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Faecal carriage rate of *Aeromonas hydrophila*

SALLY E MILLERSHIP, STEPHEN R CURNOW, B CHATTOPADHYAY

From the Public Health Laboratory and Department of Microbiology, Whipps Cross Hospital, London E11 1NR

SUMMARY Over a four and a half month period, 1004 unselected routine faecal specimens from 815 patients were cultured for *Aeromonas hydrophila*. Forty-two specimens (4·2%) representing 38 patients were culture-positive. The study specimens also yielded *Salmonella* on 116 occasions, *Shigella* on seven, *Campylobacter species* on six and other bacterial pathogens on 17 occasions respectively. Seven specimens had *A hydrophila* together with another bacterial pathogen. In only 19 of 38 patients (50%) was *A hydrophila* possibly associated with gastrointestinal symptoms. All the *Aeromonas* isolates were resistant to ampicillin but sensitive to gentamicin, piperacillin, mecillinam, chloramphenicol, ceftazidime and colistin.

The role of *A hydrophila* as a potential pathogen in the gut has yet to be determined. Cases of diarrhoea in humans have been attributed to this organism in reports from all over the world, usually when it was isolated as the predomimant aerobic faecal flora.[1–5] Symptoms are most often a short-lived, self limiting episode of diarrhoea with occasional nausea, vomiting or abdominal pain.[4] Some strains of *A hydrophila* have also been shown to be enterotoxigenic.[6]

It is known that the natural reservoir of *Aeromonas* spp is water and soil[7 8] but it has been claimed that particularly in adults the faecal carriage rate is very low.[4] Overall isolation rates of 0·7%[4] and 0·2%[9] reported from two studies of several thousand faecal specimens would seem to bear this out. However, von Graevenitz and Zinterhofer examining stools of hospital inpatients without any gastrointestinal symptoms found a 3·2% isolation rate using DNase agar,[10] and more recently an Indian study using alkaline peptone water enrichment had an isolation rate of 8%.[11]

The present study was launched to see whether the recovery rate could be improved by the introduction of better quality enrichment and selective media, and at the same time to attempt to determine whether any cases of gastroenteritis could be ascribed to the organism.

Material and methods

One thousand and four unselected stool specimens from 815 patients were examined in an 18-week period. All specimens were examined for *Salmonella, Shigella* and *Campylobacter* species by recognised methods including, where the history indicated, for enteropathogenic *Escherichia coli, Clostridium welchii, Staphylococcus aureus* and *Clostridium difficile*.

Xylose deoxycholate citrate agar (XDCA), was prepared according to a protocol from Maidstone Public Health Laboratory (personal communication). See Appendix.

All specimens were streaked directly onto ½ plate of MacConkey's agar (Oxoid) and ½ plate of XDCA and incubated overnight at 37°C. Approximately 1 g of faeces was emulsified in 10 ml alkaline peptone water (Central Public Health Laboratory, Colindale), and left overnight on the bench before subculturing from the top layers onto XDCA. The last 319 specimens were also emulsified similarly in Hajna Gram-negative broth (Bacto) and left on the bench overnight, then subcultured onto XDCA. MacConkey plates were examined for non-lactose fermenting colonies and XDCA for non-xylose fermenters. Oxidase reactions were tested directly from these plates by the method of Kovacs.[12] For the first 314 specimens non-xylose fermenting colonies were also subcultured to blood agar before oxidase testing.

Oxidase-positive colonies were tested by Hugh and Leifson's O/F test and fermentative organisms inoculated onto API 20E trays and tested for sensitivity to vibriostatic agent 0/129 with a paper disc impregnated with 150 μg. Oxidase-positive, fermentative, 0/129 resistant organisms with a biochemical profile consistent with *Aeromonas hydrophila* on API 20E were called *A hydrophila*.

β-haemolysis was observed after 18 h incubation

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on Difco horse blood agar plates and scored according to the following system:

0 = no haemolysis
\+ = haemolysis under the colony only
++ = haemolysis with a narrow margin around the colony
+++ = haemolysis with a zone greater than the radius of the colony.

Lactose fermentation was observed on MacConkey's agar after 18 h at 37°C. A lactose-fermenting strain of *E coli* and a *Proteus* spp were used as positive and negative controls.

Sensitivity testing was done by disc diffusion using the Stokes method.[13]

Results

In no case was *A hydrophila* isolated as the predominant aerobic faecal flora. There were 42 isolates from 1004 specimens giving an isolation rate of 4·2%. Some patients' faeces were cultured more than once; thus 815 patients were examined of whom 38 (4·7%) had *A hydrophila*. Two patients had the organism on three occasions. In one patient the specimens were taken two days apart and in the other over a period of 14 days. Ten (6·4%) of 157 specimens from hospital inpatients and 30 (3·6%) from 830 other sources were positive for *Aeromonas*. This was not statistically significant (null hypothesis: $\chi^2 = 2{\cdot}43$ $0{\cdot}2 > p > 0{\cdot}1$). There was also an excess of isolations from female patients—22 (58%) of the 38 patients were female, 15 (39%) male and one (3%) was unknown. *A hydrophila* was isolated in persons from one month to 88 yr of age. Assuming no age group is disproportionately represented in the unknown section, it will be seen from Table 1 that there is an excess of isolations in children under 14 yr and in the over-60s. These differences are significant (null hypothesis: $\chi^2 = 9{\cdot}45$ $0{\cdot}001 < p < 0{\cdot}010$). Nineteen (50%) of the 38 patients had other gastrointestinal disease. Eight had other enteric pathogens isolated with *Aeromonas*, a further four on another occasion and seven had tumours, drug-induced diarrhoea, inflammatory bowel disease or viral disease. Two positive isolates were from healthy persons and one from a baby negative during a diarrhoeal illness but positive three weeks later. Table 2 gives details of other pathogens isolated. There was no significant association between culture of *A hydrophila* and culture of another bacterial pathogen (null hypothesis: $\chi^2 = 0{\cdot}16$ $0{\cdot}8 > p > 0{\cdot}5$). Table 3 shows that there is an excess of isolations from liquid stool specimens, but this is not statistically significant ($\chi^2 = 2{\cdot}71$, $p = 0{\cdot}1$).

Alkaline peptone water enrichment proved to be the best method of isolation. Forty (95%) of 42 isolations were from alkaline peptone water subcultured to XDCA. Thirteen isolations (31·0%) were also obtained from direct plating onto XDCA. In one case *A hydrophila* was recovered by direct plating onto MacConkey agar from a specimen not included in the study. This specimen was from a patient who had already had *A hydrophila* isolated on XDCA and it was the sole occasion on which any strain was obtained from MacConkey medium. Three hundred and nineteen specimens were also inoculated onto G-N broth. Seven isolates were obtained in this enrichment medium compared with thirteen from alkaline peptone water.

The first 314 specimens of faeces grew non-xylose fermenters on 285 occasions. In some instances, more than one type of non-xylose fermenting colony was present, giving a total of 292 isolations altogether. Of these 207 (71%) were oxidase-negative, none of which was positive on subculture,

Table 1 *Aeromonas isolations related to age. There is an excess of isolations in children under 14 yr and in the over 60s. These differences are statistically significant ($\chi^2 = 9{\cdot}45$ $0{\cdot}0001 < p < 0{\cdot}010$)*

	Positive for A hydrophila	*All specimens*	*% Isolation of A hydrophila*
Under one year	3	37	8·1%
1–13 yr	11	152	7·2%
14–59 yr	7	385	1·8%
60 yr and over	7	117	6·0%
Unknown	10	313	3·2%

Table 2 *Other pathogens isolated in association with Aeromonas hydrophila. No significant association ($\chi^2 = 0{\cdot}16$ $0{\cdot}8 > p > 0{\cdot}5$)*

Organisms	*Positive for A hydrophila Number (% of 42)*		*All specimens Number (% of 1004)*	
Salmonella typhi	1	(2·4)	3	(0·3)
Other Salmonellae	2	(4·8)	113	(11·3)
Shigella	1	(2·4)	7	(0·7)
Campylobacter	1	(2·4)	6	(0·6)
Staphylococcus aureus	Nil	(Nil)	1	(0·1)
Clostridium welchii	2	(4·8)	14	(1·4)
Parasites	1	(2·4)	2	(0·2)
Totals	8	(19·0)	146	(14·6)

Table 3 *Isolations by type of stool. There is an excess of isolations from liquid stool specimens, but this is not statistically significant ($\chi^2 = 2{\cdot}71$, $p = 0{\cdot}1$)*

	Positive for A hydrophila	*All specimens*	*% Isolation of A hydrophila*
Formed	28	846	3·3%
Liquid	13	123	10·6%
Unknown	1	35	2·8%

which is in agreement with the work of Overman *et al.*[14]

Some characteristics of the *Aeromonas* strains relevant to isolation were studied. Thirty-six strains were tested for β haemolysis. Nine (25%) showed no haemolysis, eight (22·2%) had + haemolysis, seven (19·0%) had ++ and only 12 (33·3%) +++ haemolysis. Nearly half the strains (17 of 37, 45·9%) were non-lactose fermenting. Two strains from patients in the same family appeared to be identical in colony appearance, biochemistry and antibiogram.

The antibiotic sensitivities of 37 strains were determined. All strains were sensitive to piperacillin, gentamicin, chloramphenicol, mecillinam, colistin and ceftazidime. All the strains were resistant to ampicillin, erythromycin, clindamycin, cephaloridine and cephradine.

Discussion

We have screened a fairly large group of patients either with gastrointestinal symptoms or their asymptomatic contacts. Our isolation rate of 4·2% is comparable with that of Shread *et al*[15] who obtained an overall rate of 5% using similar techniques. It is also similar to the 3·2% rate obtained by von Graevenitz and Zinterhofer in asymptomatic hospital inpatients using DNase agar.[10] The earlier reports of very low isolation rates could be due to geographical variation but it is much more likely that poor media contributed to these results. It is established that most *Aeromonas* spp grow well on MacConkey, deoxycholate citrate or Salmonella Shigella agar and earlier investigators used these media.[4 9] Unfortunately nearly half our strains would appear as lactose fermenters. Even if an oxidase test is performed some lactose fermenting strains may appear negative.[14] Our results also suggest the organism is easily overgrown on MacConkey's medium. Thus we find that lactose containing media are highly unsatisfactory for primary isolation. Reliance on β haemolysis on blood agar as a diagnostic test is also unwise since 24% or our strains appeared non-haemolytic. Alkaline peptone water seems to be a very effective enrichment medium, certainly superior to G-N broth, and XDCA a good selective agar. The report from Australia suggesting that 2% of *Aeromonas* strains do not grow on bile[16] means that the most efficient isolation system may be alkaline peptone water enrichment with plating onto XDCA and a blood agar plate containing ampicillin.

The antibiotic sensitivities of our strains are in broad agreement with those of other workers. No strain of *A hydrophila* has been reported sensitive to ampicillin. Drugs which are most likely to be useful in serious sepsis include gentamicin, chloramphenicol, piperacillin and ceftazidime.

Aeromonas isolates are most often recovered from gastrointestinal tract and intra-abdominal sites[6 17] although some workers do not consider *Aeromonas* species to be part of the normal human faecal flora[4] from evidence based on early studies with very poor isolation rates. Acute, severe diarrhoea associated with recovery of *Aeromonas* species in the stool has been recorded[11 18] and experimental evidence from animal models suggests that at least certain strains of *Aeromonas* are enteropathogenic.

However, in our study half the patients with *A hydrophila* in their stools had another recognised cause for any gastrointestinal symptoms. Other studies have not stated the relation between isolation of *Aeromonas* and the presence of other gastrointestinal disease.[11] It is possible that an increased rate from diarrhoeal stools merely represents increased colonisation of already diseased bowel, although we were unable to show an association between isolation of other pathogens and *Aeromonas* in contrast to Shread *et al*.[15] It is interesting to note that we have found an excess of isolations in infants and the elderly which are the two groups most likely to have impaired host resistance. A recent study from Australia[16] also found a high isolation rate in younger children.

In order to determine the role of *Aeromonas* in diarrhoeal disease the faecal carriage rate in healthy adults outside hospital needs to be established, together with the relation to production of enterotoxin. It is by no means clear that *A hydrophila* is a primary pathogen in the gut of adults.

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Appendix

Xylose deoxycholate citrate agar (XDCA)

Nutrient broth (No 2 Oxoid CM67)	12·5 g
Sodium citrate	5·0 g
Sodium thiosulphate	5·0 g
Ferric ammonium citrate (brown)	1·0 g
Sodium deoxycholate (Koch-Light 84274)	2·5 g
Agar (Davis)	12·0 g
Xylose	10·0 g
Neutral red (1% solution in distilled water)	2·5 cm^3
Distilled water	1000·0 cm^3
pH 7·0	

Add distilled water and gently heat with constant mixing to 100°C; allow to simmer for 20 s; remove from heat and cool to 50°C before pouring plates.

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Requests for reprints to: Dr B Chattopadhyay, Director/Consultant Microbiologist, Public Health Laboratory, Whipps Cross Hospital, Whipps Cross Road, London E11 1NR, England.