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Epidemiology, Virulence and Public Health Significance of *Aeromonas hydrophila* in Drinking Water

G. Pandove[1]*, P. Sahota[1], S.K.Verma[2], A.P.S. Brar[3] and B.S. Sandhu[3]

[1]Department of Microbiology, Punjab Agricultural University, Ludhiana - 141 004, India.
[2]Department of Epidemiology and Preventive Medicine, [3]Department of Veterinary Pathology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, India.

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A significant number of zoonotic emerging and re-emerging waterborne pathogens like *Aeromonas hydrophila* possess virulence factors associated with human disease, and hence represent a serious public health concern. A total of 418 drinking water samples analyzed for occurrence of *Aeromonas hydrophila* and faecal coliforms (*E.coli*), detected *Aeromonas hydrophila* in 84.71% of Municipal Corporation (MC), 71.52% Submersible pump and 68.75% from Hand pumps where as *E.coli* in 53.71% of Municipal Corporation (MC), 29.16% Submersible pump and none of samples from Hand pumps. There was no positive correlation between the simultaneous occurrence of *Aeromonas hydrophila* and *E.coli* ($P < 0.005$; $R^2 = 0.84$). All the isolates of *Aeromonas hydrophila* were positive for hemolytic activity, esculin hydrolysis and congo dye uptake. It also caused significant histopathological and ultrastructural alterations in liver, lungs, kidney and intestine in experimentally infected BALB/c mice. All the isolates (n=330) from water showed multiple drug resistance (MAR), MAR indices for *Aeromonas hydrophila* isolates is 0.5 (>0.2). MIC of sodium hypochlorite (4%) required by *Aeromonas hydrophila* was 6 ppm with CT factor of 15mg/l.min. These results highlight the pathogenic potential of *Aeromonas* species which poses a public health concern.

Key words: Zoonotic, Emerging, Re-emerging, *Aeromonas hydrophila*, Histopathological, MAR.

Water is essential to sustain life and a satisfactory supply must be made available to consumers (WHO 2004). Owing to the fact that the right to drinkable water is nowadays part of human rights, one-sixth of the world population still does not have access to safe drinking water (United Nations 2008).

According to the World Health Organization, a third of the world's population suffers from water borne diseases. In developing countries 13 million people die and 1.1 billion persons lack access to an improved water source, and 2.4 billion persons lack access to adequate sanitation. As a result of infectious diseases related to unsafe water and inadequate sanitation, an estimated 3million people in developing regions of the world die each year, primarily children aged <5 years.

There is a growing need to redress the twin problem of sustainability of water resource and water quality. The average availability of water is reducing steadily with the growing population and it is estimated that by 2020 India will become a water stressed nation. Groundwater is the major source of water in India with 85% of the population dependent on it.

The provision of clean drinking water has been given priority in the Constitution of India, with Article 47 conferring the duty of providing

* To whom all correspondence should be addressed.
E-mail: gpandoveg@yahoo.co.in

clean drinking water and improving public health standards of the States in India. A total of Rs.1, 105 billion has been spent till 10th plan on providing safe drinking water but still lack of safe and secure drinking water continues to be a major hurdle and a national economic burden (Water Aid India 2005).

Aeromonas spp. are common aquatic microorganisms that occur in irrigation, river, brackish, fresh, spring, surface and ground, estuarine, sea, chlorinated and non-chlorinated and in bottled mineral water.All phenospecies are found in sewage-contaminated water. The prevalence and distribution of *Aeromonas* in aquatic environments, its role as a contaminant for drinking water supplies and potential for pathogenicity mediated by virulence factors by *Aeromonas* are all of great public health concern (Dumontet *et al.* 2000).

It has been documented in a variety of human illnesses, including septicaemia, meningitis, wound and lung infections (Janda & Abbott 1998), although the most frequent reports indicate the association of *Aeromonas* species with acute gastroenteritis. Although a strong association between diarrhoeal disease and *Aeromonas hydrophila* has been shown in children and in adults >60 years old, this organism has been isolated from cases of travellers' diarrhoea with high frequency (Yamada *et al.* 1997).

Antibiotic sensitivity of clinical isolates of *Aeromonas* spp. has been extensively studied but less is known about environmental strains (Morita *et al.* 1994). Consequently, the freshwater indigenous flora may become a reservoir for antimicrobial resistance genes, and the reuse of these waters for humans and animals may contribute to the limitation of antimicrobial efficiency. Keeping all this in view the present research was proposed with the objective to study epidemiology, virulence, antibiotic susceptibility of *Aeromonas hydrophila* isolates from in drinking water

MATERIALS AND METHODS

Research site description

Ludhiana is the largest city in Punjab, India, both in terms of area and population. The city is spread over an area of 159.37 sq.km and accomodates approximately 14.00 lacs population. The city has been divided into 70 municipal wards in which only 31 municipal wards report slums. The rapid and immense industrialization of Ludhiana city has resulted in the emergence of several slum colonies in and around the city

Sample collection, Transport and Storage

The drinking water samples were collected from endemic gastroenteritis affected suburbs of Ludhiana city with migratory population. The samples were treated with sodium thiosulfate to inactivate any residual halogen compound present in the sample ($Na_2S_2O_3$ concentration of 18mg/litre neutralizes upto 5mg of free (residual) chlorine per litre). The samples containing high concentration of zinc and copper were treated with EDTA at concentration of 372mg/litre to reduce metal toxicity (APHA 1989). The samples were analyzed within 24hrs by transporting in refrigerated container at 4°C.

Microbiological analysis of water samples

A total of 418 samples (Municipal Corporation 242, Submersible pump 144 and Hand pump 32) from endemic area of gastroenteritis infected area under Municipal Corporation were analysed by the standard methods (IS-10500-1991 BIS New Delhi, India) and Bacteriological water testing kit (BWTK), developed in the Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India for Total coliforms, Faecal coliforms and *Aeromonas hydrophila*.

Isolation of and Biochemical characterization of Aeromonas hydrophila

From primary enrichment broth (10ml of water in 10 ml MacConkey broth incubated at, 37 °C, 24-48 h) and positive BWTK, an inoculum was streaked onto Aeromonas Selective Supplement Medium (Ampicillin 2.50m g/500ml Medium), incubated at 37 °C for 24 h. Presumptive *Aeromonas* colonies, with dark green, opaque with darker centre, were further streaked on Brain Heart Agar (BHA; Himedia Laboratories Pvt. Ltd., Mumbai) or Aeromonas Selective Supplement Medium (Himedia Laboratories Pvt. Ltd., Mumbai). The bacteria isolated were identified as *Aeromonas hydrophila* on the basis of colony morphology, gram-staining, and biochemical characteristics (Konema & Winn 1992 and Brenner *et al.* 2005).

Scanning electron microscopy of bacterial cultures

Samples were removed from agar plates

and fixed with 3% glutaraldehyde at 4ºC overnight. Dehydration of the samples was then conducted by a series of 10, 25, 50, 70, 100 % ethanol solutions. Using a Critical Point Dryer the samples were dried further (CPD, Emitech). These samples were mounted on aluminum stubs and then coated with gold using a Sputter Coater (Emitech). The samples were examined using a Hitachi (S 2700).

Determination of virulence markers of *Aeromonas hydrophila*

The strains were tested for â-hemolytic activity on agar base supplemented with 5% sheep erythrocytes (Gerhardt *et al.* 1981), Congo red dye uptake (Paniagua *et al.* 1990) and Esculin hydrolysis(Himedia Laboratories Pvt. Ltd., Mumbai).

Virulence studies and Histopathology

Group of 5 mice each were deprived of drinking water for 24h and then allowed to drink from aqeuous bacterial suspension containing about 1 x 10^8 cfu/ml for 24h of *Aeromonas hydrophila*. The inocula were then withdrawn and the animals served with clean water after 24h period. Those in control were served with clean drinking water. Tissue samples, Heart, Liver, Stomach, Intestine, Spleen, and Kidney were immediately removed from dead or killed mice, rinsed in isotonic solution and were fixed in formalin, and embedded in paraffin, cut into 4 ìm sections and stained with hematoxylin-eosin.

Determination of multiple antibiotic resistance

Pure cultures were grown in brain heart infusion broth for sensitivity testing. Mueller Hinton agar (Hi Media, Mumbai, India) was used (Bauer *et al.* 1966). A total of 24 different antibiotics (Hi- Media, Mumbai, India) were used. After enrichment in BHIB at 37ºC, till the inoculum turbidity $\geq$0.10D at 620nm or 0.5Mcfarland standard is achieved, the cultures were streaked on Mueller Hinton agar plates using a cotton swab. With an antibiotic disc dispenser, ring containing the discs were placed on the agar surface. After 30 min of pre-diffusion time, the plates were incubated at 37ºC for 18-24 h. The results were recorded by measuring the inhibition zones and scored as susceptible, intermediately susceptible, and resistant, according to the Clinical and Laboratory Standard Institute (CLSI 2006). The multiple antibiotic resistance (MAR) index, when applied to a single isolate, is defined as a/b, where 'a' represents the number of antibiotics to which the isolate was resistant and 'b' represents the number of antibiotics to which the isolate was exposed. MAR index higher than 0.2 identifies organisms that originate from high-risk sources of contamination, where antibiotics are often used. MAR indices less than, or equal to 0.2, identify strains from environments where antibiotics are seldom or never used (Krumperman 1985).

RESULTS AND DISCUSSION

Isolation, Identification and Biochemical characterization of *Aeromonas hydrophila* from Drinking water.

The positive BWTK and MacConkey tubes were used for the isolation of *Aeromonas hydrophila* from drinking water. Test water sample (15ml) was inoculated in BWTK and incubated at 37ºC for 12h, and positive samples were streaked on selective media [Aeromonas Selective Supplement Medium with Ampicillin (2.50mg/500ml Medium]. Out of 418 drinking water samples, 78.94% samples were positive for *Aeromonas hydrophila.* The growth of *Aeromonas hydrophila* was observed on Aeromonas Selective Supplement Medium, colonies were dark green, opaque with darker centre. The isolates observed under microscope were Gram negative and rod shaped. The morphology was further confirmed by electron microscopy. It was found that it is rod shaped with round edges and approximately size in between 1.17-2.24µm (Plate 1).

The strains of *Aeromonas hydrophila* displayed the following characteristics tests oxidase, Indole and Voges proskauer positive. A strong correlation between cytotoxicity and positive VP reaction has been reported by Cumberbatch *et al.* (1979), Kaper *et al.* (1981) and Burke *et al.* (1984). Urease, Ornithine decarboxylase and H_2S were not produced. All the isolates produced acid as well as gas from the following sugars Arabinose, Cellobiose, Dextrose, Fructose, Galactose, Insulin, Maltose, Mannitol, Mannose, Melibiose, Rhamnose, Salicin, Sorbitol, Sucrose, and Trehalose. *Aeromonas* spp. grown on glucose or other simple sugars produces sufficient acetic acid to auto-sterilize a broth culture within 48hr. in weakly buffered systems. This metabolic activity has been called the suicide phenomenon.

Correlation between occurrence of *E.coli* and *Aeromonas hydrophila*

Out of 418 drinking water samples, *Aeromonas hydrophila* recovered in 84.71% of Municipal Corporation (MC) drinking water samples, 71.52% Submersible pump drinking water samples and 68.75% of samples from Hand pumps drinking water samples where as *E.coli* in 53.71% of Municipal Corporation (MC) drinking water samples, 29.16% Submersible pump drinking water samples and none of samples from Hand pumps

There was no positive correlation between the occurrence of *Aeromonas hydrophila* and *E.coli* ($P<0.005$; $R^2 = 0.84$).The failure of measurements of single indicator organisms to correlate with pathogens suggests, alternative indicators, should be looked for determine to determine the possible threat to public health. The detection of *Aeromonas hydrophila*, which appears to be ubiquitous in sewage, could serve as an indicator for the presence of many other pathogenic bacteria and viruses in ground water supplies.

Different authors have also found that coliform count did not correlate with *Aeromonas spp*. count. Some reports from Australia (Bruke *et al.* 1984) or from Northern America, question the suitability of coliforms as index of water quality. Similarly, an investigation about the occurrence of *Aeromonas* spp. in drinking water supplies in a mountain area in Northeast Italy (Legnani *et al.* 1998) showed that no correlation was demonstrated between the concentration of *Aeromonas* spp. and faecal indicator organisms.

Over all study revealed that *A.hydrophila* occurred in highest percentage in all the utilities. This is attributed to that Aeromonads are nutrionally versatile. It has the ability to utilize a variety of organic compounds, carbohydrates,

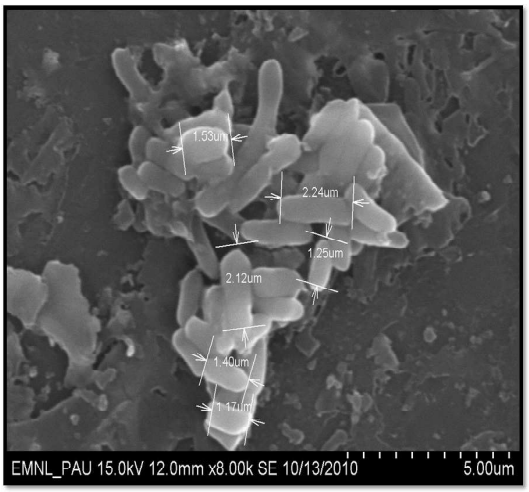

Plate 1. SEM images of *Aeromonas hydrophila*
Length: 1.17-2.24µM
Width: 0.60µM

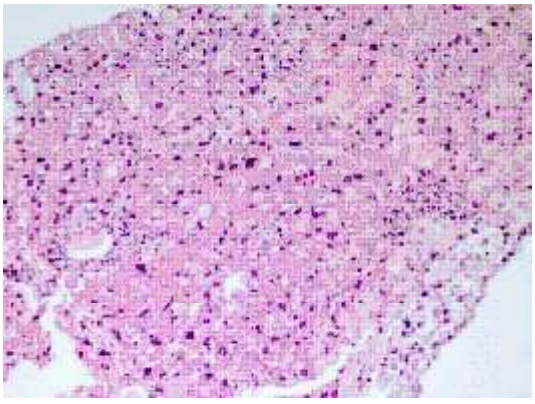

Plate 2a. Histological tissue of mice (Liver) infected with *Aeromonas hydrophia* showed hypertrophy hepatocyte nucleus, periportal mononuclear cell infiltration. (20X) (H & E Stain)

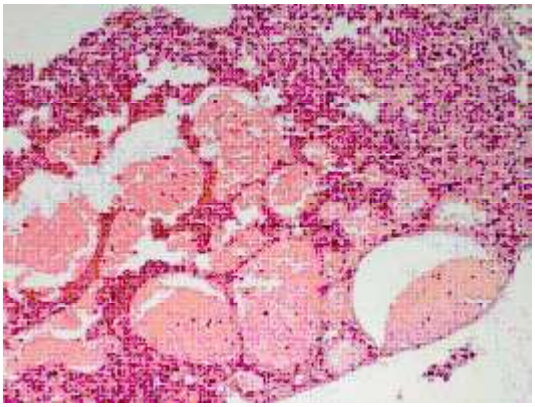

Plate 2b. Histological tissue of mice (Liver) infected with *Aeromonas hydrophia* showed Hepatic degeneration and necrosis of hepatocyte with telengiectasis, hypertrophy hepatocyte nucleus, periportal mononuclear cell infiltration (20X) (H & E Stain)

amino acids, carboxylic acids , and long chain fatty acids, even at low concentration (10µg/l) (van der Kooij 1991).Thus aeromonads are capable of growth in the presence of the low concentration of nutrients that would be available from biofilms and sediments with in distribution systems. Multiplication of *Aeromonas hydrophila* in biofilms along the distribution systems may also results in the deterioration of bacteriological quality of drinking water, the development of odour or colour as well as the acceleration of the phenomenon of corrosion with in pipe network.

A key mechanism for the control of aeromonads in drinking water is removal of biodegradable compounds. Such measures would also help to control the regrowth of heterotrophic bacteria within distribution system. Control of the development of biofilm with in water supply system will reduce, but not prevent, the proliferation of *Aeromonas*

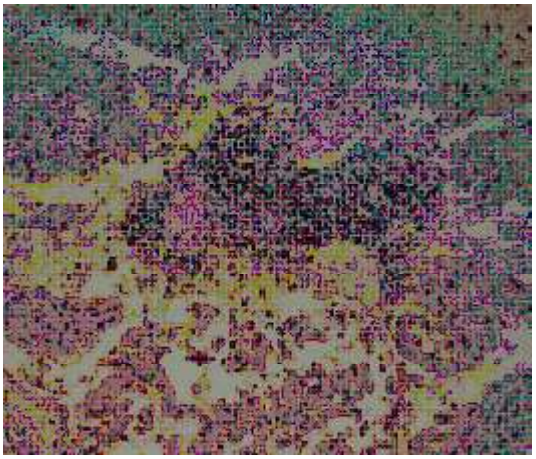

Plate 2c. Histological tissue of mice (Liver) infected with *Aeromonas hydrophia* showed necrosis and mononuclear cells infiltration in the form of follicles (20X). (H & E Stain)

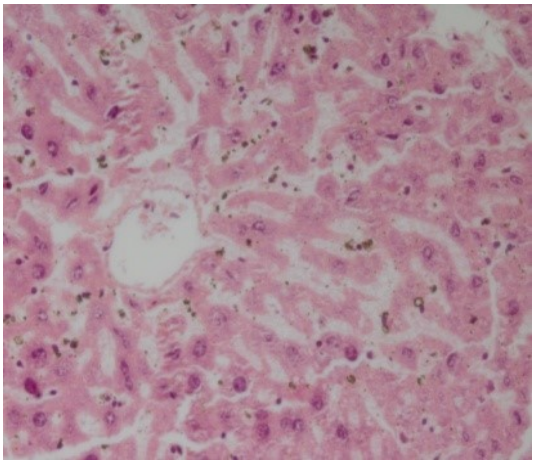

Plate 2d. Histological tissue of mice (Liver) infected with *Aeromonas hydrophia* showed necrosis of hepatocytes and increase in bile pigment (40X). (H & E Stain)

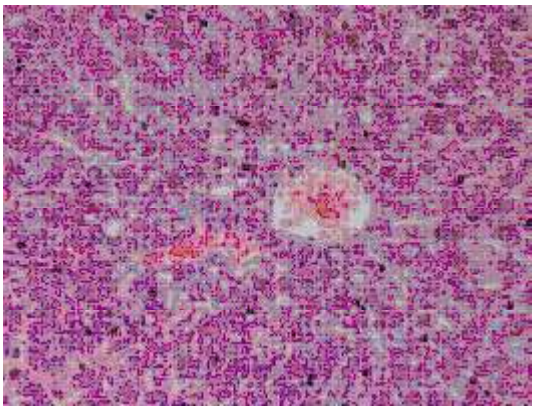

Plate 2e. Histological tissue of mice (Liver) infected with *Aeromonas hydrophia* showed hypertrophy of von kuffer cell, vacuolar degeneration, congestion (40X).. (H & E Stain)

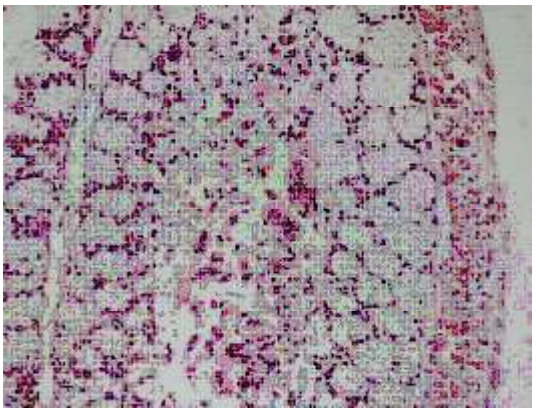

Plate 3. Histological tissue of mice (Intestine) infected with *Aeromonas hydrophia* showed catahral enteritis, mucus degeneration, increase in number of globlet cells, and sloughing of mucosal lining epithelium (20X) (H & E Stain)

Determination of virulence marker of *Aeromonas hydrophila*

Our study revealed that all the strains of *A. hydrophila* showed hemolytic activity, with a halo diameter between 0.5 and 2 mm. The higher concentration of haemolysins of *A. hydrophila* in our environmental strains agrees with the results obtained by other authors (Kirov 1993) on clincial and environmental strains. The haemolytic activity is strongly associated with enterotoxin production in members of the genus *Aeromonus* (Paniagua *et al.* 1990). Finally, according to Burke *et al.* (1984), 97% of cytotoxic *Aeromonas* spp. strains could be classified by haemolysin assay, and the correlation was stronger for drinking water isolates than for food isolates (Handfield *et al.* 1996). These results highlight the pathogenic potential of *Aeromonas* species which poses a public health concern.

All the isolates of *Aeromonas hydrophila* were positive for esculin hydrolysis and congo dye uptake; this finding suggests that all were potential enteric pathogens. The ability to take up dye is associated with the presence of a virulence plasmid. Virulence determinants are responsible for the establishment and maintenance of an infection in the host. Many plasmid-mediated properties have been used to distinguish between virulent and avirulent strains, including colony morphology (Mazigh *et al.* 1983), autoagglutination (Skurnik 1984), detachment of cells in culture (Lassen & Kapperud 1986), serum resistance (Pai & DeStephano 1982), hydrophobicity (Lachica & Zink 1984), and virulence characteristics in animals (Prpic *et al.* 1983). Most of the experimental procedures on virulence characterization are costly, time-consuming, complex, and impractical for routine diagnostic use or in field laboratories. The Congo red pigmentation assay provides a simple and efficient means of screening for virulence. Uptake of Congo red dye has been shown to be a marker for virulence in several

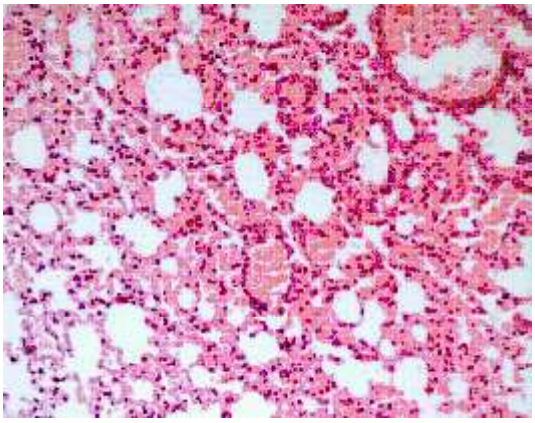

Plate 4. Histological tissue of mice (Lung) infected with *Aeromonas hydrophia* showed haemorrhage and congestion (20X) (H & E Stain)

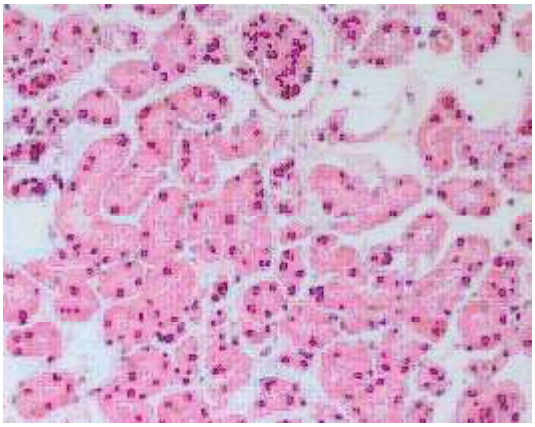

Plate 5a. Histological tissue of mice (Kidney) infected with *Aeromonas hydrophia* necrosis tubular epithelium and mild acute interstitial nephritis (40X) (H & E Stain)

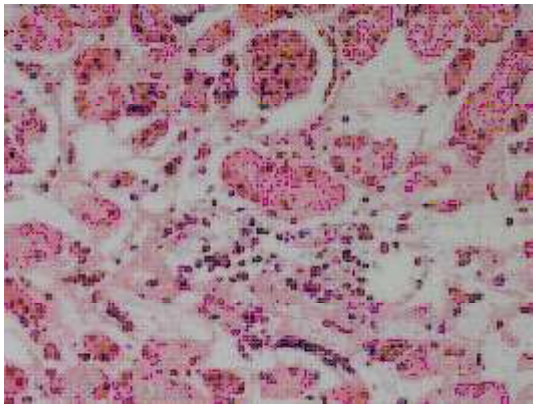

Plate 5b. Histological tissue of mice (Kidney) infected with *Aeromonas hydrophia* necrosis tubular epithelium, mild acute interstitial nephritis and increase in cellularity in the glomeruli (40X) (H & E Stain)

enteropathogenic and nonenteropathogenic bacteria.

Virulence studies and Histopathology

Animals (BALB/c mice) were deprived of drinking water for 24h and then allowed to drink from aqeuous bacterial suspension containing, 1 x 10^8 cfu/ml of the *Aeromonas hydrophila* (GP 1) for 24h. It was found that on second day after inoculation of animals with bacterial culture, signs of depression, isolation, localised skin sloughing and loss of hair (alopecia) was observed. The faecal samples showed the presence of $2X10^{14}$cfu/g of the *Aeromonas hydrophila* (GP 1). The experimental mice died on the third (2), forth (2), sixth (1) day respectively of post inoculation. Tissues from the intestine, liver, kidney and lungs were removed at postmortem of the infected animals (carcasses).

Histopathological examination of liver showed hypertrophy of hepatocytic nucleus, *swelling, hypertrophy of von kuffer cells, infilteration of mononuclear cells of periportal area, hepatic degeneration and necrosis of hepatocytes with telengiectasis, increase in bile pigment, congestion and vacuolar degeneration* (Plate 2). While Intestine showed catahral enteritis, mucus degeneration, increase in number of goblet cells, and sloughing of mucosal lining epithelium (Plate 3). The lungs revealed haemorrhage and congestion (Plate 4).

Brenden & Huizingat (1986) had also reported pulmonary congestion, oedema, haemorrhage and neutrophil infilteration of the lungs and revealed variable liver pathology, congestion, swollen kupffer cells, enlarged hepatocytes and focal necrosis due to infection from A.hydrophila in experimental mice.

Kidney showed degeneration, necrosis of tubular epithelium, mild acute interstitial nephritis and increase in cellularity in the glomeruli (Plate 5). Miyazaki *et al.* (2001) also reported that in kidney hepatocytes were either atrophied or swollen and had a granular appearance. Renal tubular cells showed vacuolar degeneration, cloudy swelling, necrosis and destruction due to infection from *A.hydrophila* in crap.

The enteropathogenicity of *Aeromonas* spp. has been ascribed to the production of exotoxins. Clinical and environmental strains of *A. hydrophila* have been reported to produce a heat-labile cytotoxin and a heat-stable cytotoxin that have enterotoxic activities. It can be inferred from this work that the strain of *A.hydrophila* have 2-3 days incubation period in the laboratory animals. This incubation period was seen to be similar to that in man (2-3 days). This could be the result of their near similar physiologic disposition as in man.

A total of 330 isolates of *Aeromonas hydrophila* were isolated from 418 drinking water samples from three different water utilities, 207/242 Municipal Corporation, 103/144 Submersible pump and 22/32 Hand pumps. These isolates were phenotyped, using antimicrobial susceptibility test against panel of 24 antibiotics.

All the isolates showed sensitivity to two out of three Penicillins i.e. Ampicillin/sulbactum 10/10 mcg, and Piperacillin 100mcg. Ampicillin/sulbactam is a combination of the common penicillin-derived antibiotic ampicillin and sulbactam, an inhibitor of bacterial beta-lactamase. Sulbactam blocks the enzyme which breaks down ampicillin and thereby allows ampicillin to attack and kill the bacteria where as Piperacillin is an extended spectrum beta-lactam antibiotic but all isolates showed resistance to Ampicillin 10mcg). Resistance to â-lactam antibiotics is due to the production of multiple inducible, chromosomally encoded â-lactames (Goòi-Urriza *et al.* 2000(a)). Resistance to the third generation cephalosporins is known to be associated with the derepression of the chromosomal enzymes (Goòi-Urriza *et al.* 2000 (b)).

The isolates of *A. hydrophila* were resistant to 60% of Cephalosporins tested i.e. all isolates were sensitive to Cephotaxime 30mcg, Ceftazidime 30 mcg where as resistant to Cefuroxime 30 mcg, Cephoxitin 30 mcg and Cephalothin 30 mcg.

Most of the isolates of *Aeromonas hydrophila* were resistant to 66% of the tested aminoglycoside i.e. sensitive to Amikacin 30mcg and resistant to Tobramycin 10mcg and Gentamicin 10mcg. Similarly all the isolates showed resistance to Co-Trimoxazole 25 mcg and Nalidixic acid 30 mcg and sensitivity to Ofloxacin 5 mcg Levofloxacin 5mcg and Ciprofloxacin 5mcg among Quinolones group.

Resistance to nalidixic acid is a function of a mutation of the gyrA, gyrB, parC, and parE genes which make up the quinolone resistance-

determining regions (Goni-Urriza *et al.* 2002).

The isolates of A. *hydrophila* showed resistance to both the Tetracyclines (Tetracycline 30 mcg and Doxicycline 30 mcg). Earlier studies also revealed resistance to Tetracycline (Subaskumar *et al.* 2006).The apparent resistance of *A. hydrophila* to antibiotics may be a result of indiscriminate or sub therapeutic use of antibiotics. Tetracyclines are the most frequently used antimicrobial agents in veterinary medicine in many parts of the word. Tetracycline resistance is most commonly mediated either by active efflux of tetracycline from the cell or by ribosomal protection from the action of tetracycline, and, in rare cases, through direct inactivation of the antibiotic or by mutations in the *16S rRNA* that prevent biding tetracycline to the ribosome.

All the isolates of *Aeromonas hydrophila* were sensitive to Imipenem 10 mcg (β-lactum: carbapenem), Nitrofurantoin 300 mcg, Aztreonam 30 mcg (Monobactams), Netillin 30 mcg (Monobactams) and resistantance to Amoxyclave 30 mcg (Monobactams)and Augmentin 30 mcg (Monobactams)

The study revealed that isolates of *A. hydrophila* isolates were found to be susceptible to Cephotaxime 30mcg, Aztreonam 30 mcg, Piperacillin 100mcg, Ceftazidime 30 mcg, Imipenem 10 mcg, Ampicillin/sulbactum 10/10 mcg, Ofloxacin 5 mcg, Netillin 30 mcg, Nitrofurantoin 300 mcg, Amikacin 30 mcg, Ceprofloxacin 5 mcg, Cephotaxime 30mcg and to be resistant to Ampicillin 10mcg, Amoxyclave 30 mcg, Gentamicin 10 mcg, Tobramycin 10mcg ,Nalidixic acid 30 mcg, Co-Trimoxazole 25 mcg, Tetracycline 30 mcg, Cefuroxime 30 mcg, Doxycycline 30 mcg, Augmentin 30 mcg, Cephoxitin 30 mcg and Cephalothin 30 mcg. The bacterial isolate from drinking water was resistant to twelve out of the twenty four antibiotics tested. The zone of inhibition produced by different isolates was measured and there was non significant difference in the size (mm) of zone produced by different isolates and all isolates showed same pattern of antibiotic susceptibility.

The MAR indices for *Aeromonas hydrophila* isolates is 0.5 (>0.2). Multiple drug resistance among *Aeromonas* spp. has been reported from many parts of the world,which is due to over expression of the EHPgp 1 and 5 genes as well as the production of superoxide dismutase (WHO 2000).

The spread of drug resistance among *Aeromonas* spp., is of concern because recent surveys indicate the emergence of these organisms as primary human pathogens and posing a public health risk and an etiological agent of gastroenteritis in epidemics. It is speculated that resistance to multiple antibiotics in *Aeromonas* isolate may be mediated by several coinducible enzymes under the selection pressure of certain widely prescribed antibiotics.

The present results are in concurrence with the reports of Yucel & Ctak (2003) and Emekdas *et al.* (2006) who reported that Cephalosporins like Cephotaxime inhibited most of the *A. hydrophila* strains and that all the strains were resistant to Penicillin.

Strains of *Aeromonas* isolated from rivers (Gon˜i-Urriza *et al.* 2000a) showed 59% resistance against Nalidixic acid. A 100% sensitivity to Amikacin, Ciprofloxacin and Imipenem was reported in a study that included a broad number of clinical and environmental strains (Ka¨mpfer *et al.* 1999). Regarding the resistance to antimicrobial agents, several authors have stated that *Aeromonas* species are rapidly adapting to new drugs commonly used in medicine, becoming a potential risk to public health (Overman &Janda 1999).

Some reports showed that isolates of *A. hydrophila* from water, food, clinical specimens and other sources are not susceptible to many antimicrobials (antibiotics) applied in clinical practice; it may become difficult to cure disease caused by *A. hydrophila*. Urban wastewater effluents are thought to contribute to the increasing rate of antibiotic resistance in environmental *aeromonads*.

In view of the high level of multiple drug resistance shown by *A. hydrophila* in this study, regulations should be enforced governing the handling and sales of antibiotics to avoid indiscriminate use of drugs that could lead to sub therapeutic dosage thereby enhancing the development of resistant mutants. Enlightenment of the public as regards to personal hygiene of individuals, foods, water and the environment is highly recommended.

CONCLUSION

This is the first surveillance study conducted in Ludhiana city, Punjab, India, showed microbial contamination of drinking water and hence its control constitutes a major issue worldwide. The use of faecal indicator bacteria raises questions regarding their reliability in assessing the bacteriological quality of water, particularly because of their poor correlation with pathogenic microorganisms. Discrepancies have been observed in terms of the occurrence of faecal indicators and emerging pathogens. Out of 418 drinking water samples, *Escherichia coli* was present in 41.14% of drinking water samples, whereas emerging pathogen *Aeromonas hydrophila* in 78.94%.The appropriate level of chlorination during storage and distribution pipe networks to customer's tap should be maintained to guarantee the water quality. New approaches to health-related monitoring are being introduced that can overcome many of the weaknesses of current methods, old age protocols, and distribution system. Update and new culture methods should be included in standard methods for monitoring, sampling and analysis of these emerging pathogens

ACKNOWLEDGMENTS

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