Comparison of direct plating with the use of enrichment culture for isolation of *Aeromonas* spp. from faeces

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Summary. Direct plating of faecal specimens on blood agar was compared with the use of enrichment culture for isolation of *Aeromonas* spp. from faeces during a large epidemiological study. Of enterotoxigenic strains isolated by direct plating, 89% were associated with acute diarrhoea and 7% with an episode of diarrhoea during the month before collection, but 79% of enterotoxigenic strains isolated only after enrichment were not associated with acute diarrhoea. With *Aeromonas* spp., as with intestinal pathogens, it appears that enrichment allows isolation of the bacteria when in low faecal concentrations likely to be found in convalescent patients, carriers and those with subclinical infection. The routine use of enrichment for isolation of faecal aeromonads, by detecting *Aeromonas* spp. in low numbers in patients without diarrhoea, is likely to confuse interpretation of epidemiological studies seeking to clarify the relationship between *Aeromonas* spp. and acute diarrhoea.

Introduction

With increased interest in *Aeromonas* spp. as potential intestinal pathogens, various media for their isolation from faeces have been described (Rogol *et al.*, 1979; Shread *et al.*, 1981; Mouldsdale, 1983; Von Graevenitz and Bucher, 1983; Millership and Chattopadhyay, 1984; Robinson *et al.*, 1984). Enrichment culture of specimens in alkaline peptone water has been recommended (Shread *et al.*, 1981; Mouldsdale, 1983; Von Graevenitz and Bucher, 1983; Millership and Chattopadhyay, 1984) and has been applied in clinical studies (Echeverria *et al.*, 1981; Millership *et al.*, 1983; Price *et al.*, 1984).

Reluctance to accept *Aeromonas* spp. as intestinal pathogens comes, in part, from reports of a high rate of faecal carriage of aeromonads in asymptomatic individuals (Pitarangsi *et al.*, 1982). Failure to observe an association between isolation of faecal aeromonads and diarrhoea is more likely when very sensitive methods are used for examination of stool specimens. Enrichment allows recovery of bacteria shed in low numbers; for example, enrichment enables the isolation of *Vibrio cholerae* from convalescent patients and from patients with subclinical infection as well as from carriers (Rennels *et al.*, 1980). Thus, if enrichment were used routinely,

many faecal isolates of *V. cholerae* would appear to be not associated with diarrhoea at the time of sampling.

Materials and methods

Faecal specimens

During a period of 6 weeks in late autumn and early winter, 1981, all faecal samples from patients with diarrhoea and from matched controls (Gracey *et al.*, 1982) submitted to the Department of Microbiology, Princess Margaret Hospital, Perth, were cultured in parallel by direct plating and with enrichment.

In this epidemiological study, patients were considered to have diarrhoea if they had had at least three loose stools per day within the previous 2 days. Patients were regarded as having no diarrhoea if stools had been normal for 2 weeks before the faecal sample was collected.

Isolation media

Blood agar containing paranitrophenyl glycerine (PNPG) 43 µg/ml w/v was used as the medium for primary plating. Nutrient broth (Cowan, 1974) and alkaline peptone water (Furniss *et al.*, 1978) were used for enrichment; they were held at room temperatures for 24 h and then plated on to the blood-agar PNPG medium.

Identification

Haemolytic and non-haemolytic colonies were tested

Table. Association between presence or absence of diarrhoea and method of isolation of *Aeromonas* spp.

Method of isolation	Number of <i>Aeromonas</i> isolates					
	<i>A. sobria</i>		<i>A. hydrophila</i>		<i>A. caviae</i>	
	D ⁺	D ⁻	D ⁺	D ⁻	D ⁺	D ⁻
Primary plating	21	3	4	1*	2*	7*
Enrichment	7	28	2	6	1*	3*

D⁺ = from patient with diarrhoea; D⁻ = from subject with no diarrhoea

* Non enterotoxigenic strains

for oxidase production (Kovacs, 1956), and oxidase-positive colonies were further identified by subculture in the medium of Kaper *et al.* (1979). *Aeromonas* spp. were classified according to the methods of Popoff (1984) as *A. sobria*, *A. hydrophila* or *A. caviae*. Enterotoxigenic strains were identified by the suckling mouse test as described previously (Burke *et al.*, 1981).

Results

During the 6 weeks of the study, 38 strains of *Aeromonas* spp. were isolated from primary plates and also after enrichment; 47 strains were isolated after enrichment but not from primary plates.

The table shows the number of strains and species of *Aeromonas* isolated by the two methods in relation to the presence of diarrhoea. Of the 43 enterotoxigenic strains isolated only after enrichment, 34 were not associated with diarrhoea. Only three of 28 enterotoxigenic *Aeromonas* strains isolated on primary plating were not associated with diarrhoea at the time of sampling and two of these were from patients who had a history of diarrhoea within the preceding month. Nine patients from whom enterotoxigenic *Aeromonas* spp. were isolated only after enrichment had diarrhoea; six strains were isolated from patients with diarrhoea of more than 2 weeks' duration. A shigella was isolated from one sample that also contained *A. sobria*.

Discussion

Rennels *et al.* (1980) compared isolation of *V.*

cholerae by direct plating with isolation after enrichment and found that in 96% of acute diarrhoeal stools that contained *V. cholerae*, the organism was detected by direct plating. In contrast, vibrios were isolated by direct plating from only 66% of formed stools that were shown to contain *V. cholerae* by enrichment. Our experience suggests that there is a similar correlation between diarrhoea and the isolation of enterotoxigenic *Aeromonas* spp. by direct plating; 89% of strains isolated by direct plating were associated with acute diarrhoea and 7% with diarrhoea within the preceding month. Of the nine patients with diarrhoea from whom enterotoxigenic *Aeromonas* spp. were isolated only after enrichment, only two had acute diarrhoea not known to be associated with another intestinal pathogen; 79% of enterotoxigenic *Aeromonas* spp. isolated only after enrichment were not associated with diarrhoea within 2 weeks of collecting the faecal sample. We have found that *Aeromonas* spp. may continue to be excreted in low concentrations by convalescent patients 2 months after symptoms have disappeared.

The data suggest that, as with *V. cholerae* (Rennels *et al.*, 1980), enrichment allows detection of faecal aeromonads present in low concentration that may be found in convalescent patients, carriers, and in cases of subclinical infection with no diarrhoea at the time of sampling. On this basis, we recommend that, as for *V. cholerae* (Gangarosa *et al.*, 1968; Rennels *et al.*, 1980), stools from patients with acute diarrhoea should be plated directly on media for the isolation of *Aeromonas* spp., without enrichment, but that enrichment should be considered for specimens from convalescent patients, possible carriers, and from those with mild or chronic diarrhoea. Alkaline peptone water or nutrient broth are satisfactory enrichment media for *Aeromonas* spp.

Epidemiological studies in which enrichment cultures are used but in which the results of *Aeromonas* spp. isolated only after enrichment are not separated from those isolated by direct plating, are likely to obscure any relationship between diarrhoeal disease and faecal *Aeromonas* spp.

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