THE ANAEROBIC CULTURE OF CLINICAL SPECIMENS: A 14-MONTH STUDY

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THE isolation rate of anaerobic bacteria from clinical specimens increased manyfold in our laboratory when the techniques for anaerobic bacteriology were revised. We present here an account of our findings with these methods applied as a routine to clinical specimens, and make recommendations concerning the techniques of incubation, media and the use of certain identification tests. The isolation of anaerobic species from different anatomical sites is described, and special attention is given to the yield of organisms from empyema fluid, the peritoneal cavity, surface lesions and blood.

MATERIALS AND METHODS

With the general exception of urines, faeces, respiratory specimens and cerebrospinal fluid (CSF), specimens of exudates received between 1 Dec. 1974 and 31 Jan. 1976 were cultured for anaerobic bacteria as described below.

Transport of specimens. Samples of pus were transported to the laboratory in sterile McCartney bottles. Swabs of purulent material were sometimes transported deep in a tube of Cary and Blair transport medium (Cary and Blair, 1964), but many swabs were received in the ordinary dry state.

Macroscopic examination. Specimens were examined for colour, odour, consistency and the presence of sulphur granules. Liquid pus samples were examined for brick-red fluorescence under an ultraviolet light (Blak-Ray UVL 21, Ultraviolet Products Inc., San Gabriel, California) at a distance of 6–9 in. (15–23 cm) for presumptive evidence of the presence of *Bacteriodes melaninogenicus*.

Microscopic examination. Samples of pus were examined by gram stain to ascertain the number and proportion of morphological forms present.

Culture of clinical material. Our complete range of media used for culture in this study were as follows:

1. *Blood agar:* Columbia Agar Base (London Analytical and Bacteriological Media) with 5% defibrinated horse-blood; this was obtained as poured plates from London Analytical and Bacteriological Media Ltd, Salford, Lancs. (LAB M).
2. *Supplemented brucella agar:* Brucella Agar (Difco Laboratories Ltd, West Molesey, Surrey) with 5% defibrinated horse blood and menadione 0.5 µg per ml (Sigma Chemical Co. Ltd, Kingston-upon-Thames, Surrey).
3. *Supplemented selective brucella agar:* supplemented brucella agar rendered selective by adding kanamycin 75 µg per ml (Kantrex solution, Bristol Laboratories, Langley, Bucks).
4. *MacConkey's agar:* obtained as poured plates from LAB M.

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5. *Egg-yolk agar*: prepared by adding 2 ml of egg-yolk emulsion (Oxoid Ltd, Basingstoke, Hants.) to 20 ml of Nutrient Agar (Southern Group Laboratories, Lewisham, London).
6. *Cooked Meat Medium*: obtained as a complete medium from Southern Group Laboratories (catalogue no. 0530).
7. *Glucose cooked-meat medium*: glucose added to cooked meat medium to a final concentration of 1%.
8. *Brewer's Thioglycollate Medium*: obtained as the complete medium from Southern Group Laboratories (catalogue no. 0586 C).
9. *Supplemented thioglycollate medium*: Brewer's thioglycollate medium supplemented with haemin (BDH Ltd, Poole, Dorset) 5 µg per ml, menadione (Sigma) 0.5 µg per ml and sodium bicarbonate (Hopkin and Williams, Romford, Essex) 1 mg per ml.
10. *Supplemented Tween thioglycollate medium*: supplemented thioglycollate with Tween 80 (Sigma) at a final concentration of 0.1% (v/v).
11. *Supplemented brucella broth*: Brucella Broth (Difco) supplemented with haemin (BDH) to a final concentration of 5 µg per ml.
12. *Carbohydrate basal medium*: for fermentation studies, prepared according to the formula described by Holdeman and Moore (1972).
13. *Supplemented carbohydrate basal medium*: carbohydrate basal medium supplemented with Tween 80 (Sigma) to a final concentration of 0.1% (v/v).
14. *Glucose Broth*: obtained as a complete medium from Southern Group Laboratories (catalogue no. 0505 B).

Specimens were cultured aerobically on blood agar (medium 1, above) and on MacConkey's agar (medium 4). Anaerobic culture was made on blood agar and on supplemented selective brucella agar (medium 3).

Samples of pus from sites normally considered to be sterile and material aspirated from abscesses were also inoculated into cooked-meat medium (medium 6) which had been pre-steamed for 30 min. with the caps loose to remove dissolved oxygen and cooled with caps tight. Inoculation was made through the rubber diaphragm of the bottle with a syringe and needle. Swabs from wounds, abscesses and deep infections were also incubated in this medium. They were inserted into the bottom of the bottle with as little aeration as possible. Anaerobic plates were incubated in steel anaerobic jars (Stuart Scientific Co., Croydon, Surrey). All plates were examined after overnight incubation and again at 48 h. Anaerobic plates were then reincubated for a further 3 days of uninterrupted incubation and read again finally on the 5th day. Some preselected specimens were inoculated on to an additional set of anaerobic plates and incubated for 48 h before the initial examination. Cooked-meat cultures were subcultured after incubation for 48 h on to blood agar and MacConkey's agar, which was incubated aerobically, and on to the supplemented selective brucella agar, which was incubated anaerobically for 3 days without interruption. These subcultures were read at the same time as the original 5-day plates.

Anaerobic jar procedure. Anaerobic jars were set up in the manner recommended by Watt and Collee (1974) with a gas mixture of 90% hydrogen and 10% carbon dioxide. Jars were fitted with the modified catalyst sachets previously described by one of us (Baldwin, 1975) containing 8.5 g of "cold catalyst D" (Engelhard Industries, Cinderford, Gloucs.). The catalyst was changed and reactivated at weekly intervals by spreading in an enamel tray and heating to 160°C for 2 h.

Blood culture. Five-ml volumes of blood were inoculated each into 50 ml of glucose broth (medium 14) and 80 ml of Brewer's thioglycollate (medium 8). The bottles were sealed tightly by a screw cap with a liner and incubated in air. These media were subcultured on to three plates of blood agar (for incubation in air, air with 10% carbon dioxide, and anaerobically), and on to a MacConkey's agar plate. Subcultures were made at 24 h, 48 h, 7 days and finally at 14 days or when turbidity was noticed on daily inspection of the cultures. Plates were incubated for 4 days before being discarded.

Sensitivity testing (disk tests). A colony of the anaerobic isolate was spread over the surface of a plate of supplemented brucella agar (medium 2). Disks of penicillin (2 units),

clindamycin (2 µg), minocycline (10 µg) and metronidazole (5 µg) were placed firmly on the agar. Sensitivity or resistance was determined by comparing diameters of zones of inhibition of clinical isolates with those of known sensitive control anaerobic organisms which were plated out and tested daily. An organism giving a zone 5 mm less than that obtained with the control organism was recorded as resistant. When organisms showed resistance to clindamycin the surface pH of the sensitivity plate was determined with pH indicator papers (Watt and Brown, 1975). The test was repeated if a pH of 6.0 or less was found.

Determination of MICs of antibiotics. These tests were performed by making doubling dilutions of antibiotic in supplemented brucella broth (medium 11). The inoculum used was one drop of a 1 in 10 dilution in this medium of an overnight culture in supplemented thioglycollate medium (medium 9).

Confirmation of anaerobic isolates. A colony of the isolate was subcultured to three plates of supplemented brucella agar. Two plates incubated anaerobically provided a purity check and a plate for sensitivity testing. The third plate was incubated in air plus 10% carbon dioxide. Only obligate anaerobes were identified.

Culture of confirmed anaerobic isolates. Gram-positive organisms were inoculated into 50 ml of supplemented Tween thioglycollate medium (medium 10). Gram-negative cocci and gram-negative bacilli were inoculated into 50 ml of supplemented thioglycollate medium, and the latter type of organism was also inoculated into 20 ml of glucose cooked-meat medium (medium 7). These media were presteamed and cooled before use.

The inoculated media were tightly sealed and incubated in air until visible growth occurred. The cultures were then used for identification tests. Viability and purity checks of these cultures were made by subculture on to two supplemented brucella agar plates. One plate was incubated anaerobically and the other in air plus 10% carbon dioxide.

Identification of anaerobic isolates. The procedures recommended by Sutter, Vargo and Finegold (1975) were followed. Identification tests used were as follows: (1) gram stain, to check morphology; (2) antibiogram patterns for gram-negative bacilli (Sutter *et al.*, 1975) to differentiate *Bacteroides* and *Fusobacterium* species; (3) inoculation of egg-yolk-agar plate (medium 5) to indicate production of lipase or lecithinase, and for subsequent tests for catalase; (4) biochemical tests as indicated after determination of microscopical and antibiogram characteristics.

The media used for biochemical tests, and the interpretation of the results, were made according to the Anaerobe Manual of the Virginia Polytechnic Institute and State University (Holdeman and Moore, 1972). Biochemical investigations of gram-negative organisms were carried out in carbohydrate basal medium (medium 12) and of gram-positive organisms in

TABLE I
Specimens cultured

Nature or source of specimen	Number of specimens yielding anaerobes	Number of specimens yielding anaerobes only	Number of specimens yielding a pure culture of an anaerobe	Total number of anaerobic isolates
Infected abdominal wound and peritonitis	230	80	23	403
Abdominal abscess	76	48	6	173
Thoracic empyema pus	10	4	2	21
Infected superficial wound	180	41	31	323
Blood culture	29	29	27	31
High vaginal swab	241	89	29	441
All	766	291	118	1392

TABLE II
Distribution of genera

Genus or group	Number of strains	Percentage of total
<i>Bacteroides</i>	739	53.0
<i>Fusobacterium</i>	39	2.8
<i>Peptostreptococcus</i>	194	14.0
<i>Peptococcus</i>	236	16.9
<i>Clostridium</i>	62	4.5
Non-spore-forming gram-positive bacilli	87	6.3
<i>Veillonella</i>	31	2.2
Other genera	4	0.3
All	1392	100.0

supplemented carbohydrate basal medium (medium 13). Charcoal gelatin disks (Oxoid Ltd) were added to the supplemented carbohydrate basal medium to detect gelatin liquefaction.

Nomenclature of isolates. Organisms that failed to fit into any recognised sugar-fermentation pattern or failed to grow in the medium used for biochemical tests (9 strains) were given a generic title only, as *Bacteroides* sp., *Peptococcus* sp., etc. We attempted speciation of all anaerobic isolates except the non-spore-forming gram-positive bacilli which require gas-liquid chromatography to place them into a genus before identification can be made. *Peptococcus magnus* and *Pc. variabilis* were not distinguished (Holdeman, 1974). Their differentiation rests on differences in gelatin liquefaction which are lost in media containing Tween 80. All biochemically inactive peptococci that liquefied gelatin in the presence of Tween 80 were named *Pc. magnus*. Non-fermenting peptococci that did not liquefy gelatin were named *Pc. prevotii*.

RESULTS

Sources and species of anaerobes isolated

A total of 1392 strains of anaerobic bacteria from clinical specimens were cultured over a period of 14 months. Table I gives details of the specimens and gives the numbers of strains isolated from each type of specimen. Of 766 specimens yielding anaerobes, 409 (53.4%) gave a pure culture of a single anaerobe or a mixed culture of anaerobes only.

Strains belonging to the genus *Bacteroides* comprised 53.0% of the total strains isolated. The anaerobic gram-positive cocci formed about one-third of the total, whilst only 4.5% of the anaerobes recovered were spore-bearers. Table II lists the distribution of the anaerobic genera isolated. *B. fragilis* was the most frequent single species, accounting for 32.3% of all anaerobes.

Pc. magnus was the most frequently isolated gram-positive coccus, accounting for 20% of gram-positive cocci and 6.1% of the total anaerobes isolated. This organism was second only to *B. fragilis* in the frequency with which it was found in pure culture. Moore, Cato and Holdeman (1974) list this organism as the most common of the peptococci.

TABLE III
Details of the anaerobic species isolated and their sources

Genus and species	Number of isolates of the stated genus* and species obtained from				
	abdominal wounds	abdominal abscesses	peritoneal exudates	superficial infections	high vaginal swabs
Bacteroides	207	103	34	154	219
<i>B. fragilis</i>	137 (16)†	61 (4)	22 (2)	79 (8)	137 (11)
<i>B. melaninogenicus</i>	46	28	9	47	37
<i>B. corrodens</i>	7	0	1	11	6
<i>B. sp.</i>	17	14	2	17	39 (3)
Fusobacterium	9	5	2	5	17
<i>F. nucleatum</i>	2	1	0	3	1
<i>F. necrophorum</i>	2	0	0	1	2
<i>F. mortiferum</i>	0	1	2	0	0
<i>F. sp.</i>	5	3	0	1 (1)	14 (1)
Peptostreptococcus	37	17	5	40	91
<i>Pst. anaerobius</i>	3	6	1	7 (1)	27
<i>Pst. intermedius</i>	6	3	3	5 (3)	21 (2)
<i>Pst. micros</i>	4	1	1	5	10 (1)
<i>Pst. parvulus</i>	2	2	0	0	3
<i>Pst. productus</i>	3	2	0	2	4 (1)
<i>Pst. sp.</i>	19	3	0	21	26 (1)
Peptococcus	52	23	5	87	61
<i>Pc. asaccharolyticus</i>	6 (1)	2	1	15 (5)	9
<i>Pc. constellatus</i>	7	2	0	4	7 (1)
<i>Pc. magnus</i>	17 (1)	11 (2)	3	36 (7)	17 (4)
<i>Pc. morbillorum</i>	3	4	0	6 (1)	10 (2)
<i>Pc. prevotii</i>	9 (1)	1	0	14 (2)	7
<i>Pc. saccharolyticus</i>	1	0	0	2	1 (1)
<i>Pc. sp.</i>	9	3	1	10 (1)	10
Clostridium	17	4	7	14	18
<i>C. perfringens</i>	17 (1)	2	7	12	17
<i>C. ramosum</i>	0	0	0	0	1
<i>C. septicum</i>	0	1	0	0	0
<i>C. sphenoides</i>	0	1	0	0	0
<i>C. sp.</i>	0	0	0	2 (1)	0
NSGPB†	12	15	10	12 (1)	27 (2)
Veillonella	3	5	1	7	6
<i>V. parvula</i>	1	5	1	7	6
<i>V. alcalescens</i>	2	0	0	0	0
<i>Propionibacterium acnes</i>	1	0	0	4	0
<i>Megasphaera elsdenii</i>	0	0	0	0	0
<i>Sarcina ventriculi</i>	1	1	0	0	2
Totals	339 (20)	173 (6)	64 (2)	323 (31)	441 (30)

* The total for each group or genus is given in bold type.

† Figures in parentheses indicate the number of strains obtained in pure culture.

† NSGPB = non-sporing gram-positive bacilli.

TABLE IV
Bacteria cultured from ten patients with pulmonary sepsis

Material cultured	Facultative flora	Anaerobic flora
(1) Empyema pus	<i>Klebsiella aerogenes</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> Group-C streptococcus	<i>Bacteroides</i> sp.
(2) Empyema pus	Group-F streptococcus <i>Streptococcus viridans</i>	<i>Bacteroides</i> sp.
(3) Empyema pus	<i>Strept. pneumoniae</i> <i>Strept. viridans</i>	<i>B. corrodens</i> <i>B. melaninogenicus</i>
(4) Empyema pus	No growth	<i>Pst. anaerobius</i>
(5) Empyema pus	Microaerophilic streptococcus <i>Strept. viridans</i>	<i>B. melaninogenicus</i> <i>Bacteroides</i> sp. NSGPB* <i>Pc. magnus</i> <i>Pc. prevotii</i> <i>Pc. morbillorum</i>
(6) Empyema pus	No growth	<i>Bacteroides</i> sp. <i>Peptostreptococcus</i> sp. NSGPB*
(7) Empyema pus	No growth	<i>B. fragilis</i>
(8) Empyema pus	<i>Escherichia coli</i>	<i>Pr. acnes</i>
(9) Empyema pus	Microaerophilic streptococcus	<i>B. fragilis</i> <i>B. melaninogenicus</i> <i>Bacteroides</i> sp. Anaerobic coccus
(10) Bronchiectasis pus	No growth	<i>B. fragilis</i>

* Non-spore-forming gram-positive bacilli.

Of the 1392 anaerobes isolated, 339 originated in abdominal wounds and 173 originated in 76 various abscesses. All abscesses yielded an anaerobic flora, but many (71%) did not yield aerobes. All cases of peritonitis yielded a predominantly anaerobic flora. A surprising number of specimens taken from superficial infections yielded anaerobic bacteria. In some superficial infections the culture probably reflected skin flora, but forms typical of the isolates in these cultures were seen in direct films of the relevant specimens and this was presumptive evidence of their pathogenic role.

Anaerobic gram-negative bacilli were the predominant species (62.3%) in exudates from abdominal wounds, abscesses and peritonitis, whilst gram-positive cocci accounted for 139 (24.1%) of the 576 strains isolated from these sources. Among a total of 323 strains from superficial wounds, 127 (39.3%) isolates were gram-positive cocci and 159 (49.2%) were gram-negative bacilli. *Peptococcus* spp. accounted for 87 (26.9%) of the 323 strains isolated from

TABLE V
*Anaerobic bacteria cultured from 29 blood cultures**

Organism culture from blood	Presumptive portal of entry
<i>C. perfringens</i>	Infected wound
<i>C. septicum</i>	Liver abscess
<i>B. fragilis</i>	Post-operative; carcinoma of colon
<i>B. fragilis</i>	Pelvic abscess
<i>B. fragilis</i>	Appendicectomy
<i>B. fragilis</i>	Appendicectomy
<i>B. fragilis</i>	Appendicectomy
<i>B. fragilis</i>	Hysterectomy
<i>B. fragilis</i>	Pressure sore
<i>B. fragilis</i>	Vulvectomy
<i>B. fragilis</i> + <i>Peptostreptococcus</i> sp.	Prostatectomy
<i>B. fragilis</i> + <i>F. necrophorum</i>	Appendicectomy
<i>Pc. magnus</i>	Gangrene of toes
<i>Peptococcus</i> sp.	Endometritis
<i>Peptostreptococcus</i> sp.	Infected ulcer
NSGPB†	Appendicectomy

* Nine other blood cultures grew *Pr. acnes*, three yielded non-sporing gram-positive bacilli and one yielded *Pc. constellatus* (from a case of osteomyelitis).

† Non-sporing gram-positive bacillus.

superficial wounds, whereas they accounted for only 52 (15.3%) of the 339 strains isolated from abdominal wounds. As was expected, anaerobic bacteria were isolated frequently from high vaginal swabs. Table III lists the anaerobic species isolated from specimens received during the study period.

A total of 21 anaerobes were recovered from the nine cases of empyema pus and one specimen of material from a bronchiectatic cavity received during the study period (table IV).

Thirty-one strains of anaerobic bacteria were cultured from 29 blood cultures (see *Methods*). The upper section of table V lists 18 isolates obtained both from blood culture and the presumed portal of entry (i.e., a body site from which the same strain was grown). Not all of these positive cultures were regarded as clinically significant. The lower section of table V lists 13 isolates that were probably contaminants. Nine of these were *Propionibacterium acnes* which frequently colonises the skin surface. In all, the 31 isolates formed 23% of potentially significant blood cultures obtained during the period of study.

Culture and identification techniques

It is estimated that cultures would not have yielded the majority of strains of non-sporing gram-positive bacilli, two-thirds of *B. melaninogenicus* and about one-third of anaerobic cocci had incubation not been continued for 5 days (table VI). Incubation periods of 5 days were often required to observe pitting of agar and formation of pigment.

The results of reading anaerobic plates at 24 h compared with continuous

TABLE VI

The influence of the time of first examination on the range of anaerobes isolated from a selected series of exudates

Specimen	Organisms cultured with first examination at 24 h		Organisms cultured with first examination at 48 h	
	24-h reading	5-day reading	48-h reading	5-day reading
Pelvic abscess pus	<i>Peptococcus</i> sp.	<i>Peptococcus</i> sp. <i>Peptostreptococcus</i> sp.	<i>Peptococcus</i> sp. <i>Peptostreptococcus</i> sp. NSGPB*	<i>Peptococcus</i> sp. <i>Peptostreptococcus</i> sp. NSGPB
Pus from peritonitis	<i>B. fragilis</i>	<i>B. fragilis</i> Anaerobic coccus <i>Pc. asaccharolyticus</i>	<i>B. fragilis</i> Anaerobic coccus <i>Pc. asaccharolyticus</i>	<i>B. fragilis</i> Anaerobic coccus <i>Pc. asaccharolyticus</i> <i>V. parvula</i>
Pus from appendix abscess	<i>B. fragilis</i>	<i>B. fragilis</i> <i>Peptococcus</i> sp.	<i>B. fragilis</i> <i>Bacteroides</i> sp. <i>Peptococcus</i> sp.	<i>B. fragilis</i> <i>Bacteroides</i> sp. <i>Peptococcus</i> sp. NSGPB
Pus from breast abscess	<i>B. fragilis</i> <i>Peptococcus</i> sp.	<i>B. fragilis</i> <i>Peptococcus</i> sp.	<i>B. fragilis</i> <i>Peptococcus</i> sp. NSGPB	<i>B. fragilis</i> <i>Peptococcus</i> sp. NSGPB
Pus from perineal abscess	None	None	<i>Fusobacterium</i> sp. NSGPB	<i>Fusobacterium</i> sp. NSGPB
Pus from pelvic abscess	<i>B. fragilis</i>	<i>B. fragilis</i>	<i>B. fragilis</i> <i>Bacteroides</i> sp. NSGPB Anaerobic coccus	<i>B. fragilis</i> <i>Bacteroides</i> sp. NSGPB Anaerobic coccus <i>V. parvula</i>
Pus from thoracic empyema	Anaerobic coccus	Anaerobic coccus	Anaerobic coccus <i>B. melaninogenicus</i> <i>Bacteroides</i> sp.	Anaerobic coccus <i>B. melaninogenicus</i> <i>Bacteroides</i> sp. NSGPB
Bronchiectasis pus	<i>Peptostreptococcus</i> sp.	<i>Peptostreptococcus</i> sp.	<i>Peptostreptococcus</i> sp. <i>Bacteroides</i> sp.	<i>Peptostreptococcus</i> sp. <i>Bacteroides</i> sp. NSGPB

* NSGPB = non-spore-forming gram-positive bacillus.

incubation for 48 h are also given in table VI. Colonies on plates incubated for 48 h before being read were larger and easier to work from. Some strains were found on the 48-h plates that never appeared, even after 5 days, on plates that had been inoculated at the same time but read at 24 h. Exposure to air at this early time apparently prevented the growth of these strains. Furthermore, the numbers of strains recovered from plates read at 48 h and 5 days were always greater than from plates read at 24 h and 5 days.

Nearly all isolates were obtained from primary plates, very few being obtained for the first time from the cooked-meat medium. Supplemented Tween thioglycollate broth gave good growth of gram-positive isolates. Most

gram-negative bacilli grew equally well in supplemented thioglycollate broth and in glucose cooked-meat medium, but there were a number of strains that grew better in the latter medium.

Positive results for indole production were obtained with Ehrlich's reagent, but not with Kovacs' reagent, with a quality-control strain of *Clostridium tetani*, two strains of *Fusobacterium* and two strains of *Pc. asaccharolyticus*. The suspending fluid from charcoal-gelatin disks was inhibitory to some organisms, consequently care had to be taken to ensure that disks were completely drained before use. The antibiograms described by Sutter *et al.* (1975) were reproducible and gave good differentiation of the gram-negative bacilli. The medium recommended by Holdeman and Moore (1972) for biochemical tests permitted the satisfactory growth of all but nine strains of anaerobes.

Sensitivity to chemotherapeutic agents

Four chemotherapeutic agents were routinely tested for inhibitory activity against each isolate. They were benzyl penicillin, clindamycin, minocycline and metronidazole. Other agents were not routinely examined. All of the 1392 anaerobic strains isolated were sensitive to metronidazole. One strain of *B. fragilis* was resistant to clindamycin. All other anaerobic isolates were sensitive to this antibiotic. Of 446 isolates of *B. fragilis*, four were sensitive to benzyl penicillin. The three isolates of *F. mortiferum* and one of 167 isolates of *B. melaninogenicus* were resistant to this antibiotic. Each of the strains of the other anaerobic species were sensitive to benzyl penicillin. Approximately 40% of strains were resistant to minocycline. Apparent resistance of *B. fragilis* to clindamycin was found occasionally. In these instances a reduction in surface pH of the medium sometimes occurred during growth of the organism (Watt and Brown, 1975). All but one of these strains were found to be sensitive when the test was repeated.

The MIC of clindamycin was determined for each strain of *B. fragilis* found to be resistant in the initial disk-sensitivity test. MICs of less than 2 µg per ml were found for each strain except one. The MIC of clindamycin to this resistant strain of *B. fragilis* was 32 µg per ml.

DISCUSSION

The present study covered a 14-month period in which a total of 1392 strains of anaerobic bacteria were cultured from clinical specimens. Sources of clinical material were pus samples from the head, neck, chest, abdomen and the extremities. Anaerobes were also cultured from blood and from superficial infections such as paronychia and bites, as well as from ulcers and pressure sores. We found material from different sites to differ in their content and average yield of anaerobic micro-organisms. Many specimens from abdominal abscesses, peritonitis, and cases of thoracic empyema contained a predominantly anaerobic flora, and we consider it essential that laboratories examine such specimens thoroughly for anaerobic bacteria.

Gorbach, Thadepalli and Norsen (1974) demonstrated the presence of

anaerobic bacteria in specimens of exudate from 40 of 46 patients with abdominal sepsis, *B. fragilis* being found most frequently. In our study, pus from 76 abscesses in the abdominal region yielded a total of 173 anaerobes and only 58 facultative bacteria (a ratio of 3:1). It is important to detect anaerobic bacteria in these situations since six of these patients developed positive blood cultures with anaerobes.

A series of studies by Bartlett and his colleagues has shown the importance of anaerobes in lung infections. They found anaerobic bacteria in 87% of cases of aspiration pneumonia (Bartlett, Gorbach and Finegold, 1974), 92% of cases of lung abscess (Bartlett, Gorbach, Tally and Finegold, 1974), and in 76% of cases of empyema (Bartlett, Gorbach, Thadepalli and Finegold, 1974). We examined pus from nine cases of empyema and one case of bronchiectasis, and found anaerobic bacteria in all of them. Strains belonging to the genus *Bacteroides* were predominant, and anaerobic isolates outnumbered facultative bacteria.

Surface infections were also a frequent source of anaerobes, and a pressure sore was an assumed portal of entry for one of the positive blood cultures. Stokes (1958) observed that sebaceous cysts were a rich source of anaerobic bacteria. We also found this, and isolated a total of 16 strains of anaerobic bacteria together with only five strains of aerobic bacteria from eight infected sebaceous cysts.

Studies by Rotheram (1974) indicated that bacteria in exudates taken from the os cervicis in septic abortion were predominantly anaerobic. Similarly, Swenson (1974) showed the predominance of anaerobic bacteria in infections of the female genital tract. We found a considerable number of high vaginal swabs to yield anaerobes either in abundance or in a heavy pure growth. A post-hysterectomy high vaginal swab yielded an anaerobic organism which was also found in the patient's blood.

This report demonstrates the large number and variety of species of anaerobes that may be recovered by improved techniques from routine specimens. However, the distinction between isolates that are commensals or contaminants and those that are true pathogens is difficult to make. Nevertheless, of the 496 specimens from abdominal sites, empyemas and superficial infections (table I), 62 yielded an anaerobe in pure culture and a further 173 specimens yielded a mixed culture of anaerobes only. Thus, in the course of 14 months a total of 235 specimens from these sites yielded anaerobes but failed to yield aerobes. Such specimens, unlike blood cultures or high vaginal swabs, were taken from sites with visible evidence of infection. It is reasonable to conclude that many of the cultures represented true anaerobic infections.

Recent studies with modern anaerobic-culture techniques have led to an increase in laboratory detection of patients with septicaemia involving anaerobic bacteria (Sonnenwirth, 1974). Anaerobic bacteria were found in 23% of positive blood cultures during the present study. An organism similar to that recovered from blood culture was found at another site in the same patient in 55% of cases. This was assumed to be the portal of entry to the blood stream. We support the findings of Shanson (1974), who found Brewer's thioglycollate

medium (Southern Group Laboratories) to be satisfactory for anaerobic blood culture. Thiol broth under vacuum and carbon dioxide is also recommended (Shanson and Barnicoat, 1975; Szawatowski, 1976).

All of the 1392 anaerobic isolates were sensitive to metronidazole and only one was resistant to clindamycin. It seems safe to assume from the results of this study that strict anaerobes are consistently sensitive to metronidazole. Benzyl penicillin was active against all but four isolates of all anaerobic species except *B. fragilis*; only 1% of the isolates of *B. fragilis* were sensitive to this antibiotic. Forty per cent of all anaerobic strains were resistant to minocycline. Other antibiotics were not tested routinely.

In our experience brucella agar gave larger colonies of anaerobic bacteria together with better development of pigment and pitting of the agar than other similar media. The use of selective media was important, especially for specimens from the abdominal region and where there was a heavy concurrent growth of coliforms. It is significant that in laboratories where a high number of anaerobes are isolated, selective media are used in the primary plating regimen (Wren, unpublished survey, 1975).

The need for an anaerobic chamber is debated by many workers. Drasar (1974) finds it essential for studies of normal flora, whereas Watt and Collee (1974) found a chamber of no advantage over conventional bench techniques for the isolation of total anaerobes from fresh faecal samples or for the recovery of exacting laboratory strains. This view is supported by the study of Rosenblatt, Fallon and Finegold (1973), who found a comparable rate of isolation of anaerobes after incubation in Gaspak jars and in an anaerobic chamber using pre-reduced, anaerobically sterilised media (provided that the specimens were transported under anaerobic conditions). Limited experience with our own anaerobic chamber seems to suggest that a chamber is unnecessary for the culture of clinical specimens for anaerobic bacteria. The results obtained by the use of the chamber were essentially the same as those obtained when anaerobic culture plates were examined first at 48 h.

We found in five instances that testing for indole production with Ehrlich's reagent was positive when a parallel test with Kovacs' reagent was negative. Extraction of indole with xylene may be more efficient and reproducible in anaerobic work than extraction with amyl alcohol. This view is supported by other workers (W. E. C. Moore, personal communication). Supplemented brucella blood agar was found to be an efficient selective primary plating medium. A second set of anaerobic plates incubated for 48 h enlarges the yield of anaerobes and we suggest that such plates should be used for important specimens. The anaerobic re-incubation of primary plates to 5 days yielded a larger number of isolates. Multiple catalyst sachets (Watt, Hoare and Collee, 1973) or an improved catalyst container (Baldwin, 1975) should be used, and the catalyst should be changed and reactivated at weekly intervals.

Anaerobes may be responsible for many types of infection (Finegold and Rosenblatt, 1973), and approximately two-thirds of specimens from appropriate sites submitted for bacteriology will contain anaerobes (Holdeman and Moore, 1972). Gorbach and Thadepalli (1974), Willis, Young and Ferguson (1974)

and a study group (Willis *et al.*, 1974) have all provided good evidence of the clinical importance of anaerobic bacteria. This paper demonstrates the variety and wide distribution of anaerobes in clinical specimens. On revision of our anaerobic techniques, the isolation rate for these bacteria increased manifold and apparently rare isolates were commonly recovered from clinical specimens.

SUMMARY

This paper presents the results of the examination of routine clinical specimens for anaerobes and gives details of the revised anaerobic culture techniques recently introduced into this laboratory. The species of anaerobic bacteria isolated from lesions in different sites of the body are described and recommendations are made concerning methods of identification and culture.

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