The isolation of anaerobic bacteria from wound swabs

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SYNOPSIS The isolation of anaerobic bacteria from routine wound swabs by three procedures was evaluated.

Recovery of anaerobic organisms was doubled by immediate incubation of seeded plates, and the recovery could be further dramatically improved by the use of prereduced media, in conjunction with an anaerobic chamber.

Recommendations for the treatment of swabs and cultures for anaerobic investigation are made.

As long ago as 1898, Veillon and Zuber noted that anaerobic fusiform bacilli were the commonest organisms associated with acute appendicitis but little attention was paid to these organisms, and since there was no reliable method of cultivating them, their significance in clinical infections was largely ignored.

With the advent of the modern anaerobic jar (McIntosh and Fildes, 1916; Brewer, 1939) and subsequent improvements (Khairat, 1964), more anaerobes have been recognized from clinical specimens. Using anaerobic jar systems, Stokes (1958) demonstrated the isolation of 496 anaerobes from 4737 positive cultures in her clinical laboratory over several years, and Gillespie and Guy (1956) isolated 117 strains of anaerobic bacteria from 111 clinical specimens.

Hungate (1950) studying oxygen-sensitive rumen bacteria devised a technique based on the production of oxygen-free culture media. Moore (1966) extended this method of production and the use of prereduced anaerobically sterilized media. McMinn and Crawford (1970) found prereduced media superior to routine laboratory methods for the isolation of anaerobes from clinical specimens.

Several workers have used prereduced media in anaerobic chambers for the study of normal flora (Spears and Freter, 1967; Drasar, 1967; Arank, Syed, Kenney, and Freter, 1969).

More recent work has concentrated on the conditions of transport of clinical specimens to the laboratory for anaerobic investigation (Attebery and Finegold, 1969). Specialized media for the isolation of anaerobes on the bench have also been devised

(eg, Watt, 1972).

In the present investigation, the isolation of anaerobes from routine wound swabs on simple solid media was examined by three methods in parallel: (1) the use of prereduced media in an anaerobic chamber; (2) anaerobic jar techniques specially performed; and (3) routine clinical laboratory techniques.

Materials and Methods

SOURCE OF SWABS

Swabs with plentiful material were selected from routine wound swabs sent for examination to the clinical laboratories of St Mary's Hospital. They had travelled from the wards by the hospital's usual collection and transport system.

BACTERIOLOGICAL PROCEDURES

Bench procedures

The swabs were seeded onto blood agar plates (Oxoid Ltd, London, SE1) which had been stored aerobically at 4°C. In each case a seeded plate was immediately placed in a standard anaerobe jar (Baird & Tatlock Ltd, Chadwell Heath, Essex), which was fitted with a palladium catalyst. The jar was evacuated, filled with hydrogen gas, and incubated at 37°C. Gas-Pak anaerobic indicator (Becton, Dickinson, UK Ltd, Wembley, Middlesex) was included in the jar. A second seeded plate was left on the bench until a suitable number of plates for incubation in an anaerobic jar had accumulated. This was the usual practice in the clinical laboratories of St Mary's Hospital. A palladium catalyst

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and Gas-Pak anaerobic indicator were included in the jar, which was evacuated, filled with hydrogen gas, and incubated at 37°C. The plates were examined after 24 and 48 hours. A third plate was seeded and incubated aerobically at 37°C.

Cabinet procedures

A flexible polyvinyl anaerobic chamber, similar to that described by Arank *et al* (1969), was employed. It was fitted with a stainless steel air lock and filled with a 90% nitrogen, 10% hydrogen gas mixture. The atmosphere was circulated by a fan over granules of palladium D catalyst (Engelhard Industries, Cinderford, Glos). The catalyst was changed weekly and was reactivated by heating at 180°C for one hour. A tray of silica gel was included in the chamber to absorb any moisture produced by the action of the catalyst.

Blood agar plates (Oxoid Ltd) were stored for three days in the chamber before use; the blood became darkened indicating reduction of the media. Swabs were introduced into the chamber via the air lock and were seeded onto the stored blood-agar plates. An electrically sterilized inoculating loop (Trotman and Drasar, 1968) was used to spread the inocula on the plates. The plates were placed in an anaerobe jar inside the chamber and the lid was screwed down. On removal from the chamber, the jar was evacuated and filled with a gas mixture containing 10% CO₂ and 90% H₂. Fresh palladium catalyst was included in all the jars. The plates were incubated at 37°C and examined on the bench after 24 and 48 hours.

Isolation of anaerobes

Colonies of suspected anaerobes were picked into Robertson's cooked meat medium (Southern Group Laboratories) and incubated at 37°C for three days. The cultures were examined for purity and for aerobic and anaerobic growth on blood-agar medium.

Identification of anaerobes

The isolates were assigned to genera on the basis of their appearance in a Gram-stained preparation (Preston and Morrell, 1962), and on the end products of glucose fermentation as detected by gas-liquid chromatography. Species were assigned on the basis of biochemical tests (Holdeman and Moore, 1972). Inhibition of lecithinase by specific antitoxin on egg yolk medium was used as a confirmatory test for the detection of *Clostridium welchii* (Nagler, 1939).

Gas-liquid chromatography

A 4-ml portion of a three-day culture in 1% glucose

broth (Holdeman and Moore, 1972) was acidified with 0.1 ml of concentrated sulphuric acid (final pH < 2). The acidified cultures were placed in the water bath of a Perkin-Elmer F.40, multifract automatic gas chromatograph for head-space analysis. The temperature of the water-bath was 95°C. The gas chromatograph was fitted with a flame-ionization detector and a 2m stainless steel column packed with Diatomite C which had been treated with phosphoric acid and impregnated with 3% polyethylene glycol. The operating temperature was 125°C and the carrier gas was oxygen-free nitrogen passing through at 30 ml/min.

Results

SELECTION OF SWABS FOR COMPARISON

Of 97 swabs cultured by the three different procedures, 21 of those processed in the anaerobic chamber, 24 of those examined on the bench with immediate incubation, and 26 of those incubated after delay yielded no bacterial growth. Within those three groups, overgrowth by spreading organisms made analysis of growth impossible in 12, six, and four cases respectively. The number of swabs remaining for comparison in each group is shown in table I. All swabs that yielded anaerobes by one or more methods are included.

	Cabinet	Bench	
		Immediate Incubation	Delayed Incubation
Total no. of swabs examined	97	97	97
No. recorded as no bacterial growth	21	24	26
No. overgrown	12	6	4
Total remaining	64	67	67
No. of patients	50	49	48

Table I Selection of swabs for comparison

COMPARATIVE RECOVERY OF ANAEROBIC ORGANISMS

Anaerobes were isolated from 42.5% of the swabs processed in the anaerobic chamber, from 13.5% of those processed on the bench and immediately incubated, and from 4.5% of the swabs processed routinely (table II). For all the methods used, bacteroides organisms were the most common isolates. Anaerobic cocci and clostridia were also isolated (table III). The recovery rates for all anaerobic organisms, including the spore-forming clostridia, were improved by the use of the anaerobic chamber with prereduced media; the recovery of bacteroides was most noticeably improved. There was a less dramatic improvement with the rapid bench procedure.

	Cabinet	Bench	
		Immediate Incubation	Delayed Incubation
Number of swabs	64	67	67
Swabs (%) yielding isolates of anaerobes	42.5	13.5	4.5

Table II *Recovery of anaerobes from wound swabs*

Organism	Cabinet	Bench	
		Immediate Incubation	Delayed Incubation
Bacteroides species	22	5	2
Anaerobic cocci	7	3	1
Clostridia	5	1	1
Total anaerobes	34	9	4
No. of patients from whom anaerobes isolated	23	9	3

Table III *Numbers of isolates of various species*

DISTRIBUTION OF ANAEROBES ISOLATED BY CABINET PROCEDURES

The 64 wound swabs that were processed in the anaerobic chamber included 33 swabs from abdominal and ischio-rectal sources, 13 of which yielded facultative organisms only and 20 of which yielded anaerobic organisms (table IV). In five cases only anaerobes were isolated. A single species of anaerobe was recovered in pure culture from three swabs and multiple species were isolated from two swabs. A single anaerobe and one or more facultative organisms were recovered from four others. Thirty-one wound swabs from other sites were also examined: these included burns, abscesses, and swabs from respiratory and renal operation wounds. Twenty-four of these yielded facultatives only. Anaerobes were isolated from only seven, which included two swabs from which a single anaerobe was isolated in pure culture. Single strains of anaerobic bacteria in mixed culture with facultative organisms were recovered from four swabs, and one swab yielded facultative organisms with two species of anaerobe.

Organism	Type of Specimen (Source of Wound Swab)						
	Total Abdominal and Rectal	Other					
		Total	Skin/Bone	Superficial Abscesses	Respiratory	Muscle	Renal
FacultatIVES only	13	24	10	7	4	2	1
Anaerobes	20	7	4	1	1	—	1
Anaerobes only	5	2	1	1	—	—	—
One species	3	2	1	1	—	—	—
Two species	2	—	—	—	—	—	—
Anaerobes and facultatives	15	5	3	—	1	—	1
One species	11	4	3	—	—	—	1
Two species	4	1	—	—	1	—	—

Table IV *Distribution of anaerobes in 64 wound swabs*¹

¹Figures represent actual numbers of specimens.

NATURE OF ANAEROBIC ISOLATES

Of the organisms isolated in the anaerobic chamber, 65% of isolates from abdominal swabs and 62.5% of isolates from other sources were bacteroides (table V). Fewer anaerobic cocci (19%) were isolated from abdominal sources than from other sources (25%). Fifteen per cent of the abdominal isolates were clostridia, as were 12.5% of isolates from other sources.

The majority of the bacteroides isolated were identified as *Bacteroides fragilis* (table VI). *Bacteroides melaninogenicus* was isolated twice, in both instances from swabs of small bowel operation wounds. The anaerobic cocci isolates were all Gram-positive organisms of the genera *Peptococcus* and *Peptostreptococcus*. The peptococci were *Peptococcus asaccharolyticus* and *Peptococcus magnus*. The anaerobic streptococci were identified as *Peptostreptococcus intermedius*. The clostridia were all *Clostridium welchii*.

	Abdominal and Rectal Source	Other Sources
No. of swabs with anaerobes	20	7
No. of anaerobes isolated	26	8
% Bacteroides	65	62.5
% Anaerobic cocci	19	25
% Clostridia	15	12.5

Table V *Anaerobes isolated from wound swabs on prereduced media*

Organism	No. of Isolates
<i>Bacteroides fragilis</i>	20
<i>Bacteroides melaninogenicus</i>	2
<i>Peptococcus asaccharolyticus</i>	2
<i>Peptococcus magnus</i>	2
<i>Peptostreptococcus intermedius</i>	2
<i>Clostridium welchii</i>	5
Total	34

Table VI *Identity of anaerobic isolates*

Discussion

The clinical significance of anaerobes in wound infections cannot be assessed from the data obtained in this study. No patients were followed up and there was no assurance that the swabs were taken in a way that avoided contamination with normal flora. Indeed the high rate of recovery of anaerobes from abdominal and rectal sources might be accounted for by such contamination. This seems less likely in the seven cases where only anaerobes and no facultative organisms were recovered. It is interesting to note here that anaerobes were isolated more frequently together with facultative organisms than on their own. Macdonald, Gibbons, and Socransky (1960) and Moore and Gross (1968) have shown cases where anaerobic bacteria are pathogenic only where they exist in a synergistic situation with other bacteria.

The results of the present study indicate that immediate incubation of plates seeded on the bench is preferable to methods which involve delayed anaerobic incubation of seeded plates. No direct comparison can be made between the results obtained by cabinet and bench procedures since the CO₂ content of the anaerobic jars differed in these two situations. However, the results of a similar study by Vargo, Michaelson, Spaulding, Vitagliano, Swanson, and Forsch (1971) in which CO₂ was included in all anaerobic jars, were comparable with those obtained in this study (table VII). Lewis, Bedell, and Rettger (1940) and Watt (1973), working with pure cultures, have shown that adding CO₂ increases both numbers and colony size of organisms incubated in anaerobic jars. The inclusion of CO₂ in the gas mixture in anaerobic jars is to be recommended, although its effect on the recovery of anaerobes in the clinical situation may not be as dramatic as with pure cultures. Even with CO₂ in the anaerobic jar, efficient isolation of anaerobes from routine clinical specimens can only be achieved with the use of prereduced media.

McMinn and Crawford (1972) found that pre-reduced liquid media were superior to solid media incubated in anaerobic jars for the isolation of anaerobes from clinical specimens known to contain anaerobes.

In contrast, Rosenblatt, Fallon, and Finegold (1973) showed that anaerobes could be isolated from clinical specimens just as effectively on the bench with anaerobic jar procedures as on prereduced solid media in an anaerobic chamber (table VII). However, in the latter study, the specimens were specially collected under anaerobic conditions, and speedily transported to the laboratory. Similarly, Dowell (1972), using prereduced solid media in an anaerobic chamber, found no improvement in the recovery of anaerobes from clinical specimens which had been submitted under anaerobic conditions.

In the present study the clinical specimens had been through the normal hospital transport system, and in many cases, several hours had elapsed between taking and cultivating the swabs. Throughout transportation the swabs were in an aerobic atmosphere. It is probable that many more organisms would have been recorded had the specimens been speedily submitted under anaerobic conditions, and it is hoped that, in future, efforts will be made to transport clinical specimens in anaerobic conditions.

It is encouraging that anaerobic organisms were isolated without employing specialized complicated media. Ordinary blood agar was used for all three procedures in this study.

Especially where it is not possible for specimens to be collected and transported anaerobically, methods are required for storing media in a reduced state and of keeping seeded plates in an anaerobic environment. To try to ensure that every culture for anaerobic incubation is put into an anaerobic jar immediately it is seeded would require extra technician time and a great many anaerobic jars. It is appreciated that many people feel that the anaerobic

No. of Specimens	McMinn and Crawford (1970)		Rosenblatt et al (1973)			Vargo et al (1971)		This Study	
	38 Specimens		23 Specimens			145 Specimens		64 Specimens	
	Bench	Prereduced Anaerobically Sterilized	Bench	Cabinet	Prereduced Anaerobically Sterilized	Bench	Cabinet	Bench	Cabinet
Anaerobic transportation	-	-	+	+	+	-	+	-	-
+ CO ₂	+	+	+	+	+	+	+	+	+
% Positive	8.2	100	100	100	100	18	41	13.5	42.5

Table VII Isolation of anaerobes from clinical specimens under various conditions

chamber and air lock system, although a useful tool for research, is too cumbersome for the ordinary clinical laboratory. However, the increased rate of isolation of anaerobes should outweigh any disadvantages. Martin (1971) has described a cupboard and jar both capable of being flushed with oxygen-free gas, and this type of apparatus may also prove useful in the routine laboratory. Prereduced, anaerobically sterilized media are now being marketed commercially in the United States, and if this service is extended to Britain it would be a further possibility for routine use.

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