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Isolation of *Aeromonas* sp from faecal specimens

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Aeromonas species, which favour moist conditions, have been implicated as a possible cause of diarrhoea, particularly in children under 2 years of age.¹ Although these organisms usually grow well on selective agar for isolation of *Salmonella* and *Shigella* species, it is difficult to distinguish them from other coliforms. Furthermore, if the pH of the medium is acid as a result of lactose fermentation a positive oxidase test is not always obtained,² and subculture to non-selective media for a repeat oxidase testing may be necessary.

Several specialised media have been suggested for the isolation of *Aeromonas* sp. The use of xylose is often recommended to aid identification of the organism^{3,4} as *Aeromonas* sp, unlike most members of the *Enterobacteriaceae*, do not ferment this sugar. Some authorities, however, prefer the use of blood agar with added ampicillin,¹ although this will preclude the isolation of small numbers of ampicillin sensitive strains. One advantage of this antibiotic containing medium is the detection of those *Aeromonas* sp that produce β -haemolysis on blood agar, and these are usually enterotoxigenic organisms. Bile

salts are often used in *Aeromonas* isolation media, although some *Aeromonas* sp have been reported to be sensitive to these substances.¹ Millership and Chattopadhyay⁵ confirmed other reports that brilliant green was a useful addition to selective media for *Aeromonas hydrophila*, and they recommended the use of a bile salt brilliant green agar.

A medium containing a high concentration of brilliant green, the disinfectant irgasan DP 300 (a brand of triclosan), xylose, and a mixture of unconjugated bile salts containing sodium cholate and sodium deoxycholate, was tested for its ability to isolate *Aeromonas* sp from children's faeces.

Material and methods

One faecal specimen from each of 2009 children presenting to this hospital with gastrointestinal symptoms—mainly diarrhoea—was investigated over 12 months (mid July 1985—mid July 1986) for the presence of *Aeromonas* sp (figure). One thousand, seven hundred and forty one of the specimens were stools and the remaining 268 were rectal swabs. All specimens were inoculated directly on to the test medium. Stool specimens alone were also inoculated into alkaline peptone water (pH 8.6) and incubated overnight before a further subculture was made on to the test medium. Over six months two temperatures of incubation of the alkaline peptone water (22°C and 37°C) were investigated.

The test medium was made up according to the following formula: proteose peptone (Difco) (5 g); irgasan DP 300 (Ciba Geigy) (0.005 g); beef extract (Oxoid) (5.0 g); xylose (10.0 g); bile salts No 3 (Difco) (8.5 g); sodium thiosulphate (8.5 g); brilliant green

Accepted for publication 29 April 1987

Figure Faecal specimens from 2009 children between July 1985 and July 1986.

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(0.005 g); neutral red (0.025 g); agar (Lab-M) (13.5 g); distilled water (1 litre). The irgasan was initially dissolved in a few ml of alcohol. The final concentrations of irgasan and brilliant green were decided on after titrations of 73 clinical isolates of *Aeromonas* sp with each inhibitor tested both separately and together in the medium. The pH of the medium was adjusted to 7.0–7.2 and it was autoclaved for 15 minutes at 15 lbs pressure (121 °C).

After overnight incubation *Aeromonas* sp presented as translucent colonies (1–2 mm in diameter) which were distinguished from *Pseudomonas* sp by Hugh and Leifson's oxidation fermentation test. The major part of the contaminating flora was either inhibited or appeared as mauve/green opaque colonies, often surrounded by a zone of precipitated bile salts. A satisfactory oxidase test could be obtained by direct testing of non-xylose fermenting colonies picked from the medium. Confirmatory biochemical tests for *Aeromonas* sp were carried out at 30 °C.⁶

Results

Aeromonas sp were isolated from the faeces of 89 of the 2009 patients tested, giving an isolation rate of 4.43%. Fifty one (57.3%) of the isolates were detected on primary culture and only one of the organisms grown on primary culture was not grown following alkaline peptone water subculture. Seventy one (79.8%) of the isolates were *Aeromonas caviae*, 11 (12.3%) *Aeromonas sobria*, and seven (7.9%) *Aeromonas hydrophila*. Other microbial pathogens were found in 20 (22.5%) of the 89 positive patients and consisted of eight *Campylobacter* sp, six *Salmonella* sp, five enteropathogenic *Escherichia coli*, two *Shigella* sp, two cryptosporidia and one *Giardia lamblia*. Simultaneous triple infection was detected in five patients. No viruses were detected by electron microscopy in any of the *Aeromonas* positive faecal samples. A seasonal influence on the isolation of the organism was noticed (figure), with a peak isolation rate during the summer months, as described by other workers.¹

The isolation rate for other microbial pathogens during this period was 2.6% for *Campylobacter* sp (an unexpected drop from 5.3% in the previous year), 2.5% for *Salmonella* sp, 4.13% for enteropathogenic *E coli*, 1.9% for *Shigella* sp, and 2.7% for parasites—mainly cryptosporidia and giardia. Investigations for the presence of *Yersinia* sp, *Clostridium difficile*, and enterotoxigenic *E coli* were not carried out on a routine basis. The identification rate on electron microscopy for viruses associated with diarrhoea, primarily rotavirus, was 30.5%.

Incubation of the alkaline peptone water was investigated at two temperatures, 22 °C and 37 °C,

over six months for 958 stool samples. During this period all *Aeromonas* sp detected on primary culture (total number 43) were also isolated after alkaline peptone water enrichment at both temperatures. Twenty *Aeromonas* sp were grown on secondary culture alone, and 12 of these were isolated from alkaline peptone water incubated at both temperatures. The remaining eight isolates growing on secondary culture alone were detected following alkaline peptone water incubation at 37 °C alone. No isolates were detected following incubation of alkaline peptone water at 22 °C alone.

Discussion

The isolation rate of 4.43% for *Aeromonas* sp using the medium and method described may be compared with isolation rates reported by other workers.^{4,7} Over half of the isolates obtained on the medium above were detected on primary culture and 98.6% were detected after subculture from alkaline peptone water. Satisfactory enrichment was achieved when the alkaline peptone water was incubated at 37 °C, although other workers have found a lower temperature of incubation of the alkaline peptone water to be more satisfactory.^{4,5}

Just over 20% of the *Aeromonas* positive patients had another microbial pathogen detected in their faeces and some had multiple pathogens. Although *Aeromonas* sp are common pathogens in immunosuppressed patients, their clinical importance with regard to gastroenteritis is not entirely clear, and the production of enterotoxin is considered by some workers to be an important factor.^{1,8} The most common species isolated in this study were *Aeromonas caviae*, and these are not normally considered to be enterotoxin producers.

The combination of the particular mixture of bile salts and the high concentration of brilliant green and the irgasan produced effective inhibition of many faecal coliforms, and a reliable oxidase test could be performed directly from the medium. Work at the University of Reading (Fricker CR, personal communication) has shown that a very similar medium (based on the medium described above and now commercially available from Difco Laboratories) gave more satisfactory isolation of *Aeromonas* sp from faecal samples than the blood agar ampicillin medium recommended by some other workers.¹ A further advantage of the medium is that it supports the growth of *Yersinia* sp, and satisfactory growth of *Yersinia pseudotuberculosis* type I was obtained on the medium from faeces of a child with an acute infection.

We thank Miss K Taylor for typing the manuscript.

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Rapid and reliable routine diagnosis of rotavirus using a commercial monoclonal antibody based immunoassay

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Traditionally, electron microscopy has been used for the detection of faecal viruses associated with gastroenteritis because it is the only "catch all" technique available. As rotaviruses are the most important and common cause of acute gastroenteritis in young children^{1,2} new assays have been developed for their detection. These assays allow a rapid throughput of large numbers of samples—more suitable for the busy diagnostic laboratory and for third world countries which do not have expensive electron microscopy units. There are a variety of assays currently commercially available including radioimmunoassay, latex agglutination, and solid phase enzyme linked immunoassay systems.³⁻⁷ These assays are supplemented by rotavirus RNA electrophoretic analysis⁸ which also provides invaluable epidemiological data but is too cumbersome for routine diagnostic use.

We describe here the use of a new rotavirus monoclonal based solid phase enzyme linked immunoassay (ELISA) produced by Cambridge Bioscience, Massachusetts, United States of America, and marketed by Boots Celltech Diagnostics Ltd, United Kingdom. We examined stool specimens from all patients up to 7 years of age with symptoms of diarrhoea or gastro-

enteritis from hospitals and general practitioners in Avon over three months during the winter.

Results obtained using this rotavirus (ELISA) were compared with those for an electron microscopy to evaluate both the specificity and sensitivity of the test. Specimens containing other identified pathogens (bacterial, viral, and parasitic) were also tested by ELISA to establish test specificity. In view of previous reports of false positive results obtained using other enzyme linked immunoassays for rotavirus in stool specimens from neonates⁹ results obtained from children less than 2 months old were of particular interest.

We also present our conclusions regarding simplicity of the test system and comment on its potential usefulness in the diagnosis of sporadic cases as well as outbreaks of rotavirus gastroenteritis.

Material and methods

Two hundred and sixty three faecal specimens from children less than 7 years of age were tested. These specimens had been submitted by local paediatric units and health centres from children presenting with acute gastrointestinal symptoms during December 1986 to February 1987. Specimens were examined by electron microscopy after concentration by ammonium sulphate precipitation¹⁰ and by the rotavirus ELISA (Boots Celltech Ltd). All tests were carried out without reference to previous results.

The ELISA was carried out in accordance with the manufacturer's instructions. It is an antigen capture test provided as a complete kit containing four strips of 12 wells coated with antirotavirus monoclonal antibody, enzyme conjugated monoclonal antibody, substrate/chromogen solution and all necessary controls and diluents. About 10% of faecal emulsions were made in dilution buffer in separate plastic tubes. About 100 µl of each sample was transferred to a single well of the test plate with the disposable pipette