Aeromonas detection and their toxins from drinking water from reservoirs and drinking fountains

Maria Tereza Pepe Razzolini, Marisa Di Bari, Petra Sanchez Sanchez and Maria Inês Zanoli Sato

ABSTRACT

Aeromonads are inhabitants of aquatic ecosystems and are described as being involved in intestinal disturbances and other infections. A total of 200 drinking water samples from domestic and public reservoirs and drinking fountains located in São Paulo (Brazil), were analyzed for the presence of *Aeromonas.* Samples were concentrated by membrane filtration and enriched in APW. ADA medium was used for *Aeromonas* isolation and colonies were confirmed by biochemical characterization. Strains isolated were tested for hemolysin and toxin production. *Aeromonas* was detected in 12 samples (6.0%). *Aeromonas* strains (96) were isolated and identified as: *A. caviae* (41.7%), *A. hydrophila* (15.7%), *A.allosacharophila* (10.4%), *A. schubertii* (1.0%) and *Aeromonas* spp. (31.2%).The results revealed that 70% of *A. caviae*, 66.7% of *A. hydrophila*, 80% of *A. allosacharophila* and 46.6% of *Aeromonas* spp. were hemolytic. The assay for checking production of toxins showed that 17.5% of *A. caviae*, 73.3% of *A. hydrophila*, 60% of *A. allosacharophila*, 100% of *A. schubertii*, and 33.3% of *Aeromonas* spp. were able to produce toxins. The results demonstrated the pathogenic potential of *Aeromonas*, indicating that the presence of this emerging pathogen in water systems is a public health concern.

Key words | *aeromonas*, drinking water, enterotoxin production, hemolytic activity

M. T. P. Razzolini (corresponding author)
School of Public Health, University of São Paulo,
São Paulo, Brazil Av. Dr Arnaldo 715 1° andar - 01246-904, São Paulo - SP,
Brazil
Tel.: 55 11 3061 7712
Fax: 55 11 3061 7732
E-mail: *razzolini@usp.br*

Marisa Di Bari
Maria Inês Zanoli Sato
CETESB - Companhia de Tecnologia de Saneamento Ambiental,
Av. Prof. Frederico Hermann Jr 346 - Prédio 5,
São Paulo - SP, 05459-900
Brazil

Petra Sanchez Sanchez
Universidade Presbiteriana Mackenzie,
Rua da Consolação 896 – Edifício João Calvino,
7° andar, 01302-907, São Paulo - SP,
Brazil

INTRODUCTION

Aeromonas genus is widely distributed in aquatic environments such as surface waters, drinking water systems, wastewater, as well as food, soil and feces. These organisms are well recognized emerging pathogens responsible for gastrointestinal disturbances (Havelaar *et al.* 1987; Joseph *et al.* 1991; Monfort & Baleux 1991*a*; Singh & Sanyal 1992*a*,*b*; Häninen *et al.* 1995; Villarruel-López *et al.* 2005), septicemia, endocarditis, conjunctivitis and wound infections (Freitas *et al.* 1993; Merino *et al.* 1995; Pin *et al.* 1995; Figueras *et al.* 2005; Hiransuthikul *et al.* 2005).

A high occurrence of *Aeromonas* was reported by Lechevalier *et al.* (1982) in chlorinated treated water in Oregon State, USA (27%) and by Araujo *et al.* (1989) in tap water in Spain (70%). In Libya, Ghenghesh *et al.* (2001) analyzed 1000 samples of water collected from wells (980) and miscellaneous sources (120) and *Aeromonas* species were isolated in 48.7% of them. They carried out *Aeromonas* speciation in 381 isolated strains that resulted in 225 (59%) being *A. hydrophila*, 103 (27%) *A. caviae*, 42 (11%) *A. sobria* and 11 (3%) atypical aeromonas. In Italy, Aulicino & Pastoni (2004) isolated *Aeromonas*, *Pseudomonas* and *Legionella* from biofilm in drinking water distribution systems and highlighted its relevance to health hazards, especially in the hospital area. An investigation conducted by Villarruel-López *et al.* (2005) in drinking water plants (DWPs) and wastewater treatment plants (WWTPs) in Mexico City reported the presence of *Aeromonas* in 31% in the both kinds of sources. It was emphasized that most samples containing *Aeromonas* had concentrations below

doi: 10.2166/wh.2007.018

0.1ppm residual chlorine and that the isolated strains were resistant to 0.3ppm of residual chlorine. This paper shows the relevance of investigating the occurrence of *Aeromonas* genus in drinking water systems, particularly in domestic reservoirs where the residual chlorine concentration is often low.

Several studies revealed that these organisms can produce exotoxins, including α- and β-hemolysins (Singh & Sanyal 1992*b*), and cytolytic enterotoxin (Sen & Rodgers 2004; Figueras *et al.* 2005). Another important aspect to take into account in relation to these bacteria is that some strains are resistant to many antibiotics, such as gentamicin, ciprofloxacin, norfloxacin, tetracycline and ceftriaxone (Pokhrel & Thapa 2004). According to Hiransuthikul *et al.* (2005), over 50% of *A. hydrophila* isolated from December 2004 tsunami survivors in Southern Thailand were resistant to amoxycilin clavanulate. Due to the fact that *Aeromonas* are commonly isolated from the aquatic environment and are associated with gastroenteritis and wound infections in humans, studies concerning the presence of these organisms in drinking water distribution systems are very important to ensure the good quality of public water, resulting in protection and promotion of public health. The World Health Organization has recently proposed that *Aeromonas hydrophila* is one of the contaminants of concern in waterborne diseases (WHO 2004)

In view of this, the present study aimed at investigating the occurrence of *Aeromonas* genus and related toxins in chlorinated drinking water from reservoirs and sanitary fountains in the city of São Paulo, State of São Paulo, Brazil.

MATERIALS AND METHODS

Sample and sampling

A total of 200 chlorinated water samples were collected in one sampling round during a period of one year from 138 domestic and public reservoirs and 62 drinking water fountains from different areas of the São Paulo such as malls and convention centers, sportive centers, clubs and fitness centers, dwellings, hospitals, transportation terminals, schools, condominiums, parks and health care centers. Residual chlorine was measured through colorimetric method using Free-chlorine Analyser Microquant® (Merck). The samples were collected according to *Standard Methods for Examination of Water and Wastewater* (APHA 1995) in sterile disposable bottles, chilled for transportation and examined within a 24-hour period.

Isolation of *Aeromonas*

Aeromonas determination was qualitative using ampicilin – dextrin agar (ADA) (Havelaar *et al.* 1987). Volumes of 500 ml were concentrated by filtration through a 47 mm diameter membrane of 0.45μm porosity. The membranes were then transferred to alkaline peptone water (APW) and incubated for 24 h at 35 + 0.5°C. A loopful from the enriched broth was streaked onto ADA plates and incubated for 24 h at 30°C. Typical colonies (yellow) were kept in Luria agar and submitted to biochemical screening: oxidase test; H_2S and gas production; fermentation of glucose, sucrose, and indole production from L-tryptophane; lysine decarboxylation; motility and urea utilization. Colonies screened as *Aeromonas* genus were confirmed using a biochemical characterization as shown in Table 1. The results were expressed as Absence (A) or Presence (P) of *Aeromonas*.

Enterotoxin assay

Enterotoxin production was tested in Y-1 mouse adrenal cells according to Sack & Sack (1975). Confirmed *Aeromonas* strains were inoculated in Brain Heart Infusion Broth (BHIB) and incubated for 4 h at 35 + 0.5°C. The bacteria growth was transferred to Tryptic Soy Broth (TSB) supplemented with 0.6% of yeast extract (TSB-YE) incubated for 18 h at 35 + 0.5°C and centrifuged at 10.000xg for 30 minutes under refrigeration (5°C). The supernatant was filtered through 0.45 μm membrane porosity with 13 mm of diameter (Swinnex or Millex®-Millipore). Volumes of 25 μl of the supernatant were inoculated in duplicate in 96 well microtiter plates with Y-1 cells; the citotonic effect was observed after 6 and 18 hours of incubation at 37°C. Positive and negative controls were performed in parallel with each assay.

Extracts showing positive results were exposed to 56°C for 15 min to check the thermostability of the toxin.

Table 1 | Expected biochemical characteristics of different species of *Aeromonas*

	Aeromonas							
Biochemical test	***Hy***	***ca***	***So***	***tr***	***ja***	***sh***	***ve***	***all***
Arginine	+	+	−	+	+	+	−	V
Lysine	+	−	+	+	+	+	+	+
Ornitine	−	−	−	−	−	−	+	V
Sucrose	+	+	+	V	−	−	+	+
Aesculin hydrolisis	+	+	−	−	−	−	+	V
Manitol	+	+	+	V	+	−	+	+
Arabinose	+	+	−	−	−	−	+	V
Inositol	−	−	−	−	−	−	−	−
Manose	+	V	+	+	+	+	+	+
Gas from glucose	+	−	+	V	+	−	+	+
ONPG	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+
Gelatinase	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+
O/129	R	R	R	R	R	R	R	R
NaCl 0%	+	+	+	+	+	+	+	+
VP	+	−	V	−	+	−	+	−

hy = *hydrophila*, *ca* = *caviae*, *so* = *sobria*, *tr* = *trota*; *Sh* = *schubertii*, *ve* = *veronii*, *all* = *allosacharophila*; + = positive reaction above 80%; V = positive reaction between 50% and 79%; R = resistant. (Matté 1995).

Hemolysin assay

Production of α and β-hemolysins was performed in Tryptic Soy Agar (TSA) plates with 5% of sheep blood incubated at 35°C + 0.5°C for 24 and 48 h.

RESULTS AND DISCUSSION

Aeromonas was detected in 12 of the 200 samples examined (6%). Of 62 samples tested from drinking fountains 1 contained *Aeromonas* (1.6%), and from 138 reservoirs 11 contained *Aeromonas* (8.0%). The results obtained from this study can be compared to those from Fuzihara's *et al.* (1995) study, which detected the presence of *Aeromonas* in 4.6% of drinking water samples collected in the city of Santo André, State of São Paulo, Brazil. However this value was lower than those reported in other countries by Lechevalier *et al.* (1982), Araujo *et al.* (1989) and Ghenghesh *et al.* (2001).

Table 2 shows the *Aeromonas* frequency according to sample origin and average and median of residual chlorine concentration in each sampled water. The median values of residual chlorine did not attend the standard established in drinking water legislation (0.2 mg/L), except at hospital reservoirs. Residual chlorine averages were not in agreement with the standard for most of the samples. These results show the fragility of these systems reinforcing the findings of Villarruel-López *et al.* (2005).

Out of 122 typical strains isolated in ADA, 96 (78.7%) were confirmed as belonging to *Aeromonas* genus. The most frequent species were *A. caviae* (41.7%), followed by A. *hydrophila* (15.6%), *A. allosacharophila* (10.4%), *A. schubertii* (1.0%), and *Aeromonas spp.* (31.2%). *A. hydrophila*, *A. caviae* and *A. sobria* have also been commonly reported in others studies conducted in drinking water (Havelaar *et al.* 1992; Chauret *et al.* 2001; Sen & Rodgers 2004), showing similar results with our study. Figueras *et al.* (2005) found *Aeromonas culicicola* in drinking water samples; it is interesting to point out that rarely is this species found in water sources.

Table 3 shows the seasonal occurrence of *Aeromonas* during the study. The percentage of isolation was similar in Spring, Summer and Autumn (7.0 to 8.0%) dropping in Winter (2.4%). These data can be explained by the low variation of the temperature throughout the year in the city of São Paulo. Burke *et al.* (1984*a*) found a remarkable seasonal pattern in the occurrence of these bacteria in drinking water samples. In Scotland, the study conducted by Gavriel *et al.* (1996) showed a gradual increase of *Aeromonas* in drinking water associated with the raise in temperature, reaching the highest level in summer months. Vilarruel-López *et al.* (2005), in Mexico, found the largest number of *Aeromonas* in the summer season, when the temperature ranges from 25 to 30°C.

Table 2 | Frequency of *Aeromonas* isolating from drinking water and average and median residual chlorine concentration

Sample origin	Samples (n)	*Aeromonas* positive samples (%)	Residual chlorine concentration (mg/L) Average	Median
Malls and convention centers	33	3 (9.0)	0.20	0.1
Sportive centers	18	1 (5.5)	0.22	0.1
Clubs and fitness centers	18	2 (11.1)	0.13	0.1
Dwellings	31	3 (9.7)	0.16	0.1
Hospitals	19	1 (5.3)	0.22	0.3
Transportation terminals	19	2 (10.5)	0.13	0.1
Schools	17	0 (0.0)	0.17	0.1
Condominiums	14	0 (0.0)	0.18	0.1
Parks	19	0 (0.0)	0.21	0.1
Health care centers	12	0 (0.0)	0.18	0.1
Total	200	6.0 (12)		

Hemolytic activity was observed in 59 of the *Aeromonas* strains isolated (61.4%). Figure 1 summarizes hemolysin production for the different species of *Aeromonas*. *A. allosacharophila* showed the highest percentage of strains with hemolytic activity (80%) followed by *A. caviae* (70%), *A. hydrophila* (66.7%) and 46.6% of *Aeromonas spp*. The *A. schubertii* strain isolated did not show any hemolytic activity. Production of exotoxin (α and β-hemolysin) is associated with enteric disturbances, as reported by Burke *et al.* (1984*b*), Daily *et al.* (1981), Handfield *et al.* (1996), Macedo *et al.* (1997), Matté (1995), Monfort & Baleux (1991*b*), Pin *et al.* (1995), Singh and Sanyal (1992*a*, *b*). These results highlight the pathogenic potential of *Aeromonas* species and pose a public health concern. According to Wong *et al.* (1996), *A. hydrophila* A6 is able to produce both hemolysins and it is the combined effect of toxins which causes hemolysis, cytotoxicity and virulence in the suckling mouse model of infection. In 1999, Heuzenroeder *et al.* carried out a study using strains that had previously been shown to be virulent in the suckling mouse model, which

Table 3 | Percentage prevalence of *Aeromonas,* according to year seasons in drinking water samples from reservoirs and drinking fountains

Season	Number of samples	% *Aeromonas* occurrence
Spring	70	7.1
Summer	51	7.8
Autumn	37	8.1
Winter	42	2.4
Total	200	

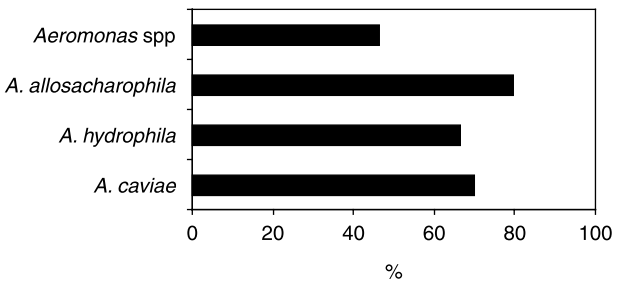

Figure 1 | Hemolytic activity observed in isolated strains of *Aeromonas*.

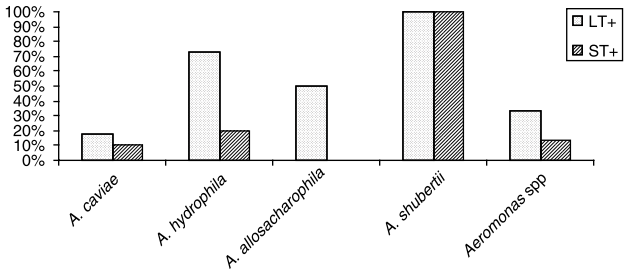

Figure 2 | Enterotoxin production by *Aeromonas* species isolated from drinking water samples, reservoirs and sanitary fountains.

showed the correlation of the presence of the genes aerA and hlyA, with cytotoxic and hemolytic activities. Their results indicated that all *A. hydrophila* isolates with hlyA + aerA + genotype were virulent in the suckling mouse model and also demonstrated β- hemolytic and cytotoxic activities. Out of four *A. veronii* biotype *sobria* strains, three were virulent and possessed the aerA gene alone, though all were β- hemolytic and cytotoxic. Takahashi *et al.* (2006) detected and purified a hemolytic toxin from *Aeromonas sobria* (ASH), which promotes fluid accumulation in the mouse intestinal loop. This work puts in evidence a novel insight into correlation between ion HCO_3^- and secretory diarrhea induced by bacterial infections, considering that ASH actively induces the secretion of HCO_3^-.

Heat-labile (LT^+) enterotoxin was produced by 35.4% of the *Aeromonas* strains isolated and 12.5% were able to produce heat-stable (ST^+) enterotoxins. Figure 2 illustrates the phenotypic characteristics of *Aeromonas* isolates for toxin production. Daily *et al.* (1981) carried out an investigation into the capacity of *A. hydrophila* and *A. caviae* to produce enterotoxins by using Y-1 cells, and found that 62.5% were able to produce such a toxin.

A study conducted by Handfield *et al.* (1996) demonstrated that 73% of *A. hydrophila* strains isolated from food and chlorinated and unchlorinated water samples were able to produce enterotoxin on a Y-1 cell culture, as well as Vero, CHO, HFF, HT-29, A-549 and HeLa. Furthermore, when the toxins were warmed up at 56°C for 10 minutes, 49% of the samples were positive against the culture cells mentioned.

Fifty nine *Aeromonas* strains showed to be hemolysin producers; out of these, 34 were LT^+ and 12, LT^+ and ST^+. Table 4 shows hemolytic activity and enterotoxin production according to the *Aeromonas* species isolated.

Sen & Rodgers (2004) studied virulence factors in *Aeromonas* strains isolated in treated water in US municipalities. These authors found that *Aeromonas* isolated from drinking water possess a wide variety of virulence in relation to genes, and consider in these municipalities studied treated water is a source of potentially pathogenic *Aeromonas* bacteria. In Australia, Snowden *et al.* (2006) studied *Aeromonas* prevalence in a major waterway of South East Queensland and their adhesion to Hep-2 and Caco-2 cells. The results showed the highest prevalence was *A. hydrophila* (43.0%) and the lowest was *A. veronii* biovar *sobria* (25.0%). Four patterns of adhesion were observed on both Hep-2 and Caco-2 cell lines.

Table 4 | Virulence factors of *Aeromonas* isolated from drinking water from reservoirs and sanitary fountains

Strains	Virulence factor expressed					
	Hac	Hac/LT + /ST +	LT + /ST +	LT +	Hac/LT +	Nv
Aeromonas spp.	10 (33.3%)	0 (0.0%)	10 (33.3%)	6 (20.0%)	4 (13.3%)	10 (33.3%)
A. shubertii	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
A. allosacharophila	4 (40.0%)	0 (0.0%)	0 (0.0%)	4 (40.0%)	4 (40.0%)	2 (20.0%)
A. hydrophila	2 (13.3%)	3 (20.0%)	3 (20.0%)	2 (13.3%)	9 (60.0%)	2 (13.3%)
A. caviae	24 (60.0%)	2 (5.0%)	2 (7.7%)	0 (0.0%)	4 (10.0%)	9 (22.5%)

Hac = only hemolytic activity; Hac/LT + /ST + = hemolytic activity and LT + and ST + producers; LT + /ST + = LT + and ST + producers; LT + = LT + producers; Hac/LT + = hemolytic activity and LT + producers; Nv = No virulence factor.

In Brazil, disturbances caused by *Aeromonas* species are not a notifiable disease; therefore this bacteria analysis is not required in clinical routine investigation. The presence of virulence factors in the *Aeromonas* isolates highlights the necessity of clinical professionals to consider the *Aeromonas* species role in gastrointestinal infections and wound infections.

CONCLUSIONS

The results obtained from this study confirm the presence of *Aeromonas* in drinking water reservoirs and drinking fountains. Most isolates were able to produce virulence markers. These emerging pathogens in the drinking water sources studied represent a public health concern, and must be taken into account for assessing the quality of drinking water.

ACKNOWLEDGEMENTS

We would like to thank FAPESP – Fundação de Amparo a Pesquisa do Estado de São Paulo for its financial support, and also CETESB – Companhia de Tecnologia de Saneamento Ambiental – for its assistance and support in lab analyses.

REFERENCES

APHA, AWWA, WEF 1995 *Standard Methods for the Examination of Water and Wastewater*, 20th edition. American Public Health Association/American Water Works Association/ Water Environment Federation, Washington, DC, USA.

Araujo, R. M., Arribas, R. M., Lucena, F. & Pares, R. 1989 Relation between *Aeromonas* and faecal coliforms in fresh waters. *Appl. Environ. Microbiol.* **67**, 213–217.

Aulicino, F. A. & Pastoni, F. 2004 Microorganisms surviving in drinking water systems and related problems. *Ann. IgJan-Apr.* **16**(1–2), 265–272.

Burke, V., Robinson, J., Cooper, M., Beaman, J. & Partridge, K. 1984*a* Isolation of *Aeromonas hydrophila* from a metropolitan water supply:seasonal correlation with clinical isolates. *Appl. Environ. Microbiol.* **48**(2), 361–366.

Burke, V., Robinson, J., Cooper, M., Beaman, J., Partridge, K., Peterson, D. & Gracey, M. 1984*b* Biotyping and virulence factors in clinical and environmental isolates of *Aeromonas* species. *Appl. Environ. Microbiol.* **47**(5), 1146–1149.

Chauret, C., Volk, C., Creason, R., Jacosh, J., Robinson, J. & Warnes, C. 2001 Detection of *Aeromonas hydrophila* in drinking water distribution system: a field and pilot study. *Can. J. Microbiol.* **47**, 782–786.

Daily, O., Joseph, S. W., Coolbaugh, J. C., Walter, R. I., Merreel, B. R., Rollins, D. M., Seidler, J., Colwell, R. R. & Lissner, C. R. 1981 Association of *Aeromonas sobria* with human infection. *J. Clin. Microbiol.* **13**(4), 769–777.

Figueras, M. J., Suarez-Franquet, A., Chacón, M. R., Soler, L., Navarro, M., Alejandre, C., Grasa, B., Martinez-Murcia, J. & Guarro, J. 2005 First record f the rare species *Aeromonas culicicola* from drinking water supply. *Appl. Environ. Microbiol.* **77**(1), 538–541.

Freitas, A. C., Milhomem, A. M., Nunes, M. & Ricciardi, I. D. 1993 Virulence factors produced by *Aeromonas hydrophila* strains isolated from a different sources. *Revista Microbiol.* **24**(3), 168–174.

Fuzihara, T. O., Murakami, K. A. & Vanucci, L. 1995 Aeromonas hydrophila: Ocorrência em águas de consumo humano. In *XVIII Congresso Brasileiro De Microbiologia, São Paulo 1995*. Resumos, São Paulo.

Gavriel, A. A, Landré, J. B & Lamb, A. J. 1996 Incidence of *Aeromonas* within a public drinking water supply. In *Health-related Water Microbiology, Mallorca 1996*, Abstracts. Mallorca, 6-10 October 1996.

Ghegehnsh, K. S., El-Ghodban, A., Dkakni, R., Abeid, S., Altomi, A., Tarhuni, A. & Maialigeti, K. 2001 Prevalence, species differentiation, hemolytic activity and antibiotic susceptibility of aeromonads in untreated well water. *Memórias do Instituto Oswaldo Cruz, Rio de Janeiro* **96**(2), 169–173.

Handfield, M., Simard, A., Couillard, M. & Letarte, R. 1996 *Aeromonas hydrophila* isolated from food and drinking water: hemagglutination, haemolysis and cytotoxicity for a human intestinal cell line (HT-29). *Appl. Environ. Microbiol.* **62**(9), 3459–3461.

Häninen, M. L., Salmi, S., Matilla, L., Taipalinen, R. & Siitonena, A. 1995 Association of *Aeromonas* sp with travellers diarrhoea in Finland. *J. Med. Microbiol.* **42**, 26–31.

Havelaar, A. H., During, M. & Versteegh, J. F. 1987 Ampicillin-dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filtration. *J. Appl. Bacteriol.* **62**, 279–287.

Havelaar, A. H., Schets, F. M., Sifhout, A. V., Jansen, W. H., Wieten, G. & Kooji, D. V. D. 1992 Typing of *Aeromonas* strains from patients with diarrhea and from drinking water. *J. Appl. Bacteriol.* **72**, 435–444.

Heuzenroeder, M. W., Wong, C. Y. F. & Flower, R. L. P. 1999 Distribution of two hemolytic toxin genes in clinical and environmental isolates of *Aeromonas* spp.: correlation with virulence in a suckling mouse model. *FEMS Microbiol. Lett.* **174**, 131–136.

Hiransuthikul, N., Tantisiriwat, W., Lertutsahakul, K., Vibhagool, A. & Boonma, P. 2005 Skin and soft-tissue infections among tsunami survivors in southern Thailand. *Clin. Infect. Dis.* **41**, e93–e96.

Joseph, S. W., Carnahan, A., Brayton, R., Fanning, G. R., Almazan, R., Brabick, C., Trudo, E. W. Jr. & Colwell, R. R. 1991 *Aeromonas jandaei* and *Aeromonas veronii* dual infection of a human wound following aquatic exposure. *J. Clin. Microbiol.* **29**(3), 565–569.

Lechevalier, M. W., Evans, T. M., Seidler, R. J., Daily, O., Merrel, D. M. & Joseph, S. W. 1982 *Aeromonas sobria* in chlorinated drinking water supplies. *Microb. Ecol.* **8**, 325–333.

Macedo, L. C., Nunes, M. & Freitas, A. C. 1997 Fatores de virulência de *Aeromonas* spp. isoladas de fezes de crianças no Rio de Janeiro, RJ. In *XIX Congresso Brasileiro De Microbiologia, Rio de Janeiro 1997*, Resumos. Rio de Janeiro.

Matté, M. H. 1995 Pesquisa de *Aeromonas* spp. potencialmente patogênicas em alguns pontos da Represa de Guarapiranga destinados à recreação e captação para abastecimento público. Dissertação de mestrado - Faculdade de Saúde Pública da Universidade de São Paulo, 113, São Paulo, 1995.

Merino, S., Rubiers, X., Knochel, S. & Tomás, J. M. 1995 Emerging pathogens:*Aeromonas* sp. *Int. J. Food Microbiol.* **28**, 157–168.

Monfort, M. & Baleux, B. 1991*a* Distribution and survival of motile *Aeromonas spp.* in brackish water receiving sewage treatment effluent. *Appl. Environm. Microbiol.* **57**(9), 2459–2467.

Monfort, M. & Baleux, B. 1991*b* Haemolysin ocurrence among *Aeromonas hydrophila, Aeromonas caviae* and *Aeromonas sobria* strains isolated from different aquatic ecosystems. *Res. Microbiol.* **142**, 95–102.

Pin, C., Marín, M. L., Selgas, D., Tormo, M. L. G. J. & Casas, C. 1995 Differences in production of several extracellular virulence factors in clinical and food *Aeromonas spp.* strains. *J. Appl. Bacteriol.* **78**, 175–179.

Pokhrel, B. M. & Thapa, N. 2004 Prevalence of Aeromonas in different clinical and water samples with special reference to gastroenteritis. *Nepal Med Coll J.* **6**(2), 139–143.

Sack, D. A. & Sack, R. B. 1975 Test for enterotoxigenic *Escherichia coli* using Y-1 adrenal cells in miniculture. *Infect. Immun.* **11**, 334–336.

Sen, K. & Rodgers, M. 2004 Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. *J. Appl. Microbiol.* **97**(5), 1077–1086.

Singh, D. V. & Sanyal, S. C. 1992*a* Haemolysin and enterotoxin production by *Aeromonas caviae* isolated from diarrhoeal patients, fish and environment. *J. Diarrhoeal Dis. Res.* **10**(1), 16–20.

Singh, D. V. & Sanyal, S. C. 1992*b* Enterotoxicity of clinical and environmental isolates of Aeromonas sp. *J. Med. Microbiol.* **26**, 269–272.

Snowden, L., Wernbacher, L., Stenzel, D., Tucker, J., McKay, D., O'Brien, M. & Katouli, M. 2006 Prevalence of environmental *Aeromonas* in South East Queensland, Australia: a study of their interactions with human monolayer Caco-2 cells. *J. Appl. Microbiol.* **101**(4), 964–975.

Takahashi, A., Nakano, M., Okamoto, K., Fijii, Y., Mawatari, K., Harada, N. & Nakaya, Y. 2006 *Aeromonas sobria* hemolysin causes diarrhea by increasing secretion of HCO_3^-. *FEMS Microbiol. Lett.* **258**, 92–95.

Villarruel-Lopez, A., Fernandez-Rendon, E., Mota-de-la-Garza, L. & Ortigoza-Ferado, J. 2005 Presence of *Aeromonas* spp. in water from drinking-water-and wastewater-treatment plants in México City. *Water Environ. Res.* **77**(7), 3074–3079.

Wong, C. Y. F., Mayrhofer, G., Heuzenroeder, M. W., Atkinson, H. M., Quinn, D. M. & Flower, R. L. P. 1996 Measurement of virulence of aeromonads using suckling mouse model of infection. *FEMS Immunol. Med. Microbiol.* **15**, 233–241.

World 2004 World Health Organization Microbial Facts Sheets. In *Guidelines for Drinking Water*, **Vol. 1**. *Recommendations*, 3rd edn. WHO, Geneva, pp. 224.

First received 15 December 2006; accepted in revised form 11 February 2007. Available online November 2007.