OXOID

Volume 2 No 2 September 1981

culture

Published as a service to microbiology
by Oxoid Limited

Slow progress in developing rapid methods and automation in clinical and public health microbiology*

C.E.D. Taylor, M.A., M.D., F.R.C. Path., Dip. Bact., Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Cambridge

"The amazing thing in life", said a seventeenth - century philosopher, "is that all the necessary things are simple and all the complicated things are useless."

During the last ten years a lot of work has been done, and much money spent, in an effort to develop rapid methods and design equipment for automating, or at least mechanising, some of the routine procedures in clinical and public health microbiology laboratories. Progress has been slow and on the whole disappointing.

Compared with clinical biochemistry and haematology, for which machines have been in routine use for some 20 years, clinical and public health microbiology is still largely a labour-intensive cottage industry. It would therefore seem timely to try to identify and discuss some of the reasons for the present state of affairs. Is it because microbiology is inherently different from the other clinical sciences? Have we been taking too large a step at a time? Are the costs of development too great for the potential reward in relation to the work load, in spite of microbiology as presently practised being a highly labour-intensive activity? Should we pack it all up or perhaps try some new ideas? Where should we go from here?

This laboratory would be as familiar to bacteriologists today as it would be to bacteriologists of the early 1900's. (*Exhibit in the Lower Wellcome Gallery, Science Museum, London).*

GENERAL CONSIDERATIONS
Is there a distinction between automation and mechanisation?

'Mechanisation' usually refers to a process which imitates a sequence of manoeuvres customarily done by a human operator. Automated procedures achieve a similar end result but not necessarily by imitating all or any of the manual manoeuvres involved. Highly developed automated procedures include self- regulating feedback systems controlled by a computer. Most automated procedures include elements of mechanisation. Throughout this article the term 'automation' will be used to include both concepts.

The problems

The problems in designing automated equipment for microbiology differ greatly from those in clinical chemistry and haematology.

1. *Maintaining sterility of the analytical process* - this is paramount in microbiology but less exacting in tests for antibiotics in serum where most progress in automation has been made.

2. *Diversity of specimens* - which in microbiology far exceeds that in clinical chemistry and haematology.

3. *Diversity of analyses* - in microbiology the diversity of organisms to be detected, identified and sometimes counted far exceeds the range of constituents determined in clinical chemistry and haematology.

4. *Nature of the analytical systems* - most culture media and many test reagents such as diagnostic antisera contain chemically undefined, variable and capricious biological materials.

5. *Preparatory stages of analyses* - to detect pathogens there is a need to 'amplify the signals' This is achieved by:
   (i) using a microscope to search for putative pathogens in stained preparations of specimens. Staining machines are available and can work well if properly maintained.
   (ii) incubating the sample on various culture media in different atmospheres for up to 18h or longer. Here there are problems in achieving an adequate 'noise to signal ratio.'

6. *Economic aspects* - the technical and engineering problems of automating microbiology and devising rapid methods may not be insuperable. The main constraints are financial and the will to afford the necessary cost.

7. *Personnel problems* - automating microbiology could lead to a loss of job satisfaction. Automating clinical chemistry has not apparently reduced the number of chemists required but the work load ratio has increased.

DETECTING AND IDENTIFYING PATHOGENS IN PUBLIC HEALTH SPECIMENS

The questions to be answered are often simpler and less technically demanding than in the case of clinical specimens, e.g. can a designated pathogen such as a salmonella be detected in this specimen?

Speed is often not as important as with clinical specimens.

Large numbers of specimens of the same kind e.g. faeces, may need to be examined for the same organism. This sort of activity should lend itself readily to automation but in practice much of the repetitive work can be done quickly by relatively unskilled and lesser paid workers.

Machines such as those mentioned in the section on the enumeration of bacteria might be cost-effective in large public health laboratories but the market would be small and may not be commercially attractive.

DETECTING AND IDENTIFYING PATHOGENS IN CLINICAL SPECIMENS

Questions to be answered

Are there any clinically relevant pathogens in the specimen? If so, what are they and what are their antimicrobial susceptibilities?

Speed and accuracy are important here, especially in emergency situations, e.g. a patient with meningitis or septicaemia.

The standard routine is:

1. *Microscopy* - Gram-stain for most organisms - Ziehl Neelsen or Auramine-phenol fluorescent stain for acid-fast organisms.
   Machines are available, and perform well if properly maintained. Attempts to design machines to scan smears of sputum microscopically for acid-fast bacilli have met with very limited success. In addition the work load of most laboratories does not justify the cost.

2. Attempts at rapid detection of bacterial antigens e.g. by *counter current immunoelectrophoresis* as an aid to detecting and identifying pathogens has very limited use.

3. Rapid detection and identification of anaerobes by *gas-liquid chromatography* has proved successful. Other forms of chromatography plus mass spectrometry have been explored but are very costly.

4. *Inoculation and incubation of various culture media.* This may include direct susceptibility tests. A machine which inoculates culture media with material on cotton-tipped swabs has been tested in routine use. The time taken to load the machine approximates to that required to spread culture plates by hand. Mechanical spreading of inoculum is not always as good as manual spreading.

5. *Selection of colonies of putative pathogens for tests of identification.* Attempts at automation have shown that it is not likely to be cost-effective. Aids to identifying putative pathogens, especially simplified means of biochemical testing (e.g. API system) have proved useful. A continuous-flow analyser adapted for biochemical testing has worked well in dedicated hands but is unlikely to be cost-effective in most laboratories.

6. Determining antimicrobial susceptibility of putative pathogens (see separate section on this topic).

7. *Blood cultures* present special problems. Often they are from seriously ill patients and there is an urgent need to know:

   (i) if there is bacterial growth,
   (ii) the nature and antimicrobial susceptibilities of the organism(s).

   Measuring changes in electrical impedance or conductivity in the cultures appears promising.

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ISOLATED PATHOGENS

1. **Disc diffusion techniques**
   Various aids for applying discs, and machines for measuring

*Adapted from a poster presentation held at the Third International Symposium on Rapid Methods and Automation in Microbiology, Washington D.C, U.S.A. May 1981.

zone diameters can improve accuracy but are not used in many clinical laboratories where, at best, comparison of test zones with zones of control organisms is made by eye with or without the aid of templates. The greatest present need is for a simple inexpensive device for rapidly obtaining standardised inocula of test and control organisms, which is essential for accurate work.

2. **Agar dilution techniques**
   Multiple inoculators allow *seeding of culture plates (each* containing a predetermined concentration of a particular antimicrobial agent) with as many as 50 test and control strains. The problems involved are mainly logistical. Strains of pathogens isolated in various parts of the laboratory need to be passed to a single work station for susceptibility testing. This may lead to transcription and labelling errors and requires additional work in collating results. Loss of job satisfaction by those who have isolated a pathogen but who are deprived of completing the work on it may arise. However, the system does work well in some laboratories.

3. **Optical density measurement of bacterial growth in liquid media**
   Some machines based on this principle perform well but the running costs are too high for most laboratories.
   Inadvertent mixed growth is not as easily detectable as in disc diffusion techniques. Uneven growth of some organisms in liquid medium may also be a problem.

4. **Other methods of detecting inhibition of bacterial growth**
   Those methods mentioned in the enumeration of living organisms section may be adapted to measure inhibition of bacterial growth in liquid media but have the disadvantages mentioned in that section.

ANTIMICROBIAL ASSAYS ON PATIENTS' SERUM

Accuracy and speed of assay (ie an answer within 6hr) are essential for proper control of drug dosage (especially aminocyclitols) administered to patients with impaired renal function or who are being dialysed.
Diffusion techniques are commonly used but clinically unacceptable *errors are all too frequent.*
Rapid enzyme and immunoassays are promising.

ENUMERATION OF LIVING BACTERIA IN URINE, WATER, MILK AND FOOD

A total undifferentiated bacterial count is usually required but a screening test for a given number of organisms may often suffice. Various approaches to automation have been made but there are problems with them all.

1. **Simple aids to manual techniques**
   A foot operated hand-set for making dilutions of bacterial suspension in agar and dispensing drops of the agar into petri dishes (droplet counter) has been most successful. Visual and electronic aids to counting bacterial colonies by hand and eye are slow and less useful, whilst electronic scanners for counting colonies are expensive and unreliable.

2. **Electronic particle counters**
   There are problems in preparing the sample in order to separate the bacteria from extraneous material and thereby achieve an adequate signal to noise ratio. In addition the aperture easily becomes blocked. It should be noted that machines designed to count blood cells give poorly reproducible results with bacteria.

3. **Bioluminescence**
   Measuring bacterial ATP as an index of bacterial content has met with limited success, and there are problems with the supply and cost of ATPase.

4. Measuring physical or chemical changes produced in liquid culture media by bacterial 'growth'

(a) *Measuring changes in pH and eH* of culture medium as a result of *bacterial activity. Both methods* are unreliable with mixed populations of unknown bacteria and are relatively insensitive.
(b) *Changes in optical properties* - several machines measure optical density as an index of bacterial growth, however there are problems with uneven growth and the technique is relatively slow. Light scattering techniques have been tried, again with limited success.
(c) *Detecting* C^{14} in CO_2 evolved by bacteria acting on substrates containing C^{14}. This method is sensitive but the correlation with numbers of colony-forming units is poor. Here there are problems with handling and disposing of the radioactive material.
(d) *Microcalorimetry*. Measuring the change in temperature resulting from bacterial metabolism and relating this to numbers of bacteria has met with some success in dedicated hands.
(e) *Measuring changes in electrical impedance or conductivity* may be useful in screening urine samples but is more promising for blood cultures.

DETECTING AND MEASURING SERUM ANTIBODIES

This aspect of microbiology is much more akin to clinical biochemistry which is now highly automated. The need to prevent bacterial contamination is not as great as in cultural methods but many of the reagents used are relatively unstable, being complex biological materials with batch to batch variation. Standard methods are therefore essential. For these reasons, and because they are often done in large batches, antibody tests lend themselves to automation.
Well-designed and efficient machines should do tests more reliably and faster than skilled manual operators. Furthermore, results can be readily fed into a computer for production of routine reports and for epidemiological analyses.
To be cost-effective, tests need to *be centralised, perhaps in* regional or state laboratories. However this poses problems with the transportation of specimens, and with ensuring that each is accompanied by adequate clinical information. In addition there is the problem of interpreting the results at a distance and distributing reports. Attempts have been made with varying degrees of success to adapt continuous-flow analysers designed for biochemical tests to perform complement fixation tests. Three major problems were found:
(1) Slow sampling rate compatible with adequate spacing between samples.
(2) Need to replenish the machine frequently with samples by hand.
(3) Need to clean and adjust the flow-cell during use.
Discrete systems of analysis seem likely to prove more suitable than continuous-flow systems for antibody tests.

DATA PROCESSING

The end product of every laboratory is information, yet most clinical and public health microbiology laboratories are still using archaic and totally manual methods of handling the data required for producing this end product and in processing the end product itself.
There is a strong case for computerised data handling in large clinical and public health microbiology laboratories.

THOUGHTS FOR THE FUTURE

The changing epidemiology of microbial diseases may profoundly affect the planning of future developments in rapid methods and automation in microbiology, e.g. the need for rapid diagnosis of smallpox has disappeared dramatically with the worldwide eradication of the disease. Can the technology of automation keep pace with such changes? If so, what of the economics? Viewed commercially, the risks are great *and the monetary rewards* doubtful. Manufacturers of laboratory equipment need competent advice and guidance from medical microbiologists.

Some suggestions

1. Select and concentrate on automating basic and repetitive aspects of the technology that are not likely to change too soon or too unexpectedly, e.g. preparation and inoculation of culture media, making serial dilutions of patients' serum, adding volumes of reagents.
2. Automate those aspects of the technology which human operators perform badly, e.g. estimating titration endpoints, microscopical scanning for organisms likely to be present in small numbers, such as acid-fast bacilli.
3. Look at novel approaches to detecting and identifying pathogens in clinical and environmental specimens, e.g. various forms of chromatography.
4. Avoid trying to automate those aspects of the technology requiring a good deal of human judgement and manual dexterity, e.g. scanning culture plates for colonies of pathogens and subculturing suspect colonies.
5. Automate data handling, including production and filing of routine reports, analysis of information on file for infection control, epidemiological studies and research.

First International Campylobacter Workshop

The first International Campylobacter Workshop organised by the Public Health Laboratory Service was held at the University of Reading on 24-26 March 1981. The 150 or so delegates present at the Workshop included leading medical and veterinary experts from many countries. The very full programme of lectures and poster *presentations included reports of* every recent finding. Considerable advances have been made in determining the special requirements of culture media and attention is currently focused on developing an efficient enrichment medium and evaluating the relative merits of various selective culture media that have been described. It is now apparent that the choice of culture medium is influenced considerably by the type of specimen.
Discussion on the special gaseous requirements indicated that much has yet to be learnt. Reports confirmed the usefulness of FBP, a mixture of ferrous sulphate, sodium metabisulphite and sodium pyruvate. This mixture increases the aero tolerance of the organism, making it possible to grow it satisfactorily in a candle jar, thus providing Third World countries with the ability to conduct Campylobacter isolation simply and inexpensively.
In addition to the lectures and poster presentations, some of Oxoid's technical staff were available to demonstrate the Company's range of products for isolating Campylobacter, including the freeze-dried antibiotic supplements devised by Dr Skirrow, Professor Butzler, and Drs Blaser and Wang who were all present at the Workshop. Oxoid have worked with these experts to develop versions of their formulae suitable for Oxoid's equipment, and is the only company in the world able to offer a complete set of Campylobacter Supplements for the growth of all the species so far discovered.
In conclusion, the Campylobacter Workshop provided an extremely useful forum for the discussion of ideas and comparison of results. Some information currently appears contradictory, but because of the degree of involvement of so many workers it is apparent that much of it will be resolved by the time of the second Workshop which has been proposed for 1983.
The following articles are based on three of the sessions at the Workshop and are a reflection of the great degree of interest currently being shown in Campylobacter.

Oxoid display at First PHL International Campylobacter Workshop.
From left to right are Dr M Skirrow, honorary consultant on Campylobacter to the PHL Service; Dr WL Wang of the Veterans Administration Medical Centre, Denver; Dr MJ Blaser of the Centers for Disease Control, Atlanta; Professor JP Butzler of the Free University, Brussels; and David Post, Oxoid's product development manager.

CAMPYLOBACTER ENTERITIS

J.P. Butzler, M.D., Ph.D., Infectious Diseases Unit, St Pierre Hospital, Free University of Brussels

During the last few years, *Campylobacter jejuni* has emerged as a common cause of acute diarrhoeal disease.

Although this organism was suspected to be a cause of acute enteritis in man as early as 1954, it was not until 1973, in Belgium that it was first shown to be a common cause of diarrhoea.[1]

Since then other workers in the United Kingdom,[2] the Netherlands,[3] Sweden,[4] the USA,[5] South Africa,[6] Canada,[7] and many other countries have reported its isolation in from 5-14% of patients suffering from diarrhoea and less than 1% of people without symptoms. The few reports that have been received from the tropics suggest that the infection is particularly common and in such areas it could well prove to be a disease of much greater importance than it is in temperate zones.[8]

Clinical features

Firstly, not all Campylobacter infections produce symptoms. Symptomless excreters commonly occur among the close contacts of infected patients, although their incidence in the total population is less than 1%. For symptomatic patients the incubation period averages from two to five days, but occasionally it extends to ten days.[5-10]

Usually, the disease starts with fever coupled with a general feeling of weakness, headache and confusion. This prodromal state lasts for up to two days and is followed by nausea and abdominal cramps.

These symptoms are rapidly followed by diarrhoea, which may be profuse, watery or slimy, and foul smelling. In 50% of cases there is fresh blood in the stools. Faecal samples examined microscopically show an inflammatory exudate with leucocytes and it is usually possible to see numerous campylobacters owing to their characteristic morphology. Sometimes nausea and vomiting cause dehydration and electrolyte imbalance which makes admission to hospital necessary. In one third of the cases the disease starts with diarrhoea without prodromal fever. The clinical features vary from those of a brief insignificant gastroenteritis to an enterocolitis with abdominal pain and bloody diarrhoea which may last for several weeks. Patients presenting with signs of acute colitis have been mistakenly thought to have ulcerative colitis. The distinction is important because treatment with steroids can have serious consequences. The help of the laboratory is therefore required in order to establish the correct diagnosis.[11-13]

Complications - the "acute abdomen"

In a few patients the abdominal pain is so severe that they are admitted to hospital as cases of acute appendicitis, cholecystitis, or peritonitis. Some undergo emergency surgery, and occasionally they do indeed have peritonitis from an acute appendicitis, but most of those that undergo laparotomy have inflammation of some part of the ileum and jejunum coupled with mesenteric adenitis which has more than once been mistaken for the lesions of typhoid fever. Most of the patients who end up in hospital are young adults and teenagers. Another potential surgical complication may arise in young infants. Diarrhoea is commonly mild at this age yet blood may appear in the stools and mislead the clinician into thinking there is an intussusception. Six instances are known where babies underwent laparotomy on suspicion of this condition.[9] Some patients develop reactive arthritis after campylobacter enteritis and there have been cases both with and without HLA B27 antigen.[14] Others have had acute cholecystitis in which *C. jejuni* has been isolated from the bile in pure culture.[9-15] An association of meningism with campylobacter enteritis has been described, and very occasionally a genuine *C. jejuni* meningitis may occur.[16]

Persistence of organisms in stools

In the absence of chemotherapy, the faeces of patients remain positive for about two to seven weeks after the illness.[7] However, a mild case may excrete the organism for only a few days and there is always the odd patient who excretes for a much longer period.

Prognosis and therapy

In general, campylobacter enteritis has a very good prognosis and the isolation of campylobacter from the stools does not necessarily warrant chemotherapy. Indeed, by the time a bacteriological diagnosis is made, it is common to find that the patient is already recovering.

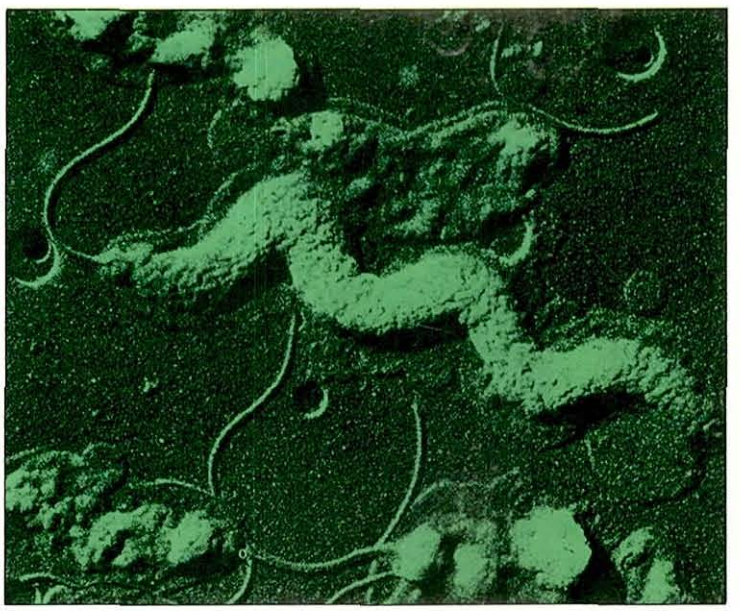

Figure 1. Electron micrograph of *C. jejuni* showing a spiral form with a flagellum at each end of the rods (x 25,000). Electron microscope studies from the Department of Prof. Dekegel, Pasteur Institute, Brussels.

Campylobacter enteritis can be treated successfully by diet with or without antidiarrhoeal compounds, but if the abdominal pain is severe or there is the possibility of a complication, it is preferable to administer antibiotics.

Campylobacters have rather unusual antimicrobial sensitivities. The minimum inhibitory and bactericidal concentrations for a variety of antimicrobial agents have been described by Belgian investigators.[17, 18, 19] The aminoglycosides, erythromycin, doxycycline, minocycline, chloramphenicol and furazolidone are the most active compounds.

Almost all strains are resistant to penicillin G, cephalothin, lincomycin, colistin and trimethoprim. Erythromycin stearate 500 mg b.i.d. for adults, or erythromycin ethylsuccinate 50 mg/kg/day for children are treatment regimens that have been used successfully.

It is preferable to carry out sensitivity tests because cases of erythromycin and tetracycline resistance have been described.

In campylobacter septicaemia, tetracyclines, erythromycin, and chloramphenicol can be used. It should be noted that penicillins and cephalosporins are totally ineffective. The prognosis in septicaemic cases is difficult to evaluate, but most patients recover after a few days of treatment. An unfavourable course is likely to be due to the presence of an underlying disease. Chloramphenicol should be considered in patients with meningitis, since it is difficult to attain an adequate concentration of aminoglycosides in the cerebro-spinal fluid.

Pathology and pathogenesis

It is clear that the main site of infection is the jejunum and ileum: *C. jejuni* has been cultured from ileal aspirates in four children with campylobacter enteritis,[20] and post-mortem examinations of patients who died of campylobacter enteritis showed hemorrhagic lesions in the jejunum and ileum.[21] Recently, several investigators showed that campylobacters can, like shigellae, salmonellae, and *Yersinia enterocolitica*, cause acute inflammation of the colonic mucosa.[11 13] Thus campylobacter colitis has to be considered in the scheme of differential diagnosis of acute colitis. In these cases a moderate inflammation and congestion of the mucosa but without ulceration can be seen on sigmoidoscopy. Mucopus is often present and the mucosa bleeds on contact. Rectal biopsy shows a flattened surface epithelium with diminution of the goblet cells.[11] The colonic mucosal crypts are infiltrated with leucocytes and sometimes there are crypt abscesses. There is a mixed inflammatory infiltrate containing lymphocytes, leucocytes and plasma cells in the lamina propria. These histological changes are sometimes difficult to differentiate from those of ulcerative colitis.

In many documented cases, campylobacter has been isolated from both the blood and the faeces of patients with enteritis. These patients developed high specific antibody titres towards their infecting organism. In some animals (monkeys, sheep and rabbits) campylobacter has been shown to invade caecal epithelium and to cause enteritis.[22]

A human volunteer suffered a typical attack of campylobacter enteritis a few days after swallowing a living culture of *C. jejuni* that had recently been isolated from a patient with the disease.[23] Experiments at the St Pierre Hospital in Brussels suggest that campylobacter produce a predominantly invasive type of infection like salmonellae and yersiniae. They have been shown to invade chicken embryo cells as well as the caecal walls of 8-day-old chicks following intragastric inoculation.[9] It is nevertheless possible that both toxic secretory and invasive mechanism operate in campylobacter infections. The pathogenesis of the disease should be further studied and a suitable animal model should be sought.

The full list of references have been omitted due to lack of space, but are available on request from the publishers, Oxoid Limited.

Campylobacter jejuni - the serological approach

Joan R Davies, M.D., Dip. Bact., Public Health Laboratory Service, 61 Colindale Avenue, London NW9 5EQ.

A session of the International Workshop on Campylobacter Infections was devoted to the problems of serotyping strains of *Campylobacter jejuni* and to techniques for the serological diagnosis of infection. The following is an attempt to summarise the current "state of the art".

Serotyping

There is clearly a need for a definitive serotyping scheme for *C. jejuni,* analogous to the Kauffmann-White scheme for salmonellas. Without such a scheme, only limited advances can be made in epidemiological investigations and in assessment of the significance of the large number of strains which can be isolated from farm animals and poultry, and from environmental sources. It is likely that *C. jejuni* strains possess a number of heat-stable and heat-labile antigens and these can be demonstrated by traditional serological techniques.

The approach used by several groups of workers has been to select a number of "representative" strains, to raise antisera and to investigate the reactions of these sera with each of the strains. After eliminating sera which give identical cross-reactions, a set of typing sera are used to investigate field strains. Generally the typing sera so selected - usually 20 to 30 - will enable about 60 to 70% of wild strains to be "typed". Strains believed to be epidemiologically related are generally found to be the same type.

On the basis of the work reported so far it seems likely that a scheme will be developed which divides strains into groups and further subdivides these groups into types. There are many technical problems involved in the production of suitable specific antisera and in the preparation of strains so that they give clear-cut results in antigen-antibody reactions. The ideal system should use the minimum of reagents and lend itself to mechanisation. A wide range of type strains from human infections, from animals and from food and environment sources will be needed to give an adequate spectrum of antigens.

Workers in this field are encouraged to exchange strains and to include the following details in their publications:

Preparation of antisera
Treatment of strains - live, formolised, heated (time/temp) etc.
Immunisation schedule
Treatment of sera - if absorbed, details of procedure
Preparation of strains for typing
Growth - media, time/temp. etc.
Treatment - live, formolised, heated (time/temp.) etc.
Standardisation
Details of technique
System - slide agglutination, tube agglutination, passive haemagglutinatination, immunofluorescence, CFT, bactericidal, etc.
Reagents, diluent etc.
Time and temperature criteria for reading end-points
Results
Specificity, sensitivity, technical problems (and their solutions)

Epidemiological investigation

The investigation of suspected outbreaks of *C. jejuni* infection cannot wait until a definitive typing scheme is available. Essentially the problem is to determine whether a number of strains, isolated from patients, animals, suspected source materials and so on, are identical. For this purpose, strains which show a characteristic pattern of reactions with sera raised against them or against strains from other sources may be assumed to be related. Useful epidemiological information may thus be obtained. Strains from such outbreaks should be preserved so that, when a typing scheme is developed, the relative importance of different serotypes in human infection can be established.

Serological diagnosis

The use of serological techniques to examine sera for evidence of past or current infection is a further area of activity. One approach has been to use a broadly reactive antigen to look for significant changes in antibody titre in sera collected early in an illness and in convalescence. An alternative is to use the strain isolated from an outbreak and to examine sera by a technique which is highly strain-specific or which detects the presence of specific antibody of the IgM class. Those who had the opportunity to examine pairs of sera from confirmed suspected cases were seldom able to demonstrate a significant rise in antibody titre. This may merely reflect the fact that the first serum was often collected relatively late after infection.

Conclusion

The interest shown by participants from many countries at this Workshop indicated that *C. jejuni* infection is a common cause of infective diarrhoea world-wide. It is to be hoped that resources will be made available to enable serological techniques to be developed. This is essential if the problems related to sources and routes of infection are to be solved and appropriate control measures introduced.

THE TAXONOMY OF CAMPYLOBACTER

M.B. Skirrow, M.B., Ph.D., F.R.C. Path., Worcester Royal Infirmary, Worcester WR1 3AS.

Campylobacter taxonomy has been bedevilled by two systems of nomenclature: that of Véron and Chatelain (1973),[1] and that of Smibert (1974).[2] Smibert's system is probably the most widely used since it appears in *Bergey's Manual of Determinitive Bacteriology,* but the French system has precedence and it was the one accepted for the *Approved Lists of Bacterial Names* (1980).[3] This article attempts to show how the two systems differ and which is most appropriate.

To begin with there are several points of agreement. It is recognized that campylobacters differ sufficiently from the classical vibrios to warrant inclusion in a separate genus, and the name *Campylobacter*[4] (Greek, curved rod) is generally accepted. So too is the placing of the genus in the family *Spirillaceae* alongside the genus *Spirillum*. There are many points of similarity between the two genera, notably in morphology e.g., curved or hellical form, flagellar arrangement, cell wall structure, and tendency to coccal transformation. There is also agreement on the nomenclature of the catalase-negative group (*C. sputorum*) except that subspecies *mucosalis*[5] is not included in the Approved Lists. The problems arise with the catalase-positive group, which includes the classical organisms of 'vibrionic' abortion of cattle and sheep (*C. fetus*) and the enteropathogenic organisms now familiar to medical microbiologists (*C. jejuni* group).

The type species

Firstly, there is disagreement over which organism constitutes the type subspecies (*C. fetus* subsp. *fetus*) - or more correctly the neotype subspecies since no examples of Smith and Taylor's original *Vibrio fetus*[6] remain. Véron and Chatelain reasoned that Florent's *V.fetus* subsp. *intestinalis*[7] was most likely to have been the organism described by Smith and Taylor in 1919, but Smibert held the alternative view and chose Florent's *V. fetus* subsp. *venerealis* as the neotype. Recent observations by a Canadian team[8] have helped to vindicate Véron and Chatelain's choice (and hence that of the Approved Lists). They showed that the wavelength and amplitude of the spirals of the two subspecies were clearly distinct and that the mean dimensions recorded by Smith matched those of Florent's *V. fetus* subsp. *intestinalis* rather than the subspecies *venerealis*.

The enteropathogenic group

Secondly, there is the question of the status of the enteropathogenic group first described in detail by Elizabeth King as 'related vibrios'[9] and shown by her to be thermophilic relative to *C. fetus*. In Smibert's classification these organisms were regarded as a subspecies of *C. fetus* (*C. fetus* subsp. *jejuni*), but in Véron and Chatelain's work[1] they appeared as two separate species: *C. jejuni* and *C. coli* (Figure 1). This arose by simply changing the generic name of strains from the *Collection of the Institut Pasteur* (CIP) that had already been designated *V. jejuni* and *V. coli* after organisms of the same name described many years before by Jones *et al*[10] and Doyle.[11] Unfortunately there were too few strains for a clear distinction to be drawn between these two 'species', hence Smibert's preference for a single grouping.

This situation suddenly changed when it was discovered that these organisms were a common cause of diarrhoea in man.[12, 13] Almost overnight we had more strains than we knew what to do with, but gradually some order began to emerge. By extending King's growth temperature tests and adapting some of the tests described by Véron and Chatelain, Skirrow and Benjamin[14] showed groupings that conformed to the CIP *C. jejuni* and *C. coli* type strains as well as two extra groupings among organisms conforming to King's 'related vibrios'. Moreover, it was apparent that *C. jejuni*-like strains were associated with cattle and *C. coli*-like strains with pigs, which was in accordance with the origins of the original *V. jejuni* and *V. coli* strains. More recently these groupings have been clarified by the introduction of tests for hippurate hydrolysis and the production of hydrogen sulphide in an iron-containing medium.[15] It is now possible by means of a few simple tests to divide these 'thermophilic' campylobacters into four groups. Clearly it no longer makes sense to regard these organisms as a single subspecies of *C. fetus* (subsp. *jejuni*). Moreover there is now ample evidence that they constitute a separate species or group of species. They have been shown to differ from *C. fetus* in morphology, cultural characteristics, modes of anaerobic respiration, cellular fatty acid composition, phage specificity, and antigenic make up.[16]

But what of the taxonomic relationship of these four groups to each other? The nalidixic acid-resistant strains (NARTC),[14] which are found mainly as commensals in seagulls, form a tightly knit group with characteristics that place them in a position intermediate between *C. fetus* and the *C. jejuni group;* in fact they probably represent a separate species. The relationship of *C. jejuni* to *C. coli* is more difficult to assess. Phenotypically they differ in only minor respects and *C. coli* might well be regarded as a biotype of *C. jejuni;* but judgment on this must await the results of DNA hybridization tests. If it turns out that *C. coli* warrants species status a further problem of nomenclature will arise. Doyle stated that his *V. coli* did not reduce nitrate (methods were not stated), but the CIP strains designated *C. coli* and the great majority of catalase-positive campylobacters do so. It is possible that Doyle's nitrate results were wrong, but it is also possible that strains conforming to Doyle's description may yet be found, so the name *C. coli* should perhaps be reserved for such a usage. This would mean that another name would have to be found for what we now call *C. coli.*

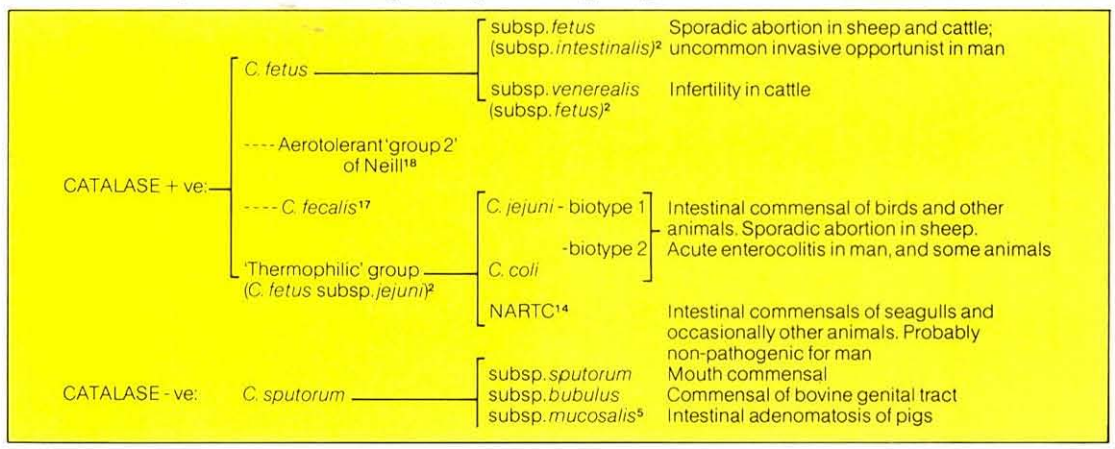

Figure 1. Classification of genus Campylobacter. Nomenclature is that of *Approved Lists of Bacterial Names* (1980)[3] except where indicated with reference number.

Miscellaneous groups

Lastly two groups of uncertain status must be mentioned. The one is *C. fecalis*, which was first described by Firehammer in 1965,[17] and the second is the aerotolerant 'group 2' of Neill and Ellis.[18] Both are probably more nearly related to *C. fetus* than *C. jejuni.*

Conclusions

To summarize we can say that there is every reason to follow the nomenclature of the *Approved Lists of Bacterial Names* (1980).[3] Apart from being the first major attempt at international standardization, the choice of the French system of nomenclature has been vindicated. Yet the clinical microbiologist is still faced with the problem of what to call the commonly isolated campylobacters of acute enteritis, particularly as biotyping is seldom routinely done. The term *C. fetus* subsp. *jejuni* is no longer tenable and the composite *C. jejuni/C. coli* is cumbersome. In view of the uncertainty about the name *C. coli* (see above), a reasonable course would be to use *C. jejuni* pro tem for all nalidixic acid sensitive thermophilic strains.

The full list of references have been omitted due to lack of space, but are available on request from the publishers, Oxoid Limited.

Culture is published as a service to microbiology by Oxoid Limited, Wade Road, Basingstoke, Hampshire RG24 0PW, England