Introduction
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Standing at this new millenium, it is fascinating to note that a number of exotic infections unknown previously have made an appearance in the global disease scenario and many infectious diseases are emerging and becoming serious global concern. Among the infections of the gastrointestinal tract, the severe diarrhoeal scourge accounts for excruciating number of deaths in several parts of the world, particularly in developing countries (Guarrant et al., 1990). Among them, bacteria represent approximately 61% having ability to cause diarrhoea (Gascon et al., 1993). Of the diarrhoeagenic bacteria E. coli, Vibrio and Shigella spp. are most common and extensively studied, followed by Salmonella and Aeromonas.

Over the last few years, the interest on Aeromonas species has gone beyond the boundaries of fish pathology; this is due to the increase of disease that is caused by these agents in man, as they can often act as opportunistic pathogens in ipoergic individuals, or in patients with chronic and weakening diseases (Janda, 1991). Only in recent years, the clinical importance of motile aeromonads have been recognized and implicated in clinical cases of diarrhoea, where they have been isolated as the sole pathogen (Janda and Abbott, 1998; Isonhood and Drake, 2002).

A. hydrophila is commonly found in wide range of aquatic system and foods. Strains belonging to the genus Aeromonas have been isolated from lakes, rivers, drinking water and a variety of foods (Buchanan and Palumbo, 1985). The growth potential of A. hydrophila in drinking water and food with low concentration of organic compounds is well established (vander Kooij and Hijnen, 1988). It has been isolated from chlorinated drinking water, which seems less sensible to chlorine compared to coliforms (Chamorey et al., 1999). Aeromonas associated diarrhoea has a distinct seasonal pattern with a sharp summer peak (von Gravenitz and Mensch, 1968). This may be related to one of the
reasons for the prevalence of the organism in environmental and clinical sources during the summer months (Trust et al., 1980).

The high prevalence in the environment lends support to the hypothesis that infections are mainly acquired through the consumption of contaminated food and water, although no major outbreak has been documented (Chopra and Houston, 1999). Other severe illnesses, such as systemic infections are less frequent (Janda and Abbott, 1998) and normally associated with immuno-compromised patients (Ko et al., 2000). The genus Aeromonas has high diversity and 18 hybridization groups (HG) have been recognized (Pidiyar et al., 2002). Among these HG groups A. hydrophila, A. caviae and A. veronii bv. sobria are clinically significant (Janda, 1991; Ko et al., 2000).

The spectrum of infectious disease caused by A. hydrophila includes gastrointestinal infections as well as extraintestinal infections such as cellulitis, wound infections, septicaemia, urinary tract infections and hepatobiliary and ear infections, among other species of Aeromonas (Janda et al., 1995). Among the diarrhoeal species of Aeromonas, A. hydrophila has been recorded as the major and significant organism and its prevalence and virulence factors have been studied by several researchers throughout world (Kuijper et al., 1987; Khardori and Fainstein, 1988; Janda, 1991; Koe et al., 1991; Teka et al., 1999; Nzeako and Okafor, 2002; Urbino et al., 2003; Sen and Rodgers, 2004; Aslani and Hamzeh, 2004).

Many studies have resulted in the isolation of several species of Aeromonas from patients with gastroenteritis, and these have been extensively reviewed (Altwegg and Giess, 1989; Joseph, 1996). Mild diarrhoea has been developed after a dose of $10^9$ organisms and moderate diarrhoea will develop after the dosage of $10^7$ (Morgan et al., 1985). However, there have been reports of laboratory-acquired infections among microbiologists who ingested significant doses of Aeromonas and developed self limiting diarrhoea (Joseph, 1996).
The strains of *A. hydrophila* were highly susceptible to tetracycline, chloramphenicol, polymyxin-B, gentamicin and trimethoprim-sulfamethoxazole (Fainstein *et al.*, 1982; Ramteke *et al.*, 1993). Increased incidence of multiple antibiotic resistant *Aeromonas* isolated from clinical and environmental sources have been reported worldwide and they may pose a serious problem in chemotherapy (Pettibone *et al.*, 1996).

Species of *Aeromonas* are capable of expressing a number of virulence factors such as haemolysin, aerolysin, cytotoxic enterotoxin, cytotoxic enterotoxin, endotoxin lipopolysaccharide, outer membrane protein and enzymes such as protease, lipase, DNase, elastase and gelatinase (Gosling, 1996; Howard *et al.*, 1996; Castro-Escarpulli *et al.*, 2003). The primary toxin haemolysins are produced, of which the most significant is aerolysin, a heat-labile β-haemolysin, expressed by many strains of *A. hydrophila* (Chopra *et al.*, 1991; Janda, 1991; Gosling, 1996; Howard *et al.*, 1996). It was reported as a pore forming cytolysin, able to cause damage to the cell membrane bilayer causing leakage of cytoplasmic contents.

An interesting approach for the direct detection of potential pathogenic *A. hydrophila* isolates is the use of virulence determinants as genetic markers. In addition it has been suggested that variation in the distribution of potential virulence genes amongst *A. hydrophila* isolates might contribute to their degree of virulence factors including haemolytic toxin (aerA and hlyA), heat labile cytotoxic enterotoxin (Act), heat stable cytotoxic enterotoxin (Ast), flagellin (fla) and elastase (ahyB). PCR technique is used for studying the specific detection of an *A. hydrophila* virulence gene (Howard and Buckley, 1986).

*Aeromonas* is being isolated with increasing frequency throughout the world from variety of focal and systemic infections of varying severity persons who are apparently immunologically normal. Infections due to *Aeromonas* in immunocompromised hosts are generally severe. As the list of new genomospecies to the
genus *Aeromonas* is still expanding, the clinical syndromes due to this organism are also evolving rapidly. Since the taxonomy of the genus *Aeromonas* is still confused (Carnahan and Altwegg, 1995; Aslani and Hamzeh, 2004), the need of a good typing system for the identification and classification of *Aeromonas* isolates to justify their ecological and clinical importance is needed. Random amplification of polymorphic DNA (RAPD) PCR and enterobacterial repetitive intergenic consensus sequence (ERIC) PCR are widely used in bacteriology for epidemiological and taxonomic studies (Davin-Regli *et al.*, 1998; Szczuka and Kaznowski, 2004). Outer membrane proteins (OMP) and lipopolysaccharides (LPS) have also been used to correlate the phenospecies and serotyping of *Aeromonas* species (Maruvada *et al.*, 1992). It is clearly evident that *Aeromonas* possesses the requisite potential pathogenic determinants and virulence factors to be an aetiologic agent of diarrhoea.

**Scope of the work**

Despite the number of studies on the incidence of infectious diarrhoeal agents were undertaken in our region, reports on *A. hydrophila* in children diarrhoea are not available in this part of the state and hence the study was undertaken to find out the incidence of *A. hydrophila* in diarrhoeal samples. Attention has been given to the incidence of haemolytic as well as multiple antibiotic resistant strains of *A. hydrophila* among diarrhoeal samples of children. Further, molecular techniques have been applied to check the genetic diversity of the strains, isolated from four selected places of this region. In the above juncture, this work has been planned and carried out with the following objectives.

**Objective of the study**

The objective of the present investigation was to find out the incidence of *A. hydrophila* in children diarrhoeal samples and to identify the cytotoxin virulent *(act)* gene responsible for diarrhoea. The present study was planned as given below:
1. Incidence of diarrhoeagenic bacteria and *A. hydrophila* in children diarrhoeal stool samples,

2. Assessment of multiple antimicrobial resistant patterns of diarrhoeal isolates of *A. hydrophila*,

3. Determining the putative virulence factors such as haemolysin, protease, DNase, slime, cytotoxic activity and serum resistance of diarrhoeal isolates of *A. hydrophila*,

4. Identification of the virulence gene (*act*) using PCR methods, which is responsible for cytolytic enterotoxin (Act) and

5. Molecular typing of selected haemolytic strains of *A. hydrophila* using outer membrane proteins (OMPs), lipopolysaccharides (LPSs), random amplification of polymorphic DNA (RAPD) PCR and enterobacterial repetitive intergenic consensus sequence (ERIC) PCR to assess their genetic diversity.