Aeromonas spp as a potential cause of diarrhoea in children

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Abstract

Aims: To determine the prevalence of *Aeromonas* spp in the faeces of children and the association with symptoms of gastroenteritis.

Methods: Faecal specimens (n = 1026) were cultured for *Aeromonas* spp using three selective media and an enrichment broth at both 30°C and 37°C. The isolation of *Aeromonas* spp was correlated with symptoms of gastroenteritis, previous antibiotic use, and environmental temperature.

Results: Aeromonas spp (n = 28) from 26 (2·5%) patients were recovered. Bile salt, Irgasan, and brilliant green agar was the most efficient selective culture medium. Eleven of the patients had symptoms of gastroenteritis, usually mild diarrhoea of two to three days' duration, in the absence of other recognised enteropathogens. *A caviae* was a particularly frequent isolate (nine out of 11 cases) in symptomatic individuals. Only one out of seven *Aeromonas* spp recovered by enrichment culture alone was possibly associated with symptoms of gastroenteritis. There was a close correlation between the environmental temperature and isolation of *Aeromonas* spp.

Conclusions: Aeromonas spp and particularly *A caviae* may cause gastroenteritis in children, most often during warmer months of the year. Culture for these potential enteropathogens could be confined to summer and autumn months. Bile salt, Irgasan, and brilliant green selective agar, but not alkaline peptone water enrichment, is an efficient culture medium for recovering possibly clinically important isolates.

(*J Clin Pathol* 1992;45:959–963)

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The potential role of mesophilic aeromonads as a cause of gastroenteritis remains controversial.[1] Geographically, there seems to be a pronounced variation in the isolation rate of *Aeromonas* spp from human faeces. For example reports from Australia,[2] Djibouti,[3] India,[4] Peru[5] and the USA[6] note isolation rates of 11%, 3%, 2%, 58%, and 1% from diarrhoeal stools, respectively. *Aeromonas* spp were detected in the faeces of controls in the same studies in 1%, 0%, 0%, 9%, and 0% of cases. However, the use of varied isolation procedures and different study populations (chil-

dren, adults, community and hospital patients, for example) makes comparison of these rates difficult. Furthermore, as the detection of *Aeromonas* spp has been shown to be seasonally associated,[2 5 6] studies should ideally report isolation rates over 12 month periods, or multiples thereof.

Of the three commonly recognised phenotypes, *A hydrophila* and *A sobria* have generally been considered to be the most virulent, with *A caviae* rarely being implicated as a pathogen.[7–9] However, recent studies have indicated that *A caviae* may indeed be an enteropathogen, particularly in children.[5 10 11] One of these[11] noted an association between high stool pH and the isolation of *A caviae*, possibly related to the poor tolerance (suicide phenomenon) of acidic conditions by this species.[12]

We report the results of a hospital-based study lasting 12 months to determine the isolation rate of *Aeromonas* spp from the faeces of children in Sheffield. Clinical evidence of enteropathogenicity for each of the major species was determined by recording symptoms and signs of gastroenteritis. Evidence to support the association between stool pH and *A caviae* isolation was also sought. To maximise the isolation rate and to determine the most efficient means of detecting *Aeromonas* spp in faeces, we used three different selective media at both 30°C and 37°C, and also an enrichment medium.

Methods

A total of 1026 faecal specimens from children with gastrointestinal symptoms, submitted to the Bacteriology Department at the Sheffield Children's Hospital between November 1990 and October 1991, were examined. This represented 60% of all the faecal specimens received, as only those samples that could be processed within 24 hours of receipt were included in the study. Eighty five per cent of the specimens were from inpatients with the remainder equally divided between attenders at the Accident and Emergency Department and outpatients.

Routine investigations for enteropathogens were performed on all the study specimens. These included wet film microscopy, a modified Ziehl-Neelsen stain for cryptosporidia, culture on MacConkey's, deoxycholate, brilliant green and charcoal-cefaperazone agars, and selenite broth enrichment. Specimens were also examined by electron microscopy if virological investigation was requested by the clinician; 40% of the study specimens were
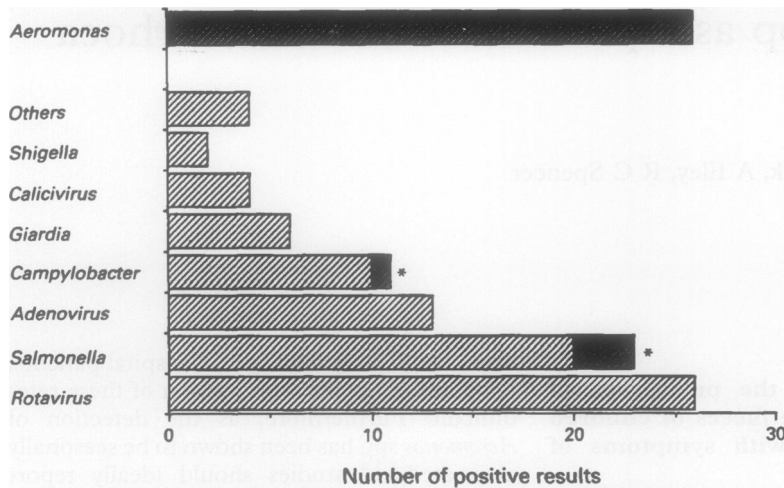
Figure 1   Enteropathogens and aeromonads isolated from 1026 faecal specimens.
*Aeromonas isolated together with one campylobacter and three salmonellae.

examined as such.

For the isolation of *Aeromonas* spp a sterile swab was used to inoculate faeces on to two whole plates of each of the following media: ampicillin (30 g l^{-1}) blood agar, comprising 7% horse blood and Oxoid Columbia agar base (ABA)[13]; Difco Aeromonas agar (bile salt, Irgasan, brilliant green agar) (DAA)[14]; and xylose deoxycholate agar (XDCA) (Life Technologies Ltd, Scotland).[15] One of each pair of plates was incubated overnight at 30°C and the other at 37°C. A loopful of faeces was also inoculated into alkaline peptone water (pH8·6), which was incubated overnight at 37°C and then subcultured on to half-plates of ABA, DAA, and XDCA.

After incubation, potential Aeromonas colonies (translucent colonies on DAA and pale yellow colonies on XDCA) were screened for oxidase production using filter paper impregnated with tetramethyl-p-phenylenediamine. All oxidase positive colonies were subcultured for purity, and a Hugh and Leifson (oxidation/fermentation) test was done. Fermentative, oxidase positive isolates were tested for resistance to the vibriostatic agent 0129 by disc diffusion using 10 µg and 150 µg discs (Oxoid). Those isolates resistant to the higher level of 0129 were then phenospeciated by three methods: conventional biochemistry according to the criteria of Janda *et al*[16]; the suicide phenomenon and aesculin production[12]; API 20 NE strips and haemolysin production (Wilcox MH, Cook AM, Geary I, Spencer RC, abstract 729; 17th International Congress of Chemotherapy 1991).

The pH of each study specimen was measured by direct contact between Whatman pH paper (BDH) and faeces.

Maximum and minimum temperatures for each day of the study period were obtained from the local meteorological office. This is situated immediately opposite the Sheffield Children's Hospital. The data were converted to mean weekly temperatures by averaging 14 daily figures.

Patients' details, including an antibiotic history, were collected to determine the potential significance of specimens positive for *Aeromo-*

nas spp. The former data were collated without knowledge of the culture results. Diarrhoea was defined as multiple loose stools of more than one day's duration. Gastroenteritis was considered to be present if there was diarrhoea plus or minus additional symptoms such as nausea, vomiting, and abdominal pain.

The χ^2 test with Yates' correction for small numbers was used to compare the numbers of *A caviae* in symptomatic and asymptomatic patients; the Wilcoxon rank sum test was used to compare their ages.

Results

A total of 89 accepted enteropathogens were recovered from faecal specimens, equivalent to an isolation rate of 8·7% (fig 1). Twenty six specimens (2·5%) contained 28 *Aeromonas* spp isolates (two each containing two different isolates). In four of these 26 cases *Aeromonas* spp were isolated together with known enteropathogens (three salmonellae and one campylobacter). The 28 *Aeromonas* spp comprised 17
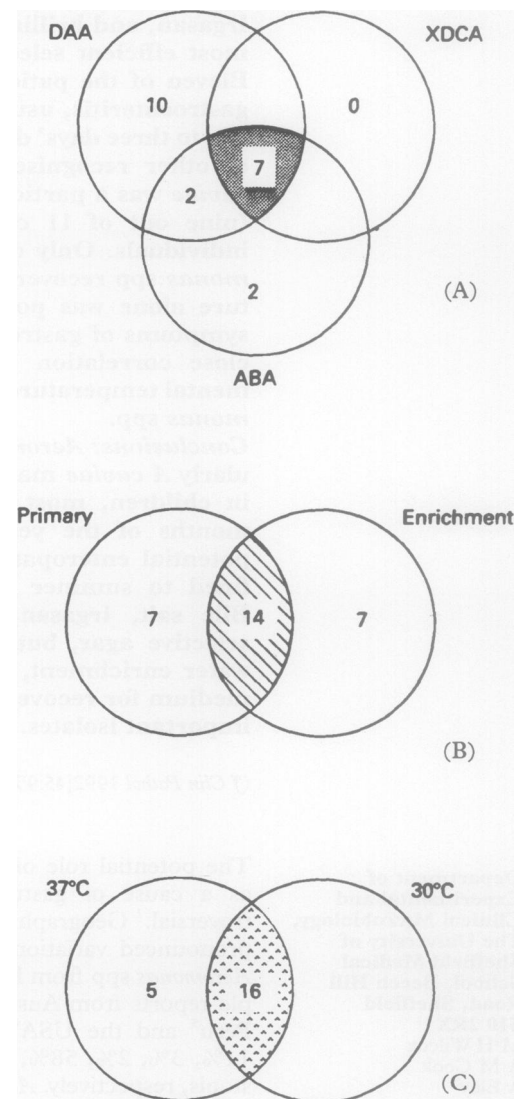

Figure 2   Isolation of aeromonads according to primary media type (A), primary or enrichment culture (B), and temperature of primary culture (C). Figures refer to number of isolates.
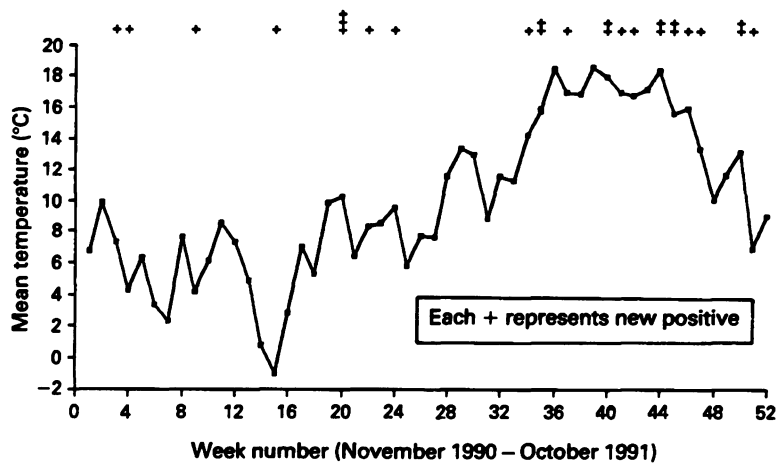
Figure 3 Correlation between mean weekly atmospheric temperature over 12 months and isolation of Aeromonas spp from faeces.

(61%) *A caviae*, eight (29%) *A hydrophila*, two (7%) *A sobria*, and one isolate that could not be identified. Apart from the latter isolate, the three different methods of phenospeciation gave concordant results.

Figure 2 shows the relation between culture media and temperature conditions with the isolation of *Aeromonas* spp. Of the 21 isolates recovered on primary culture plates, 19 grew on DAA, 11 on ABA, and seven on XDCA. All the isolates grew on either DAA or ABA, although a quarter were recovered only after alkaline peptone water enrichment. Notably, only two of the seven isolates recovered by enrichment alone came from patients with symptoms of gastroenteritis (see below); in one of these cases an accepted enteropathogen was also isolated. Incubation of primary cultures at 37°C increased the yield of positive results by five isolates compared to 30°C. The routine laboratory methods failed to detect *Aeromonas* spp in any of the study faecal specimens.

Interestingly, of the 28 isolates, 17 were later found to be lactose positive on MacConkey's agar; only three out of 17 *A caviae* isolates were lactose negative. The pHs of faeces from which *A caviae* was isolated (range 6·0–8·5, median 7·0) were similar to those containing other *Aeromonas* spp (6·0–8·5, median 7·0), and also to negative specimens (6·0–8·5, median 6·5).

The isolation of *Aeromonas* spp was more common during periods of warmer weather

(fig 3). There were only two positive faecal specimens when the mean weekly environmental temperature was less than 7°C. After a cluster of isolations in March–April 1991 (weeks 20–24), two months (weeks 25–33) elapsed without any positive specimens. In March 1991 (weeks 17–21) the mean temperature was 8°C compared with a 30 year (1951–1980) historical average of 5·5°C. Conversely, the month of June (weeks 30–34) saw a mean temperature of 12·2°C which was 2·2°C lower than the historical average.

The age range (and median age) of all study patients and of those with *Aeromonas* spp in their faeces was 1 week to 15 years (11 months) and 1 month to 9 years (16 months), respectively. Symptomatic Aeromonas positive patients were generally younger (1 month–3 years; median 1 year) than asymptomatic individuals (5 months–9 years; median 2 years); $0.05 < p < 0.1$. Of the 26 patients from whom *Aeromonas* spp were isolated, 11 had no diarrhoea nor any other symptoms of gasteroenteritis (table 1). Six of them, however, were either receiving antibiotics at the time of isolation or had done so within the previous two weeks; three patients received amoxycillin, two had penicillin and flucloxacillin, and one was given cefuroxime and metronidazole. Faecal specimens were sent to the laboratory in five of these 11 cases because of the passage of a single loose/offensive stool. Three of the 11 *Aeromonas* positive patients who did have diarrhoea (but no accepted enteropathogen) had a recent history of antibiotic treatment— one with amoxycillin, one with penicillin, and one with erythromycin. The duration of diarrhoea ranged from two to 21 days, with a median of three days. Two patients had associated abdominal pain and three had vomiting, of which one (a 15 month old baby with an ileostomy) became clinically dehydrated. Although the numbers are small, the isolation of *A caviae* was particularly common in symptomatic (nine out of 11) compared with asymptomatic patients (five out of 11); $p > 0.1$.

Discussion

Accumulating evidence suggests that in at least a proportion of cases *Aeromonas* spp can cause gastroenteritis.[1][2][5] There are several weaknesses in this hypothesis: a lack of a suitable animal model; inconsistent results in human volunteer challenge studies[17]; and an absence of reported outbreaks of infection. As the major virulence determinants of *Aeromonas* spp have still to be clarified, it is difficult to perform adequate animal and human challenge studies. Strains used in such work may lose their pathogenicity during subculture and storage. A recent report documented for the first time that an identical strain of *A hydrophila* was isolated from a patient with acute gastroenteritis and from recently consumed prawns.[18]

This study is the second largest United Kingdom report of the prevalence of *Aeromonas* spp in the faeces of paediatric inpatients.

Relation of Aeromonas spp isolated to clinical setting

Clinical setting	No of cases positive for Aeromonas spp	Antibiotic use within previous two weeks	Identity of Aeromonas spp
Diarrhoea (no accepted enteropathogen)	11	3	9 A caviae 2 A hydrophila
Diarrhoea (with accepted enteropathogen)	4	0	3 A caviae 1 A sobria
No diarrhoea	11*	6	5 A caviae 6 A hydrophila 1 A sobria 1 Unknown

* In two cases both *A caviae* and *A hydrophila* were isolated; one of the isolates could not be speciated.

An earlier report noted that 4·4% of specimens contained *Aeromonas* spp, but did not correlate isolation with clinical details. The isolation rate of *Aeromonas* spp from routine faecal specimens in this study was 2·5%, similar to that for the accepted enteropathogens salmonellae (2·2%) and rotavirus (2·5%). We considered the possibility of collecting specimens from a control population of asymptomatic children, but following earlier unsuccessful results in a pilot study this proved impracticable. Our use of three culture media, an enrichment broth, and two culture temperatures to isolate *Aeromonas* spp makes it unlikely that we had false negative specimens. Several different commercially available selective media and ampicillin blood agar have been suggested for isolating *Aeromonas* spp.[19 20] In our hands DAA performed very well, and in only two out of 21 cases did *Aeromonas* spp fail to grow on it on primary culture; these two isolates were recovered on ABA. DAA contains brilliant green, bile salts, and the disinfectant Irgasan as selective agents. The usefulness of brilliant green has been noted earlier,[21] and Irgasan has also been incorporated successfully into another selective medium (CIN; cefsulodin, irgasan, novobiocin).[20] DCA inhibits commensal flora by virtue of bile salts, but it performed poorly in the present study and in this formulation cannot be recommended.

We confirmed the findings of others that the use of alkaline peptone water enrichment increases the yield of faecal specimens positive for *Aeromonas* spp.[21 22] Studies that do not incorporate such enrichment techniques are likely to underestimate significantly the prevalence of *Aeromonas* spp in faeces. However, the clinical importance of isolates recovered by enrichment alone must be considered to be extremely doubtful. Robinson *et al* found that the use of enrichment culture frequently detected *Aeromonas* spp in asymptomatic individuals.[23] Similarly, we observed that only one out of seven isolates detected by alkaline peptone water enrichment alone came from a patient with gastroenteritis, in the absence of a recognised pathogen.

We were prompted to compare isolation at 30°C and 37°C, because preliminary studies with a collection of 30 *Aeromonas* spp had shown two strains that failed to grow on at least one of the media at 37°C. However, no isolates were recovered from faeces on media incubated at 30°C compared with those incubated at 37°C.

Clinical assessment of *Aeromonas* spp positive individuals not otherwise infected with a known enteropathogen suggested that half of the cases had symptoms attributable to these micro–organisms. Undoubtedly, *Aeromonas* spp are carried asymptomatically by some individuals.[5 24] However, this is also true for well recognised enteropathogens such as salmonellae. In this study children with gastroenteritis and *Aeromonas* spp in their faeces generally had non-profuse diarrhoea of short duration (median three days). Vomiting and abdominal pain were present in only a few cases. The affected children were 3 years old or

younger, while four (36%) of those carrying *Aeromonas* spp asymptomatically were at least 5 years old. An association between antibiotic use and isolation of *Aeromonas* spp noted elsewhere was most apparent in asymptomatic children in the present study.[25] *A caviae* predominated among the clinical isolates, particularly in those from symptomatic individuals (82%). These results support the recent findings that *A caviae* seems to be a relatively common isolate from the faeces of young children with gastroenteritis.[5 10 11] We were unable to confirm an association between alkaline faecal pH and isolation of *A caviae*.[11] However, the patients positive for *A caviae* reported by Namdari and Bottone[11] were mostly less than 1 year old. In our series the three infants of less than 1 year old with gastroenteritis possibly caused by *A caviae* had stool pHs of 8·0 (twice) and 7·5; all were bottle fed. The alkaline faecal pH found in bottle fed babies may indeed help to select for *A caviae* and explain the relatively high isolation rate of this species in infants.

Aeromonas spp are widely distributed in fresh water, sewage, marine environments close to freshwater outlets, and drinking water.[26–28] Maximal levels of these bacteria in fresh water occur during summer and early autumn. *Aeromonas* spp have also been suggested as potential foodborne pathogens (see above), which is supported by their high prevalence in supermarket meat samples (58–74%) and raw milk (27%).[29] Our data confirm the close relation between environmental temperature and isolation of *Aeromonas* spp from faeces.[5 6 25] Laboratories could focus their efforts to isolate these bacteria during the warmer months to limit the additional costs of the extra media required.

We would encourage attempts to isolate *Aeromonas* spp from well defined populations to determine more closely their prevalence and potential to cause gastroenteritis. Selective (DAA) but not enrichment media are recommended. Uncertainties concerning the taxonomy of *Aeromonas* spp and the virulence of given species will not be resolved without such studies.

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