Pathogenicity of the *Bacteroides fragilis* Group*

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**ABSTRACT**

The *Bacteroides fragilis* group is one of the most important pathogens in polymicrobial infections. The distribution of the different members of the *B. fragilis* group in clinical infections varies. *Bacteroides fragilis* accounts for 63 percent of all the group isolates, *Bacteroides thetaiotaomicron* for 14 percent, *Bacteroides vulgatus* and *Bacteroides ovatus* for seven percent each, *Bacteroides distasonis* for six percent and *Bacteroides uniformis* for two percent. All members of the group induced bacteremia that was associated with an average mortality of 27 percent. The *B. fragilis* group resist beta lactam antibiotics by producing the enzyme beta-lactamase. This enzyme can be detected in abscess fluid, and can interfere with the eradication of other bacteria that are susceptible to penicillins and cephalosporins. All members of the *B. fragilis* group can become encapsulated during an inflammatory process as was demonstrated in a subcutaneous abscess model in mice. Non-encapsulated strains can become encapsulated with the assistance of their aerobic counterparts. These encapsulated strains are more virulent to the host than non-encapsulated strains. 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 infection. Antimicrobial therapy directed only at the eradication of the aerobic bacteria did not prevent encapsulation, or reduce the number of *Bacteroides* species. The virulence of all members of the *B. fragilis* group highlights the need to direct antimicrobial therapy against all members of this group.

**Introduction**

The polymicrobial nature of abdominal, pelvic and skin and soft tissue (proximal to the oral or rectal areas) infections is apparent in the majority of patients, where the number of isolates in an infectious site varies between 2 and 6.\(^14,35\) The average number of isolates is 3.6 in skin and soft tissue infections (2.6 anaerobes and 1.0 aerobes) per specimen,\(^23,47\) five in intraabdominal infection (3.0 anaerobes and 2.0 aerobes) per speci-
men,9 and four in pelvic infections (2.8 anaerobes and 1.2 aerobes) per specimen.66 In some types of infection, polymicrobial infections are known to be more pathogenic for experimental animals than those involving single organisms.2

The organisms recovered in polymicrobial infections vary and depend on the location site and the circumstances leading to the infection. Most polymicrobial aerobic-anaerobic infections originate from the mucus flora adjacent to the infected site. The recovery of anaerobes in these infections is not surprising since they outnumber aerobic and facultative bacteria in ratios of 10:1 to 10,000:1 in these normal flora sites.14,35 The Bacteroides species predominate in most polymicrobial infection sites. The Bacteroides fragilis group are generally isolated in abdominal infections. The Bacteroides melaninogenicus group and Bacteroides oralis are mainly found in oral infections and B. bivius and B. diversis are most often recovered in pelvic infections.14,35 Enterobacteriaceae are predominant in abdominal and rectal infections; staphylococci and streptococci are important in wound and skin and subcutaneous tissue abscesses, and Neisseria gonorrhoeae and chlamydia are found in pelvic infections (table I).

Bacteroides fragilis is one of the most important pathogens in polymicrobial infections. It possesses several virulence factors which facilitate its pathogenicity to humans. Included among the factors is the production of a capsule. In addition to a capsule, anaerobic bacteria possess other important virulence factors. These include the production of superoxide dismutase and catalase, immunoglobulin proteases, coagulation-promoting and -spreading factors (such as hyaluronidase, collagenase, and fibrinolysin), and adherence factors.35 Other factors that enhance the virulence of anaerobes include mucosal damage, oxidation-reduction potential drop, and the presence of hemoglobin or blood in an infected site.

This review of our work describes evidence supportive of the importance of all members of the Bacteroides fragilis group in mixed infections. It is based on clinical as well as animal studies, showing synergy between the aerobic and anaerobic bacteria, and the importance of encapsulation of anaerobic bacteria.

Recovery Rate of B. fragilis Group in Clinical Specimens

The recovery rate of members of the B. fragilis group varies in different sites. These organisms predominate in abdominal infections and their sequel and in skin and soft tissue infections that originate from the gut flora.14,35 They are less frequently isolated in pelvic infections and respiratory infections.35,66

The B. fragilis group is comprised of several subspecies that were recently elevated to a species level.31 Data presented in this review will support the statement that all of these can participate in polymicrobial infections and are also capable of causing infections by themselves.

The recovery rate of the different members of the B. fragilis group varies

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**TABLE I**

Predominate Isolates at Various Body Sites

<table>
<thead>
<tr>
<th>Infection Site</th>
<th>Anaerobic Bacteria</th>
<th>Facultative and Miscellaneous Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin and subcutaneous wounds and abscesses</td>
<td>B. fragilis group (rectal)</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>B. melaninogenicus group (oral)</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>Abdomen</td>
<td>Peptostreptococcus</td>
<td>E. coli (rectal)</td>
</tr>
<tr>
<td></td>
<td>Clostridia</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Pelvis</td>
<td>B. bivius</td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>B. diveris</td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>B. fragilis group</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td></td>
<td>Chlamydia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>trachomatis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enterobacteriaceae</td>
<td></td>
</tr>
</tbody>
</table>
in different infection sites. The relative distribution of the different members of the B. fragilis group has important clinical implications on the management of infections involving anaerobic bacteria. This is because of the different antimicrobial susceptibility of the various Bacteroides species. Although most members of B. fragilis groups produce beta-lactamase and resist penicillins, their susceptibility to second and third generation cephalosporins is variable but predictable. While B. fragilis is generally the most susceptible, Bacteroides thetaiotaomicron and Bacteroides distasonis generally are more resistant.

The distribution of the different members of the B. fragilis group was illustrated in three recent studies of intraabdominal infections (table II). Among the B. fragilis group, B. fragilis accounted for 40 percent to 57 percent of the Bacteroides isolates recovered from intraabdominal infections. However, another important pathogen that belongs to the B. fragilis group is B. thetaiotaomicron, which accounts for 13 percent to 23 percent of the isolates; other members of the B. fragilis group account for a total of 23 percent to 37 percent.

A review of 12 years experience (1973 to 1985) of the Walter Reed Army Medical Center in Washington, DC and the Navy Hospital in Bethesda, MD revealed certain trends regarding the distribution of the different Bacteroides species in clinical isolates (table III). Bacteroides fragilis accounted for 63 percent of all B. fragilis group isolates; B. thetaiotaomicron for 14 percent; Bacteroides vulgatus and Bacteroides ovatus for seven percent each; B. distasonis for six percent; and Bacteroides uniformis for two percent. The highest frequency of recovery of B. fragilis compared to other members of B. fragilis group was in blood cultures (78 percent of all B. fragilis group isolates), wounds (69 percent), abscesses (65 percent), and abdominal infection (59 percent). Bacteroides thetaiotaomicron was most frequently isolated in chest infections (35 percent) (data not shown in table III), cysts (22 percent), and tumors (19 percent). Bacteroides vulgatus was mostly recovered from pelvic infections (20 percent), and B. ovatus from bile infections (19 percent), B. distasonis from pelvic (10 percent) and abdominal infections (nine percent), and B. uniformis in wounds (three percent).

The incidence of recovery of the different members of the B. fragilis group in Walter Reed Army Hospital and Navy Hospital varied. Of the total of 800 isolates of B. fragilis, 183 (23 percent) were recovered in abscesses, 153 (19 percent) in wounds, 139 (17 percent) in abdomen, 129 (16 percent) in blood, and 103 (13 percent) in pelvic infections. Of 181 isolates of B. thetaiotaomicron, 42 (23 percent) were isolates in abscesses, 34 (18 percent) each in abdomen and wounds, 23 (13 percent) in blood, 13 (seven percent) in pelvic infections, and 12 (seven percent) in chest infections. Of 92 isolates of B. vulgatus, 36 (39 percent) were recovered in pelvic infections, 18 (20 percent) in abdomen, and 13 (14 percent) in abscesses. Of 86 B. ovatus, 26 (30 percent) were found in abscesses, 22 (26 percent) in abdomen and nine each (10 percent) in pelvic infections and wounds. Of 81 B. distasonis, 21 (25 percent)
PATHOGENICITY OF THE BACTEROIDES FRAGILIS GROUP

TABLE III

Distribution of Bacteroides fragilis Group Isolates in Clinical Specimens from Five Major Sites (Percent)*

<table>
<thead>
<tr>
<th>Site</th>
<th>Number Isolates</th>
<th>B. uniformis</th>
<th>B. vulgatus</th>
<th>B. ovatus</th>
<th>B. distasonis</th>
<th>B. thetaiotaomicron</th>
<th>B. fragilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abscesses</td>
<td>283</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>15</td>
<td>65</td>
</tr>
<tr>
<td>Wounds</td>
<td>232</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>15</td>
<td>69</td>
</tr>
<tr>
<td>Abdomen</td>
<td>236</td>
<td>1</td>
<td>8</td>
<td>9</td>
<td>6</td>
<td>14</td>
<td>59</td>
</tr>
<tr>
<td>Pelvic</td>
<td>183</td>
<td>2</td>
<td>20</td>
<td>5</td>
<td>10</td>
<td>7</td>
<td>56</td>
</tr>
<tr>
<td>Blood</td>
<td>165</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>14</td>
<td>78</td>
</tr>
<tr>
<td>Total</td>
<td>1,099</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>14</td>
<td>63</td>
</tr>
</tbody>
</table>

Recovered at Walter Reed Hospital, Washington, DC and the Navy Hospital, Bethesda, MD, 1973-1985.

cent) were isolates in abdomen, 19 (23 percent) in pelvic infections, and 13 (16 percent) each in abscesses and wounds. Of the 21 B. uniformis, seven (33 percent) were isolates in wounds and six (29 percent) in abscesses.

The virulence of all members of B. fragilis group is highlighted by their recovery in blood cultures, especially when they were associated with mortality. Although more cases of bacteremia caused by B. fragilis were noted, the mortality rate following B. thetaiotaomicron bacteremia was higher (38 percent) compared to B. fragilis (24 percent) (table IV). In many of these cases, the B. fragilis group isolates were the sole organisms recovered from these patients, which further illustrates their potential virulence.7 Similar data were described in a smaller study by Chow and Guze.33

The Clinical Significance of Production of Beta-lactamase by the B. fragilis Group

The B. fragilis group are generally resistant to penicillins, mainly by the production of beta-lactamase. The production of the enzyme beta-lactamase (mostly cephalosporinase) enables members of the B. fragilis group to hydrolyze beta-lactam antibiotics, including all first generation and most second and third generation cephalosporins. A growing resistance of other Bacteroides species previously susceptible to penicillins has been noticed in the last decade.31,50 These are members of the pigmented Bacteroides, including the B. melaninogenicus group, B. oralis, B. disiens, B. bivius, and B. oris-buccae. The main mechanism of resistance is through the production of the enzyme beta-lactamase. However, other mechanisms of resistance to penicillin, such as alteration in the penetration of penicillins into the microbial cell, have also been observed.53

Various aerobic and anaerobic organisms can produce the enzyme beta-lactamase.

TABLE IV

Bacteremia and Mortality Owing to B. fragilis Group (Death/Total [Percent])

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B. fragilis</td>
<td>5/16 (31%)</td>
<td>26/115 (24%)</td>
</tr>
<tr>
<td>B. vulgatus</td>
<td>3/8 (37%)</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>B. ovatus</td>
<td>0/3 (0%)</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>B. distasonis</td>
<td>1/2 (50%)</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>B. thetaiotaomicron</td>
<td>3/3 (100%)</td>
<td>8/21 (38%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>12/32 (37.5%)</td>
<td>40/148 (27%)</td>
</tr>
</tbody>
</table>

mase. The distribution of the different beta-lactamase producing bacteria (BLPB) in infectious sites is similar to their distribution in the normal flora adjacent to the infected site. Staphylococcus aureus that resides in the skin was mostly found in skin and subcutaneous tissue infections. The B. fragilis that is part of the colonic flora was found in infections adjacent to that area; the B. melaninogenicus group and B. oralis, which predominate in the oral cavity, were recovered in infections proximal to that area.\(^{14,35}\)

The beta-lactamase producing B. fragilis group as well as other Bacteroides sp. and aerobic bacteria were recovered from a variety of infected sites. Over the past decade, the aerobic and anaerobic microbiology and the recovery rate of beta-lactamase-producing Bacteroides were studied in many infections. The beta-lactamase-producing B. fragilis group were isolated in infections of the skin and subcutaneous tissue (26 percent of patients), of the upper respiratory tract (20 percent), of the lungs (36 percent), and following surgery (98 percent).\(^{14,16}\)

The beta-lactamase produced by Bacteroides sp. can protect the enzyme producing organisms as well as its partners in mixed infections from beta lactam antibiotics. Several animal studies demonstrated the ability of the enzyme beta-lactamase to influence polymicrobial infections. Hackman and Wilkins\(^{36}\) showed that penicillin resistant strains of B. fragilis, B. melaninogenicus, and B. oralis protected a penicillin-sensitive Fusobacterium necrophorum from penicillin therapy in mice. Brook et al.\(^{25}\) utilizing subcutaneous abscess models in mice, demonstrated protection of Staphylococcus pyogenes from penicillin by B. fragilis and B. melaninogenicus.

Several studies demonstrate the activity of the enzyme beta-lactamase in polymicrobial infections. De Louvois and Hurley\(^{34}\) demonstrated degradation of penicillin, ampicillin, and cephaloridine by purulent exudates obtained from four of 22 patients with abscesses. Studies by Matsuda and Tomioka\(^{46}\) demonstrated beta-lactamase activity in empyema fluid. Most infections were polymicrobial and involved both Klebsiella pneumoniae and Pseudomonas aeruginosa. O'Keefe et al.\(^{54}\) demonstrated inactivation of penicillin G in an experimental B. fragilis infection model in the rabbit peritoneum.

The presence of beta-lactamase in clinical specimens was reported by several investigators. Bryant et al.\(^{29}\) studied the beta-lactamase activity in samples of pus obtained from 12 patients with polymicrobial intraabdominal abscesses or polymicrobial empyema. Using the chromogenic cephalosporin nitrocefin, these investigators were able to show strong enzyme activity in four of the 11 abscess fluids. Brook\(^{15}\) studied beta-lactamase activity in pus obtained from 109 abscesses. Beta-lactamase-producing organisms were recovered in 84 (77 percent) specimens. These included all 28 isolates of B. fragilis, 18 of 30 B. melaninogenicus, 42 of 43 S. aureus, and 11 of 14 Escherichia coli. Using the chromogenic nitrocefin methodology, beta-lactamase activity was detected in 14 of 25 (56 percent)
of abscesses where members of the *B. fragilis* group were isolated. The 14 members of *B. fragilis* group included eight *B. fragilis*, four *B. thetaiotaomicron*, two *B. vulgatus*, and one *B. distasonis*.

Three examples are presented of abscesses caused by polymicrobial flora (table V). In each abscess, there was at least one organism that produced beta-lactamase in sufficient quantities to be detected by the chromogenic cephalosporin nitrocefin assay. The survivability of penicillin-susceptible bacteria in these abscesses, despite penicillin therapy, highlights and substantiates this phenomena.

The recovery of penicillin-susceptible bacteria mixed with BLPB in patients who have received penicillin or cephalosporin therapy and failed to respond to therapy suggests the ability of BLPB to protect penicillin- or cephalosporin-susceptible organisms from the activity of those drugs.

The emergence of BLPB following penicillin therapy may account for many of the therapeutic failures. A recent report described five adults with clinical failures after penicillin therapy associated with the isolates of anaerobic BLPB. In a study of 185 children with orofacial and respiratory infections who failed to respond to penicillin, BLPB were recovered in 75 (40 percent). The predominant BLPB were *S. aureus*, *B. melaninogenicus* group, *B. fragilis* group, and *B. oralis*.

Several clinical studies showed the superior efficacy of therapeutic modalities that are effective against BLPB over penicillins. These laboratory and clinical data support, therefore, the use of such therapy in mixed infections.

**Capsule as a Virulence Factor**

The *B. fragilis* group, the most prevalent among the anaerobes recovered in mixed infections, has several virulence factors. These include possession of a capsule and excretion of succinic acid, both of which inhibit phagocytosis and production of other enzymes and metabolic by-products. A mucopolysaccharide capsule can be found in all *Bacteroides* sp. including all of the members of the *B. fragilis* group.

Several recent studies demonstrated the pathogenicity of encapsulated *B. fragilis* and their ability to induce abscesses in animals even when injected alone. Onderdonk et al. correlated the virulence of *B. fragilis* strains with the presence of capsule. Inoculations of encapsulated *B. fragilis* alone resulted in abscess formation in most animals, whereas unencapsulated *Bacteroides* sp. seldom caused abscesses unless they were combined with an aerobic organism. Heat-killed encapsulated strains of *B. fragilis* or purified capsular polysaccharide alone, but not capsular polysaccharides from *Escherichia coli*, resulted in formation of intraabdominal abscesses. Simon et al. showed that encapsulated strains of *Bacteroides* were resistant to neutrophil-mediated killing, compared with unencapsulated strains. These investigations provide evidence that the capsular polysaccharide of *B. fragilis* is an important virulence factor for this organism.

The susceptibility of pathogenic bacteria to phagocytosis and killing by polymorphonuclear leukocytes and macro-

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**TABLE V**

Organisms Isolated from Three Patient Abscesses†

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Organisms Producing Beta-Lactamase</th>
<th>Organisms Not Producing Beta-Lactamase and Surviving Penicillin Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Interautobdominal abscess</td>
<td><em>B. distasonis</em></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>2. Pelvic abscess</td>
<td><em>B. evisus</em></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>3. Rectal abscess</td>
<td><em>E. coli</em></td>
<td><em>Peptostreptococcus</em></td>
</tr>
</tbody>
</table>

phages is of major importance in determining the outcome of the host-pathogen interaction. Casciato et al.\textsuperscript{30} and Bjornson et al.\textsuperscript{6} demonstrated phagocytosis and killing of \textit{B. fragilis} by human leukocytes \textit{in vitro}. Phagocytosis of \textit{B. fragilis} is the presence of serum occurred in aerobic and anaerobic conditions. Ingham et al.\textsuperscript{40} investigated the effect of \textit{Bacteroides} sp. on the phagocytic killing of facultative species. Killing of \textit{B. fragilis} and \textit{Proteus mirabilis} in mixtures \textit{in vitro} was impaired when the concentration of \textit{B. fragilis} was greater than $1 \times 10^7$ colony forming units per ml in the phagocytic system. Tofte et al.\textsuperscript{67} and Jones and Gemmell\textsuperscript{42} reported that both phagocytic uptake and killing of facultative species were impaired at high concentrations of encapsulated \textit{Bacteroides}. This inhibitory effect of \textit{Bacteroides} could be related to the effect of capsule on phagocytosis.

The implication of capsular polysaccharide as a factor associated with virulence is not unique to the \textit{Bacteroides} sp., but it has also been described in \textit{Streptococcus pneumoniae}, where the capsular material was shown to inhibit phagocytosis.\textsuperscript{68} The presence of capsule in \textit{B. fragilis} was shown to provide the organism with growth advantage \textit{in vivo} over unencapsulated isolates.\textsuperscript{55} Furthermore, encapsulated strains survived better \textit{in vitro} than unencapsulated variants when they were grown in an aerobic environment. Thus, the presence of a capsule apparently enables a strain of \textit{Bacteroides} to resist exposure to oxygen as well as host defenses.

The importance of the capsular polysaccharide of \textit{B. fragilis} as an immunogen was demonstrated when antibodies against it protected animals from early bacteremia.\textsuperscript{43} However, prevention of intraabdominal abscess formation by this encapsulated organism was found to be T-cell mediated.\textsuperscript{62} Recovery of Encapsulated \textit{B. fragilis} Group from Abscesses and Normal Flora

Subcutaneous abscesses in and around the oropharynx and the rectal area contain many species of aerobic and anaerobic bacteria, which are also found in high numbers in the normal flora adjacent to their location.\textsuperscript{23,47} It is believed, therefore, that most of these organisms originate from that source and become pathogenic during the inflammatory process, perhaps by selection. An evaluation was recently done of the role of encapsulation among the most frequently recovered anaerobes, including \textit{B. fragilis} group. A comparison was made between the recovery rates of encapsulated organisms in chronic inflammatory conditions in and around the oral cavity and in the pharynx of normal individuals.\textsuperscript{17} Specimens were obtained from 182 individuals with infections and 26 who served as a control group. Most individuals did not harbor encapsulated \textit{Bacteroides} in their mucus flora (encapsulated \textit{Bacteroides} were found in about 25 percent). However, 81 percent of abscesses or chronically infected sites harbored encapsulated \textit{Bacteroides} including all members of the \textit{B. fragilis} group.

Since most \textit{Bacteroides} sp. recovered from infected sites probably originate from the non-encapsulated endogenous mucus flora, they probably express their capsule during the inflammatory process.

Importance of Capsule in Mixed Infections

In an attempt to define the important pathogens among isolates recovered from clinical specimens, the virulence and importance of encapsulated bacterial isolates recovered from 13 clinical abscesses was studied.\textsuperscript{26} This was done by injecting each of the 35 isolates (30
anaerobes and five aerobes) subcutaneously (s.c.) into mice alone or in all possible combinations with the other isolates recovered from the same abscess. Sixteen of the isolates were encapsulated; 15 of them were able to cause abscesses by themselves and were recovered from the abscesses even when inoculated alone. The other organisms, which were not encapsulated, were not able to induce abscesses when inoculated alone. However, some were able to survive when injected with encapsulated strains. Therefore, the possession of a capsule by an organism was associated with increased virulence, compared with the same organism's non-encapsulated counterparts, and might have allowed some of the other accompanying organisms to survive. This phenomenon occurred in Bacteroides sp., and E. coli. Detection of a capsule in a clinical isolate may therefore suggest a pathogenic role of the organism in the infection.

Emergence of Capsule in Mixed Infections

The ability of the aerobic component in mixed infections to enhance the appearance of encapsulated anaerobic bacteria in these infections was studied in an s.c. abscess model in mice. The anaerobic bacteria with which they were inoculated were those commonly recovered in mixed infections. The B. fragilis group did not induce abscess when isolates that contained only a small number of encapsulated organisms (< one percent) were inoculated. However, when these relatively non-encapsulated isolates were inoculated, mixed with abscess-forming viable or nonviable bacteria ("helpers"), members of all studied B. fragilis group except one strain of B. distasonis survived in the abscesses and became heavily encapsulated (>50 percent of organisms had a capsule) (figures 1 and 2). A capsule emerged in 10 of 20 (50 percent) members of the B. fragilis group that were recovered in the subcutaneous abscess (table VI). About half of all tested members of B. fragilis group (regardless of species) are therefore capable of producing capsular material in vivo. Once encapsulated, a member of the B. fragilis group (B. fragilis, B. ovatus, B. thetaiotaomicron, B. vulgatus) can produce subcutaneous or intraperitoneal abscesses, even when

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**Figure 1.** An electron microscopic picture of B. fragilis where the cells are stained with ruthenium red. Unencapsulated cells (on the left side) can become encapsulated (on the right side) after animal passage. These two cells (unencapsulated and encapsulated) are from the same isolate of B. fragilis before and after animal passage (×55,000).

The capsule is a clinically important virulence factor of the entire Bacteroides group*
injected along. This highlights their potential virulence in infectious processes.

Most of the “helper” strains were encapsulated; however, several of the strains were not encapsulated but were able to induce abscesses when inoculated alone. The helper organisms used in conjunction with the B. fragilis group were E. coli, K. pneumoniae, S. aureus, S. pyogenes, enterococci, and Neisseria gonorrhoeae. The presence of encapsulation was only noticed in abscesses older than seven days. The process of encapsulation over time explains the emergence of Bacteroides as an important pathogen in chronic infection.

Suppression of the aerobic component of mixed infections using antimicrobials effective only against these bacteria did not prevent the encapsulation of eight tested members of B. fragilis group (five B. fragilis, two B. vulgatus, and one B. thetaiotamicron), nor did this prevent the formation of subcutaneous abscesses in mice.

For example, single therapy of mixed infection of B. fragilis (with metronidazole) and E. coli (with gentamicin) directed at the elimination of only one organism induced significant reduction in the number of treated organisms and also a smaller yet significant decrease in the number of untreated organisms. However, the abscesses were not eliminated after such therapy. Combination therapy or use of single agent (cefoxitin) directed against the aerobic and anaerobic components of the infection was more effective. The non-encapsulated B. fragilis group became encapsulated after passage in mice mixed with E. coli. Therapy directed at the elimination of E. coli did not prevent the emergence of encapsulated Bacteroides. These data demonstrate the synergy between all members of the B. fragilis group and reiterate the need to direct antimicrobial therapy at the eradication of the aerobic and anaerobic components of mixed infections.

**Role of a Capsule of B. fragilis in Bacteremia**

Anaerobic bacteremia account for five to 15 percent of cases of bacteremia and are especially prevalent in polymicrobial bacteremia, associated with abscesses. Onderdonk et al., who studied the model of intraabdominal sepsis and abscesses in rats, were able to detect bacteremia owing to B. fragilis only during the first few hours after inoculation of B. fragilis and E. coli. Bennion et al., who induced colonic ischemia in dogs, were able to induce prolonged and persistent bacteremia owing to B. fragilis and other anaerobic bacteria.

The role of possession of capsular material in the systemic spread of B. fragilis was investigated in mice following

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**TABLE VI**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. Species Tested</th>
<th>No. with Emergence of Capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. fragilis</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>B. thetaiotamicron</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>B. ovatus</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>B. vulgatus</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>B. distasonis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>10 (50%)</td>
</tr>
</tbody>
</table>

s.c. inoculation of non-encapsulated as well as encapsulated strains alone or in combination with aerobic or anaerobic facultative bacteria. Encapsulated \textit{B. fragilis} were isolated more frequently from infected animal blood, spleen, liver, and kidney than were non-encapsulated organisms.

After inoculation with a single encapsulated \textit{B. fragilis} strain, encapsulated organisms were recovered in 31 of 60 (52 percent) animals, whereas non-encapsulated \textit{B. fragilis} were recovered in only three of 60 (five percent) animals. Following inoculation of \textit{B. fragilis} mixed with aerobic or facultative flora, encapsulated \textit{B. fragilis} was isolated more often and for longer periods of time than was the non-encapsulated strain. Furthermore, encapsulated \textit{B. fragilis} was recovered more often after inoculation with other flora than it was when inoculated alone.

These data demonstrate the synergy between \textit{B. fragilis} and the other flora present with them in mixed infections. Further studies are warranted to explain the pathophysiology of increased virulence of encapsulated \textit{B. fragilis} expressed by persistent bacteremia and spread to different organs. Encapsulated strains were more virulent than non-encapsulated strains and highlight the importance of encapsulated \textit{B. fragilis} over non-encapsulated \textit{Bacteroides} species (table VII). On the other hand, encapsulated \textit{B. fragilis} group organisms were found to be of equal or greater importance than \textit{E. coli} and group D streptococci in the induction of abscesses. This demonstrates that encapsulated \textit{B. fragilis} are a factor that should be considered in the treatment of mixed infections with antibiotics.

Synergy Between \textit{B. fragilis} Group and Other Bacteria

Synergy between aerobic and anaerobic bacteria has long been recognized in

<table>
<thead>
<tr>
<th>Capsule Positive Bacteroides</th>
<th>Relative Virulence</th>
<th>Other Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{B. fragilis}</td>
<td>&gt;</td>
<td>\textit{K. pneumoniae}, \textit{E. coli}, Group D Strept., \textit{S. aureus}</td>
</tr>
<tr>
<td>\textit{B. vulgatus}</td>
<td>&gt;</td>
<td>\textit{E. coli}, Group D Strept., Group A Strept.</td>
</tr>
<tr>
<td>\textit{B. ovatus}</td>
<td>&gt;</td>
<td>\textit{E. coli}, \textit{K. pneumoniae}, Group D Strept.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Capsule Negative Bacteroides</th>
</tr>
</thead>
</table>

* Two isolates of each \textit{Bacteroides} sp. were tested, with one isolate of 16 other organisms.
† \textit{Infect. Immun. 44:12-15, 1984.}
‡ As determined by abscess size after selective antimicrobial therapy.

Significance of \textit{Bacteroides fragilis} Group in Mixed Infection

Although anaerobic bacteria, including the \textit{B. fragilis} group, are often recovered mixed with other aerobic and facultative flora, their exact role in these infections, and their relative contribution to the pathogenic process is unknown. The relative importance of the \textit{B. fragilis} group and facultative anaerobic pathogens in mixed infections was investigated in a s.c. abscess model in mice. This was determined by measuring the size of the abscess induced following antimicrobial therapy directed against one or both organisms present in the abscess. In almost all instances, the aerobic counterparts in the infection were more important than the unencapsulated \textit{Bacteroides} species (table VII).
a variety of clinical infections. In several animal studies, a mixed inoculum of anaerobic and aerobic bacteria produced sepsis that could not be induced by either component alone. Several studies documented the synergistic effect of intestinal bacteria in experimental infection. Altemeier demonstrated the pathogenicity of bacterial isolates recovered from peritoneal cultures after appendiceal rupture. Pure cultures of individual isolates were relatively innocuous when implanted subcutaneously in animals, but combinations of facultative and anaerobic strains greatly increased virulence. Similar observations were reported by Meleney et al. and Hite et al.

The synergistic potentials were evaluated between the B. fragilis group and aerobic and anaerobic bacteria commonly recovered in mixed infections. Three in vivo models were used to demonstrate the bacterial synergy: ability to reduce LD$_{50}$, reduction in the number of organisms required to produce abscesses, and enhancement of growth of all organisms in subcutaneous abscess.

**Reduction of LD$_{50}$**

The potential for synergy between aerobic, facultative, and anaerobic bacteria was studied by s.c. inoculation of mixtures of these organisms into mice and observation of subsequent mortality (table VIII). The anaerobic bacteria tested included six strains of anaerobic cocci and one strain each of B. fragilis and B. asaccharolyticus. The facultative and aerobic bacteria (FAB) included one strain each of S. aureus, P. aeruginosa, E. coli, K. pneumoniae, and P. mirabilis. The parameter measured was LD$_{50}$. The LD$_{50}$ was defined as the number of organisms that killed 50 percent of the mice by the seventh day after inoculation. Synergy was defined as a significant increase in mortality when two organisms were inoculated as compared to one. Mortality increased significantly when each aerobic organism was inoculated along with either of the Bacteroides species. A similar increase occurred when the anaerobic gram-positive cocci were inoculated along with P. aeruginosa (four of six combinations) or S. aureus (four of six). The results demonstrate synergistic potential between Bacteroides species and all aerobic bacteria tested and between most anaerobic gram-positive cocci and P. aeruginosa or S. aureus.

**Reduction in AB$_{50}$**

The potential for synergy between different anaerobic bacteria was studied by s.c. inoculation of mixtures of these organisms into mice and observing the abscess formation (table IX). The organisms tested were two species each of Bacteroides, Fusobacterium, and Clostridium and 12 anaerobic cocci.

The parameter determined was the median abscess formation dose (AF$_{50}$), that was defined as the number of organisms that induced s.c. abscess at least 10 mm in diameter in 50 percent of mice five days after inoculation. Synergy was defined as a significant decrease in AF$_{50}$ when two organisms were inoculated as
TABLE IX
Synergy Between Anaerobic Bacteria†

<table>
<thead>
<tr>
<th>Pepto-</th>
<th>F.</th>
<th>C.</th>
<th>C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strepto-</td>
<td>varium</td>
<td>perfringens</td>
<td>butyricum</td>
</tr>
<tr>
<td>coccius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. fragilis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>B. asaccharolyticus</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>P. varium</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>No</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>C. butyricum</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Synergy was demonstrated by reduction in the number of bacterial needed to induce subcutaneous abscess in mice by subcutaneous inoculation of bacteria.
‡Yes, synergy present
§No, synergy absent

 Compared to one. A significant decrease in AF_{50} was noted when 10 of the 12 anaerobic gram-positive cocci were inoculated with B. fragilis, when nine of the 12 were inoculated with B. asaccharolyticus, when 10 were inoculated with F. varium or F. nucleatum, and when three were inoculated with C. butyricum.

**Enhancement of Bacterial Growth**

The potential for mutual enhancement of growth of the B. fragilis group and the aerobic and facultative organisms commonly isolated with them in mixed infections was evaluated.

Enhancement was studied by measuring the relative increase in CFU of the two bacterial components, inducing s.c. abscesses in mice. Single bacterial isolates of each organism were used. Synergy was defined as a significant increase in growth of a bacterial component in mixed infection, as compared to the CFU of the organism when inoculated alone.

A mutual significant enhancement of growth of both E. coli and B. fragilis was noted in mixed infection, as compared to the number of these isolates when each was inoculated alone (P < 0.05) (figure 3). Similar mutual increases were also noticed in combinations of B. ovatus and B. vulgatus with E. coli (table X). An increase in the number of aerobic and facultative bacteria when combined with three members of the B. fragilis group was illustrated in almost all instances except for the one between H. influenzae and B. fragilis group (table X). However, this is not surprising since this combination is rarely seen in clinical infections.

**Effect of Capsule on Synergy**

When encapsulated members of B. fragilis group were coinoculated subcutaneously with E. coli (table XI), the encapsulated bacteria showed a significantly higher number of organisms in the abscesses that were formed than were non-encapsulated organisms. This highlights the increased virulence of encapsulated organisms.

![Figure 3. Number of Bacteroides fragilis and Escherichia coli (Log_{10} colony-forming unit) in subcutaneous abscesses induced by single and combined bacteria in mice.](Brook, I.: Infect. Immun. 50:929, 1985.)

TABLE X

| Average Number of Facultative and Aerobic Bacteria (CFU) in SC abscesses Induced by Each Organism Alone Combined with B. fragilis Group* |
|---|---|---|
| S. aureus | 8.0 | 11.9† | 12.2† |
| Group A streptococci | 9.2 | 12.1† | 12.7† |
| Group B streptococci | 7.1 | 8.9† | 8.8† |
| E. coli | 9.0 | 12.1† | 11.8† |
| K. pneumoniae | 7.2 | 10.4† | 10.6† |
| P. aeruginosa | 6.1 | 11.7† | 10.0† |
| H. influenzae | 6.3 | 6.9 | 5.4 |

†P < 0.05, between single and mixed infections
The effects of encapsulation of B. fragilis on facultative and aerobic bacteria (FAB) were evaluated using a s.c. abscess model in mice. The change in number of FAB was studied by comparing their number when injected with non-encapsulated, encapsulated B. fragilis and capsular material of Bacteroides species (Table XII). In seven combinations of FAB mixed with non-encapsulated B. fragilis, an increase in the number of non-encapsulated B. fragilis and an increase in the number of FAB occurred in two instances (E. coli and K. pneumoniae). However, an increase in the number of FAB occurred in five of seven combinations with encapsulated B. fragilis. No change in the bacterial numbers was observed when the FAB were inoculated with capsular material of B. fragilis. These data demonstrated the ability of viable encapsulated Bacteroides species to enhance the growth of FAB.

The effect of antimicrobial therapy in mixed infection has been studied. When selective antimicrobial therapy was administered effective against either component or both components of the infection, a significant decrease in the number of the susceptible organisms occurred. In Table XIII are illustrated the response in abscesses caused by B. thetaiotaomicron and E. coli. Similar data were obtained with B. fragilis and B. vulgatus. Metronidazole was effective against the B. fragilis group, and gentamicin reduced the number of E. coli. Although the decrease in number of the susceptible organisms was the largest one and occurred in all instances, a reduction in the number of the other non-susceptible organisms occurred in a

**TABLE XI**

Average Number of Encapsulated and Non-Encapsulated Bacteroides in Mixed Abscesses with E. coli*

<table>
<thead>
<tr>
<th>Bacteroides</th>
<th>Encapsulated</th>
<th>Non-Encapsulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. fragilis</td>
<td>10.8 ± 1.1</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td>B. thetaiotaomicron</td>
<td>11.3 ± 1.3</td>
<td>7.6 ± 0.7</td>
</tr>
<tr>
<td>B. vulgatus</td>
<td>10.2 ± 0.8</td>
<td>7.3 ± 0.9</td>
</tr>
</tbody>
</table>


**TABLE XII**

Changes (Expressed in log_{10} CFU) in Number of Bacteria in Polymicrobial Abscesses with B. fragilis (BF) 5 days after Inoculation†

<table>
<thead>
<tr>
<th>Aerobic or Faculative Bacteria (FAB)</th>
<th>No. of Organisms when Injected Alone (log_{10} CFU ± SD)</th>
<th>Change in No. of FAB Organisms when Injected with Non-encapsulated Bacteroides§</th>
<th>Change in No. of FAB Organisms when Injected with Encapsulated Bacteroides§</th>
<th>Change in No. of FAB Organisms when Injected with Capsular Material from Bacteroides§</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>8.6 ± 0.8</td>
<td>0.5</td>
<td>2.1§</td>
<td>0.7</td>
</tr>
<tr>
<td>Gr A streptococci</td>
<td>9.0 ± 0.7</td>
<td>0.4</td>
<td>2.0§</td>
<td>0.8</td>
</tr>
<tr>
<td>Gr D streptococci</td>
<td>7.3 ± 1.6</td>
<td>0.7</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>E. coli</td>
<td>9.2 ± 0.4</td>
<td>1.4§</td>
<td>2.6§</td>
<td>1.5</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>7.6 ± 1.4</td>
<td>1.6§</td>
<td>1.9§</td>
<td>0.8</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>8.3 ± 1.5</td>
<td>0.8</td>
<td>2.6§</td>
<td>0.7</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>6.5 ± 1.0</td>
<td>-0.4</td>
<td>-1.0</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

*Abscesses were induced by subcutaneous injection in the right groin of a 0.1 ml of suspension containing 10^9 of each bacterium per ml. The abscess cultures and bacterial counts were performed on day 5 of inoculation. The CFU (± SD) of encapsulated B. fragilis when injected alone were 8.6 ± 0.8 and 8.2 ± 0.7 concomitantly. Six mice were included in each experimental group.

§Compared to number of organisms when injected alone.
¶Compared to number of organisms when injected with non-encapsulated B. fragilis.
**Significant difference present P < 0.05
TABLE XIII
Average Number of Organisms in Abscesses Induced by Encapsulated B. thetaiotaomicron and E. coli After Five Days of Antimicrobial Therapy

<table>
<thead>
<tr>
<th>CFU/Abscess</th>
<th>Bacteroides</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Therapy</td>
<td>11.3 ± 1.3</td>
<td>10.8 ± 1.2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>9.6 ± 0.7</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>4.6 ± 0.6</td>
<td>8.5 ± 1.1</td>
</tr>
<tr>
<td>Gentamicin and metronidazole</td>
<td>3.7 ± 0.3</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>2.8 ± 0.3</td>
<td>3.6 ± 0.6</td>
</tr>
</tbody>
</table>

* Eight animals were included in each experimental group
† The log_{10} CFU of E. coli when injected as single organism was 9.1 ± 0.7
‡ Significant change compared to control (P < 0.5)
§ The log_{10} CFU of B. thetaiotaomicron when injected as single organism was 10.2 ± 1.6

 quarter of the combinations. This effect may be due to the interruption in the bacterial synergistic effect between E. coli and the B. fragilis group. However, for successful eradication of the infection, therapy directed at the eradication of both components of the infection was needed.

Several hypotheses have been proposed to explain microbial synergy. When this phenomenon occurs in mixtures of aerobic and anaerobic flora, it may be due to protection from phagocytosis and intracellular killing, production of essential growth factors, and lowering of oxidation-reduction potentials in host tissues. Obligate anaerobes can interfere with the phagocytosis and killing of aerobic bacteria. The ability of human polymorphonuclear leukocytes to phagocytose and kill Proteus mirabilis was impaired in vitro when the human serum used to opsonize the target bacteria was pretreated with live or dead organisms of various Bacteroides sp. The B. gingivalis cells or supernatant culture fluid was shown to possess the greatest inhibitory effect among the Bacteroides sp. Supernatants of cultures of the B. fragilis group, the B. melaninogenicus group, and B. gingivalis were capable of inhibiting the chemotaxis of leukocytes to the chemotactic factors of P. mirabilis. Another possible mechanism that explains the synergistic effect of aerobic-anaerobic combinations is the lowering of local oxygen concentrations and the oxidation-reduction potential by the anaerobic component of the infection. Such environmental factors are known to be critical for anaerobic growth in vitro and may apply with equal relevance to in vivo experimental animal studies. Mergenhagen et al. noted that the infecting dose of anaerobic cocci was significantly lowered when the inoculum was supplemented with chemical reducing agents. A similar effect may be produced by facultative bacteria, which may provide the proper conditions for establishing an anaerobic infection at a previously well-oxygenated site.

The demonstration of the synergistic potentials of the B. fragilis group, when mixed with various aerobic and anaerobic bacteria, further indicates their pathogenic role. These findings provide further support for the important pathogenic role of all members of the B. fragilis group in mixed infections and highlight the need to use therapy directed at their eradication in the earlier stages of the infectious process. These data also reiterate the usefulness of prophylactic therapy and specific therapy of mixed infection that will include coverage for the Bacteroides sp. as well as its aerobic or facultative counterparts. Since encapsulated Bacteroides are more virulent than non-encapsulated organisms, earlier institution of antimicrobials effective against these organisms may prevent their subsequent encapsulation.

The importance of surgical drainage also is highlighted by these studies because none of the antimicrobials or their combinations were effective in completely eradicating the infection. Therefore, surgical drainage supplemented by antimicrobials should be used in conjunction in the management of mixed infection.
Although the distribution of the members of the *B. fragilis* group varies, all members of the *B. fragilis* group are potential pathogens capable of causing serious localized and/or systemic infections, including bacteremia associated with mortality. The *B. fragilis* group is resistant to penicillin through production of beta-lactamase, which may shield other pathogens from penicillin therapy.

Conclusions

These studies showing the process of encapsulation of *Bacteroides* sp. which are assisted by aerobic or facultative “helpers” provide insight into the synergistic relationship between these organisms. Since even killed “helpers” provided protection for the unencapsulated anaerobic bacteria, antimicrobial therapy should be directed against both the aerobic and anaerobic components of infection.

The virulence of the *B. fragilis* group can be illustrated in vivo by enhancement of growth of their aerobic and facultative bacterial partners. It is evident that the possession of a capsule is an important mechanism of virulence of all *Bacteroides* species, including all members of the *B. fragilis* group. The emergence of a capsule occurs during polymicrobial infection and explains the predominance of this pathogen in chronic infection.

*Bacteroides fragilis* is the most susceptible among the *B. fragilis* family to antimicrobials. Other members (i.e., *B. thetaiotaomicron* and *B. vulgatus*) are generally more resistant. For successful elimination of polymicrobial infections involving the *B. fragilis* group, therapy has to be directed at the eradication of both the *B. fragilis* group and their aerobic or facultative counterparts.

References

18. BROOK, I.: Recovery rate of anaerobic bacteria in


