A 15-year study of the role of *Aeromonas* spp. in gastro-enteritis in hospitalised children

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Summary. During a 15-year period, 146 strains of *Aeromonas* spp. were isolated from 32810 faecal specimens from 13820 hospitalised patients up to 13 years of age. These isolates constituted 4% of all the pathogenic bacterial strains cultured. For the years 1978–1988, the files of children with gastro-enteritis revealed 81 whose faeces yielded *Aeromonas* spp. Most of them (94%) were < 3 years of age, 78% < 1 year old. The peak incidence was at 2–6 months, involving severe morbidity including dehydration and vomiting with acidemia and azotemia; the mean duration of illness and length of hospitalisation at this age were longer than at other ages. Bloody diarrhoea was found in 7% of the children. Almost all the strains of *Aeromonas* were resistant to ampicillin. We conclude that *Aeromonas* spp. are of aetiological significance in gastro-enteritis in small children; culture for this pathogen should be routine in the bacteriological examination of faeces.

Introduction

Aeromonas spp. are ubiquitous in soil and untreated water, causing diseases of fish and amphibians.¹ These facultatively anaerobic, rod-shaped bacteria in the family Vibrionaceae are considered today as potential enteric human pathogens around the world.^{2–4} They were considered by some authors as opportunist pathogens in immunocompromised patients,^{3,5,6} but today they are recognised as causes of a spectrum of gastrointestinal diseases from self-limiting diarrhoea to acute, persistent dysentery.^{3,7–10} They have been isolated from children with acute diarrhoea and from adults with traveller's diarrhoea.^{11,12} Enterotoxins, cytotoxins and haemolysins were described as possible virulence factors of aeromonas^{3,13} although their role in pathogenesis is not clear. Drinking of untreated water is the most probable manner of exposure to aeromonas.¹⁴

The present retrospective study of aeromonas infections was undertaken to investigate the bacterial species involved, their sensitivity to antibiotics, the seasonal distribution of the disease, the age and sex incidence, and the range of patients' symptoms.

Materials and methods

Patients

During the 15 years, 1974–1988, faecal specimens

from 13820 hospitalised children with acute gastro-enteritis were cultured. The patients, up to 13 years of age, had been admitted with diarrhoea often associated with dehydration, weight loss, vomiting or acidosis. To evaluate the significance of aeromonas as the possible cause of the disease, we analysed only the files of children whose faecal specimens yielded this organism as the sole pathogen.

Statistical analysis of data was performed by the χ^2 method.

Bacterial culture and identification

A faecal specimen was collected from each patient immediately after admission, before treatment; sometimes additional specimens were taken. Undiluted samples were inoculated routinely on MacConkey and Salmonella-Shigella agar plates for the isolation of enteroinvasive, enteropathogenic and enterotoxigenic *Escherichia coli*, salmonella and shigella. For each, confirmative agglutination tests were performed.

Since 1974 we also examined for *Aeromonas* spp. Colonies were screened for oxidase by Kovacs' method,¹⁵ bacteria from one colony being applied to filter paper moistened with two drops of reagent. Oxidase-positive, shiny colonies were transferred to a tube of Kligler iron agar and to semi-solid urea medium. Motile organisms that produced indole but not urease, and fermented glucose rapidly, but lactose only slowly, were further tested with API 20E (API System GA, La Balme Les Grottes, Montalieu – Vercieu, France): identification as *Aeromonas* spp. was confirmed by hydrolysis of arginine and liquefaction of gelatin but absence of ornithine decarboxylase.

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Fig. 1. Yearly distribution of enteric pathogens isolated during a period of 15 years: aeromonas ■, *E. coli* ▨, salmonella ▤, shigella □, campylobacter ▦.

Table I. Patients from whom one or more enteric pathogens were isolated during 15 years (1974–1988)

Pathogen isolated	Number (%) of patients excreting pathogen in one or more specimens (n = 2567)	Number (%) of specimens that yielded		
		one or more pathogens (n = 4045)	one pathogen (n = 3926)	more than one pathogen (n = 119)*
<i>E. coli</i>	1272 (50)	1840 (45)	1750	116
Shigella	759 (30)	1257 (31)	1196	68
Salmonella	496 (19)	781 (19)	747	42
Aeromonas	103 (4)	146 (4)	141	5
Campylobacter	76 (4)†	101 (4)†	92	9
Total isolates	...	...	3926	240

* Number of specimens with two or more pathogens of the same genus or of different genera.

† Derived from 1757 patients since the year 1982, whose 2392 specimens yielded one or more pathogens.

Lack of sensitivity to the vibriostatic agent O129 was also used to differentiate *Aeromonas* from *Vibrio*.

Identification of aeromonas to species level was performed by the Central Laboratories of the Ministry of Health, Jerusalem, and was based on production of gas from glucose, of acetoin, H_2S and lysine decarboxylase, and on aesculin hydrolysis and fermentation of arabinose and salicin.¹⁸

Antibiograms were performed by the Kirby-Bauer disk method on Mueller-Hinton agar.¹⁷ The strains were defined as susceptible, resistant or of intermediate resistance, as recommended by Barry and Thornsberry.¹⁸

Since 1982, specimens were also examined for campylobacter, on Butzler medium prepared with Columbia agar and containing human blood 6%.

Results

During the period 1974–1988, 32810 faecal specimens from 13820 children were cultured for enteric pathogens. The yearly distribution of the isolates is

shown in fig. 1; 4045 specimens yielded a pathogen; 1840 (45%) contained specific strains of *E. coli*, 1257 (31%) shigella, 781 (19%) salmonella, and 146 (4%) had *Aeromonas* spp.; since 1982, 101 (4%) contained campylobacter. The number of isolates of *Aeromonas* spp. was highest (22) in 1980, and was lowest during 1977 and 1982–1985 (one or two isolations each year).

The isolates from 4045 specimens containing one or more pathogens are shown in table I. Most yielded only one pathogen; but 119 yielded two or three, giving a total of 240 isolates. *Aeromonas* spp. were associated with other pathogens in five specimens: with *E. coli* (3), campylobacter (1) and shigella (1). Fig. 2 shows that aeromonas was isolated throughout the year, with a somewhat higher incidence in the summer months.

During the last 11 years of the study (1978–1988), 117 isolates of *Aeromonas* spp. were obtained as the sole pathogen from the faeces of 81 patients. The charts of these patients were reviewed, and clinical information was analysed and processed on computer-compatible data sheets. Of the 81 patients, 54 were

Fig. 2. Seasonal distribution of *Aeromonas* spp. isolated from samples of faeces during a 15-year period.

Fig. 3. Age and sex of cases of enteritis caused by *Aeromonas* spp.: girls, □, boys ▨.

Fig. 4. Mean duration (and SEM) of illness ▨ and hospitalisation □ in different age groups.

boys and 27 girls; the peak incidence for both was at 2–6 months, an age group in which 52% of the male and 48% of the female cases were clustered (fig. 3). The mean duration of illness and hospitalisation of this group was significantly longer than for other age groups (fig. 4). The mean duration of illness increased from 12.2 days (0–1 month) to 20.1 days (2–6 months)

Table II. Susceptibility of isolates of *Aeromonas* spp. to antibiotic and chemotherapeutic agents

Antibiotic or chemotherapeutic agent (number of strains tested)	Number (%) of strains		
	susceptible	of intermediate resistance	resistant
Ampicillin (81)	3 (4)	5 (6)	73 (90)
Chloramphenicol (81)	79 (98)	0	2 (2)
Tetracycline (81)	73 (90)	4 (5)	4 (5)
Cefoxitin (41)	34 (83)	1 (2)	6 (15)
Cotrimoxazole (79)	61 (77)	3 (4)	15 (19)

and then decreased gradually to 5.2 days for children over 3 years old ($p < 0.01$ and $p < 0.05$, respectively). The mean duration of hospitalisation was 9.9 days (0–1 month), 15.6 days (2–6 months), and 3.8 days (> 3 years old).

The Central Laboratories of the Ministry of Health examined 46 of the *Aeromonas* isolates: 24 (52%) were identified as *A. hydrophila*, 11 (24%) as *A. caviae* and seven (15%) as *A. sobria*; four (9%) were atypical.

Among the symptoms of aeromonas infection, the most frequent was diarrhoea (91%). Stools were watery in 39 (48%), mucous in 29 (36%) and bloody in six (7%) of the patients. Vomiting occurred in 59 (73%); 34 (42%) showed signs of dehydration. The average weight-loss of the dehydrated patients was 8% of the last known previous weight; 18 of the dehydrated children showed metabolic acidosis in venous blood samples (pH decreased to < 7.3 and $\text{HCO}_3^- < 15$ meq/L).

The susceptibility of *Aeromonas* spp. to antibiotic and chemotherapeutic agents is shown in table II: most strains were resistant to ampicillin but susceptible to the other agents tested. We did not find significant differences in antibiotic sensitivity pattern among the three species ($p > 0.5$).

Discussion

Previous studies have shown that aeromonas is isolated more frequently from the faeces of patients with diarrhoea^{7,8} than from normal stools. In the only published study from our area, none of 500 specimens from healthy adults yielded aeromonas.¹⁰ In a preliminary study to the present one, the isolation rate from 461 children without gastro-intestinal symptoms was only 1%: four isolates from children aged 7–12 months and one from a child over 3 years old (unpublished observations).

The reported prevalence of aeromonas in human faeces varies widely, from 0.1% to 27%, depending on the climate, the quality of drinking water, the hygiene of the population, and the methods of bacterial culture.^{8,9} Therefore, it is difficult to make valid comparisons of isolation rates. In the USA, an association was shown between the drinking of untreated water and the isolation of aeromonas from cases of diarrhoea.¹⁴ A study in Israel found large

numbers of the organism in the water of an uncovered reservoir;¹⁹ but usually, drinking water is not screened for aeromonas. The water for the patients in the present study came from the national system and from local reservoirs. The quality of water in Israel is generally acceptable, and the population is notified if it becomes unsatisfactory.

In our experience, the culture methods did not present difficulties: the organism grew well on the media used routinely. The oxidase test provided an easy and efficient screening method for colonies resembling *E. coli*, though the occurrence of "oxidase-variable" strains has been reported.²⁰ Treatment with ampicillin or cotrimoxazole before hospitalisation is unusual, so it is doubtful if this influenced the isolation rates.

In Australia,¹² 127 of 975 children with diarrhoea yielded *Aeromonas* spp., compared with only 20 matched controls. After peptone water enrichment of unselected routine faecal specimens from 1004 patients in an English hospital, aeromonas was found in 4%, but it was possibly associated with gastrointestinal symptoms in only half of these; the isolation rate was higher in children: 8% in children under 1 year, 7% in those aged 1–13 years.²¹ Moyer²² found an isolation rate of 7% among patients with diarrhoea in Iowa, USA; and it was as high as 30% in children under 3

years and in middle-aged adults. Our isolation rate of only 4% may have been influenced by the severity of enteric disease that led to hospitalisation.

Studies in different countries have shown a peak isolation rate of *Aeromonas* spp. in the summer and autumn.^{12,21} In the present study, and in a previous study in adults,¹⁰ more cases of aeromonas gastroenteritis were found during the spring, summer and autumn, but the differences were not statistically significant; this could be due to the relatively slight differences in weather between our seasons.

Few of our patients were in the first month of life, probably due to lack of exposure and to the protective effect of maternal antibodies. Children aged 1–6 months showed a peak incidence, and also the highest duration of illness and of hospitalisation. *Aeromonas* diarrhoea was frequently associated with vomiting, dehydration, weight-loss and acidosis. No correlation was found between the species of *Aeromonas* and the clinical features—bloody, watery or mucous diarrhoea, or dehydration ($p > 0.5$). It is noteworthy that 42% of the children in our study were dehydrated and 7% had bloody diarrhoea. We conclude that *Aeromonas* spp. are important causes of intestinal infection especially in small children.

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