Pathogenicity of capsulate and non-capsulate members of *Bacteroides fragilis* and *B. melaninogenicus* groups in mixed infection with *Escherichia coli* and *Streptococcus pyogenes*.

I. BROOK

Departments of Pediatrics and Surgery, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-5145, USA

Summary. The relationships between capsulate and non-capsulate *Bacteroides fragilis* strains and *Escherichia coli*, and between capsulate and non-capsulate strains of the *B. melaninogenicus* group and *Streptococcus pyogenes*, were studied in a subcutaneous abscess model in mice. Selective antimicrobial agents directed against either aerobic or anaerobic bacteria were used alone or in combination to explore the effect of eradication of one component of the mixed infection. Single agent therapy effective against both aerobic and anaerobic flora was also employed. Single therapy of mixed infection directed at the elimination of only one organism (*S. pyogenes*, *E. coli* or *Bacteroides* sp.) caused significant reductions in the numbers of sensitive organisms and also smaller yet significant decreases in the numbers of insensitive organisms. However, the abscesses were not eliminated after such therapy. Combination therapy or use of a single agent (cefoxitin) directed against the aerobic and anaerobic components of the infection was more effective. Non-capsulate *Bacteroides* spp. became capsulate after passage in mice mixed with either *S. pyogenes* or *E. coli*. Therapy directed at the elimination of *S. pyogenes* and *E. coli* did not prevent the emergence of capsulate *Bacteroides* spp. These data demonstrate the synergy between all members of the *B. fragilis* group and *E. coli* and between the *B. melaninogenicus* group and *S. pyogenes*, and reiterate the need to direct antimicrobial therapy at the eradication of the aerobic and anaerobic components of mixed infections.

Introduction

Members of the *Bacteroides fragilis* group mixed with *Escherichia coli* are often isolated from intra-abdominal infections (Brook, 1988), while members of the *B. melaninogenicus* group and *Streptococcus pyogenes* are frequently isolated from orofacial and respiratory infections (Brook, 1981). Although the virulence of capsulate strains of *B. fragilis* is well established (Onderdonk *et al.*, 1977; Brook *et al.*, 1984a, b) the pathogenicity of other members of the *B. fragilis* and *B. melaninogenicus* groups requires further investigation.

We have previously demonstrated the ability of “helper” organisms to assist in the emergence of capsulate *Bacteroides* spp. *in vivo* (Brook *et al.*, 1983,

1984a). However, since this phenomenon depended on the presence of viable “helper” bacteria, it was important to ascertain whether elimination of these bacteria by selective antibacterial agents would interfere with the production of capsulate *Bacteroides* spp. and the formation of abscesses.

In this study, the relationship between capsulate and non-capsulate members of the *B. fragilis* group and *E. coli*, and between capsulate and non-capsulate strains of the *B. melaninogenicus* group and *S. pyogenes* was studied in experimental subcutaneous abscesses in mice. Selective antimicrobial agents directed against either aerobic or anaerobic bacteria were used alone or in combination to explore the effect of eradication of one component of the mixed infection. This model enabled study of the interactions between the organism and also the effect of antimicrobials on the process of capsulation of the *Bacteroides* spp.

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Offprint requests should be sent to Dr I. Brook, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145, USA.

Materials and methods

Organisms

All aerobic and anaerobic bacterial strains used in the experiments were recent clinical isolates. They included one strain each of *B. fragilis*, *B. thetaiotaomicron*, *B. vulgatus*, *B. melaninogenicus*, *B. asaccharolyticus*, *B. intermedius*, *E. coli*, and *S. pyogenes*. The bacteria were kept frozen in skim milk at -70°C . They were identified by standard criteria (Lennette *et al.*, 1985; Sutter *et al.*, 1985) and processed as previously described (Brook *et al.*, 1983). Each species of *Bacteroides* contained two forms: one non-capsulate ($<0.1\%$ of the organisms had a capsule) and the other capsulate ($>50\%$ of the organisms had a capsule). The capsulate form was induced by passage of the non-capsulate form mixed with *Klebsiella pneumoniae* in subcutaneous (s.c.) abscesses in mice (Brook *et al.*, 1983). The presence of a capsule was established by Hiss's staining method (Lennette *et al.*, 1985) and confirmed by electronmicroscopy after staining with ruthenium red (Kasper, 1976). Ruthenium red staining demonstrated a homogeneous polysaccharide capsule external to the cell wall. Capsular stains revealed the presence of a capsule also in *S. pyogenes*.

Frozen bacterial suspensions were thawed to room temperature, subcultured on to Schaedler blood agar, and incubated for 48 h at 37°C in an anaerobic glove box (Sutter *et al.*, 1985) for *Bacteroides* spp. or in $5\% \text{CO}_2$ for other bacteria; 24 h before injection into mice, the bacterial strains were inoculated on to Trypticase Soy Agar containing sheep blood 5% (BBL Microbiology Systems, Cockeysville, MD). Colonies were harvested with cotton swabs from these media and transferred to normal (0.9%) saline. The suspensions of organisms prepared were equivalent to a 0.5 McFarland standard. Viable counts (cfu) were determined by plate counts on brain-heart infusion agar enriched with vitamin K₁ and haemin to support the growth of the *Bacteroides* spp.

Animals

The mice used were male Swiss albino mice, weighing 20–25 g, obtained from the Naval Medical Research Institute mouse colony. The mice were raised in conventional conditions. Each mouse was inoculated s.c. in the right groin with 0.1 ml of each appropriate bacterial suspension, in saline, that contained 10^8 cfu of each organism. To induce a combined infection, two inocula were mixed and each animal received 0.2 ml containing 0.1 ml of each bacterial suspension.

Cultivation of abscess contents

Animals were killed by cervical dislocation on day 5 after injection of bacteria. The sizes of the abscesses were estimated from measurements by caliper of two perpendicular diameters, corresponding to maximum length and width. The product of these two measurements, expressed as mm^2 , was approximately proportional to

the outer surface of the abscess. For the determination of the bacterial contents of the abscesses, the abscess material was removed aseptically and homogenised, in an anaerobic glove box, in 1 ml of sterile saline in a ground-glass tissue homogeniser. Tenfold serial dilutions of the homogenates were made in sterile saline, and 0.1-ml volumes of each dilution were spread in triplicate on brain heart infusion enriched blood-agar plates. No attempt was made to inactivate the antimicrobial agents in the homogenised abscess material, since a considerable dilution was achieved before plating, especially with the smaller abscesses. Colonies were counted after incubation at 37°C in an anaerobic chamber for 48 h, and the results are presented as $\log_{10}$ of viable bacteria (cfu) per abscess.

Antimicrobial therapy

Antimicrobial therapy was directed against either species or both species present in the abscess. Metronidazole was directed against the *Bacteroides* spp. only and gentamicin was directed against *E. coli* only, whereas spiramycin was directed against *S. pyogenes*.

Dosages of antimicrobial agents were selected on the basis of pharmacokinetic studies on untreated animals (table I). Serum levels of antibiotics were measured by agar diffusion assay (Lennette *et al.*, 1985) at 30, 60, 120 and 180 min after administration of the last dose of the agents. Abscess levels were measured 1 h after administration of the last dose of antimicrobial agents on day 5. In most instances, the doses of antimicrobial agents approximated (in mg/kg) to the usual recommended dose for human patients. The total dosages (in mg/kg of body weight) administered intramuscularly (i.m.) at intervals of 8 h were: gentamicin (Schering, Kenilworth, NJ) 7.5; metronidazole (Searle, Chicago, IL) 50; spiramycin (Rhone-Poulenc, SA, Paris) 100; and cefoxitin (Merck

Table I. Blood and abscess concentration of metronidazole, spiramycin, gentamicin and cefoxitin in mice infected with *B. fragilis* and *E. coli* (for metronidazole, gentamicin and cefoxitin) and *B. melaninogenicus* and *S. pyogenes* (for spiramycin)

Antimicrobial agent (Daily dose, mg/kg)	Serum level (mg/L)* at		Abscess level (mg/ L)* at 1 h
	0.5 h	8 h	
Metronidazole (50)	27.6 (7.1)	11.4 (3.1)	13.6 (4.1)
Spiramycin (100)	8.0 (2.5)	1.8 (0.8)	6.1 (1.3)
Gentamicin (75)	6.4 (1.6)	1.2 (0.5)	3.9 (1.6)
Cefoxitin (200)	56.0 (9.1)	18.2 (1.8)	12.4 (1.2)

*Mean (SEM) of 10 determinations.

Sharp and Dohme, West Point, PA) 200. Treatment was started 2 h after inoculation and continued for 5 days. The concentrations of metronidazole were assayed by high-performance liquid chromatography (Wheeler *et al.*, 1978). The concentration of all other agents was determined by diffusion assays (Lennette *et al.*, 1985); for spiramycin and cefoxitin with *Micrococcus luteus* ATCC 9341 and for gentamicin with *Bacillus subtilis* ATCC 6633.

Susceptibility tests

The MICs of the test antibiotics against the bacterial isolates were determined by agar dilution (Thornsberry and Svenson, 1978; Lennette *et al.*, 1985).

Experimental design

To determine relationships between the various organisms in mixed infections, non-capsulate and capsulate *Bacteroides* spp. were injected in combination with one species at a time: *B. fragilis* group with *E. coli* and *B. melaninogenicus* group with *S. pyogenes*.

A group of 32 mice was challenged with single species or mixtures of *Bacteroides* spp. with the facultative anaerobes. Each infected group was divided into four groups of eight mice and given no treatment (1), or therapy with single agents (2 and 3), or combination therapy (4). Each experiment was done three times. Statistical analysis was Student's *t* test of independent means.

Results

In-vitro susceptibility to antibiotics

Members of the *B. fragilis* and *B. melaninogenicus* groups were susceptible to metronidazole (MIC 0.1 mg/L) and cefoxitin (MIC < 4 mg/L) and were resistant to gentamicin (MIC > 256 mg/L) and spiramycin (MIC > 32 mg/L). All facultative anaerobes were resistant to metronidazole (MIC > 128 mg/L). *E. coli* was susceptible to gentamicin (MIC < 0.5 mg/L) and cefoxitin (MIC 2 mg/L). *S. pyogenes* was susceptible to spiramycin and cefoxitin (MIC < 0.5 mg/L).

Antibiotic concentrations in serum

The concentrations of antimicrobials were determined in animals challenged with *B. fragilis* and *B. melaninogenicus*. This was done to ascertain the level of antimicrobials achieved in an infected animal. The serum concentrations of metronidazole, spiramycin, and gentamicin were at least four

times higher than the MIC for all susceptible bacteria (table I).

Abscess formation

Non-capsulate *Bacteroides* spp. did not induce abscesses when injected alone. All other test organisms caused abscesses when injected alone. The viable counts of each are noted in tables II and III. Abscesses usually were formed within 24–72 h of inoculation. When left alone, they reached a maximum diameter of 12–14 mm within 5–7 days, and began to drain between 10 and 20 days. As a consequence of mixed infections, the maximum diameter of an abscess was larger (22–24 mm) than those caused by single organisms (12–15 mm).

Histological examination of abscesses showed a central area of necrotic cells, fibrin, and bacteria, surrounded by a band of leucocytes and a distinct collagen capsule. There was no mortality associated with abscess formation.

Number of organisms in abscesses. The differences in the numbers of facultative and anaerobic bacteria found in untreated and treated animals were calculated. The differences in numbers ($\log_{10}$ cfu) of facultative bacteria and the differences in numbers of *Bacteroides* spp. are presented in tables II and III.

The number of organisms in mixed infections of capsulate *Bacteroides* spp. and *S. pyogenes* or *E. coli* was always significantly larger than the number of each of the participating organisms in abscesses induced by a single species (tables II and III).

Therapy of infections caused by single species

Therapy with drugs to which the organisms were susceptible caused a significant decrease in the viable counts of all organisms. Metronidazole reduced the viable counts of the *Bacteroides* spp. and the sizes of the abscesses, spiramycin was effective against *S. pyogenes*, and gentamicin was active against *E. coli*. Cefoxitin was effective against all of the organisms. The combination of spiramycin and metronidazole reduced the viable count of *B. melaninogenicus* more than metronidazole alone. The combination of gentamicin and metronidazole showed no increased efficacy.

Therapy of infections caused by two species

Single therapy. Therapy with metronidazole was universally effective in reducing the viable counts of all non-capsulate and capsulate strains of *B.*

Table II. Number of organisms in abscesses and size of abscesses induced by capsulate and non-capsulate members of the *B. fragilis* group and *E. coli* after 5 days of antimicrobial therapy

<i>Bacteroides</i> species	Antimicrobial therapy	Results with capsulate bacteroides			Results with non-capsulate bacteroides		
		<i>Bacteroides</i> cfu/abscess §	<i>E. coli</i> † cfu/abscess §	size (mm ²) of abscess (mean ± SD)	<i>Bacteroides</i> cfu/abscess §	<i>E. coli</i> † cfu/abscess §	size (mm ²) of abscess (mean ± SD)
<i>B. fragilis</i> (9.2 ± 0.8)†	No therapy	10.8 (1.1)	11.9 (1.6)	346 ± 32	6.8 (0.9)	10.4 (0.7)	204 ± 38
	Gentamicin	9.0 (1.0)	3.3 (0.6)*	132 ± 39*	5.6 (0.8)	2.5 (0.4)*	66 ± 38*
	Metronidazole	2.7 (0.8)*	7.5 (0.8)*	112 ± 25*	NG*	9.1 (0.7)	84 ± 31*
	Gentamicin and metronidazole	3.1 (0.5)*	3.6 (0.4)*	34 ± 12**	NG*	3.1 (0.6)*	42 ± 16
	Cefoxitin	2.8 (0.6)*	3.8 (0.6)*	31 ± 8*	NG*	2.6 (0.5)*	40 ± 9
<i>B. thetaiotaomicron</i> (10.2 ± 1.6)†	No therapy	11.3 (1.3)	10.8 (1.2)	348 ± 41	7.6 (0.7)	9.3 (1.2)	236 ± 52
	Gentamicin	9.6 (0.7)	3.1 (0.6)*	108 ± 18*	6.1 (0.9)	3.4 (0.5)*	90 ± 18*
	Metronidazole	4.6 (0.6)*	8.5 (1.1)*	150 ± 26*	1.3 (0.4)*	8.2 (0.9)	102 ± 28*
	Gentamicin and metronidazole	3.7 (0.3)*	3.2 (0.5)*	62 ± 18**	NG*	1.5 (0.4)**	48 ± 22**
	Cefoxitin	2.8 (0.5)*	3.8 (0.6)*	46 ± 12	1.6 (0.2)*	1.3 (0.3)*	28 ± 12
<i>B. vulgatus</i> (8.8 ± 0.9)†	No therapy	10.2 (0.8)	11.5 (1.4)	382 ± 40	7.3 (0.9)	9.8 (1.1)	220 ± 28
	Gentamicin	9.1 (1.1)	3.1 (0.5)*	98 ± 21*	6.8 (0.8)	4.1 (0.8)*	88 ± 27*
	Metronidazole	3.4 (0.5)*	9.3 (0.8)*	110 ± 24*	2.0 (0.3)*	7.6 (1.0)*	75 ± 20*
	Gentamicin and metronidazole	4.1 (0.6)*	2.9 (0.5)*	30 ± 15**	0.8 (0.2)*	1.3 (0.2)*	23 ± 12**
	Cefoxitin	2.8 (0.7)*	3.2 (0.8)*	22 ± 10	NG*	1.2 (0.4)*	29 ± 15

Each experimental group comprised 8 animals and each experiment was done three times.

* Significant difference from control ($p < 0.05$).

** Significant difference between both single therapies and combined therapy ($p < 0.05$).

† The viable count ($\log_{10}$ cfu) of *E. coli* injected alone was 9.1 SD 0.7.

‡ The numbers in parentheses indicate the viable count ($\log_{10}$ cfu and SD) of organisms in s.c. abscesses induced by capsulate *Bacteroides* strain alone.

§ Mean (SEM) of 24 abscesses.

Table III. Number of organisms in abscesses and size of abscesses induced by capsulate and non-capsulate *B. melaninogenicus* group and *S. pyogenes* after 5 days of antimicrobial therapy

<i>Bacteroides</i> species	Antimicrobial therapy	Results with capsulate bacteroides			Results with non-capsulate bacteroides		
		<i>Bacteroides</i> cfu/abscess§	<i>S. pyogenes</i> † cfu/abscess§	size (mm ²) of abscess (mean ± SD)	<i>Bacteroides</i> cfu/abscess§	<i>S. pyogenes</i> † cfu/abscess§	size (mm ²) of abscess (mean ± SD)
<i>B. melaninogenicus</i> (8.4 ± 0.9)‡	No therapy	9.4 (1.0)	10.9 (1.2)	410 ± 69	7.2 (0.8)	9.2 (1.0)	276 ± 56
	Spiramycin	8.2 (0.5)	3.2 (0.6)*	141 ± 35*	6.7 (0.9)	3.8 (0.4)*	105 ± 22*
	Metronidazole	3.5 (0.4)*	9.6 (1.1)	130 ± 24*	0.8 (0.2)*	7.1 (0.8)*	116 ± 28*
	Spiramycin and metronidazole	1.8 (0.5)**	3.0 (0.4)*	49 ± 23**	0.6 (0.1)	2.0 (0.6)*	53 ± 23**
	Cefoxitin	2.3 (0.8)*	2.0 (0.5)*	53 ± 14	NG	NG	48 ± 13
<i>B. asaccharolyticus</i> (7.9 ± 0.9)‡	No therapy	9.2 (1.0)	11.0 (1.1)	345 ± 66	6.6 (0.6)	8.7 (0.9)	262 ± 75
	Spiramycin	7.8 (0.8)	4.0 (0.5)*	161 ± 41*	6.0 (0.6)	2.9 (0.4)*	78 ± 30*
	Metronidazole	3.2 (0.6)*	9.8 (0.8)	182 ± 38*	0.4 (0.1)*	7.1 (1.1)	92 ± 23*
	Spiramycin and metronidazole	2.2 (0.4)*	2.5 (0.6)*	72 ± 24**	NG*	NG**	18 ± 5**
	Cefoxitin	1.4 (0.6)*	1.6 (0.2)*	65 ± 18	NG*	0.8 (0.5)	22 ± 7
<i>B. intermedius</i> (9.0 ± 1.6)‡	No therapy	9.8 (0.9)	11.3 (0.9)	326 ± 78	5.5 (0.7)	8.4 (1.0)	231 ± 76
	Spiramycin	8.2 (0.7)	4.5 (0.5)*	126 ± 25*	5.6 (0.5)	2.6 (0.7)*	102 ± 30*
	Metronidazole	4.1 (0.5)*	8.6 (0.7)*	123 ± 36*	2.8 (0.6)*	7.9 (1.2)	86 ± 27*
	Spiramycin and metronidazole	3.3 (0.3)*	2.0 (0.2)**	64 ± 24**	1.0 (0.3)*	1.4 (0.4)*	31 ± 23**
	Cefoxitin	2.8 (0.4)*	2.8 (0.8)*	72 ± 12	NG*	1.8 (1.0)	17 ± 6

Each experimental group comprised 8 animals and each experiment was done three times.

* Significant change compared to control ($p < 0.05$).** Significant change between single and combined therapy ($p < 0.05$).† The viable count ($\log_{10}$ cfu) of *S. pyogenes* injected alone was 8.2 SD 1.1.‡ The numbers in parentheses indicate the viable count ($\log_{10}$ cfu and SD) of organisms in s.c. abscesses induced by capsulate *Bacteroides* strain alone.

§ Mean (SEM) of 24 abscesses.

fragilis and *B. melaninogenicus* groups in all combinations. Spiramycin was effective in reducing the numbers of *S. pyogenes*, and gentamicin decreased the numbers of *E. coli* (tables II and III). Although the decrease in viable count of the susceptible organisms was the largest and occurred in all instances, the count of the other non-susceptible organisms was also reduced by a small but significant extent in 6 of the 24 combinations. Cefoxitin was as effective as combination therapy with gentamicin and metronidazole or spiramycin (see below).

Combination therapy. Combined therapy with gentamicin and metronidazole showed greater reduction of viable counts of *B. thetaiotaomicon*, compared with reduction by either agent alone, when administered to animals with abscesses induced by non-capsulate *B. thetaiotaomicon* and *E. coli* (table II). Increased elimination of *S. pyogenes* was noted with the combination of spiramycin and metronidazole in mice with abscesses induced by capsulate *B. intermedius* and *S. pyogenes* (table III). Similarly, an increase in the eradication of *B. melaninogenicus* was noted in combined infections with *S. pyogenes*.

Emergence of capsule

Culturing of abscesses induced by non-capsulate *Bacteroides* spp. and *S. pyogenes* or *E. coli* revealed that the *Bacteroides* spp. became heavily capsulate (i.e., > 50% of cells showed the presence of capsule). This phenomenon occurred even in animals treated with antibiotics effective against *S. pyogenes* or *E. coli*, but not in those treated with metronidazole or cefoxitin, which are effective against the *Bacteroides* spp.

Discussion

The ability of members of the *B. fragilis* group, other than *B. fragilis*, to induce an abscess by themselves strengthens the evidence supporting their pathogenic role. *B. fragilis* is the most commonly isolated member of the *B. fragilis* group, accounting for 40–50% (Brook, 1980; Sutter *et al.*, 1985) of clinical isolates. However, the other members of the group account for the rest of the isolates and have frequently been isolated from blood cultures and other infected sites as the only isolates (Brook, 1988). Strains of *B. thetaiotaomicon*, *B. vulgatus*, and the *B. melaninogenicus* group possess capsular material that is morphologically similar to that of *B. fragilis* and all manifest the ability to express their capsule material after

passage in an animal assisted by a “helper” organism (Brook *et al.*, 1983, 1984). The inability of antimicrobial therapy directed at the eradication of the “helper” organism to prevent the emergence of the capsule and subsequent formation of a subcutaneous abscess strengthens the need to direct antimicrobial therapy at the eradication of both the aerobic and anaerobic components of mixed infections.

Although most members of the *B. fragilis* group possess the enzyme β -lactamase, and all are therefore resistant to penicillin, their susceptibility to cephalosporins varies (Tally *et al.*, 1983). Of all the *B. fragilis* group, *B. fragilis* is the most susceptible to second- and third-generation cephalosporins (Wexler and Finegold, 1988). Among the second- and third-generation cephalosporins, cefoxitin is the most active against all members of the *B. fragilis* group (Tally *et al.*, 1983; Wexler and Finegold, 1988). In this study, single-agent therapy with cefoxitin was shown to be as effective as combination therapies against single and combined infection. These data are supported by recent clinical studies in which therapy with cefoxitin was found to be equally effective as clindamycin plus an aminoglycoside (Gentry *et al.*, 1984; Hofstetter *et al.*, 1984; Nichols *et al.*, 1984; Solomkin *et al.*, 1984) and superior to cefamandole (Gentry *et al.*, 1984; Jones *et al.*, 1985) in the management of intra-abdominal infection after trauma.

This study highlights the synergic relationship between the *Bacteroides* spp. and their aerobic and facultative counterparts in mixed infection. Such a relationship has been demonstrated in other studies, and it has been documented by increased mortality (Altemeier, 1942; Brook *et al.*, 1984), abscess formation (Brook *et al.*, 1983, 1984a, 1984b), and mutual enhancement of growth (Brook, 1985).

The interruption of such synergy by antimicrobial agents aimed at one component of the infection, the anaerobic or aerobic, had a significant effect on the untreated bacterial partner. This was especially apparent when therapy directed only at the facultative component (gentamicin or spiramycin) or the anaerobic component (metronidazole) was also effective in reducing the number of untreated bacteria. Despite this phenomenon, in no instance was such therapy effective in completely eliminating the infection and eradicating the untreated organisms, and the abscesses were still present.

The ability of metronidazole to reduce the viable counts of aerobes and facultative anaerobes when present in mixed infection with *Bacteroides* spp. could be due to its ability to reduce the numbers of the anaerobe, thus interfering with the synergic

relationship between the two. A similar effect of metronidazole was previously noted in mixed infection with *B. fragilis* and *E. coli* (Onderdonk *et al.*, 1979). They suggested that metabolites of metronidazole generated by *B. fragilis* impaired the viability of *E. coli*. Subsequently, Ingham *et al.* (1980) and Chrystal *et al.* (1980) demonstrated that, in anaerobic conditions, metronidazole temporarily reduces the number of *E. coli*. It seems, therefore, that metronidazole by itself may have direct antibacterial activity against *E. coli* in an anaerobic environment. Studies are warranted to further explore this phenomenon.

Our findings provide further support for the important pathogenic role of all members of the *B. fragilis* and *B. melaninogenicus* groups in mixed infections, and emphasise the need to use therapy directed at their eradication in the earlier stages of the infectious process. The advantages of eradicating non-capsulate *Bacteroides* spp. also reiterate the value of prophylactic therapy and specific therapy of mixed infection that includes coverage for the

Bacteroides spp. as well as its aerobic or facultative counterparts.

The importance of surgical drainage also is emphasised by this study 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.

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