The prevalence of *Aeromonas hydrophila*-induced diarrhoea in the pig, buffalo and human in Pune area, India

Rahimi-Larki, E.[1*] **and Nene, S. S.**[2]

[1]Department of Production, Razi Vaccine and Serum Research Institute, Shiraz, Iran; [2]Department of Microbiology, School of Medicine, University of Pune, Pune, India

[*]**Correspondence:** E. Rahimi-Larki, Department of Production, Razi Vaccine and Serum Research Institute, Shiraz, Iran. E-mail: rahimilarki@yahoo.com

Summary

Aeromonas hydrophila is pathogen for several vertebrates. The bacteriological, clinical and epidemiological evidences for the role of *A. hydrophila* have been described in human infections. The presence of this pathogen in contaminated water is well-established and ingestion of such water may cause infection. There are many reports of acute diarrhoea associated with *A. hydrophila* transmitted by animals. In this study, 100 faecal samples of patients suffering from diarrhea and 33 faecal specimen from healthy individuals who served as control, were examined for presence of *A. hydrophila.* The faeces of pigs and buffaloes and the drinking water in this area were also examined for isolation and characterization of the bacteria. The results showed that in this area, the role of *A. hydophila* in development of acute human diarrhoea (1%) was less significant. The organism was sensitive to erythromycin, chloramphenicol, kanamycin, and gentamycin, but resistant to penicillin and ampicillin. *A. hydrophila* was present in faeces of buffaloes. Five samples of contaminated water were found toxigenic, too.

Key words: *Aeromonas hydrophila*, Diarrhoea, Pune, India

Introduction

Aeromonas hydrophila has been known as a pathogen associated with several human infections such as acute gastroenteritis (Kindschu *et al*., 1987; Hanninen *et al*., 1995; Chopra and Houston, 1999; Huys *et al*., 2003); wound infections caused by contamination of water (Phillips *et al*., 1974); septicaemia in immunocompromized hosts (Harris *et al*., 1985); and other less frequently encountered infections, including urinary tract infections (McCracken and Barkley, 1972), myositis (Deepe and Coonrod, 1980), peritonitis (Sitto and Schiks, 1973), meningitis (Quadri *et al*., 1976), endocarditis (Davis *et al*., 1978) and aspiration pneumonia (Reines and Cook, 1981). Various virulence factors have been described for *A. hydrophila*, including cytotoxin (Turnbull *et al*., 1984; Krovacek *et al*., 1994; Xu *et al*., 1998), haemolysin (Wadstrom *et al*., 1976; Janda *et al*., 1996), haemagglutinins (Burke *et al*., 1984; Thorney *et al*., 1997), and the ability to adhere to and invade epithelial cells (Watson *et al*., 1985; Janda, 1991; Chopra *et al*., 1996). *A. hydrophila* was cultured from human sources as early as 1937 (Miles and Halnan, 1937) and their presence in drinking water is well-established (Le-Chevallier *et al*., 1982; Kuhn *et al*., 1997). Ingestion of such contaminated waters may cause severe infections (Picard *et al*., 1984; Haque *et al*., 1996). The significance of *A. hydophila*, as intestinal pathogens, is still controversial (Figura *et al*., 1986).

The correct diagnosis of infective diarrhoea is imperative in view of correct therapy against the infective agent and for subsequent planning for prevention programs. As few reports are available on *A. hydrophila* in relation to acute cases of diarrhoea in India and since no data is available from Pune area, this study was undertaken to determine the isolation rate of *A. hydrophila* from patients who were presented with diarrhoea to the Department of Infectious Diseases of Pune Hospital.

Materials and Methods

Stool samples taken from 100 patients

with diarrhoea and 33 normal individuals (controls) were collected and studied for isolation of *A. hydrophila*. Patients were randomly selected among those who were admitted to the Department of Infectious Diseases of Pune Hospital.

Patients with loose or watery stool who had increased frequency of defecation were included in this study. The clinical details, age, sex and socio-economic status of patients were also noted.

Stool samples in Pediatrics Department were collected by two sterile rectal swabs and care was taken to obtain the samples free from urine contamination. The materials on swabs were examined for presence of any pus, mucus or blood. One of the swabs was transferred into alkaline peptone water (pH = 8.6) and the other was sent for microscopic examination. Children and adults stool samples were collected in sterile petri dishes and then transferred into alkaline peptone water for microscopic examination.

The procedure planned for isolation of *A. hydrophila* was that of Millership *et al*., (1983).

In this study, 68 and 55 fresh faecal samples of buffaloes and pigs were also collected. Furthermore, 45 samples of stagnant and five samples of tap water from various parts of Pune were collected for isolation of *A. hydrophila*.

Stagnant water was collected from ponds near the drinking water sources. Some samples were taken from river bed where the flow of water is stagnated and water may be contaminated by decaying tree leaves, birds and animals. Water samples were received in sterile glasses. The samples were processed and examined as described by Millership *et al*., (1983).

Antibiotic sensitivity test was carried out on isolated samples of *A. hydrophila* by disc diffusion method as shown by Baur *et al*., (1966).

Enterotoxigenicity test for the isolates was carried out on suckling mice as recommended by Burke *et al*., (1981).

Results

The age range of the affected children was two months to 14 years. Fifty-two children were under the age of two years, 17 between two and five years and nine between five to 14 years of age. Twenty-two patients were older than 14 years.

A. hydrophila was isolated only from the stool specimen of a 6-month-old patient (1%). In the control group, *A. hydrophila* was not isolated from any stool specimen.

The stool in patients with diarrhoea was watery with flakes of mucus. However, none had pus or blood. Patients had mild colicy pains, but none complained of vomiting, fever or tenesmus. Mild to moderate dehydration was seen in patients at Pediatric Department. Parasitic examination revealed the presence of *Ascaris lumbricoides* eggs in 33% of children; a few were positive for giardiasis.

A. hydrophila was isolated in 1.47% of faecal samples of the buffaloes but in none of the pigs.

A. hydrophila was isolated only from unchlorinated water (11.11%); three samples were isolated during summer and two during winter.

The chlorinated supplies were negative for the bacteria.

Table 1: The results of toxigenicity test by suckling mice

No.	Strain No.	IW/BW ratio		Scoring
		Min	Max	
1	34 (W)	0.063	0.095	+++
2	42 (W)	0.040	0.072	+
3	160A (Pt.)	0.066	0.093	+++
4	160B (Pt.)	0.069	0.078	+
5	229 (B)	0.11	0.0183	++++
6	Control strain	0.061		

IW/BW- Ratio of intestinal weight to remaining body weight; Ratio of IW/BW, if less than 0.070 = 0; between 0.070 and 0.079 = +; between 0.080 and 0.089 = ++; between 0.090 and 0.099 = +++; above 0.1 = ++++

Results of the toxigenicity test by suckling mice are shown in Table 1 and Fig. 1. The toxigenicity test was further extended to haemolysin test by using rabbit red blood cells and also by cytopathic effects on green monkey cell lines. Table 2 shows the comparison of various toxigenic tests for human, animal and water sources.

A complete correlation was only observed in human isolates. *A. hydrophila* isolated in the present study was found to be

Table 2: Comparison of the results of various toxigenic tests

Strain No.	Source	Scores of enterotoxin on suckling mice assay	Result of haemolysin on rabbit (RBCs)	Cytotoxin effet on B.G.M. cell line*
34	Water	+++	-ve	-ve
42	Water	+	+ve	+ve
160A	Human	+++	+ve	+ve
160B	Human	+	-ve	+ve
229	Buffaloes	++++	+ve	-ve

*B.G.M. cell line: Buffalo green monkey cell line

Table 3: Antibiotic sensitivity test for *A. hydrophila*

No.	Agent	Symbol	Strength in MCG	4	27	29	34	42	160 A	160 B	229
1	Ampicillin	AM	10 mcg	R	R	R	R	R	R	R	R
2	Cephaloridine	CR	30	S	ND	ND	R	R	R	R	R
3	Chloramphenicol	C	30	S	S	S	S	S	S	S	S
4	Carbenicillin	CN	50	ND	ND	ND	R	R	R	R	R
5	Kanamycin	K	30	S	S	S	S	S	S	S	S
6	Cotrimoxazole (Buctrim)	BA	25	ND	ND	ND	S	S	S	S	S
7	Sulphatriad	ST	300	ND	ND	ND	S	S	S	S	S
8	Tetracycline	TC	30	S	S	S	S	S	S	S	S
9	Gentamycin	GM	10	S	S	S	S	S	S	S	S
10	Streptomycin	S	10	S	S	S	S	S	S	S	S
11	Erythromycin	E		S	S	S	S	S	S	S	S
12	Penicillin	P		R	R	R	R	R	R	R	R

Impression: *A. hydrophila* was found resistant to ampicillin and penicillin. It was sensitive to kanamycin, gentamycin, chloramphenicol, erythromycin. ND: Not done; R: resistant; S: sensitive

resistant to ampicillin and penicillin but sensitive to kanamycin, streptomycin, tetra-cycline, chloramphenicol and genta mycin (Table 3).

Fig. 1a: Normal cell growth of BGM cell line, BGM = buffalo green monkey cell line

Fig. 1b: Cytopathogenic effect of *A. hydrophila* toxin (75%)

Discussion

A. hydrophila has been recognized in soil and natural water sources for many

years (Le-Chevallier *et al*., 1982; Picard *et al*., 1984; Haque *et al*., 1996; Kuhn *et al*., 1997). They have been isolated from pigs (Gray, 1984; Figura and Marri, 1985), horses, sheep and cows (Gray, 1984). This organism has been recovered from stools of asymptomatic children (Von Graevenitz and Mensch, 1968) and has also been reported as a cause of acute diarrhoea in small children (Taylor *et al*., 1985), and travellers' diarrhoea in both adults and children (Taylor *et al*., 1985; Kindschuch *et al*., 1987; Hanninen *et al*., 1995; Chopra and Houston, 1999; Huys *et al*., 2003).

In Pune, India, pigs and buffaloes are constantly seen around surface and drinking water supplies and can be regarded as sources of contamination of water. Sanyal *et al*., (1975), and Annapurna and Sanyal (1977) isolated the bacteria from faeces of domestic animals. The results of this study showed that pigs and buffaloes can be regarded as carriers of *A. hydrophila*, because the bacteria was not isolated from pigs and only one positive faecal specimen was seen in buffaloes.

In this study, *A. hydrophila* was isolated from five surface water samples; the bacteria was however not isolated from chlorinated tap water. The results were similar to Bhat *et al*., (1974), Annapurna and Sanyal (1977), Gray (1984), Krovacek *et al*., (1994), Hanninen and Siitonen (1995) and Kuhn *et al*., (1997), who have reported the isolation of *A. hydrophila* from water sources.

Gray (1984) obtained the bacteria from two samples of chlorinated water which is not in keeping with our findings.

Isolation of *A. hydrophila* in 1% of the patients in this study is similar to the reports of Aggar *et al*., (1985), Saraswathi and Deodhar (1986), Kindschuch *et al*., (1987), Hanninen *et al*., (1995), Chopra and Houston, (1999), Albert *et al*., (2000) and Huys *et al*., (2003).

Isolation of *A. hydrophila* from diarrheaic stools is not a proof that the diarrhoea is caused by this organism. Toxigenicity test is imperative to confirm the diagnosis. In the present study, the single isolation of the bacteria from a 6-month-old child was found toxigenic by suckling mouse test. The toxigenicity test was further extended from haemolysin and cytotoxin tests to cytopathic effects to confirm the toxigenicity of the bacteria. There are still controversy on the pathogenicity tests of *A. hydrophila*. Some authors reported no correlation (Kindschuch *et al*., 1987), while others substantiated it (Chakroborty *et al*., 1987).

It seems that in Pune area, India, *A. hydrophila* is not a major cause of human diarrhoea. More large-scale studies are needed to shed light over its role as a human enteropathogen in this place.

Acknowledgements

We would like to thank the Microbiology staff of Pune University for doing laboratory procedures, and Dr. D. Mehrabani, from the Center for Development of Clinical Research of Nemazee Hospital, for editorial and statistical assistances.

References

1- Aggar, WA; McCornick, JD and Guerwith, MJ (1985). Clinical and microbiological features of *Aeromonas hydrophila*. Associated diarrhea. J. Clin. Microbiol., 21(6): 909-913.

2- Albert, MJ; Ansaruzzaman, M; Talukder, KA; Chopra, AK; Kuhn, I; Rahman, M; Faruque, ASG; Islam, MS; Sack, RB and Mollby, R (2000). Prevalence of enterotoxin genes in *Aeromonas* spp. isolated from children with diarrhea, healthy controls, and the environment. J. Clin. Microbiol., 38(10): 3785-3790.

3- Annapurna, E and Sanyal, SC (1977). Enterotoxicity of *Aeromonas hydrophila*. J. Med. Microbiol., 10: 317-323.

4- Baur, AW; Kirby, WVM; Sherris, JC and Turck, M (1966). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin. Pathol., 45: 493-496.

5- Bhat, P; Shanthakumari, S and Rajan, D (1974). The characterization and significance of Plesiomonas shigelloides diarrhea. Indian J. Med. Res., 62: 1051-1059.

6- Burke, V; Cooper, M; Robinson, J; Gracey, M; Lesmana, M; Echeverria, P and Jandan, JM (1984). Hemagglutination patterns of *Aeromonas* spp. in relation to biotype and source. J. Clin. Microbiol., 19(1): 39-43.

7- Burke, V; Robinson, J; Berry, RJ and Gracy, M (1981). Detection of enterotoxin of

Aeromonas hydrophila by a suckling mouse test. J. Med. Microbiol., 14: 401-408.

8- Chakroborty, T; Katharion, S; Hacker, J; Hof, H; Huhle, B; Wagner, W; Kuhn, M and Goebel, W (1987). Molecular analysis of bacterial cytolysins. Rev. Infect. Dis., 9: 46.

9- Chopra, AK and Houston, CW (1999). Enterotoxins in *Aeromonas*-associated gastroenteritis. Microb. Infect., 1: 1129-1137.

10- Chopra, AK; Peterson, JW; Xu, XJ; Coppenhaver, DH and Houston, CW (1996). Molecular and biochemical characterization of a heat-labile cytotonic enterotoxin from *Aeromonas hydrophila*. Microb. Pathog., 21: 357-377.

11- Davis, WA; Kane, JG and Garagusi, VF (1978). Human *Aeromonas* infections: a review of the literature and a case report of endocarditis. Medicine. 57: 267-277.

12- Deepe, GS and Coonrod, JD (1980). Fulminant wound infection with *Aeromonas hydrophila*. South Med. J., 73: 1546-1547.

13- Figura, N; Marri, L; Verdiani, S; Ceccherini, G and Barberi, A (1986). Prevalence, species differentiation, and toxigenicity of *Aeromonas* strains in cases of childhood gastroenteritis and in controls. J. Clin. Microbiol., 23(3): 595-599.

14- Figura, N and Marri, L (1985). Isolation of *Aeromonas* species from animals. Eur. J. Clin. Microbiol., 4: 354-355.

15- Gray, SJ (1984). *Aeromonas hydrophila* in livestock. Incidence and biochemical characteristics and antibiotic sensitivity. J. Hyg., 92: 365.

16- Hanninen, ML and Siitonen, A (1995). Distribution of *Aeromonas* phenospecies and genospecies among strains from water, foods or from clinical samples. Epidemiol. Infect., 115: 39-50.

17- Hanninen, ML; Salmi, S; Mattila, L; Taipalinen, R and Siitonen, A (1995). Association of *Aeromonas* spp. with travellers' diarrhoea in Finland. J. Med. Microbiol., 42: 26-31.

18- Haque, QM; Sugiyama, A; Iwada, Y; Midorikawa, Y and Yamaguchi, T (1996). Diarrheal and environmental isolates of *Aeromonas* spp. produce a toxin similar to Shiga-like toxin 1. Curr. Microbiol., 32: 239-245.

19- Harris, RL; Fainstein, V; Eltting, L; Hopfer, RL and Bodey, GP (1985). Bacteremia caused by *Aeromonas* species in hospitalized cancer patients. Rev. Infec. Dis., 7(3): 314-320.

20- Huys, G; Cnockaert, M; Janda, JM and Swings, J (2003). *Escherichia albertii* sp. nov., a diarrhoeagenic species isolated from stool specimens of Bangladeshi children. Int. J. Syst. Evol. Microbiol., 53: 807-810.

21-Janda, JM (1991). Recent advances in the study of the taxonomy, pathogenicity, and infectious syndromes associated with the genus *Aeromonas*. Clin. Microbiol. Rev., 4: 397-410.

22- Janda, JM; Abbott, SL; Khashe, S; Kellogg, GH and Shimada, T (1996). Further studies on biochemical characteristics and serologic properties of the genus *Aeromonas*. J. Clin. Microbiol., 34: 1930-1933.

23- Kindschuch, HM; Pickering, LK; Cleary, TG and Ruiz-Palacios, G (1987). Clinical and biochemical significance of toxin production by *Aeromonas hydrophila*. J. Clin. Microbiol., 25(5): 916-921.

24- Krovacek, KV; Pasquale, SB; Baloda, V; Soprano, M; Conte, M and Dumontet, S (1994). Comparison of putative virulence factors in *Aeromonas hydrophila* strains isolated from the marine environment and human diarrheal cases in southern Italy. Appl. Environ. Microbiol., 60: 1379-1382.

25- Kuhn, IMJ; Albert, M; Ansaruzzaman, NA; Bhuiyan, SA; Alabi, G; Huys, M; Islam, S; Janssen, P; Kersters, K; Neogi, PKB and Mollby, R (1997). Characterization of *Aeromonas* spp. isolated from humans with diarrhea, from healthy controls and from surface water in Bangladesh. J. Clin. Microbiol., 35: 369-373.

26- Kuhn, IG; Allestam, G; Huys, P; Janssen, K; Kersters, K; Krovacek, K and Stenstrom, TA (1997). Diversity, persistence, and virulence of *Aeromonas* strains isolated from drinking water distribution systems in Sweden. Appl. Environ. Microbiol., 63: 2708-2715.

27- Le-Chevallier, MW; Evans, TM; Seidler, RJ; Daily, OP; Merri, BR; Rollins, DM and Joseph, SW (1982). *Aeromonas sobria* in chlorinated drinking water supplies. Microbiol. Ecol., 8: 325-333.

28- McCracken, AW and Barkley, R (1972). Isolation of *Aeromonas* species from clinical sources. J. Clin. Pathol., 25: 970-975.

29- Miles, AA and Halnan, ET (1937). A new species of microorganisms causes black rot in eggs. J. Hyg., 37: 79.

30- Millership, SE; Curnow, SR and Chattopadhyay, B (1983). Faecal carriage rate of *Aeromonas hydrophila*. J. Clin. Pathol., 36: 920-923.

31- Phillips, JA; Bernhardt, HE and Rosenthal, SG (1974). *Aeromonas hydrophila* infections. Pediatrics. 53: 110-112.

32- Picard, B; Arlet, G and Goullet, PH (1984). Septicemies of *Aeromonas hydrophila*. Aspects epidemiologiques. Quinze Obser-

vations La Presse Medicale, 13: 1203-1205.
33- Quadri, SMH; Gordon, LP; Wende, RD and Williams, RP (1976). Meningitis due to *Aeromonas hydrophila*. J. Clin. Microbiol., 3: 102-104.
34- Reines, HD and Cook, FV (1981). Pneumonia and bacteremia due to *Aeromonas hydrophila*. Chest. 80: 264-267.
35- Sanyal, SC; Singh, SJ and Sen, PC (1975). Enteropathogenicity of *Aeromonas hydrophila* and Plesiomonas shigelloides. J. Med. Microbiol., 8: 195-198.
36- Saraswathi, K and Deodhar, LP (1986). Diarrhoea associated with *Aeromonas hydrophila*. Indian J. Med. Res., 84: 571-573.
37- Sitto, RI and Schiks, S (1973). *Aeromonas hydrophila* peritonitis. Cancer Chemoth. Rep., 57: 489-491.
38- Taylor, DB; Echevarria, P; Blaser, MN; Blacklam, N; John, PC and Weniger-Bruce, G (1985). Polymicrobial aetiology of travellers' diarrhoea. Lancet. 1: 381-383.
39- Thorney, JP; Shaw, JG; Gryllos, IA and Eley, A (1997). Virulence properties of clinically significant *Aeromonas* species: evidence for pathogenicity. Rev. Med. Microbiol., 8: 61-72.
40- Turnbull, PCB; Lee, JV; Milliotis, MD; Van de Walle, S; Koornhof, HJ; Jeffery, L and Bryant, TN (1984). Enterotoxin production in relation to taxonomic grouping and source of isolation of *Aeromonas* species. J. Clin. Microbiol., 19(2): 175-180.
41- Von Graevenitz, A and Mensch, AH (1968). The genus Aeromonas in human bacteriology. Report of 30 cases and review of the literature. N. Engl. J. Med., 278: 245-249.
42- Wadstrom, T; Ljungh, A and Wretlind, B (1976). Enterotoxin hemolysin and cytotoxic protein in *Aeromonas hydrophila* from human infections. Acta Pathol. Microbiol. Scand., Sect. B, 84: 112-114.
43- Watson, IM; Robinson, JO; Burke, V and Gracey, M (1985). Invasiveness of *Aeromonas* spp. in relation to biotype, virulence factors, and clinical features. J. Clin. Microbiol., 22: 48-51.
44- Xu, XJ; Ferguson, MR; Popov, VL; Houston, CW; Peterson, JW and Chopra, AK (1998). Role of a cytotoxic enterotoxin in *Aeromonas*-associated infections: development of transposon and isogenic mutants. Infect. Immun., 66: 3501-3509.