CLINICAL ANAEROBIC MICROBIOLOGY

Bacteroides infections in children

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Summary. From 1974 to 1990, 336 Bacteroides isolates were obtained from 312 specimens from 274 patients. They comprised 180 (54%) B. fragilis isolates, 55 (16%) B. theta-tiotaomicron, 36 (11%) B. vulgatus, 34 (10%) B. distasonis, 21 (6%) B. ovatus and 10 (3%) B. uniformis. Infections in 253 (92%) patients were polymicrobial, but in 21 (8%) children, a Bacteroides sp. was isolated in pure culture. Most Bacteroides isolates were from peritoneal fluid (114), abscesses (110), wound infections (20), blood cultures (10) and from patients with pneumonia (14) or chronic otitis media (8). Predisposing conditions were present in 145 (53%) children; these were previous surgery (46), trauma (28), malignancy (21), prematurity (19), immunodeficiency (18), steroid therapy (12) foreign body (10), diabetes (9) and sickle cell disease (7). The micro-organisms isolated most commonly mixed with Bacteroides spp. were anaerobic cocci (221), Escherichia coli (122), Fusobacterium spp. (38) and Clostridium spp. (30). All patients received antimicrobial therapy in conjunction with surgical drainage or correction of pathology in 197 (72%) cases. All but 12 (5%) patients recovered. These data illustrate the importance of Bacteroides spp. in infections in children.

Introduction

The species of Bacteroidaceae that occur with greatest frequency in anaerobic infections in children, as they do in adults, belong to the genus Bacteroides. These micro-organisms are resistant to penicillins by virtue of β-lactamase production and by other unknown mechanisms.1 They were formerly classified as five subspecies of B. fragilis (ss. fragilis, ss. distasonis, ss. ovatus, ss. theta-tiotaomicron and ss. vulgaris) and were reclassified in 1976 into distinct species on the basis of DNA homology studies,2 and then became the only species remaining in the genus Bacteroides sensu stricto in 1990.3 B. fragilis is the anaerobe most frequently isolated from clinical infections.

Recognition of the role of Bacteroides spp. in paediatric infection has become more important in recent years because of their increased resistance to some commonly used antimicrobial agents that were previously very effective against them,4 e.g., clindamycin, cephalosporins, metronidazole and chloramphenicol.1

This retrospective review summarises my experience of the isolation of Bacteroides spp. from infections in children during a period of 16 years. Some of the data have been published before in articles describing the role of anaerobic bacteria in various paediatric infections,5 but cases not previously presented are also included and the unique nature of bacteroides infections in children is emphasised.

Patients and methods

Patients

The children included in this review were studied by the author between June 1974 and June 1990. They were seen in the following hospitals: University of California Medical Center, Los Angeles County Medical Center and Serra Memorial Hospital in Los Angeles, CA; Children's Hospital National Medical Center and South-East Medical Center in Washington, DC; and National Naval Medical Center in Bethesda, MD. Records from the clinical microbiology laboratory were reviewed to identify patients with bacteroides infections. The available case histories of all patients from whom Bacteroides spp. had been isolated were reviewed to ascertain the presence and site of infection, associated micro-organisms, underlying disease processes, and possible predisposing or associated conditions.

Microbiological examination

Only specimens that were properly collected without...
contamination by the normal skin or mucosal surface flora and submitted in transport media appropriate for anaerobic bacteriological investigation were accepted by the microbiology laboratories. These were specimens obtained during surgery or by aseptic needle or biopsy aspiration of abscesses or fluid from body cavities. Lung aspirates were obtained by transtracheal aspiration or biopsy. Urine was collected by suprapubic aspiration. When possible, pus and fluids were collected and transported in syringes. Most specimens, except blood, were submitted on a Port-A-Cul transport swab or in liquid systems (BBL, Cockeysville, MD, USA). However, precise records of the transport media used were not available. Blood was collected aseptically from patients suspected of having bacteraemia and was inoculated (10% v/v) into one bottle each of two commercially produced broth media, both under vacuum and with CO₂ 5% in the atmosphere. The sources of blood-culture media varied during the period of review.

Specimens were inoculated on to pre-reduced vitamin K₁-enriched Brucella Blood Agar (BBL), blood agar with kanamycin (75 mg/L) and vancomycin (7.5 mg/L), and blood agar with colistin sulphate (0.01 mg/L) and nalidixic acid (50 mg/L), and into an enriched thioglycolate broth containing haemin (5 mg/L) and vitamin K (0.1 mg/L). The cultures were incubated in GasPak jars (BBL) and examined after 48 and 96 h<sup>6</sup>. Plates that showed any growth were held until the micro-organisms had been identified. All cultures that showed no growth were held for at least 5 days. Micro-organisms were identified by the API anaerobic system (Analytab Products, Plainview, NY, USA). When complete identification was not possible by this method, other carbohydrate tests (Scott Laboratories, Fiskeville, RI, USA) and gas-liquid chromatography<sup>6</sup> were performed as needed to identify the organisms. The criteria for identification were according to guidelines published previously.<sup>6,7</sup>

**Results**

**Patients**

From a total of 1658 specimens examined for anaerobic bacteria, 336 *Bacteroides* strains were isolated from 312 (20%) specimens obtained from 274 patients. Clinical records were not available for review from another 18 patients; these were not included in the final analysis. Patients’ ages ranged from one day to 17.5 years (mean 7 years 9 months); 164 were male and 110 female. Predisposing or underlying conditions were present in 145 (53%) of the patients. A single underlying condition was noted in 120 cases, and two conditions were recorded in 25; these conditions were previous surgery (46), trauma (28), malignancy (21), prematurity (19), immunodeficiency (18), steroid therapy (12), presence of a foreign body (10), diabetes (9) and sickle cell disease (7).

**Bacteroides isolates**

The 336 *Bacteroides* isolates comprised 180 (54%) *B. fragilis*, 55 (16%) *B. thetaiotaomicron*, 36 (11%) *B. vulgatus*, 34 (10%) *B. distasonis*, 21 (6%) *B. ovatus* and 10 (3%) *B. uniformis* (tables I and II). Infections were polymicrobial in 253 (92%) patients, but in 21 (8%) children, *Bacteroides* spp. were isolated in pure culture (table III).

There were 749 other bacterial isolates with the *Bacteroides* spp. in the 290 specimens from mixed infection; 429 of these were anaerobes and 320 were facultative or aerobic species. The number of isolates in the mixed cultures was 2–7 (average 2.6 isolates/specimen, 1–5 anaerobes and 1–1 facultative or aerobic). The micro-organisms isolated most commonly with *Bacteroides* spp. were anaerobic coccii (211 isolates), *Escherichia coli* (122), *Fusobacterium* spp. (40) and *Clostridium* spp. (31). Most *E. coli* and *Clostridium* spp. were isolated with *Bacteroides* spp. from intra-abdominal infections, and skin and soft tissue infections around the rectal area. Most pigmented *Prevotella* and *Porphyromonas* isolates were from head and neck infections.

Most *Bacteroides* isolates were from peritoneal fluid specimens (114 isolates, 34%), abscesses (110, 33%), wounds (29, 9%), pneumonia (14, 4%), blood cultures (13, 4%), decubitus ulcers (10, 3%) and chronic otitis media (8, 2%).

**Types of infections**

**Peritonitis.** The 114 peritoneal isolates of *Bacteroides* spp. were from 108 children and were mixed with other bacteria, mostly *Peptostreptococcus* spp. (96 isolates) and *E. coli* (65), but also *Fusobacterium* spp. (23), group D streptococci (19), *Eubacterium* spp. (17) and *Clostridium* spp. (14). In 79 of the children, peritonitis was associated with a ruptured appendix, nine cases followed trauma, and 10 rupture of a viscus.

**Abscesses.** Of the 321 specimens from abscesses, 101 (32%) yielded a growth of *Bacteroides* spp. (table II). The most common abscesses from which the *Bacteroides* spp. were isolated were abdominal (23 isolates), perirectal (14), pilonidal (12) and subphrenic (9). The most common other organisms in these abscesses were *E. coli* and *Peptostreptococcus* spp.

**Wound infections.** The 29 isolates from wounds were from post-surgical infection of the abdomen (13 isolates), head and neck (six) and rectal area (five). In all cases, the *Bacteroides* isolates were mixed with other bacteria—*E. coli* (eight isolates), enterococci (four) and *Staphylococcus aureus* (four). The 10 isolates from decubitus ulcers were from ulcers of the buttocks (four isolates), lower extremities (three) and head (three); all were mixed with other micro-organisms—*Peptostreptococcus* spp. (eight isolates), *E. coli* (four), *S. aureus* (three) and *Clostridium* spp. (two).
Table I. *Bacteroides* isolates from 312 specimens from 274 children

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Number of specimens</th>
<th>Number of <em>Bacteroides</em> isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with Bacteroides spp.</td>
<td>with B. fragilis</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>115</td>
<td>108</td>
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<tr>
<td>Abscess</td>
<td>321</td>
<td>101</td>
</tr>
<tr>
<td>Wounds</td>
<td>75</td>
<td>24</td>
</tr>
<tr>
<td>Decubitus ulcers</td>
<td>58</td>
<td>9</td>
</tr>
<tr>
<td>Omphalitis</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>Burn</td>
<td>180</td>
<td>5</td>
</tr>
<tr>
<td>Bites, human</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Blood</td>
<td>334</td>
<td>13</td>
</tr>
<tr>
<td>Ear: mastoiditis</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>otitis, chronic</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>otitis, cholesteatoma</td>
<td>38</td>
<td>6</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>80</td>
<td>13</td>
</tr>
<tr>
<td>Empyema</td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td>Sinusitis, chronic</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>148</td>
<td>1</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>1658</td>
<td>312</td>
</tr>
</tbody>
</table>

Table II. *Bacteroides* isolates from 101 abscesses

<table>
<thead>
<tr>
<th>Abcess site</th>
<th>Number of specimens</th>
<th>Number of <em>Bacteroides</em> isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with Bacteroides spp.</td>
<td>with B. fragilis</td>
</tr>
<tr>
<td>Abdomen</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Buttocks</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Breast</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Hand</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Fingers</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Head</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>Intracranial</td>
<td>39</td>
<td>5</td>
</tr>
<tr>
<td>Liver</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Pilonidal</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Perirectal</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>Scap</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>Subphrenic</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Renal</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Vulvovaginal</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Spleen</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Trunk</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>321</td>
<td>101</td>
</tr>
</tbody>
</table>

Seven isolates were from omphalitis and all were isolated with other flora, mostly *E. coli* (four). Five *Bacteroides* isolates were from burns around the rectal area (three) and lower extremities (two); all were mixed with Enterobacteriaceae or *Pseudomonas aeruginosa*. The single isolate of *B. ovatus* was from a bite wound and was mixed with *Pstr. magnus* and a-haemolytic streptococci.

*Bacteraemia.* Of the 13 isolates from blood cultures, 12 were in pure culture (table III) and one was mixed with *E. coli* in a patient with a ruptured appendix. The single isolates were from two patients with immunodeficiency, three with perforated appendix, two with meningitis and one each with prematurity and necrotising enterocolitis, and leukaemia, empyema and rectal abscess.

*Other infections.* Seventeen isolates were from chronic otitis media and its complications (mastoiditis and cholesteatoma); all were mixed with other bacteria, the most common being *Peptostreptococcus* spp. (10 isolates) and pigmented *Prevotella* or *Porphyromonas* spp. (seven). One isolate was from a maxillary sinus infection, in a mixed infection with two anaerobic cocci. Fourteen isolates were from aspiration pneumonia, of which three were in pure culture (table III). The other 11 isolates were from mixed
infection with pigmented Prevotella or Porphyromonas spp. (five isolates), Peptostreptococcus spp. (four), Fusobacterium spp. (three) and Klebsiella pneumoniae (two). Seven isolates were from empyema, of which three were in pure culture (table III), all in patients with a primary intra-abdominal infection (one with a hepatic abscess); the other four isolates were from mixed infections with Peptostreptococcus spp. (four isolates) and enterobacteria (one).

Four osteomyelitis isolates were from three patients with mixed infections: one neonate with infection of the occipital bone after fetal monitoring, and two children with infection of the coccyx and occipital bone that complicated decubitus ulcers of the overlying skin. Of the three urinary isolates, one was in pure culture (table III) in a girl with cystitis, and the two others were mixed with E. coli and Pr. melaninogenica. The single Bacteroides isolate from the conjunctiva was from a 6-year-old girl without any predisposing condition (table III).

**Discussion**

This retrospective review demonstrates the importance of Bacteroides spp. in various infections in children. The 336 Bacteroides isolates were from 312 (20%) of 1658 specimens submitted for culture for anaerobic bacteria. Bacteroides spp. are especially prevalent in abscesses (mostly abdominal, perirectal and subphrenic), peritonitis, aspiration pneumonia and bacteraemia.

Because of its presence in the normal flora of the gastrointestinal tract, B. fragilis predominates in bacteraemia associated with intra-abdominal infections, peritonitis and abscesses following rupture of a viscus, and subcutaneous abscesses or burns near the anus. Although Bacteroides spp. are not generally found in the normal oral flora, they can colonise the oral cavity of patients with poor oral hygiene or
of those who have received antimicrobial therapy, especially with a β-lactam agent. Colonisation of the oropharyngeal cavity can lead to the isolation of these species from paediatric infections that originate in this area, such as aspiration pneumonia, lung abscesses, chronic otitis media, brain abscesses, and subcutaneous abscesses or burns near the oral cavity.

_Bacteroides_ spp. can be isolated from infections in neonates. The newborn infant is at risk of developing these infections when born to a mother with amnionitis or premature rupture of membranes, or through aspiration or contamination of wounds during passage through the birth canal. _B. fragilis_ has been isolated from neonates with aspiration pneumonia, bacteremia, omphalitis, and subcutaneous abscesses and occipital osteomyelitis following fetal tumours (1.9% of all obstetric and gynaecological infections). The isolation rate of the different _Bacteroides_ spp. varies. In a recent survey in two military hospitals, _B. fragilis_ accounted for 63% of all _Bacteroides_ isolates. _B. thetaiotaomicron_ for 14%, _B. vulgatus_ and _B. ovatus_ for 7% each, and _B. distasonis_ for 6%. The highest frequency of isolation of _B. fragilis_ compared to other _Bacteroides_ spp. was in blood cultures (78% of all _Bacteroides_ isolates), wounds (69%), abscesses (65%) and abdominal infection (59%). _B. thetaiotaomicron_ was most frequently isolated from chest infections (35%), infected cysts (22%) and infected tumours (19%). _B. vulgatus_ was mostly isolated from obstetric and gynaecological infections (20%) and _B. ovatus_ from biliary tract infections (18%).

_Bacteroides_ spp. are generally isolated from mixed infections with other aerobic and anaerobic bacteria, forming a polymicrobial infection where the number of isolates varies from two to six. The microorganisms mixed with _Bacteroides_ spp. in polymicrobial infections vary and depend on the location and circumstances leading to the infection. Most polymicrobial infections originate from the mucosal flora adjacent to the infected site. Enterobacteria and _Clostridium_ spp. predominate in mixed infections with _Bacteroides_ spp. in abdominal and rectal infections; _Prevotella_ and _Porphyromonas_ spp. are common in head and neck infections.

Although _B. fragilis_ is the most common species found in clinical specimens, it is only a minor _Bacteroides_ spp. in faecal flora, comprising only 0.5% of the cultivable bacteria in stool samples. The pathogenicity of this species probably results from several virulence characters, including its ability to produce capsular material and the metabolite succinic acid which protect against phagocytosis. Most _Bacteroides_ strains are more virulent than the non-capsulate forms. This increased virulence can be demonstrated by a higher rate of induction of bacteremia, and a greater enhancement of growth of other bacteria in mixed infections. The emergence of capsule production in polymicrobial infection may explain the predominance of _B. fragilis_ in chronic infection.

The relationship between the _Bacteroides_ spp. and the aerobic and facultative bacteria in intra-abdominal infections has been shown to be synergic. Altemeir, Meloney et al., and Brook et al. demonstrated such synergy in animal models. Brook et al. evaluated the synergic potential between _Bacteroides_ spp. and other aerobic and anaerobic bacteria inoculated subcutaneously into mice; the synergic effects were determined by observing abscess formation, animal mortality and the number of organisms present in the abscesses. The bacteria tested included _Fusobacterium_ spp., _Peptostreptococcus_ spp., _S. aureus_, _P. aeruginosa_, _E. coli_, _K. pneumoniae_, _Proteus mirabilis_ and enterococci. _B. fragilis_ significantly enhanced the virulence of most of the other organisms. The most virulent combinations were between _P. aeruginosa_ or _S. aureus_ and _B. fragilis_. Enhanced growth of all the aerobic or facultative species was found in most of these combinations. Several hypotheses have been proposed to explain this microbial synergy. It may be due to mutual protection from phagocytosis and intracellular killing, production of essential growth factors, lowering of oxidation-reduction potentials in host tissues, or any combination of these.

The _Bacteroides_ spp. are generally resistant to penicillins, mainly through the production of β-lactamase. Other mechanisms of resistance to penicillin include alteration in the penetration of penicillins and the resistance of group β-haemolytic streptococci to penicillin increased 8500-fold. Several animal studies have demonstrated the ability of β-lactamase to influence polymicrobial infections. Hackman and Wilkins showed that penicillin-resistant strains of _B. fragilis_, "B." _melanoenygenus_ and "B." _oralis_ protected a penicillin-sensitive _F. necrophorum_ strain from penicillin therapy in mice. Brook et al. demonstrated protection of _Streptococcus pyogenes_ from penicillin by _B. fragilis_ in a subcutaneous abscess model in mice. Brook also studied β-lactamase activity in pus from 109 abscesses;
β-lactamase-producing organisms were isolated from 84 (77%) specimens, including all 28 isolates of *B. fragilis*. β-Lactamase activity was detected by the nitrocefin method in 14 (56%) of 25 pus samples from abscesses that harboured *Bacteroides* spp.

Almost all (> 99%) *Bacteroides* strains are susceptible to metronidazole, imipenem and the combination of a penicillin and a β-lactamase inhibitor, and > 90% are generally susceptible to clindamycin, cefoxitin and ticarcillin. However, as most infections due to *Bacteroides* spp. are polymicrobial, therapy has to be directed also towards the other bacterial components of the infections. Management of mixed infections caused by aerobic and anaerobic bacteria, including *Bacteroides* spp., requires surgical correction of pathology, drainage of pus and the administration of antimicrobial agents effective against both components of the infection. When such therapy is not given, the infection may persist and lead to complications such as intra-abdominal abscesses. Antimicrobial therapies effective against mixed infections are combinations of clindamycin, cefoxitin or metronidazole for the anaerobes plus an aminoglycoside, cephalosporin or quinolone for the enterobacteria. Single agent therapy with cefoxitin, a penicillin plus a β-lactamase inhibitor, or imipenem may also be effective.

The laboratory assistance of the staff of the microbiology laboratories at the University of California Medical Center, Los Angeles County Medical Center and Serra Memorial Hospital in Los Angeles, and Children's Hospital National Medical Center and South-East Medical Center in Washington, D.C., and the Navy Hospital in Bethesda, MD, and the secretarial assistance of Sarah Biaisdell are gratefully acknowledged.

References


