Occurrence of *Aeromonas hydrophila* in acute gastroenteritis among children

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*Background & objectives:* The interest on the occurrence multidrug resistance and pathogenicity of *Aeromonas hydrophila* is increasing worldwide since it causes gastroenteritis to children. Though reports on the occurrence of gastroenteritis among children due to *A. hydrophila* in Tamil Nadu are available from certain areas, no information is available from Coimbatore. Hence, this study was undertaken to find out the occurrence of the pathogenic *A. hydrophila* in diarrhoeal stool of children, particularly in Coimbatore region.

*Methods:* Isolation and identification of *A. hydrophila* was carried out from stool samples collected from children with acute diarrhoea. Multiple antibiotic resistance was determined by disc diffusion method. The pathogenicity of *A. hydrophila* was confirmed by production of haemolysin, protease and slime.

*Results:* Of the 216 samples, 21 (9.7%) were positive for *A. hydrophila*. Among them 20 isolates were resistant to bacitracin. Most of the isolates showed multiple antibiotic resistance. Among the 21 isolates, protease and haemolysin producers were 100 and 95 per cent respectively. About 76 per cent of the isolates produced slime.

*Interpretation & conclusion:* The results of the present study indicated the presence of pathogenic *A. hydrophila* in the study area causing diarrhoea among children.

**Key words** *Aeromonas hydrophila* - haemolysin - MAR index - protease - slime

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Aeromonas hydrophila is one of the causative agents for diarrhoeal infections in children and immunocompromised patients. These are ubiquitous water borne organisms and have gained importance as human pathogens causing gastrointestinal and extraintestinal infections. Clinical and environmental isolates secrete many extracellular products, such as haemolysins, enterotoxins, aerolysin, haemagglutinins, protease and slime. Among these toxins, aerolysin is released as a protoxin, which can be activated by protease released by the bacteria. Aeromonas infection may occur by taking faecal contaminated water and food.

In recent years, extensive studies have been done on the presence of Escherichia coli, Salmonella, Shigella, Vibrio and Enterobacter in diarrhoeal stool samples. Though reports are available on the presence of A. hydrophila in diarrhoeal stool samples of children in certain areas in Tamil Nadu, no report is available from Coimbatore. The present study was therefore carried out to document the presence of pathogenic A. hydrophila in diarrhoeal stool samples in children at Coimbatore.

Material & Methods

Collection of samples: A total of 216 stool samples were collected from children (<6 yr) who had infected with acute diarrhoea attending Massonic Child Care Hospital, Coimbatore, south India during January - December 2003, before starting antibiotic therapy. The stool specimens were collected, and transported into laboratory by using Stuart’s transport medium (Hi-media, Mumbai, India).

Isolation and identification: The stool specimens were enriched in alkaline peptone water (APW) and a loopful of enriched culture was streaked on to starch ampicillin agar medium (SAA) (Hi-Media, Mumbai, India) and Rimler Shotts medium (Hi-Media, Mumbai, India). Yellow to honey coloured, oxidase, catalase positive colonies were taken and tested in Kaper’s multitest medium. Appearance of alkaline surface and acid butt after 24 h at 37°C demonstrated the presence of A. hydrophila. Other biochemical tests for confirmation of identification were also done. A control strain A. hydrophila MTCC-646 (Microbial Type Culture Collection Centre, Chandigarh, India) was used in parallel.

Determination of multiple antibiotic resistance: Disk diffusion method was used to assess the resistance of A. hydrophila to various antibiotics, and multiple antibiotic resistance (MAR) index was calculated.

Haemolysin assay: Haemolysin production was determined using blood agar plates (Hi-Media, Mumbai, India) and also cell free haemolytic method.

Proteolytic activity: Proteolytic activity was determined by modified method proposed by Charney and Tomerelli.

Slime test: Brain heart infusion agar (Hi-Media, Mumbai, India) plates were prepared containing 0.8 g/l Congo red. A. hydrophila isolates were inoculated onto the surface of the medium and the plates were incubated at 30°C for 24 h. Slime producing bacteria appeared as black colonies, whereas non-slime producers remained non-pigmented.

Results & Discussion

Of the 216 stool samples tested, 21 (9.7%) were positive for A. hydrophila. Our results were higher than the findings of 4.7 per cent incidence in Chennai.
Table. MAR index, resistance pattern and protease production of *A. hydrophila*

<table>
<thead>
<tr>
<th>Culture no.</th>
<th>MAR index</th>
<th>Resistance pattern</th>
<th>Protease (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHD1</td>
<td>0.68</td>
<td>A, B, Cz, E, K M, N, Nv, R, T, Va</td>
<td>113.43</td>
</tr>
<tr>
<td>AHD2</td>
<td>0.87</td>
<td>A, B, Cz, C, E, K, M, Na, N, Nv, R, T, Tr, Va</td>
<td>125.67</td>
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<td>AHD3</td>
<td>0.81</td>
<td>A, B, Cz, E, K, M, Na, N, Nv, R, T, Tr, Va</td>
<td>123.46</td>
</tr>
<tr>
<td>AHD4</td>
<td>0.25</td>
<td>A, B, N, Va</td>
<td>116.34</td>
</tr>
<tr>
<td>AHD5</td>
<td>0.62</td>
<td>A, B, Cz, E, M, N, Nv, R, T, Va</td>
<td>125.38</td>
</tr>
<tr>
<td>AHD6</td>
<td>0.31</td>
<td>A, B, Na, N, Va</td>
<td>115.42</td>
</tr>
<tr>
<td>AHD7</td>
<td>0.75</td>
<td>A, B, Cz, E, K, M, Na, N, Nv, T, Tr, Va</td>
<td>115.64</td>
</tr>
<tr>
<td>AHD8</td>
<td>0.75</td>
<td>A, B, Cz, E, K, M, Na, N, Nv, R, T, Tr, Va</td>
<td>117.13</td>
</tr>
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<td>AHD9</td>
<td>0.87</td>
<td>A, B, C, E, K, M, Na, N, Nv, R, T, Tr, Va</td>
<td>125.78</td>
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<tr>
<td>AHD10</td>
<td>0.62</td>
<td>A, B, Cz, E, K, M, Nv, R, T, Va</td>
<td>151.66</td>
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<tr>
<td>AHD11</td>
<td>0.56</td>
<td>A, B, Cz, K, M, N, Nv, R, Va</td>
<td>146.73</td>
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<tr>
<td>AHD12</td>
<td>0.75</td>
<td>A, B, E, G, K, M, Na, N, Nv, R, T, Va</td>
<td>146.32</td>
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<tr>
<td>AHD13</td>
<td>0.93</td>
<td>A, B, Cz, C, E, K, M, Na, N, Nv, Pb, R, T, Tr, Va</td>
<td>116.95</td>
</tr>
<tr>
<td>AHD14</td>
<td>0.87</td>
<td>A, B, Cz, E, G, K, M, Na, N, Nv, R, T, Tr, Va</td>
<td>135.5</td>
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<tr>
<td>AHD15</td>
<td>0.68</td>
<td>A, B, Cz, E, K, M, N, Nv, R, T, Va</td>
<td>137.92</td>
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<td>AHD16</td>
<td>0.62</td>
<td>A, B, Cz, E, K, M, Na, Nv, R, T</td>
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<td>AHD17</td>
<td>0.50</td>
<td>A, B, Cz, K, M, Na, Nv, Va</td>
<td>123.03</td>
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<td>AHD18</td>
<td>0.56</td>
<td>A, Cz, E, G, K, M, Na, Nv, Va</td>
<td>138.65</td>
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<td>AHD19</td>
<td>0.75</td>
<td>A, B, Cz, E, K, M, Na, N, Nv, R, T, Va</td>
<td>122.83</td>
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<tr>
<td>AHD20</td>
<td>0.18</td>
<td>A, B, Cz</td>
<td>129.92</td>
</tr>
<tr>
<td>AHD21</td>
<td>0.75</td>
<td>A, B, Cz, E, K, M, N, Nv, R, T, Va</td>
<td>135.67</td>
</tr>
</tbody>
</table>

A. ampicillin (10 µg); B, bacitracin (10 units); Cz, cefazoline (30 µg); C, chloramphenicol (30 µg); E, erythromycin (10 µg); G, gentamicin (10 µg); K, kanamycin (30 µg); M, methicillin (5 µg); Na, nalidixic acid (30 µg); N, neomycin (30 µg); Nv, novobiocin (30 µg); Pb, polymyxin-B (300 units); R, rifampicin (30 µg); T, tetracycline (30 µg); Tr, trimethoprim (10 µg); Va, vancomycin (10 µg); MAR, multiple antibiotic resistance

India⁵, 1.28 per cent¹⁹ and 1.4 per cent²⁰ of *A. hydrophila* from Mumbai, India. Alavandi and Anandhan²¹ reported *Aeromonas* associated diarrhoea in 1 to 13 per cent samples in Chennai, while Kuijper et al²² and Ogunsanya et al²³ reported 3.7 per cent in Netherlands and 1.4 per cent in Lagos, Nigeria respectively. However, higher prevalence of 17.7 and 28.1 per cent were recorded during 2000 and 2001 in Kolkata, India²⁴. It is believed that gastroenteritis caused by *A. hydrophila* occurred more commonly in children with acute diarrhoea and adults with traveller’s diarrhoea (2%)¹. Usually it remains as a self-limiting watery diarrhoea but could be more severe in children²² and immunocompromised patients²⁵.

*A. hydrophila* isolates from children with acute diarrhoea exhibited resistance to bacitracin (95.2%), novobiocin (95.2%), vancomycin (90.5%), methicillin (85.7%), cefazoline (85.7%), kanamycin (81%), rifampicin (76.2%), erythromycin and tetracycline (71.4% each) and nalidixic acid (62%). All the isolates were resistant to ampicillin as has been reported earlier²⁶. The isolates exhibited
susceptibility to polymyxin B (95.3%), chloramphenicol (90.5%) and gentamicin (76.2%). Earlier studies revealed the incidence of chloramphenicol resistance strains\textsuperscript{27}. The MAR indices of the 21 isolates ranged between 0.18 and 0.87 (Table). Most of the isolates were from the high risk source contamination like faecal-oral contamination. Due to indiscriminate use of antibiotics the microorganisms might have developed resistance towards several antibiotics.

Of 21 isolats of \textit{A. hydrophila} tested 20 (95.2%) of them were haemolysin producers. The isolates varied in their ability to lyse the red blood cells of human origin. Overall 90.47, 4.76 and 4.76 per cent isolates were beta, alpha and gamma haemolytic, respectively. Attention has been given on the haemolysin of motile \textit{A. hydrophila} because the production of hemolytic toxin has been regarded as indication of pathogenic potential, though non-haemolytic aeromonads have also been implicated as human pathogens\textsuperscript{28}. As defined by Wong \textit{et al}\textsuperscript{29}, all \textit{A. hydrophila} isolates with haemolysin positive genotype were virulent in the suckling mouse assay model. Burke \textit{et al}\textsuperscript{30} reported 97 per cent correlation between haemolysin and enterotoxin production determined by suckling mouse test. It was found that all enterotoxigenic \textit{A. hydrophila} isolates produced haemolysins.

The protease activities of the 21 samples were tested in neutral pH conditions at 37°C for 30 min. Casein was easily digested (at 30 min) by all the isolates because of the production of protease enzyme (Table). The digestive time did not exhibit major differences in protease production among all the samples. The maximum production was in sample AHD 10 at 30 min of digestion time. Castro-Escarpulli \textit{et al}\textsuperscript{31} reported that 61 per cent of the \textit{Aeromonas} isolates produced protease.

The haemolysin and protease production was found more frequently in the clinical isolates, which may be important in colonization through the disruption of the intestinal barrier\textsuperscript{5}. The slime production test was positive for 16 (76.2%) of the \textit{A. hydrophila} isolates. The slime production was also one of the virulence properties, which indicates the high-risk source contamination\textsuperscript{5}. They also found that 50 per cent of the clinical and 35.3 per cent of the environmental isolates were positive for slime production. The frequency of slime producing clinical isolates was more.

In conclusion, our results confirmed the presence of pathogenic \textit{A. hydrophila} in children with gastroenteritis in the study area. High prevalence of multiple drug resistant, haemolysin and protease producing \textit{A. hydrophila} was noticed.

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References


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