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Campylobacters and enteritis

M. B. Skirrow, M.B., Ch.B., Ph.D., M.R.C. Path., D.T.M. and H. Consultant Microbiologist, Department of Pathology (Microbiology), Worcester Royal Infirmary

The recent emergence of campylobacters as a cause of enteritis might be regarded as something of an embarrassment to medical microbiologists. Organisms that were thought to be extremely rare in man have suddenly become commonplace, yet we know little about the bacteria themselves or their epidemiology. The obscurity that campylobacters have so long enjoyed can be attributed to their unusual growth requirements, which are not provided by methods traditionally used in clinical laboratories; moreover their isolation from faeces depended on the development of a suitable selective culture technique. The initial breakthrough was made by Butzler and his colleagues[1] in Brussels in 1973, but the significance of their work was not generally appreciated until after the publication of Skirrow's paper four years later[2].

FIGURE 1. Overnight primary culture of thermophilic campylobacter showing typical effuse colonies. VPT selective agar incubated at 42-43°C.

Classification of Campylobacters

When they were first discovered in 1913[3] these organisms were classified as vibrios on account of their curved shape and rapid motility; and because they were associated with infectious infertility and abortion in cattle and sheep they were called *Vibrio fetus*[4]. During the ensuing years it became clear that several types were involved, and in 1963 Sebald and Véron[5] showed that they were sufficiently different to warrant separation into a new genus – hence the name *Campylobacter* (Greek, curved rod). Apart from being non-saccharolytic and microaerophilic they were shown to have a DNA base composition far removed from the true vibrios (G + C content 30-35 mols% and 48% respectively). Moreover their morphology is now recognised to be more akin to that of the Spirilla than to the vibrios, and in Bergey's Manual[6] the genus *Campylobacter* is included in the family *Spirillaceae*. Biochemically campylobacters are rather inactive, but all are oxidase positive, and some produce catalase – a property that serves to divide the genus into two groups.

Catalase-negative group. As far as we know members of this group are non-pathogenic to man. *C. sputorum* constitutes part of the normal mouth flora and can be found in about 3% of faecal samples from normal people. A subspecies of this organism, *C. sputorum mucosalis*, has recently been described as a cause of intestinal adenomatosis of pigs[7]. Colonies on horse blood agar are smooth, entire, and may produce slight greening of the medium. The organisms appear as slender irregularly bent rods rather than spirals as in the catalase-positive group. The other member of this group, *C. bubulus* is a non-pathogenic organism found in the prepucial secretions of bulls where it may be confused with *C. fetus*.

Catalase-positive group (Table 1). This group is divided into *C. fetus* (two subspecies) and a heterogenous sub-group characterised by a high optimum growth temperature; it is these thermophilic organisms that are associated with acute enteritis. Elizabeth King[9] was the first to recognise that the latter constituted a distinct group, and it was she who devised the temperature tolerance test for their differentiation from *C. fetus* – a test which is still the most reliable for this purpose. She called these organisms "related vibrios" in recognition of their similarity to *C. fetus* (then *Vibrio fetus*). Subsequently Véron and Chatelain divided them into the two species *C. coli* and *C. jejuni*, but they are listed by Smibert[6] in Bergey's Manual as a subspecies of *C. fetus* (*C. fetus jejuni*). The names adopted by Véron and Chatelain have historical precedence in the *V. jejuni* of Jones *et al* (1931)[11] isolated from calves with winter scours, and the *V. coli* of Doyle (1948)[12] isolated from pigs with swine dysentery – a disease not known to be caused by a treponeme. These authors are probably correct in their subdivision of this group, but their criteria for differentiating the two species need further clarification.

Pathogenicity

The two subspecies of *C. fetus* are the organisms principally responsible for infertility and abortion in cattle and sheep, but the thermophilic group have also been implicated in outbreaks of bovine abortion. *C. fetus venerealis* does not appear to infect man; *C. fetus intestinalis* does, though infections are rare and virtually limited to those who are immunodeficient or have some other predisposition to infection. These patients generally suffer an ill-defined febrile relapsing type of illness, sometimes with an associated localised infection such as arthritis, endocarditis, or meningitis. Thus, as far as human disease is concerned, our concern is almost entirely with the thermophilic group as a cause of acute enteritis in normally fit people.

Campylobacter Enteritis

In some laboratories, particularly those with a large intake of general practitioner specimens, campylobacters are the commonest organisms to be isolated from diarrhoeic faeces. Some have reported isolation rates as high as 14%[13] but about 6% is more usual. Reports from England and Wales to the Communicable Disease Surveillance Centre, Colindale, exceeded 200 per week on several occasions during the summer of 1978. As with salmonellosis the incidence seems to be high during the warm months. Also like salmonellosis the infection is a zoonosis with a wide range of animal hosts, but with man-to-man transmission also playing a part in the spread of infection. Attempts to find the source of infection by working back from a patient is often unrewarding, but some cases have been traced to contact with chickens, including raw carcasses and to young dogs themselves suffering from campylobacter enteritis. Campylobacters have also recently been implicated in water borne and milk borne outbreaks of enteritis.

TABLE 1 Catalase-Positive Campylobacters (based on Berg *et al.*[8]).

Organism	Serotype	Biotype	Habitat	Disease
C. fetus venerealis (*C. fetus fetus* – Smibert[6])	A	1 Sub-1	Genital tract of cattle	Infectious infertility of cattle
C. fetus intestinalis (*C. fetus fetus* – Véron & Chatelain[10])	A B	2	Intestinal tract of cattle and sheep	Infectious abortion of cattle and sheep
Thermophilic group				
syn. "Related vibrio" (King[9]) / *C. fetus intestinalis* serotype C (Berg *et al.*[8]) / *C. fetus jejuni* (Smibert[6]) / *C. coli*/*C. jejuni* (Véron & Chatelain[10])	"C" heterogenous	Several undefined	Intestinal tract of many types of animals, particularly birds	Infectious abortion of sheep. Enteritis in man, dog, and probably others

Clinical manifestations

The disease has been described elsewhere,[2] but the main features are summarised in Table 2. Mild and asymptomatic infections also occur. All ages are affected and although adults account for most of the cases seen, the true incidence is highest in infants.

Pathology

The fact that these organisms are sometimes isolated from the blood of infected patients and that mesenteric adenitis has been observed in those who have undergone laparotomy suggests an invasive process. The rigors that some patients experience during the prodromal phase certainly suggests a transient bacteraemia. The ileum and jejunum appear to be the parts of the bowel principally involved, but endoscopy has shown the presence of procto-colitis in some patients. Specific agglutinins appear in the sera of most patients by about the fifth day of illness. The organisms usually disappear spontaneously from the stools within 1-4 weeks of the illness.

Isolation of organisms

Selection can be achieved in two ways:

1) Filtration of suspension of faeces (or other material) through 0.65 µm Millipore membrane. This method is rather tedious and less sensitive than selective media, but it has the advantage that it can be used with non-inhibitory media. It is necessary for the isolation of *C. sputorum* which does not grow on the selective media listed below.

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☐ *continued from page 1*

2) Selective media:
   A. Oxoid BA Base No. 2 with 5-7% lysed horse blood containing vancomycin 10 µg/ml, polymyxin B sulphate 2.5 I.U./ml, trimethoprim lactate 5 µg/ml† (Skirrow's medium[2]).
   B. Thioglycollate agar with 15% sheep blood containing bacitracin 25 I.U./ml, novobiocin 5 µg/ml, actidione 50 µg/ml, colistin 10 units/ml, and cephalothin 15 µg/ml. (Butzler's medium[14]).

Incubate in reduced O_2, preferably about 6%; an anaerobic jar (without catalyst) is convenient for this; additional CO_2 is beneficial. A recent paper[15] described an iron containing supplement that increases aerotolerance and this may permit isolation in a candle jar. An incubation temperature of 37°C is satisfactory but selectivity is increased and quicker results obtained at 42-43°C – but to the exclusion of *C. fetus*.

Identification of organisms

A basic identification scheme is given in Table 3. The morphology of these organisms is so characteristic that for routine purposes additional tests are unnecessary.

Antibiotic sensitivities may also help in identification. In general these organisms are resistant to trimethoprim, novobiocin, cephalothin, polymyxins, and penicillin (some to ampicillin) and sensitive to macrolides, aminoglycosides, tetracyclines and chloramphenicol; a few streptomycin, tetracycline and erythromycin resistant strains have been found. Erythromycin is an effective form of chemotherapy.

† A combined additive is available commercially (Oxoid Ltd) and several firms market ready-poured media to this formula.

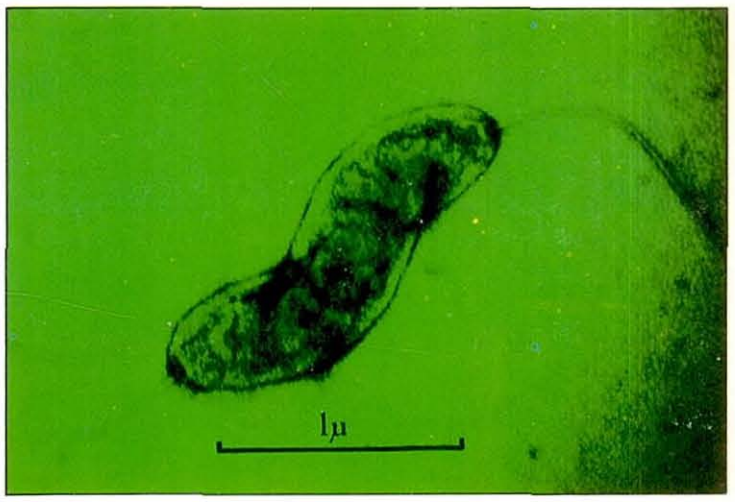

FIGURE 5. Electron micrograph of thermophilic campylobacter showing amphitrichate configuration.

TABLE 2 Main features of campylobacter enteritis

Prodromal phase: a few hours to a few days – not always present

"Flu-like" – fever, malaise, headache, general aches, sometimes rigors

Diarrhoeic phase: 1 to 3 days

Abdominal cramps, profuse diarrhoea, prostation in severe cases

Cellular exudate in stools, sometimes frank blood

Nausea, but vomiting transient or absent

Recovery phase: Several days

Bowel actions less frequent

Abdominal pain persists

Dehydration, weight loss, lassitude

Note: Severe abdominal pain → hospital as "acute abdomen"; sometimes genuine appendicitis

In infants, blood in stools may mimic intussusception

Strain identification

1) **Serology.** There are many serotypes within the thermophilic group but a classification has not yet been worked out. Formalinised suspensions exhibit specific agglutinins; slide agglutination with live organisms seems to be less specific.

2) **Cultural tests.** Tests based on those described by Véron and Chatelain and developed in this laboratory have shown differences within the thermophilic group. Among the more useful tests are: finer degrees of temperature tolerance (Table 3), sensitivity to nalidixic acid, tolerance to triphenyl tetrazolium chloride (TTC), and grading of H_2S production. Analysis of results is incomplete, but useful information is beginning to emerge. For example it is clear that most of the organisms obtained from pigs conform to a recognisable pattern (*C. coli?*), and that this pattern is seen in only about 5% of human isolates.

This, of course, is only the beginning. The next few years will doubtless see a reclassification of the thermophilic campylobacters, and hopefully the development of methods, such as phage typing, for the finer differentiation of strains. Only then will the epidemiology be understood and with that, the possibility of control.

Acknowledgments

The photomicrographs were taken by Mr G.H. Green and the electron micrograph by Mr D. Bruce.

TABLE 3 Identification of enteric campylobacters

Colonies:	typically effuse with tendency to spread along direction of streak (Fig 1); may swarm on wet plates; some strains form discrete domed colonies like *C. fetus* (Fig 2).
Morphology:	Slender (0.2-0.5 µm) Gram-negative spiral or S-shaped rods with tapering ends (Fig 3), occasionally bacillary; coccoid forms predominate in post-mature cultures (Fig 4); amphitrichate (Fig 5)
Motility:	very rapid, darting and oscillating, spin on axis; can be detected in fresh stools by dark ground microscopy.
Oxidase +	Catalase +

Differentiation from C. fetus:-

		C. coli/jejuni	C. fetus
Growth on nalidixic acid agar 40 µg/ml (or up to 30 µg disc)		–*	+
Growth on blood agar at (°C)	45	+ or –	–
	42	+ (vigorous)	– or ±
	37	+	+
	30	± or –	+
	25	–	+

* except for one uncommon biotype

Note: *C. fetus* has more open undulations (longer "wavelength"), and thus short forms appear as curved bacilli (Fig. 6); ends rounded rather than tapering; often monotrichate.

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FIGURE 2. Thermophilic campylobacter showing discrete non-effuse type of colony – indistinguishable from *C. fetus*

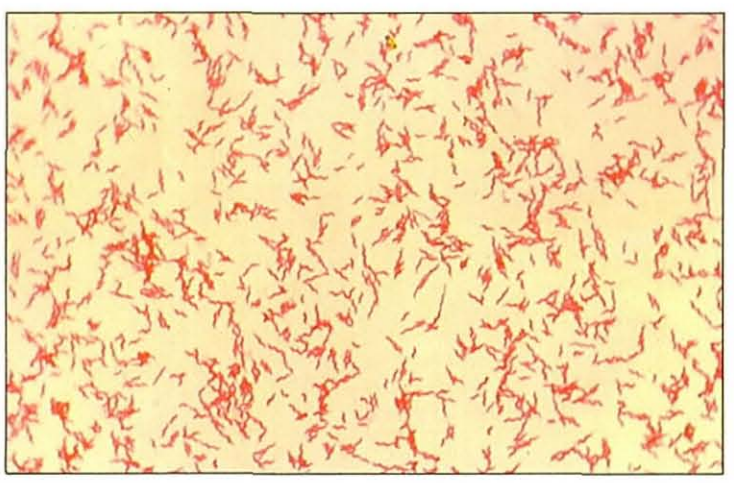

FIGURE 3. Gram stained smear of thermophilic campylobacter x 1100

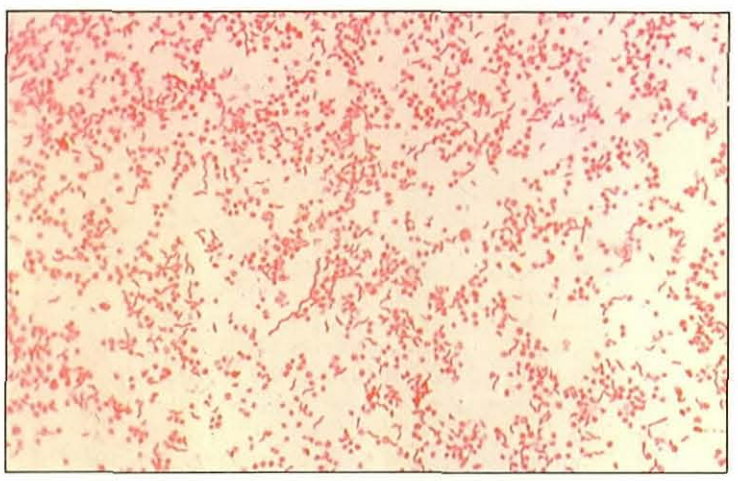

FIGURE 4. Coccoid forms in post mature culture x 1100

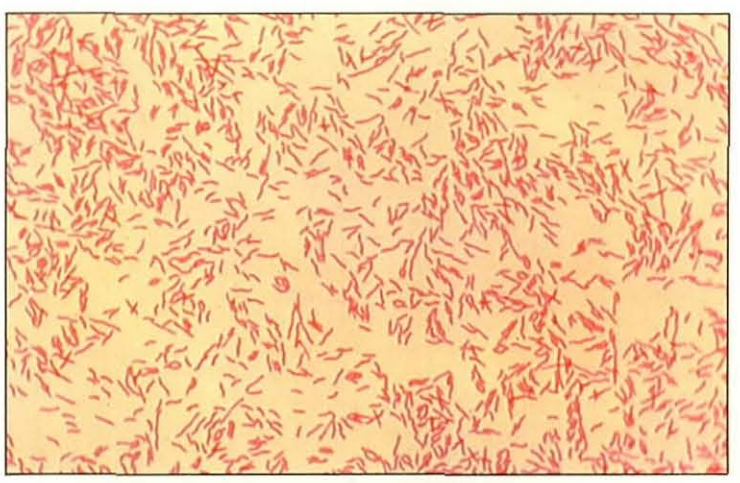

FIGURE 6. Gram stained smear of *C. fetus intestinalis* x 1100

Antibiotics as selective agents in anaerobic bacteriology

K. D. Phillips, B.Sc. Senior Scientific Officer, Public Health Laboratory, Luton and Dunstable Hospital

The use of selective media in clinical anaerobic bacteriology is particularly appropriate by virtue of the fact that obligate anaerobes are commonly encountered in pathological material and in normal floras as mixtures of species often in association with facultatively anaerobic organisms.
Inhibitory agents such as bile, dyes and a variety of other chemicals have a long history of empirical inclusion in bacteriological media for the elective cultivation of anaerobic bacteria. However, with the advent of antibiotics, a more rational approach to the problems of qualitative assessment of anaerobic populations was made possible. Early applications of antibiotics as selective agents were directed mainly towards the clostridia which at the time were the obligate anaerobes of major concern to the clinical microbiologist. However, an increasing awareness of the significance of non-sporing anaerobes in pyogenic infections of man, coupled with appreciation of the role of these organisms as important components of the normal human bacterial flora stimulated further exploitation of antibiotics as selective agents in anaerobic bacteriology. Table 1 refers to some of the antibiotic agents that may be used in culture media for the selective isolation of different anaerobic bacterial species. The use of many of these antibiotics or antibiotic combinations was developed by Finegold and his co-workers and have been reviewed by Finegold *et al.*[1]

Although the primary concern in this article is with antibiotics, the great value of certain non-antibiotic substances as selective agents should not be overlooked. Outstanding examples of these include the addition of phenylethyl alcohol to solid and fluid media for the isolation of heat sensitive strains of *Clostridium botulinum,* the use of dyes such as gentian violet and brilliant green for the isolation of fusobacteria and inclusion of sodium azide and bile salts in media for the selective culture of bacteroides.

Table 1

Antibiotic	Anaerobes Selected	Facultative Anaerobes Inhibited	Major Selective Use
Neomycin	Clostridia, Bacteroides, anaerobic cocci, Gram positive non-sporing bacilli	Gram negative bacilli	Selective for all obligate anaerobes
Kanamycin/ Vancomycin	Bacteroides, Fusobacterium, Veillonella	Gram negative bacilli Streptococci, Staphylococci	Bacteroides
Neomycin/ Vancomycin	Bacteroides, Fusobacterium, Veillonella	Gram negative bacilli Streptococci, Staphylococci	Fusobacterium, Veillonella
Rifampicin	*F. varium* and *F. mortiferum,* some strains of clostridia and Eubacterium	Most facultative bacteria	*F. varium* and *F. mortiferum*
Nalidixic acid	Bacteroides, Fusobacterium, Gram positive anaerobic cocci	Gram negative bacilli	Most non sporing anaerobes

Media

For the vast majority of anaerobes isolated from clinical material, a good quality horse blood agar, which has been freshly prepared, is entirely appropriate as the basis of a selective medium. However, the addition of other growth factors such as menadione, haemin or cysteine hydrochloride may occasionally be advantageous in some circumstances.

Neomycin

Neomycin, as neomycin sulphate was introduced for the isolation of *Clostridium perfringens* Type A by Lowbury and Lilley[2] and its use was later extended by Willis and Hobbs[3] for the isolation of the commonly occurring clostridia (*see also* Willis[4]). Egg yolk agar containing 100 µg/ml of neomycin is of particular value for the separation of clostridia from many aerobic contaminants and allows ready recognition of strains of *Cl. perfringens* and *Cl. botulinum* by their lecithinase or lipase reactions. Cooked meat broth containing similar concentrations of neomycin is effective for primary enrichment of clostridia and is of value in facilitating, for example, the isolation of *Clostridium tetani* on subsequent subculture to solidified media. Neomycin blood agar and neomycin egg yolk agar are unsurpassed for the selective isolation of clostridia and commonly encountered strains are relatively easily isolated and purified by virtue of rapidity of growth and their distinctive colonial appearance.
Neomycin, in concentrations of 70-100 µg/ml is also eminently suitable for the detection and isolation of the non-sporing anaerobes. This group of organisms includes bacteroides, fusobacterium and the Gram positive anaerobic cocci, and forms a large part of the normal flora of the gastrointestinal tract, the female genital tract and the oropharynx. Under appropriate conditions those anaerobes may

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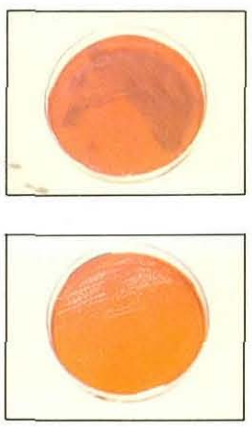
OXOID NEWSLINES

Campylobacter supplement

CODE SR69

The development of a selective culture medium has now made the isolation of campylobacters from faeces a simple matter. All that is required is a blood agar medium, the Oxoid Antibiotic Supplement (SR69), an Oxoid Gas Generating Kit, the Oxoid Anaerobic Jar and an incubator set at 43°C.
The illustrations of growth on the culture plates show the remarkable selective effect of the supplement. Subculture and identification of *C. jejuni–C. coli* from the medium containing the supplement is made very simple.



Without Supplement
Enteric isolate containing Campylobacter species will normally be overgrown by commensurates such as *E. coli, Strep. faecalis* and *Proteus spp.* when grown on lysed blood agar.

With Supplement
By adding Oxoid Campylobacter Supplement (SR69) a pure culture of campylobacter can be obtained from the same isolate.

Cary-Blair transport medium

CODE CM 519

The transport medium of Cary and Blair was developed from Stuart's medium for transport of rectal swabs to a central diagnostic laboratory in *field epidemiological* surveys. Cary and Blair reported recovery of salmonellae and shigellae after 49 days storage at high ambient temperatures (*J. Bact.* 1964, **88,** 96-98).

The high pH and low Eh also makes the medium particularly suitable for the transport of fastidious anaerobic bacteria. It may be prepared as a pre-reduced anaerobic sterilized medium (PRAS). For the transport of *Neisseria gonorrhoeae* Amies Medium is preferred (see Newslines, September 1978).

Antibiotic supplements Staph/Strep CODE SR70 and Strep CODE SR74

Antibiotics are now widely accepted selective agents in culture media and the ever-increasing range allows many combinations to be used to tailor selectivity for particular organisms or groups of organisms.
The two latest in the range of Oxoid freeze dried supplements for addition to Blood Agar Bases are designed to select Gram positive cocci.

Oxoid Antibiotic Supplement – Staph/Strep (SR 70) selects both *Staphylococcus aureus* and streptococci. It is inhibitory to *Staph. albus* and *Micrococcus spp.* as well as Gram positive and Gram negative rods, making isolation from mixed flora a simple matter (Fig. 1).

Antibiotic Supplement – Strep (SR 74) is more selective, allowing growth of streptococci only. It may be used to assist detection of beta-haemolytic streptococcal carriage in throats as well as their isolation from wound and burn sites (Fig. 2).
Haemolytic patterns on media containing blood are clearly defined; colonial size and recovery of streptococcal groups A.B.C.D.& G. are comparable to that on unsupplemented media.

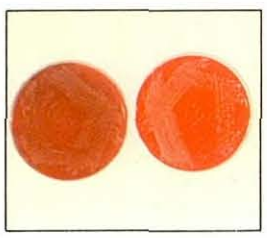


Fig. 1 Selection of staphylococci and streptococci from mixed growth of Gram positive and Gram negative bacteria.

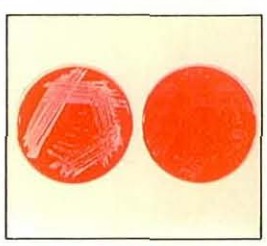


Fig. 2 Selection of streptococci from commensurate bacteria.

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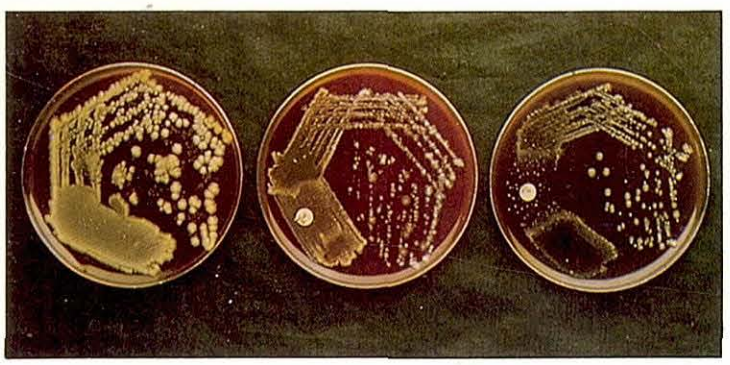

FIGURE 1. Exudate from an abdominal abscess cultured (left to right) aerobically and anaerobically on unselective blood agar and anaerobically on neomycin blood agar. Discs containing 5 µg of metronidazole are placed on the inoculated areas of the anaerobically incubated plates.

cause endogenous infection by invading adjacent tissues. Notable examples of infective processes in which non-sporing anaerobes have been implicated are intra-abdominal and pelvic sepsis. Anaerobic sepsis of this type is characterised by the formation of large deep seated abscesses from which mixtures of non-sporing anaerobes can be isolated, usually in association with passenger aerobic, and facultatively anaerobic species. Laboratory diagnosis of infection due to non-sporing anaerobes is achieved by direct plating of exudate on unselective blood agar for incubation aerobically and anaerobically; a selective blood agar should be included for anaerobic incubation in parallel with the unselective medium. The cultures are subsequently examined for identity of isolates and compared with one another for the relative proportions of aerobic, facultatively anaerobic and obligately anaerobic growth. Fig. 1 illustrates the culture of a pus obtained from a post-appendicectomy abdominal abscess under aerobic and anaerobic conditions using a neomycin blood agar as a selective medium. Heavy growth of *E. coli, Streptococcus faecalis* and staphylococci occurred on the unselective aerobic and anaerobic blood agar plates largely obscuring growth of *Bacteroides fragilis, Bacteroides melaninogenicus* and anaerobic cocci which were readily visible on neomycin blood agar.

Neomycin is a good general purpose selective agent for use in clinical anaerobic bacteriology, although the growth of some commonly encountered organisms notably *B. melaninogenicus* and *Bacteroides corrodens* can be partially or completely inhibited at concentrations above 70 µg/ml. Moreover, neomycin does not suppress growth of streptococci or staphylococci although growth of facultative Gram negative bacilli is effectively prevented. The use of discs containing 5 µg of metronidazole to which obligate anaerobes are universally sensitive is a valuable aid for discriminating between colonies of obligate and facultative anaerobes on both selective and unselective blood agar.

Kanamycin

Kanamycin may be used with effect in selective media at a concentration of 100 µg/ml of kanamycin base as an alternative to neomycin; it shares with neomycin a similar range of selective properties, although for strains of bacteroides which exhibit reduced growth in the presence of neomycin, Finegold[5] found kanamycin to be less inhibitory. A concentration of 75 µg/ml of kanamycin is favourable for *B. melaninogenicus*.

Vancomycin

Vancomycin, as vancomycin hydrochloride, is employed at a concentration of 7.5 µg/ml in combination with appropriate concentrations of either kanamycin or neomycin. Vancomycin completely inhibits the growth of streptococci and staphylococci, organisms which are frequently encountered in mixed bacterial populations of human origin. Neomycin plus vancomycin is a marginally favoured combination for veillonella and fusobacterium; kanamycin plus vancomycin selects for the majority of Gram negative non-sporing anaerobes. Sutter *et al.*[6] recommend kanamycin (75 µg/ml) plus vancomycin–laked blood agar as a general purpose selective medium in clinical anaerobic bacteriology; the laked blood promotes earlier detection of the characteristic black pigment of *B. melaninogenicus*. Fig. 2 illustrates an abdominal wound exudate plated directly on unselective blood agar and on kanamycin/vancomycin blood agar. The predominant growth comprised *B. fragilis, Fusobacterium varium, E. coli* and facultatively anaerobic streptococci. The kanamycin/vancomycin combination has selected completely for the Gram negative obligately anaerobic bacilli as revealed by the absence of growth around the metronidazole disc.

Rifampicin

Rifampicin is highly selective for *Fusobacterium varium* and *Fusobacterium mortiferum* at a concentration of 50 µg/ml. Because of its inhibitory nature to most other obligate anaerobes, it is unsuitable for general use in clinical bacteriology.

Nalidixic acid

A recent report by Ingham *et al.*[7] demonstrated the use of nalidixic acid (50 µg/ml) as a selective agent for bacteroides, fusobacterium and Gram positive anaerobic cocci encountered in otogenic cerebral abscesses. This selective agent has a potential for routine use, but Finegold *et al.*[1] do not consider nalidixic acid to have advantages over neomycin or kanamycin in this context.

General Considerations

Selective media are never perfect; they frequently suppress to some degree the growth of organisms whose selection is required. Moreover resistant strains of species which the medium is designed to suppress are by no means uncommon. On neomycin or kanamycin containing media, resistant strains of *Proteus spp.* will occasionally be encountered and the growth of staphylococci and streptococci is commonly unaffected by the usual concentrations of these agents.

In clinical bacteriology, the cultural procedure aims to reproduce and elucidate the bacterial constituents as they occur in the infective lesion. Although selective agents are valuable for the "qualitative" isolation of anaerobes from mixed cultures, the bacteriological diagnosis of anaerobic sepsis is made primarily on a value judgement of qualitative and quantitative results of culture. In choosing a selective medium which strikes a balance between selectivity for anaerobes and suppression of their growth, some loss of selectivity is inevitable; this loss can usefully be offset by the use of metronidazole discs in conjunction with the selective medium.

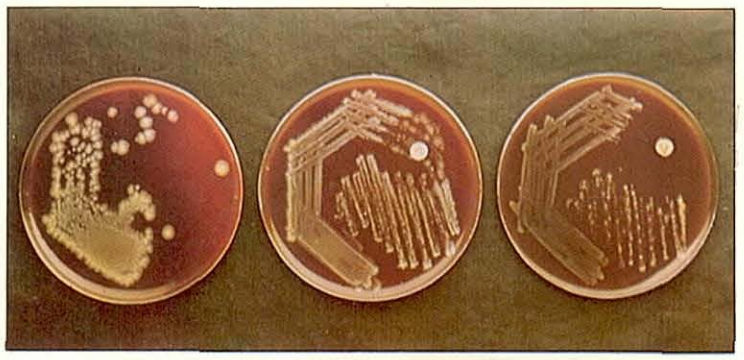

FIGURE 2. Abdominal wound drainage on (left to right) aerobic and anaerobic unselective blood agar and an anaerobic kanamycin/vancomycin blood agar. The isolation of obligate anaerobes only on the selective medium is revealed by the absence of growth round the metronidazole disc.

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