Incidence and isolation of *Bacteroides* species from clinical material and their sensitivity to antibiotics

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SYNOPSIS One thousand and sixty-seven strains of anaerobic Gram-negative bacilli were isolated from routine clinical material between January 1971 and December 1972. Most of the strains were isolated from sites allied to the lower intestine and genito-urinary tract. Resistance to tetracycline was present in 39.2% of strains tested. In contrast only one strain was found resistant to clindamycin. It is suggested that the recovery of anaerobes from material sent to the laboratory depends largely on the efficiency of the anaerobic system used.

Bacteroides are a group of Gram-negative, non-sporing anaerobic bacilli (Cowan and Steel, 1965). Gillespie and Guy (1956), in a review of the earlier literature, showed that they are the predominating organism in the lower intestine and had been isolated from appendicitis, postpartum infections, septicaemia, lung abscesses, and abdominal infections. Tillotson and Lerner (1968), reviewing mainly the American literature, pointed out the increased awareness that tonsillar cysts, carious teeth, the intestines, and vagina meet the fastidious anaerobic requirements of bacteroides and that this is corroborated by the isolation of bacteroides from sinusitis, otitis media, meningitis, bacteraemia, endometritis, pelvic thrombophlebitis, intraabdominal abscesses, and empyema. Rotherman and Schick (1970) stated that *B. fragilis* was the most common anaerobic Gram-negative bacillus isolated in cases of septic abortion and suggest that this finding establishes the role of these organisms as major pathogens. In a study of the antibiotic sensitivity of *B. fragilis*, Ingham, Selkon, Codd, and Hale (1968) isolated five out of 55 representative strains from abscesses. Felner and Dowell (1970) reported the isolation of *B. fragilis* from nine patients suffering from anaerobic bacterial endocarditis, and Mitchell and Simpson (1973) reported bacteroides septicaemia in five patients following abdominal surgery.

This study was carried out between January 1971 and December 1972, the object being to determine the frequency of isolation of bacteroides from routine clinical specimens received in this laboratory.

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Material and Methods

Specimens from the upper respiratory tract were omitted, as were specimens of urine (except for a small selected series). No special precautions were taken to preserve anaerobic organisms during transit of specimens of pus to the laboratory. Vaginal swabs were normally delivered to the laboratory in Stuart's transport medium (Oxoid) and this was found to preserve bacteroides satisfactorily. From mid 1972 this medium has been used to preserve specimens of pus collected during the night when the laboratory is closed.

No attempt was made to isolate bacteroides on primary culture routinely. All specimens were incubated overnight in Robertson's cooked meat broth. These were subcultured onto either (a) blood agar containing 100 µg/ml neomycin, which will also permit the growth of *Cl. welchii*, *Str. faecalis*, anaerobic streptococci, and actinomyces sp, and/or (b) blood agar containing 100 µg/ml neomycin and 10 µg/ml vancomycin which normally inhibits all Gram-positive bacteria. The plates were then incubated anaerobically overnight, using the Gas-Pak system (BBL) which contains a mixture of hydrogen + 5% carbon dioxide.

BLOOD CULTURES

In this laboratory the routine medium used is tryptose phosphate broth (50 ml), to which 5-10 ml blood is added into a rubber sealed bottle. Bacteroides can be isolated from this medium after four to five days' incubation.

BIOCHEMICAL TESTS

One per cent peptone water sugars containing glucose, lactose, sucrose, mannitol, and maltose in bijoux bottles with loose fitting caps were incubated anaerobically for 48 to 72 hours.

ADDITIONAL TESTS

Growth on bile salt medium using MacConkey agar no. 2 (Oxoid) and the effect of haematin on growth using blood agar base no. 2 (Oxoid) and X factor discs (Oxoid) were also estimated.

ANTIBIOTIC SENSITIVITY TESTS

These were carried out by the disc diffusion method (Multidisk, Oxoid) on blood agar plates which were incubated overnight anaerobically. The following antibiotics were tested: penicillin 1.5 µg, streptomycin 10 µg, tetracycline 10 µg, methicillin 10 µg, gentamicin 10 µg, neomycin 10 µg, fusidic acid 10 µg, clindamycin 2 µg, erythromycin 2 µg, rifampicin 30 µg, chloramphenicol 50 µg, carbenicillin 100 µg, cephalothin 30 µg, cephalixin 30 µg, cephaloridine 30 µg, and colistin 10 µg.

OTHER ANTIBACTERIAL AGENTS

Filter paper discs impregnated with 1% solutions of Dettol and Savlon were prepared and stored at 4°C, as were discs containing 10 µg metrodiazole (Flagyl).

Results

In this study, the isolation of Gram-negative, anaerobic bacilli was sufficient for them to be named as *Bacteroides* species. Further detailed attempts at identification were initially based on biochemical activity and subsequently on the susceptibility to various antibiotics.

BIOCHEMICAL TESTS

The first 42 strains isolated were examined in some detail. All grew in 1% peptone water sugars anaerobically and acid was produced from glucose, lactose, maltose, and sucrose. Mannitol was not attacked nor was indole produced. No haemolysis or pigment was observed in blood agar. All strains grew on MacConkey agar. Growth was poor on blood agar base no. 2 (Oxoid), but was markedly improved by the addition of haematin (X factor discs, Oxoid). Morphologically these isolates were small Gram-negative coccobacilli. Such strains were resistant to penicillin, neomycin, cloxacillin, and gentamicin and sensitive to clindamycin and erythromycin. These strains and subsequent isolates with the same antibiotic resistance pattern were considered to be *Bacteroides fragilis*. Strains which

deviated from this antibiotic resistance pattern were examined in more detail. Nine such strains were isolated and produced larger colonies on blood agar than did *B. fragilis* and microscopically appeared as large Gram-negative pleomorphic bacilli with numerous filaments. In addition to being sensitive to penicillin, these isolates were all uniformly resistant to fusidic acid and rifampicin (to which *B. fragilis* is normally sensitive) and sensitive to colistin to which *B. fragilis* is resistant. These strains were identified as *Sphaerophorus* species.

SOURCE OF STRAINS

Table I shows the source of strains isolated during the two-year period. The sites from which anaerobic Gram-negative bacilli were isolated from pus are shown in table II.

Source	No. of Specimens Examined	No. of Specimens Containing Gram-negative Anaerobic Bacilli
Pus (all sources)	6977	256
Vaginal swabs	4414	762
Ear swabs	422	26
Urines	114	17
Cerebrospinal fluid	252	1
Blood cultures	612	5
		1067

Table I Sources from which *Bacteroides* were isolated

Site	No. of Isolations
Lower abdominal wounds	109
Appendicectomies	80
Ischio-rectal abscesses	11
Bed sores	24
Varicose ulcers	1
Amputation wounds	6
Pilonidal sinus	6
Chest-wall sinus	2
Leg sinus	2
Recto-vaginal sinus	1
Abdominal abscess	3
Pelvic abscess	1
Perianal abscess	4
Thigh sinus	1
Groin abscess	1
Cheek abscess	1
Pleural cavity	1
Scrotal abscess	1
Buttock abscess	1
	256

Table II Sources of pus from which *Bacteroides* were isolated

Seven hundred and fifty-nine isolations of *bacteroides* and three isolations of *sphaerophorus* were made from 4414 vaginal swabs. Routine antenatal swabs produced 251 positive cultures and 307 were isolated from postnatal patients who were pyrexial and/or had a foul-smelling lochia. The majority of these cultures were obtained from women who had

either an induced rupture of the membranes or a Caesarian section. The remaining 204 isolates came from women suffering from 'vaginitis'.

Bacteroides were isolated on 26 occasions from 422 patients with infected ears. Eighteen gave a history of chronic suppurative otitis media. In many instances the production of 'foul-smelling pus' was noted. One patient who had suffered a head injury subsequently developed meningitis, and *Proteus* and *B. fragilis* were isolated from cerebrospinal fluid.

One hundred and fourteen midstream urine specimens, 77 from females, were examined for the presence of bacteroides. Seventeen strains were isolated from 16 female patients; no bacteroides were isolated from males. In seven instances there were a significant number of aerobic organisms (more than 100 000/ml), but in the remaining 10 isolations were made in the absence of a significant number of aerobic bacteria, and in six cases the centrifuged urine had less than five leucocytes present per high-power field. In this series of urines, 21 specimens were from antenatal patients. Vaginal swabs from the patients were examined for bacteroides on the same day as the urine specimens. Three vaginal swabs produced positive cultures and two urines were also positive. In only one instance was the urine and the vaginal swab from the same patient positive. Both urines had neither leucocytes nor a significant number of aerobic bacteria.

Anaerobic Gram-negative bacilli were isolated from five out of 612 blood cultures examined. In each instance septicaemia followed abdominal surgery in patients over 65 years of age. In each case

growth of anaerobic bacteria was only detected after four to five days' incubation.

ANTIBIOTIC SENSITIVITY

Table III shows the results of testing a number of strains of anaerobic Gram-negative bacilli against various antimicrobial drugs.

Dettol and Savlon were tested as an example of antiseptics which are commonly used in obstetrics and gynaecology. Flagyl is generally used in the treatment of trichomonal infections but has also been shown to be bactericidal to bacteroides (Nastro and Finegold, 1972).

Discussion

As a result of being part of the normal bacteriological flora of the lower intestine, it may often be difficult to assess if and when bacteroides are acting as commensals or potential pathogens. If they are isolated in pure culture and the patient responds clinically to specific chemotherapy, their pathogenic role in these instances is proven. However, the technical difficulties involved in isolating bacteroides may be considerable and time consuming. As a result, their presence may be missed and only the more easily isolated aerobic bacteria will be reported. In the five instances where bacteroides were isolated from blood cultures their presence was not detected until the culture bottles had been incubated for four or five days (Mitchell and Simpson, 1973). Therefore, the recommendation by Gunn (1957) that if bacteroides infection is suspected treatment should be commenced before the bacteriological report is available seems to be justifiable.

A feature of anaerobic culture work which is not always appreciated is that it takes several hours for an anaerobic jar, especially a metal one, to heat up to 37°C. This means that if a jar which has been kept at room temperature all day is incubated late in the afternoon, and then re-opened first thing the next morning, the contents of the jar may have been at 37°C for less than 12 hours. This might be satisfactory for rapidly growing organisms like *Cl. welchii* but it is probably not long enough for bacteroides. The habit of storing empty anaerobic jars in the incubator when not in use is recommended, as the temperature lag is reduced.

Gillespie and Guy (1957) reported that all the strains they tested were sensitive to tetracycline. Bodner, Koenig, Treanor, and Goodman (1972) found that only 40% of strains of *B. fragilis* were sensitive to tetracycline. In this series, 60.8% were found to be sensitive. This increase in tetracycline resistance since 1957 is unfortunate as it reduces the number

Antibiotic	No. Strains Tested	No. Strains Sensitive
Penicillin	1067	9 ¹
Streptomycin	1067	Nil
Gentamicin	1067	Nil
Neomycin	1067	Nil
Methicillin	1067	Nil
Tetracycline	1067	651
Fusidic acid	794	779 ¹
Clindamycin	1067	1066
Erythromycin	275	272
Rifampicin	63	54 ¹
Chloramphenicol	25	25
Carbenicillin	44	41
Cephaloridine	6	Nil
Cephalothin	6	Nil
Cephalexin	6	Nil
Colistin	46	9 ¹
1% Dettol	25	25
1% Savlon	25	25
10 µg Flagyl	25	25

Table III The susceptibility of *Bacteroides* to various antimicrobial agents

¹The nine strains sensitive to penicillin were also sensitive to colistin but resistant to fusidic acid and rifampicin and were regarded as being *Sphaerophorus* sp.

of broad-spectrum antibiotics which can be used to treat both coliform and bacteroides infections where there is doubt as to which organism is responsible for the septic condition. Chloramphenicol would seldom be used because of its dangerous side effects, and at present there is no information as to the effectiveness *in vivo* of carbenicillin against bacteroides.

Ingham, Selkon, Codd, and Hale (1970) showed that 0.3 µg-1.25 µg clindamycin was bactericidal to strains of *B. fragilis*. The high sensitivity of bacteroides to clindamycin and to a slightly lesser extent, the parent compound, lincomycin, has been confirmed by Kislak (1972), Bodner *et al* (1972), and Martin, Gardner, and Washington (1972). In this series only one of 1067 strains tested was found to be resistant to clindamycin. Geddes, Munro, Murdoch, Begg, and Burns (1967) and Tracy, Gordon, Moran, Love, and McKenzie (1972) have reported the successful use of lincomycin in treating bacteroides infections, and Mitchell and Simpson (1973) have reported on the use of clindamycin in treating bacteroides septicaemia.

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