

Incidence and clinical symptoms of *Aeromonas*-associated travellers' diarrhoea in Tokyo

S. YAMADA¹, S. MATSUSHITA², S. DEJSIRILERT³ AND Y. KUDOH⁴

¹ Tama Branch Laboratory, Tokyo Metropolitan Research Laboratory of Public Health, 3-16-25, Shibazaki-cho, Tachikawa, Tokyo 190, Japan

² Department of Microbiology, Tokyo Metropolitan Research Laboratory of Public Health, Tokyo

³ Division of Clinical Pathology, Department of Medical Science, Nonthaburi, Thailand

⁴ School of Health Science, Kyorin University, Tokyo

(Accepted 10 April 1997)

SUMMARY

In a survey examining the causes of travellers' diarrhoea treated in Tokyo between July 1986 and December 1995, *Aeromonas* species were isolated from 1265 (5·5%) of 23215 travellers returning from developing countries. *Aeromonas* species were the fourth most frequent enteropathogen isolated, following enterotoxigenic *E. coli* (8·5%), *Salmonella* spp. (7·6%) and *Plesiomonas shigelloides* (5·6%). *Aeromonas* species were found in 1191 (5·6%) of 21257 patients with diarrhoea and in 74 (3·8%) of 1958 healthy individuals without diarrhoea. Mixed infection was observed in 512 (40·5%) cases. No significant difference in the prevalence of *Aeromonas* by year, season, age distributions, or sex was observed, but a slight difference was noted depending on the country where the travellers visited. Of the 1265 *Aeromonas* isolates, 893 strains (70·6%) were *A. veronii* biovar *sobria*, 330 (26·1%) were *A. hydrophila*, and 42 (3·3%) were *A. caviae*. The clinical symptoms of patients from whom *Aeromonas* species was isolated as the only potential enteric pathogen were almost similar, which were watery diarrhoea (about 60%), abdominal cramps (43%), fever (around 15%), and nausea or vomiting (13%). Although the severity of illness was milder than that of enterotoxigenic *E. coli* alone, these data suggest that *Aeromonas* species are important enteric pathogens in travellers' diarrhoea.

INTRODUCTION

Aeromonas species occur widely in the environment, particularly in water, and certain species have been reported to be an important cause of acute diarrhoea in children [1–3] and adults, especially in those older than 60 years of age [4–6]. Although the significance of *Aeromonas* species as a cause of travellers' diarrhoea has been shown [6, 7], the incidence of this organism among Japanese travellers visiting developing countries has been unclear.

The clinical features of gastroenteritis caused by *Aeromonas* usually present as acute self-limiting diarrhoea, which is more frequent in young children

and older adults. Fever, vomiting and leukocytes or erythrocytes in faeces may or may not be present [8]. However, these characteristics have not been clearly elucidated at the species level. Thus, the present study was undertaken to assess the incidence and significance of different *Aeromonas* species in travellers' diarrhoea, and to characterize the clinical features of intestinal infection caused by *Aeromonas*.

MATERIALS AND METHODS

Stool specimen

A total 23215 faecal specimens obtained from travellers returning to Japan from other countries, mostly

Asian countries, during the period July 1986 to December 1995 were examined. Stool specimens collected from persons under quarantine or receiving medical care were received in transportation medium from Public Health Centers in Tokyo and transferred within 2 days to our laboratory with the 'Health cards' of the travellers examined. This card was declared by the traveller at the time of collecting the stool sample, and included the following information: visiting country(s); day of departure from Japan; day of entry into Japan; onset day or place of illness; presence or absence of diarrhoea at presentation of faecal sample; character of stool (watery, loose or bloody); maximum number of diarrhoea episodes per day; presence or absence of abdominal cramps, nausea or vomiting, and fever; and confirmation of treatment with antibiotics or other drugs.

Isolation and identification of *Aeromonas* strains and other enteropathogens

For the isolation of *Aeromonas* spp., stool specimens were inoculated directly onto sheep blood agar plate with 10 µg/ml ampicillin (ASBA) which was recommended by Kay and colleagues [9]. In addition, the stools were inoculated in alkaline peptone water, pH 8.6 with 2% NaCl (APW) and incubated overnight at 37 °C, after which the cultures were streaked on ASBA. The plates were incubated overnight at 37 °C. Grey colonies with hemolysis on ASBA plates were picked up as suspicious *Aeromonas* spp. colonies and inoculated on nutrient agar slants, triple-sugar-iron agar slants (Eiken Chemical Co. Tokyo) and lysin-indol-motility medium (Nissui Pharmaceutical Co. Tokyo), and incubated at 37 °C overnight. Presumptive identification of *Aeromonas* spp. was made on the basis of oxidase positivity on nutrient agar slants. Oxidase positive colonies were identified and confirmed as *Aeromonas* spp. by the AP120E system (La Balme LesGrottes, France) and the ability to grow in peptone water without NaCl. *Aeromonas* isolates were identified at the species level by the following biochemical tests, most of which chosen from those proposed by Popoff [10] or Janda and colleagues [11]: gas production from glucose, acid production from salicin, aesculin, hydrolysis, and arbutin hydrolysis. All tests were performed at both 37 °C and 25 °C, and results were read daily for 1 week.

All faecal samples were also cultured for other bacterial-enteropathogens including vibrios, *Plesiomonas shigelloides*, *Shigella* spp., *Salmonella* spp.,

enteropathogenic serotype *E. coli* enteroinvasive *E. coli*, enterotoxigenic *E. coli*, *Yersinia* spp. and *Campylobacter* spp. For the isolation of these pathogens, thiosulphate-citrate-bile salt-sucrose agar (Eiken), vibrio agar (Nissui), salmonella-shigella agar (Eiken), deoxycolate-hydrogen sulfide-lactose agar (Nissui) and Skirrow's medium (Oxoid, Hampshire, England) were used. These enteric media, except Skirrow's medium, were incubated overnight at 37 °C. Skirrow's medium was incubated in an atmosphere of 5% O₂, 10% CO₂, 85% N₂ at 42 °C for 48 h. In addition, subcultures for vibrios and *Salmonella* spp. were made on the respective agar media after overnight enrichment at 37 °C in alkaline peptone water, pH 8.6 with 2% NaCl and selenite F-broth, respectively. The procedure for isolation and identification of these pathogens was according to standard bacteriological methods [8]. No attempt was made to detect enterovirus, rotavirus, *Giardia lamblia* or *Cryptosporidium*.

Susceptibility of *Aeromonas* strains to antimicrobial agents

A total of 857 strains isolated during the period 1989–95 were examined for susceptibility to nine antimicrobial agents by the disk diffusion method [12] using Muller Hinton agar (disk and agar from BBL). The agents and concentrations were as follows: 30 µg chloramphenicol (CP), 30 µg tetracycline (TC), 10 µg streptomycin (SM), 30 µg kanamycin (KM), 10 µg ampicillin (AM), sulfamethoxazole-trimethoprim (1.25 µg trimethoprim + 23.5 µg sulfamethoxazole, ST), 30 µg nalidixic acid (NA), 50 µg fosfomycin (FOM), and 10 µg norfloxacin (NFX).

RESULTS

Incidence and epidemiological aspects of *Aeromonas* spp. in travellers' diarrhoea

The travellers examined were mostly adults who visited Asian country(s). Table 1 shows the results of detection of enteropathogens from overseas travellers in Tokyo over 9.5 years. *Aeromonas* species were found in 1265 (5.5%) of 23215 samples, and was the fourth highest, following enterotoxigenic *E. coli* (8.5%). *Salmonella* spp. (7.6%) and *Plesiomonas shigelloides* (5.6%).

Of travellers examined, 21257 were patients with diarrhoea and the remaining 1958 were healthy individuals without any diarrhoea, abdominal cramps, etc. *Aeromonas* strains were isolated from 1191 (5.6%)

patients and 74 (3.8%) healthy individuals [$\chi^2 = 13.5 > \chi^2 (0.05) = 3.84$]. Among healthy individuals also, 11 different kinds of enteropathogens were identified in 318 positive cases, and *Aeromonas* species were predominant following *Salmonella* spp. (100 cases).

Of the 1265 isolates characterized biochemically, 893 (70.6%) were identified as *A. veronii* biovar *sobria*, 336 (26.1%) as *A. hydrophila*, and 42 (3.3%) as *A. caviae*. None of the strains was *A. veronii* biovar *veronii* (an ornithine-positive strain).

There was no significant difference in the prevalence of *Aeromonas* spp. by year, season, age distribution, or sex. However, the isolation rate was slightly different depending on the country visited. As shown in Table 2, higher isolation rates were recorded among travellers to Malaysia (7.5%), Taiwan (7.1%) and Indonesia (6.6%), whereas those among travellers to Korea, Nepal or Sri Lanka were below 2.0%.

Mixed infection with the isolation of two or more enteropathogens was detected in 495 (39.1%) of *Aeromonas*-positive cases. This ratio was the fourth highest following those of *Plesiomonas* spp. (45.5%), *Vibrio cholerae* non 0-1 (45.5%), and *Vibrio fluvialis/furnissii* (45.2%).

Associated clinical symptoms of travellers infected with *Aeromonas* species

To clarify the severity of the illness and clinical symptoms at the species level of patients infected with *Aeromonas* spp., retrospective investigations were carried out on 696 patients from whom *A. hydrophila*, *A. veronii* biovar *sobria*, or *A. caviae* was found as the only potential enteric pathogen. In addition, those patients infected with enterotoxigenic *E. coli* alone were also investigated as a control of travellers' diarrhoea. As shown in Table 3, the severity of the illness and clinical symptoms of patients infected with *A. hydrophila* and *A. veronii* biovar *sobria* were almost identical. Both showed a predominance of mild cases with diarrhoea less than four times per day. Although bloody diarrhoea was observed in three persons infected with each organism, more than 60% had watery diarrhoea. Abdominal cramps were also observed in 43% cases in both groups. A few of the cases were accompanied by fever (around 15%), and nausea or vomiting (about 13%). In 21 patients infected with *A. caviae* alone, watery diarrhoea (67%), abdominal cramps (57%), and nausea or vomiting (38%) were the main symptoms. In contrast, most patients infected with enterotoxigenic *E. coli* alone

Table 1. Detection of enteropathogens from overseas travellers in Tokyo (July 1986-Dec 1995)

Number examined	23215
Number positive for pathogens (%)	7166 (30.9)
No. of cases with (%)	
Enterotoxigenic <i>E. coli</i>	1967 (8.5)
<i>Salmonella</i> spp.	1762 (7.6)
<i>Plesiomonas shigelloides</i>	1302 (5.6)
<i>Aeromonas</i> spp.	1265 (5.5)
<i>Campylobacter</i> spp.	1152 (5.0)
<i>V. parahaemolyticus</i>	773 (3.3)
<i>Shigella</i> spp.	524 (2.3)
Enteropathogenic <i>E. coli</i> , serotype	466 (2.0)
<i>V. cholerae</i> non 0-1	192 (0.8)
<i>V. cholerae</i> 0-1	107 (0.5)
<i>V. fluvialis/furnissii</i>	57 (0.3)
Enteroinvasive <i>E. coli</i>	53 (0.2)
<i>V. mimicus</i>	5
<i>Y. enterocolitica</i>	1

Table 2. Suspected countries of *Aeromonas* infection

Country	Number of travellers examined	Number of positive cases (%)
East Asia		
Taiwan	480	34 (7.1)
China	164	8 (4.9)
Hong Kong	142	8 (5.6)
Korea	125	1 (0.8)
Southeast Asia		
Indonesia	5231	343 (6.6)
Thailand	4748	266 (5.6)
Philippines	2066	90 (4.4)
Singapore	624	33 (5.2)
Malaysia	467	35 (7.5)
South Asia		
India	2459	132 (5.4)
Nepal	166	4 (2.4)
Sri Lanka	61	1 (1.6)
Africa		
Egypt	103	6 (5.8)
Kenya	94	3 (3.2)
Europe	41	1 (2.4)
South America	259	12 (8.3)

were moderate or severe with watery diarrhoea four or more times per day, and about 50% of the cases had abdominal cramps.

Susceptibility of *Aeromonas* strains isolated

Out of the *Aeromonas* strains isolated from overseas travellers, 857 strains isolated between 1989 and 1995

Table 3. Summary of symptoms and severity of travellers' diarrhoea in patients from whom *Aeromonas* spp. was found as the only potential enteric pathogen

Characteristics of patients	% of symptoms with			
	<i>A. hydrophila</i> (n = 198)	<i>A. sobria</i> * (n = 477)	<i>A. caviae</i> (n = 21)	ETEC† (n = 328)
Character of stool				
Watery	62	64	67	96
Loose	35	35	33	4
Bloody	2	1	0	0
Unknown	2	1	0	0
Maximum number of diarrhoea episodes per day				
Mild (1-3)	48	51	67	22
Moderate (4-6)	28	31	19	49
Severe (> 7)	11	8	10	29
Unknown	13	10	5	9
Abdominal cramps	43	43	57	54
Nausea/vomiting	13	13	38	17
Fever	19	16	14	23

* *Aeromonas veronii* biovar *sobria*.

† Patients infected with ETEC alone during 1990-4.

Table 4. Antibiotic susceptibility patterns of *Aeromonas* spp. isolated from overseas travellers in Tokyo during 1989-95

Species	Number tested	% Resistant to antibiotics*								
		CP	TC	SM	KM	AM	ST	NA	FOM	NFX
<i>A. hydrophila</i>	217	0.0	12.0	4.6	0.9	96.8‡	2.8	3.2	0.0	0.0
<i>A. sobria</i> †	612	0.7	25.0	9.8	0.7	98.4‡	2.5	3.1	0.0	0.2
<i>A. caviae</i>	28	0.0	7.1	7.1	0.0	100	0.0	0.0	0.0	0.0

* CP, chloramphenicol; TC, tetracycline; SM, streptomycin; KM, kanamycin; AM, ampicillin; ST, sulphamethoxazole/trimethoprim; NA, nalidixic acid; FOM, fosfomycin; NFX, norfloxacin.

† *Aeromonas veronii* biovar *sobria*.

‡ Seven strains of *A. hydrophila* and 10 strains of *A. veronii* biovar *sobria* showed an intermediate value.

were tested for susceptibility to nine antibiotics. Except for 7 strains of *A. hydrophila* and 10 strains of *A. veronii* biovar *sobria*, which showed an intermediate value for ampicillin, all strains were resistant to this agent (Table 4). Twenty-six (12.0%) of *A. hydrophila* were resistant to TC, 10 (4.6%) to SM, 7 (3.2%) to NA, 6 (2.8%) to ST and 2 (0.9%) to KM. In contrast, 153 (25.0%) strains of *A. veronii* biovar *sobria* were resistance to TC, 60 (9.8%) to SM, 19 (3.1%) to NA, 15 (2.5%) to ST, 4 (0.7%) each to CP and KM. In addition, an NFX-resistant strain was

detected in 1995. Two strains of *A. caviae* were resistant each to TC and SM. All strains were susceptible to FOM.

DISCUSSION

In this study, we found a high incidence of *Aeromonas* species in overseas travellers' diarrhoea in Tokyo. *Aeromonas* species were isolated from 1265 (5.5%) of 23215 faecal samples, which was the fourth highest, following those of enterotoxigenic *E. coli* (8.5%),

Salmonella spp. (7.6%) and *Plesiomonas shigelloides* (5.6%). In another study of travellers' diarrhoea [14], *A. hydrophila* was found in 31% of American Peace Corps volunteers in rural Thailand. These results indicate that *Aeromonas* species have occasionally been associated with traveller's diarrhoea, although it is not as common as enterotoxigenic *E. coli*.

The overall incidence of *Aeromonas* species in diarrhoeal disease is 1–27% [1, 3, 7, 13–15], whereas the asymptomatic faecal carriage rate in children is 0.5–2.1% [2, 3, 16, 17]. In the present study, *Aeromonas* strains were found in 5.6% of the patients with diarrhoea and 3.8% of healthy individuals without diarrhoea. These rates may reflect actual differences or may be influenced by the selection of patients and control group.

As mentioned above, *Aeromonas* species are frequently isolated from diarrhoeal disease of humans, but the role of *Aeromonas* spp. as a potential human enteric pathogen is still unclear. This may be due to the lack of its clinical significance, and confusion over the taxonomy of the *Aeromonas* genus. In the present study, we identified *Aeromonas* strains by biochemical tests, and compared at the species level the clinical data in patients from whom *Aeromonas* species was found as the only potential enteric pathogen with those of enterotoxigenic *E. coli*. Of 1265 isolates, 893 (70.6%) were identified as *A. veronii* biovar *sobria*, 330 (26.1%) as *A. hydrophila*, and 4% (3.3%) as *A. caviae*. In studies in other countries, the most frequent species in human diarrhoeal disease was *A. hydrophila* in India [13], and *A. caviae* in France [18], Italy [2], Netherland [19], or India [20]. Furthermore, Daily and colleagues [21] reported that most *A. sobria* strains were clinical isolates, and most *A. hydrophila* strains were isolated from water. These differences may depend on the geographical location.

In retrospective investigations of the clinical symptoms of 696 patients from whom *Aeromonas* species alone was detected, it was found that the severity of the illness and clinical symptoms of patients with *A. hydrophila* and *A. veronii* biovar. *sobria* were similar, but not more severe than the cases with enterotoxigenic *E. coli*. Both showed a predominance of mild cases with diarrhoea less than four times per day, and clinical symptoms included abdominal cramps (43%), nausea or vomiting (13%), and fever (around 15%). Diarrhoea was generally characterized as watery (60%) and loose (35%), but few patients with bloody diarrhoea were observed. In American travellers who visited Thailand, the main symptoms of the patients

from whom *A. hydrophila* was found were diarrhoea of less than five times per day and abdominal cramps [7]. Cholera-like illness was also observed in a 67-year-old woman infected with *A. sobria* [4]. These data suggest that *A. hydrophila* and *A. veronii* biovar *sobria* are important enteric pathogens in traveller's diarrhoea, and that clinically, *Aeromonas*-associated diarrhoea is not distinct from other types of diarrhoea commonly encountered in developing countries.

Although *A. caviae* has been recognized as non-haemolytic and non-enteropathogenic, this organism has been predominantly detected from stool samples in paediatric patients less than 3 years of age [16, 22]. In this study also, 42 strains isolated were identified as *A. caviae*. Except for 2 strains, all strains were isolated from stools of patients, of which 21 were found as the only potential enteric pathogen. The symptoms of these patients were also watery diarrhoea and abdominal cramps. The virulence factor of *A. caviae* is unclear; however, all strains exhibited very weak haemolysis on blood agar plate different from that of other *Aeromonas* species. Recently, Namdari and Bottone [23] reported a cytotoxic factor on HEp-2 cells in the culture filtrate of *A. caviae*.

It is interesting whether *Aeromonas*-induced diarrhoea is toxigenic. The clinical spectrum of the patients from whom enterotoxigenic *E. coli* was isolated was shown to be evidently toxigenic, and most patients had watery diarrhoea more than 4 times per day, whereas the character of the stools of patients infected with *Aeromonas* sp. was varied. This difference may have been caused by several virulence factors of *Aeromonas* spp. To define the enteropathogenicity of *Aeromonas* spp, the examination of the correlation between clinical symptoms of gastroenteritis due to *Aeromonas* spp. and some virulence factors will be necessary. Furthermore, volunteer studies or intestinal biopsies of patients with diarrhoea may be required to establish whether *Aeromonas* species are gastrointestinal pathogens in humans. We are now reexamining the biological activities of the *Aeromonas* strains isolated in this study.

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