | | | | | | | | 2 (100) |
| Tumors | 61 | 1 | 0.02 | | | | | | | | | 1 (100) |
| Wounds | 622 | 124 | 0.20 | | 2 (2) | | | | 1 (1) | 2 (2) | 59 (48) | 60 (48) |
| Miscellaneous | 51 | 3 | 0.06 | | | | | | | | 3 (100) | |
| Total | 4,458 | 471 | 0.11 | 2 (0.5) | 3 (1) | 3 (1) | 4 (1) | 5 (1) | 7 (1) | 16 (3) | 227 (48) | 202 (43) |

[a] Number in parentheses is percentage of all *Clostridium* spp. in source indicated.

TABLE 7. Relative incidence of *Fusobacterium* species in various infections (Walter Reed Army Medical and Naval Medical Centers, 1973 to 1985)

| Specimen source | Total no. of specimens | Total no. of fusobacterial isolates | No. of fusobacterial isolates per specimen | Relative incidence | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | *F. necrophorum* | *F. varium* | *F. mortiferum* | *F. nucleatum* | *Fusobacterium* spp. |
| Abdomen | 359 | 43 | 0.12 | | 3 (7)[a] | 4 (9) | 11 (26) | 25 (58) |
| Abscess | 820 | 97 | 0.12 | 4 (4) | 4 (4) | 4 (4) | 43 (44) | 42 (43) |
| Bile | 66 | 1 | 0.02 | | | | | 1 (100) |
| Bites | 9 | 1 | 0.11 | | | | | 1 (100) |
| Blood | 587 | 24 | 0.04 | 3 (13) | 1 (4) | 1 (4) | 14 (58) | 5 (21) |
| Bone | 37 | 4 | 0.11 | | | | 1 (25) | 3 (75) |
| CNS | 220 | 2 | 0.01 | | | | 2 (100) | |
| Chest | 191 | 31 | 0.16 | 2 (6) | 1 (3) | 2 (6) | 19 (61) | 7 (23) |
| Cysts | 206 | 5 | 0.02 | | | 1 (20) | 2 (40) | 2 (40) |
| Ear | 25 | 1 | 0.04 | 1 (100) | | | | |
| Eye | 55 | 3 | 0.05 | | | | 1 (67) | 2 (33) |
| Genitourinary tract | 30 | 2 | 0.07 | | | | | 2 (100) |
| Grafts | 13 | | | | | | | |
| Joints | 63 | | | | | | | |
| Lymph glands | 70 | 3 | 0.04 | 1 (33) | 1 (33) | | | 1 (33) |
| OBG infections | 871 | 42 | 0.05 | | 2 (5) | 2 (5) | 24 (57) | 14 (33) |
| Sinuses | 102 | 11 | 0.11 | 1 (9) | | | 6 (55) | 4 (36) |
| Tumors | 61 | 1 | 0.02 | | | | | 1 (100) |
| Wounds | 622 | 20 | 0.03 | | 2 (10) | | 13 (65) | 5 (25) |
| Miscellaneous | 51 | 3 | 0.06 | | | | 1 (33) | 2 (67) |
| Total | 4,458 | 294 | 0.07 | 12 (4) | 14 (5) | 14 (5) | 137 (47) | 117 (40) |

[a] Number in parentheses is percentage of all *Fusobacterium* spp. in source indicated.

Medical Center between 1972 and 1980 (17), and at Temple University and the University of Minnesota in 1973 (2). However, the frequency of members of *B. fragilis* group and pigmented *Bacteroides* spp. was reported in only one of these studies (22). The slight differences in distribution may be due to the larger number of specimens in this report (4,458 specimens) compared with the one by Sutter et al. (1,290 specimens) (22). They may also be due to the use of different identification methodologies and transport systems in the various studies.

The recovery rate of *Bacteroides* spp. in infected sites is similar to their distribution in the normal flora (7, 14, 21). While members of the *B. fragilis* group were more often isolated from sites proximal to the gastrointestinal tract (abdomen and bile), strains of pigmented *Bacteroides* spp. were more prevalent in infections proximal to the oral cavity (bones, sinuses, and chest), and *B. disiens* and *B. bivius* were more often isolated from OBG infections. Knowledge of this distribution allows a logical choice of antimicrobial agents adequate for the therapy of infections in these sites. Following the recent description of *B. disiens* and *B. bivius*, they were recognized as important bacteroides isolated in OBG infections (15, 20).

AGPC were the second most frequently recovered anaerobes and accounted for 26% of anaerobic isolates, a finding similar to reports in previous studies (2, 14, 17, 18, 22). The species most frequently recovered are *P. magnus*, *P. asaccharolyticus*, *P. anaerobius*, and *P. prevotii*, which account for 64% of AGPC isolates. The predominance of *P. magnus* was previously established (4). As a group, these organisms were the most frequently recovered anaerobes in bone, ear, graft, and joint infections. They have been shown to possess virulence in animals infected with them alone (12) but more so in synergy with other aerobic and anaerobic bacteria (11). Clostridia and fusobacteria were recovered mostly from patients with wounds, abscesses, abdominal infections, and OBG infections. While clostridia were mostly found in abscesses and wounds around the rectal or vulvovaginal area, fusobacteria were isolated from those in and around the oral area. This corresponds to their presence as part of the normal host flora in these sites (21).

Anaerobic, gram-positive, nonsporeforming rods accounted for 17% of all anaerobic isolates. These data are similar to those reported in most other centers (2, 18, 22). Propionibacteria were the largest group among them. Although propionibacteria were isolated in 13% of the specimens, their pathogenic role is not clear. Propionibacteria ordinarily are not pathogens but can be found in association with implanted cardiac or neurogenic prostheses or as a cause of endocarditis on previously damaged valves (13). Many nonpathogenic organisms may be involved in infection under these circumstances. Because these organisms are part of the normal skin flora, they are common laboratory contaminants or may grow in blood cultures from skin contamination if the skin surface has been improperly decontaminated before the blood sample is drawn. Therefore, interpretation of the significance of an isolate must be undertaken with caution. However, there are several well-documented cases of infection among our patients. *P. acnes* can cause bacteremia, especially in association with shunt infections (3).

The results of this 12-year retrospective study reflect the importance of anaerobic bacteria and illustrate the distribution of these organisms in different infections. With growing instances of bacterial resistance to antimicrobial agents, knowledge of the distribution of these organisms may assist in the selection of empiric therapy of anaerobic infections.

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