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

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**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. hydrophila* 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); septicemia 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 (Tumbull 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. hydrophila*, 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 colicky 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**

<table>
<thead>
<tr>
<th>No.</th>
<th>Strain No.</th>
<th>IW/BW ratio</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>1</td>
<td>34 (W)</td>
<td>0.063</td>
<td>0.095</td>
</tr>
<tr>
<td>2</td>
<td>42 (W)</td>
<td>0.040</td>
<td>0.072</td>
</tr>
<tr>
<td>3</td>
<td>160A (Pr.)</td>
<td>0.066</td>
<td>0.093</td>
</tr>
<tr>
<td>4</td>
<td>160B (Pr.)</td>
<td>0.069</td>
<td>0.078</td>
</tr>
<tr>
<td>5</td>
<td>229 (B)</td>
<td>0.11</td>
<td>0.0183</td>
</tr>
<tr>
<td>6</td>
<td>Control strain</td>
<td>0.061</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Source</th>
<th>Scores of enterotoxin on suckling mice assay</th>
<th>Result of haemolysin on rabbit (RBCs)</th>
<th>Cytotoxin efft on B.G.M. cell line*</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Water</td>
<td>+++</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>42</td>
<td>Water</td>
<td>+</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>160A</td>
<td>Human</td>
<td>+++</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>160B</td>
<td>Human</td>
<td>+</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>229</td>
<td>Buffaloes</td>
<td>+++</td>
<td>+ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

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

Table 3: Antibiotic sensitivity test for A. hydrophila

<table>
<thead>
<tr>
<th>No.</th>
<th>Agent</th>
<th>Symbol</th>
<th>Strength in MCG</th>
<th>4</th>
<th>27</th>
<th>29</th>
<th>34</th>
<th>42</th>
<th>160</th>
<th>160</th>
<th>229</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ampicillin</td>
<td>AM</td>
<td>10 mcg</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
</tr>
<tr>
<td>2</td>
<td>Cephaloridine</td>
<td>CR</td>
<td>30</td>
<td>S</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>Chloramphenicol</td>
<td>C</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>Carbenicillin</td>
<td>CN</td>
<td>50</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>Kanamycin</td>
<td>K</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>Cotrimoxazole</td>
<td>BA</td>
<td>25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>Sulphatrad</td>
<td>ST</td>
<td>300</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<tr>
<td>8</td>
<td>Tetracycline</td>
<td>TC</td>
<td>30</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>9</td>
<td>Gentamycin</td>
<td>GM</td>
<td>10</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>10</td>
<td>Streptomycin</td>
<td>S</td>
<td>10</td>
<td>S</td>
<td>S</td>
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<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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</tr>
<tr>
<td>11</td>
<td>Erythromycin</td>
<td>E</td>
<td></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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</tr>
<tr>
<td>12</td>
<td>Penicillin</td>
<td>P</td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

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, tetracycline, chloramphenicol and genta mycin (Table 3).

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 from diarrheic 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

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