Occurrence of *Aeromonas* species and *Plesiomonas shigelloides* in patients with and without diarrhoea in Lagos, Nigeria

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Summary. The prevalence of *Aeromonas* spp. and *Plesiomonas shigelloides* was determined in patients attending the enteric laboratory of the Department of Medical Microbiology and Parasitology, Lagos University Teaching Hospital, Nigeria. During the 12-month study (October 1986–September 1987), *Aeromonas* spp. were isolated from 53 (2.26%) of 2350 patients with diarrhoea and only 2 (0.4%) of 500 patients without diarrhoea ($p < 0.01$). Similarly, *P. shigelloides* was isolated from 16 (0.68%) patients with diarrhoea and none of the controls ($p > 0.05$). The seasonality, age and sex distribution of diarrhoea associated with *Aeromonas* spp. and *P. shigelloides* in this study were similar to those of diarrhoea associated with other recognised enteropathogens in Nigeria. Both species may play a role in the aetiology of acute diarrhoeal disease in that environment.

Introduction

In recent years, reports of the isolation of *Aeromonas* spp. or *Plesiomonas shigelloides* from patients with diarrhoea in different parts of the world have increased. This development has no doubt increased the level of awareness of their potential pathogenic role and geographical spread. Some authors have proposed *Aeromonas* or *Plesiomonas* spp. as causes of acute diarrhoeal disease (Sanyal *et al.*, 1975; Rutala *et al.*, 1982; Agbonlahor, 1983; Ljungh and Wadstrom, 1985; Reinhardt and George, 1985), but others have been unable to ascribe any clinical significance to these organisms (Pitarangsi *et al.*, 1982; Figura *et al.*, 1986). Their importance as agents of diarrhoea is, therefore, still controversial and subject to continuing investigation in many parts of the world.

In Nigeria, as in most other West African countries, little is known about diarrhoea associated with *Aeromonas* spp. or *P. shigelloides*. In this paper, we report on the prevalence of these species in patients attending the Lagos University Teaching Hospital (LUTH) in Nigeria and comment on their possible roles in diarrhoeal episodes in that environment.

Materials and methods

Patients and sources of isolates

The study included 2350 patients with diarrhoea and 500 patients without diarrhoea (controls) who submitted faecal specimens for culture at the enteric laboratory of the Department of Medical Microbiology and Parasitology, LUTH, from October 1986 to September 1987. Diarrhoea in this study was defined as three or more loose stools a day within 2 days of specimen collection. Patients in the control group had no diarrhoea in the 2 weeks before specimen collection. Other data obtained on each patient included age, sex, general medical history and accompanying clinical features.

Isolation and identification of Aeromonas and Plesiomonas spp.

Freshly collected faecal specimens were plated on to xylose desoxycholate citrate agar (XDCA) and sheep-blood agar containing ampicillin 15 µg/ml (ABA). These media are recommended for the isolation of these organisms from specimens such as faeces (Millership and Chattopadhyay, 1984; Agger *et al.*, 1985). In addition, alkaline peptone water, pH 8.6 (APW), was used as an enrichment medium for both organisms.

Cultures were incubated at 37°C for 18–24 h, after which non-xylose-fermenting colonies on XDCA and all colonies on ABA were screened for oxidase production

(Kovacs, 1956). Oxidase-positive colonies were subcultured on to nutrient-agar plates (Oxoid). They were further confirmed as *Aeromonas* spp. or *P. shigelloides* according to the criteria outlined by von Graevenitz (1985).

Isolation of other bacterial enteropathogens

Specimens were also inoculated on to MacConkey agar (MAC), desoxycholate citrate agar (DCA), and thiosulphate-citrate-bile salt-sucrose agar (TCBS). In addition, appropriate enrichment broths were used to enhance the isolation of *Salmonella* spp., *Shigella* spp., *Vibrio cholerae* and *Yersinia enterocolitica*. Incubation was at 37°C for 18–24 h except for cultures for *Y. enterocolitica* which were incubated at room temperature (22–25°C) for 48 h.

On primary isolation, suspicious colonies were screened by subculture on Kligler iron agar (KIA) and motility-indole-urea medium (MIU). Further identification of isolates was by standard biochemical tests as described by Cowan (1974). Serological confirmation of identification was by slide agglutination with Wellcome antisera (Wellcome Reagents Ltd, Wellcome Research Laboratories, Beckenham).

For economic reasons, culture for *Campylobacter* spp. was not performed.

Statistical analysis

Results were analysed statistically by the χ^2 test (Colton, 1974).

Results

Of the 2350 faecal specimens from patients with diarrhoea processed during this study, *Aeromonas* spp. were isolated from 53 (2.26%), *P. shigelloides* from 16 (0.68%), *Shigella* spp. from 4 (0.17%), *Salmonella* spp. from 3 (0.13%) and *Y. enterocolitica* from 2 (0.09%). Enteropathogenic *Escherichia coli* (EPEC) strains were isolated from 39 (2.85%) of 1368 specimens examined. *V. cholerae* was not isolated from any of the specimens processed.

Only two strains of *Aeromonas* spp. and no other recognised bacterial pathogens were isolated from 500 faecal specimens from control patients included in this study. Statistically, *Aeromonas* spp. were isolated significantly more often from patients with diarrhoea than from controls ($\chi^2=7.5$, $p<0.01$). For *P. shigelloides*, the difference between the two groups was not statistically significant ($\chi^2=3.43$, $p>0.05$).

In this study, clinical features of diarrhoea associated with *Aeromonas* spp. and *P. shigelloides* were similar to those of diarrhoea due to other known bacterial enteropathogens. There was abdominal pain in 3 (5.7%), anorexia in 4 (7.6%),

vomiting in 5 (9.4%) and weight loss in 3 (5.7%) of 53 patients from whom *Aeromonas* spp. were isolated. Similarly, there was abdominal pain in 2 (12.5%), anorexia in 2 (12.5%), vomiting in 1 (6.5%) and weight loss in 1 (6.5%) of 16 (patients with diarrhoea associated with *P. shigelloides*).

The distribution of cases of diarrhoea associated with *Aeromonas* spp. and *P. shigelloides* according to age and sex of patients is shown in the table. There were 20 (37.7%) episodes related to *Aeromonas* spp. in males and 33 (62.2%) in females. On the other hand, more diarrhoea associated with *P. shigelloides* occurred in males (10; 62.5%), than in females (6; 37.5%). However, the difference in sex distribution of isolates was not statistically significant ($\chi^2=3.5$, $p>0.05$). Furthermore, diarrhoea due to these two organisms affected all age groups. However, 23 (43.4%) of cases of diarrhoea associated with *Aeromonas* spp. occurred in children under 5 years of age, whereas 7 (13.2%) of such episodes occurred in patients over 70 years old. Similarly, 3 cases (18.8%) of diarrhoea associated with *P. shigelloides* occurred in the very young and 5 cases (31.3%) occurred in the very old. Also, the majority of our strains were isolated during the wet months of July–October.

Discussion

This prospective 12-month study has shown that *Aeromonas* spp. and *P. shigelloides* are found in association with diarrhoea more often than in controls in our environment. In fact, *Aeromonas* spp. were isolated significantly more often from patients with diarrhoea than from controls ($p<0.01$). Furthermore, isolation rates of 2.26% for *Aeromonas* spp. and 0.68% for *P. shigelloides* from patients with diarrhoea were similar to those of recognised enteric pathogens obtained in this study. These findings suggest that both organisms, but particularly *Aeromonas* spp., can cause acute diarrhoea in patients in Lagos.

In similar studies in other parts of the world, workers have reported an isolation rate of less than 1% for *Aeromonas* spp. in normal subjects (Paukova and Fukalova, 1968; Burke *et al.*, 1983). Similarly, Arai *et al.* (1980) found *P. shigelloides* in the faeces of only 3 (0.01%) of 38454 normal subjects examined in Japan. However, both Echeverria *et al.* (1981) and Pitarangsi *et al.* (1982) found no difference in the isolation of these organisms from normal controls and patients with diarrhoea in Thailand. Therefore, we agree with an earlier suggestion by Agger *et al.* (1985) that these divergent results may be related to geographical location,

Table. Distribution of cases of diarrhoea associated with *Aeromonas* spp. and *P. shigelloides* by age and sex

Age (years)	<i>Aeromonas</i> spp.			<i>P. shigelloides</i>		
	Male	Female	Total	Male	Female	Total
0-5	8	15	23	2	1	3
6-10	2	4	6	1		1
11-20	1	3	4	1	2	3
21-30	3	4	7	2	...	2
31-40	...	1	1	1	...	1
41-50	1	...	1	1	...	1
51-60	...	1	1	...	...	...
61-70	1	2	3	...	...	...
71-80	1	3	4	1	...	1
81-90	1	...	1	...	1	1
91-100	2	...	2	1	2	3
Total	20	33	53	10	6	16
(%)	(37.7%)	(62.3%)	(100)	(62.5%)	(37.5%)	(100)

season of collection, and the microbial media used for isolation.

Diarrhoea associated with *Aeromonas* and *Plesiomonas* spp. appeared to affect all age groups, but particularly children under the age of 5 years (table). Also, more *Aeromonas* spp. were isolated from females than males in this study, while the reverse was the case for *P. shigelloides*. In an earlier report by Agbonlahor (1983), two out of six *Aeromonas* strains isolated from patients in Lagos with acute diarrhoea were from children.

Clinical features of diarrhoea associated with *Aeromonas* and *Plesiomonas* spp. in this study included abdominal pain, anorexia, vomiting and weight loss. These features were not distinct from those of diarrhoea due to recognised enteropathogens. Furthermore, most of our strains were isolated during the warm and wet months of the year. This is in agreement with earlier suggestions that the association of *Aeromonas* and *Plesiomonas* spp. with water may be responsible for the apparent seasonality in the incidence of diarrhoea associated with these agents (Burke *et al.*, 1983; Agger *et al.*, 1985; Reinhardt and George, 1985). There have been some reports that *Aeromonas* spp. and *P. shigelloides*

cause a more severe form of diarrhoea in immunocompromised hosts, thereby suggesting an opportunist role for these organisms (Ljungh and Wadstrom, 1985). In this study, clinical conditions that might have predisposed to *Aeromonas*-associated diarrhoea were less frequent, and these were peptic ulcer in 3 (5.7%) and kwashiorkor in 1 (1.9%) of our patients. Similarly, there was only one case each of diabetes mellitus and kwashiorkor in a total of 16 patients with *Plesiomonas*-associated diarrhoea.

In conclusion, results of this study strongly suggest an aetiological role for both organisms, but particularly *Aeromonas* spp. in diarrhoeal disease in Nigeria. However, for a better understanding of the epidemiology of diarrhoea that may be due to these organisms, there is a need for related community-based studies. Meanwhile, routine culture of these organisms from faecal specimens of patients with diarrhoea is advised to determine their level of involvement in such infections.

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