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Full Length Research Paper

Epidemiology, virulence and public health significance of *Yersinia enterocolitica* in drinking water

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***Yersinia enterocolitica*, an important food and water-borne enteropathogen cause acute diarrhoea, terminal ileitis and mesenteric lymphadenitis. This study investigated the occurrence, antibiotic sensitivity and virulence factors of yersinial isolates from drinking water samples from Ludhiana, Punjab, India. A total of 418 drinking water samples from various water utilities were analyzed for occurrence of *Y. enterocoliitica* and faecal coliforms (*Escherichia coli*). *Y. enterocolitica* was detected in 78.09% of Municipal Corporation (MC) drinking water samples, 59.02% Submersible pump drinking water samples and 53.12% of Hand pump samples where as *E. coli* was found in 53.71% of Municipal Corporation (MC) drinking water samples, 29.16% Submersible pump drinking water samples and 8% of samples from Hand pumps. There was no positive correlation between the simultaneous occurrence of *Y. enterocolitica* and *E. coli* ($P < 0.005$; $R^2 = 0.88$). All the isolates of *Y. enterocolitica* were positive for virulence marker test Congo dye uptake. Significant histopathological and ultrastructural alterations in liver, lungs, kidney and intestine were reported in experimentally infected mice. All the isolates (n = 291) from water showed multiple antibiotic resistance (MAR). The MAR indices for *Y. enterocolitica* isolates is 0.5 (> 0.2). These results highlight the pathogenic potential of *Y. enterocolitica* which poses a public health concern.**

Key words: Enteropathogen, *Yersinia enterocolitica,* histopathological, *Escherichia coli*, multiple antibiotic resistance (MAR).

INTRODUCTION

Water is essential to sustain life and its satisfactory supply must be made available to consumers (WHO, 2004). Owing to the fact that “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 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 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 inadequate sanitation, an estimated 3 million people in developing regions of the world die each year, primarily children aged < 5 years.

Demand of water at present exceeds the available re-newable water resources with the current high population growth rate, increased modernization, and higher standard of living. The gap between water supply and demand is expected to widen. In industrialized countries drinking water is ranked as food and high standards are set for quality and safety. The provision of clean drinking water has been given priority in the Constitution of India, with Article 47 conferring the duty of providing clean

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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).

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. The spectrum of water-borne diseases is expanding and majority of diseases once believed to be conquered are on the rise (Marshall et al., 1997). A significant number of zoonotic emerging and re-emerging waterborne pathogens like *Yersinia enterocolitica* have been recognized now. Zoonoses are of increasing concern for human health; next to pathogens with human-to-human transmission, they pose the greatest challenges to ensure the safety of drinking water.

Y. enterocolitica, an emerging waterborne bacterial pathogen is associated with wide spectrum of clinical and post immunological manifestations including acute gastroenteritis, mesenteric adenitis, septicemia, arthritis and erythema nodosum, predominantly affecting young children (Fredriksson-Ahomaa et al., 2006). *Y. enterocolitica* a heterogeneous species, is divided into six biotypes, eight phage-types and more than 60 serotypes. *Y. enterocolitica* is a Gram-negative bacterium, belonging to the family Enterobacteriaceae. It is widespread throughout the environment. Humans can be carriers; even pigs can carry the same serotypes found in man.

The spread of drug resistance among *Y. enterocolitica* is also of concern for public health appraisal. The World Health Organization (WHO) report on infectious diseases in 2000 declared that antibiotic resistance poses a severe threat to human health, and that the problem is growing globally. Keeping all this in view the present research was proposed with the objective to study epidemiology, virulence, antibiotic susceptibility of *Y. enterocolitica* isolates from 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 16.1400 lacs population (Wikipedia, 2011). 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 randomly from endemic gastroenteritis affected suburbs of Ludhiana 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 18 mg/L neutralizes up to 5 mg of free (residual) chlorine per litre). The samples containing high concentration of zinc and copper were treated with EDTA at concentration of 372 mg/L to reduce metal toxicity (APHA, 1989) and were analyzed within 24 h by transporting in refrigerated container at 4°C.

Microbiological analysis of water samples

A total of 418 drinking water samples (Municipal Corporation 242, Submersible pump 144 and Hand pump 32) from endemic area of gastroenteritis infected area were analysed by the standard methods (Bureau of Indian Standards, IS-10500-1991, New Delhi, India; www.indiawaterportal.org/sites/indiawaterportal.org/files/Drinking%20Water%20Standards_IS%2010500_1991_BIS.pdf) and Bacteriological water testing kit (BWTK), developed in the 'Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India (Sahota et al., 2010) for total coliforms, faecal coliforms and Emerging pathogens.

Isolation, identification and biochemical characterisation of *E. coli*

From BWTK and MacConkey broth *E. coli* was isolated after primary enrichment, followed by streaking onto Eosine methyl blue agar. Typical *E. coli* colonies with green metallic sheen were isolated, restreaked to ensure purity and biochemically confirmed by Indole, methyl red Voges Proskauer and Citrate (IMViC) test using KB002 HiAssorted trade mark (TM) biochemical test kit (Himedia Laboratories Private Limited, Mumbai).

Isolation, identification and biochemical characterization of *Y. enterocolitica*

The pre-enriched water samples from BWTK were streaked on Yersinia Selective Supplement Medium (Cefsulodin7.50 mg/500 ml Medium, Triclosan 2.0 mg/500 ml Medium, Novobiocin 1.25 mg/500 ml Medium) (Himedia Laboratories Private Limited., Mumbai) and incubated at 37°C for 24 to 48 h. Colonies with characteristic bull's eye morphology with deep red centres and white to translucent periphery, were isolated, restreaked to ensure purity and maintained on nutrient agar slants for further biochemical characterization. The bacteria isolated were identified as *Y. enterocolitica* on the basis of colony morphology, Gram-staining, and biochemical characteristics (Koneman et al., 1992; Brenner et al., 2005).

Scanning electron microscopy of bacterial cultures

Samples were removed from agar plates and fixed with 3% glutaraldehyde at 4°C overnight. Dehydration of the samples was then conducted by a series of 10, 25, 50, 70 and 100% ethanol solutions. Using a critical point dryer the samples were dried further (Polaron E300 CDS, Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana-141004, India). These samples were mounted on aluminum stubs and then coated with gold using a Sputter Coater (Hitachi Model E-1010, Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana-141004, India). Finally the samples were examined using a Scanning Electron Microscope (SEM, Hitachi S-3400N, Electron Microscopy and Nanoscience

Laboratory, Punjab Agricultural University, Ludhiana-141004, India).

Determination of virulence markers of *Y. enterocolitica*

The strains were tested for β-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 Private Limited., Mumbai).

Virulence studies and histopathology

In group A, 5 mice were taken. They were deprived of drinking water for 24 h after that they were allowed to drink from aqueous bacterial suspension containing about 1×10^8 cfu/ml of *Y. enterocolitica* for 24 h. The inocula were then withdrawn and the animals served with clean water after 24 h period. In group B, 5 mice were taken as control and 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 µm sections and stained with hematoxylin-eosin.

Determination of multiple antibiotic resistances

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 37°C for 6 to 8 h, till the inoculum turbidity is achieved > 0.10D at 620 nm or 0.5 Mcfarland standard, 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 37°C for 18 to 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 *E. coli* and *Y. enterocolitica* from drinking water

A total of 418 drinking water samples were randomly collected from urban, suburban, rural and gastroenteritis infected area of Ludhiana city from three different water utilities, Municipal Corporation (242), Submersible pumps (144) and Hand pumps (32). All the drinking water samples were analyzed by bacteriological water testing kit (BWTK) and standard methods (BIS 10500:1991) and the positive samples were streaked on selective media for confirmation followed by biochemical identification.

Out of 418 drinking water samples, 41.14% samples were positive for *Escherichia coli.* Colonies of *E. coli*, on MacConkey agar were smooth glossy, translucent and rose pink in colour, on urinary tract infection (UTI) Hichrome Agar (Himedia Laboratories Private Limited., Mumbai) colonies were also glossy and rose pink in colour. The isolates observed under microscope were gram negative and rod shaped. Isolates were further streaked on eosin methylene blue (EMB) medium; produced metallic sheen with green coloration. Bio-chemically all the isolates were found positive for indole production test, methyl red test while voges-proskauer, citrate utilization, urease test were found negative.

Out of 418 drinking water samples, 69.61% samples were positive for *Y. enterocolitica.* Isolates with characteristic bull's eye morphology, deep red centres and white to translucent periphery typical of *Y. enterocolitica* were identified on Yersinia Selective Supplement Medium. 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 1.37 to 2.34 µm (Plate 1).

All the isolated strains of *Y. enterocolitica* depicted following biochemical properties typical of genus *Y. enterocolitica*: urease, ornithine decarboxylase and Voges proskauer positive negative for oxidase, lysine decardoxylase and H_2S. *Y. enterocolitica* fermented 10/21 tested sugar, produced acid as well as gas from the following sugars; arabinose, cellobiose, dextrose, fructose, galactose, mannitol, mannose, sorbitol, and trehalose and whereas weak reaction was observed against Maltose sugar.

All cultures of *Y. enterocolitica* were positive for urease indicating clinical importance of these isolates. De Koning-Ward and Robins-Browne (1995) also reported the production of urease by all clinical isolates of *Y. enterocolitica* and are encoded by the urease gene complex (*ure*) on the chromosome.

Bacterial survival depends on the adaptability of organism in the extreme environment such as tempera-ture, pH, osmolarity, and nutrient availability. Studies have implicated urease as a factor that is necessary for survival and pathogenesis of some bacteria. *Y. enterocolitica* is able to grow over a wide pH range from approximately 4 to 10, with an optimum pH of around 7.6. Survival of the high acidity of some foods and the passage through the stomach suggests that *Y. enterocolitica* species are relatively acid-resistant. Although the mechanism of acid tolerance is unknown, it may be due to the activity of urease, which catabolizes urea to release ammonia, which in turn elevates the cytoplasmic pH.

All the isolates *of Y. enterocoitica* were positive for haemolytic activity whereas negative for esculin. The aesculin hydrolysis test is used to determine the ability of

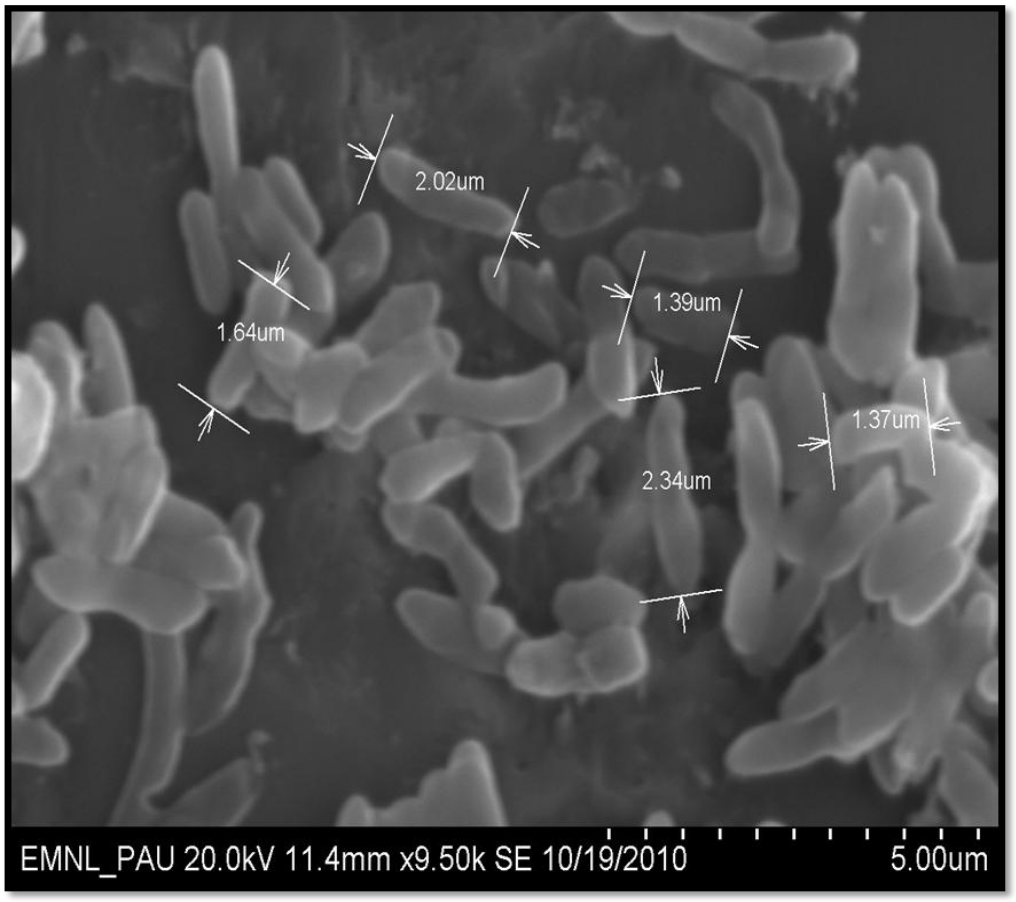

Plate 1. SEM image of *Yersinia enterocolitica.* Length: 1.37 to 2.34 µm, Width: 0.60 µm.

an organism to hydrolyse the glycoside aesculin toaesculetin and glucose in the presence of 10 to 40% bile.

Correlation between occurrence of *E. coli* and *Y. enterocolitica*

Y. enterocoitica was reported in 78.09 % of Municipal Corporation (MC) drinking water samples, 59.02% of Submersible pump drinking water samples and 53.12% of samples from Hand pump where as *E. coli* was found in 53.71% of Municipal Corporation (MC) drinking water samples, 29.16% of Submersible pump drinking water samples and 8% of samples from Hand pump.

There was no positive correlation between the simultaneous occurrence of *Y. enterocolitica* and *E. coli* ($P < 0.005$; $R^2 = 0.88$). In a study of 120 river samples, Massa et al., 1988 has also reported that no correlation between the presence of *Yersinia* and the total and fecal coliform bacteria. The direct search for *Y. enterocolitica* is not recommended by BIS for microbiological examination of water supplies. In India much reliance is placed on the faecal coliform indicator bacteria. Thus over all study revealed that, *E. coli* do not adequately warn or reflect the occurrence of pathogens in drinking water. Thus public health is not protected by using the indicator concept method laid by BIS.

Similarly the possible reason for occurrence of *Y. enterocolitica* in drinking water samples from Municipal Corporation supply, Submersible pumps and Hand Pumps may be breaches in the system's physical integrity, intrusions through leaks, as well as main breaks, which can provide a pathway for contaminants outside of the pipe to enter the distribution system during low negative pressure events. It may be also through pipeline fracture cracks, leaking joints, leaking adaptors, deteriorating seals and backflow through cross connection, untreated water containing bacteria to enter the water distribution system. Sewage overflow from sewer collection system may also be the source of contamination. Nutrients in the distribution system also support growth of microorganism present in the distribution system. There is a substantial amount of pathogenic and non pathogenic bacteria in the fecal matter of animals and when faecal matter spread on soil surface, could be reason of contamination of ground water either through seepage, surface run off, or through contamination of

water with faeces.

Y. enterocolitica also occurs in biofilm, which is a dense aggregate of surface adherent microorganisms embedded in a polysaccharide matrix. Hydrophobicity of *Y. enterocolitica* also plays a role in its adhesion to surfaces. Bacteria have lipid side chains, as well as portions of surface proteins that are hydrophobic. Usually, protein fold in such a way that hydrophilic amino acid regions are exposed, they play a role in hydrophobic interactions (Newby et al., 2000). Attachment of pathogenic bacteria such as *Y. enterocolitica* to surface in water distribution systems also has been noted by Camper et al. (1985). These pathogens may detach or slough form biofilms, cause persistent detection and even waterborne diseases.

Only few *Yersinia* outbreaks have occurred in which these pathogens have been isolated from water systems and implicated as the source of the infection. This is probably because of inappropriate or late sample examination and to the fact that health authorities are probably not vigilant when it comes to seeking out waterborne *Y. enterocolitica*. Moreover, public health laboratories do not normally carry out special isolation and diagnostic procedures required for these particular pathogens.

Our study is consistent with the results of earlier studies, which also reported the occurrence of *Y. enterocolitica* in water source. Falcão et al., (2004) also reported *Yersinia* spp. from water sources and sewage in Brazil. From 416 *Yersinia* strains isolated from wells and drinking water plants during the period 1982 to 1987, 82% were *Y. enterocolitica* and 11% were *Y. intermedia* (Aleksic and Bockemuhl, 1988).

Thus, *Y. enterocolitica* remains at the apex of bacterial versatility in being finely honed to make its transition from natural reservoir to human host. Endowed with an array of temperature-inducible, chromosomal and plasmid-mediated virulence factors, this bacterial species is primed to establish gastrointestinal tract pathology or to cause fulminant systemic disease in the appropriate host setting for example, immunosuppression, iron overload, or the extreme of age. Acquired either by the oral route in contaminated food or water, *Y. enterocolitica* has raised public health awareness due to its presence in water.

Determination of virulence marker of *Y. enterocolitica*

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 enteropathogenic and non-enteropathogenic bacteria.

All the isolates of *Y. enterocolitica* were positive for virulence marker test congo dye uptake. The ability to take up dye is associated with the presence of a virulence plasmid. Plasmids are well known carriers of several virulence determinants and play important role in the pathogenesis of several bacteria. Presence of plasmid also helps bacteria in intracellular survival within the host macrophages (Libby et al., 2000) and encodes for proteins that destabilize the cytoskeleton of eukaryotic cells (Lesnick et al., 2001). In several bacteria, plasmids play intrinsic role in the induction of host cell apoptosis. Analysis of different mutants of *Yersinia* spp. have shown macrophage apoptosis depends on proteins encoded by the virulence plasmids present in these bacteria (Waterman and Holden, 2003). The plasmids also confer resistance to antimicrobials which can be transferred along with transfer of these plasmids (Casas et al., 2005).

This finding suggests that all isolates were potential enteric pathogens. Virulence determinants are responsible for the establishment and maintenance of an infection in the host.

Virulence studies and histopathology

Y. enterocolitica is an enteric bacterium and infections by this organism are mostly water and foodborne. It has been implicated to cause enterocolitis, terminal ilitis, diarrhoea, mesenteric lymphadenitis and arthritis in man. Due to paucity of information regarding histopathological and especially ultra structural alterations in tissues affected, this study was planned with mice as the experimental model as infection in mice closely resembles that in humans. One biochemically characterized strain of *Y. enterocolitica* (GP 2), isolated from drinking water, was selected to study the course of *in vivo* infection in mice (5). Animals (mice) were deprived of drinking water for 24 h and then allowed to drink from aqeuous bacterial suspension containing, 1×10^8 cfu/ml of the *Y. enterocolitica* (GP 2) for 24 h. The inocula were then withdrawn and the animals served with clean water after 24 h period. Incubation period observed was five days as on sixth day animals showed signs of depression, isolation, progressive ruffling of the fur as well as steady loss of weight leading to gross emaciation. Profuse diarrhoea was noticed after 7^{th} day of inoculation. The signs of diarrhoea shown by mice infected with *Y. enterocolitica* could be attributed to the heat stable enterotoxin produced by the organism which resembles the ST toxin of *E. coli.*

The faecal samples showed the presence of 1×10^{14} cfu/g of the *Y. enterocolitica* (GP 2). The post inoculation experimental mice died on the eighth (1), tenth (2), eleventh (2) day respectively. Tissues from the intestine, liver, kidney and lungs were removed at post-mortem of the infected animals (carcasses).

Histopathological examination of liver showed granular degeneration, hypertrophy of hepatocyte nucleus,

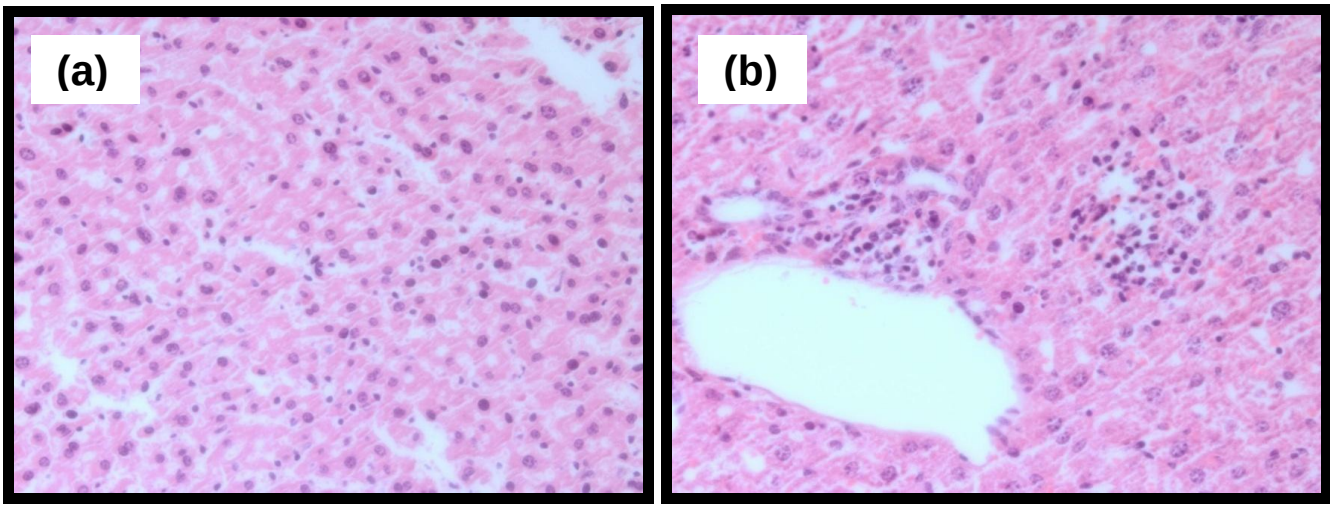

Plate 2. Histological tissue of mice (Liver) infected with *Yersinia enterocolitica* showing granular degeneration, hypertrophy of (a) haepathocyte nucleus (b) haepathocyte nucleus, periportal hepatitis, proliferation of von kuffer cells, diffused infilteration of mononuclear cells and necrosis (40×) (haematoxylin and eosin stain).

periportal hepatitis, proliferation of von kuffer cells, diffused infilteration of mononuclear cells and necrosis (Plate 2). Lal et al. (2004) also reported congestion, hepatocellular degeneration, necrosis, atrophy of hepatocytes and microabcesses of liver due to infection of *Y. enterocolitica* through oral and intraperitoneal route. Zhao et al. (2000) also reported periportal inflammation in the liver, necrosis, atrophy of hepatocytes and microabcesses of liver in mice.

The lungs revealed oedema and congestion (Plate 3). Kidney showed degeneration, necrosis of tubular epithelium, focal area of interstitial nephritis, increase in cellularity mild granular, vacuolar degeneration, and desquamation of tubular epithelium: (Plates 4a, b and c). Lal et al. (2004) also reported congestion in lungs and necrotic changes in kidney due to infection of *Y. enterocolitica* through oral and intraperitoneal route.

Intestine showed necrotic enteritis and infilteration of mononuclear cells in lamina propria (Plate 5) and heart showed myocardial degeneration, mononuclear cell infilteration, epicarditis and congestion (Plate 6). Hablolvarid et al. (2008) also reported acute hepatitis and enteritis in liver and intestine in monkey due to infection of *Y. enterocolitica*. Orally administered *Y. enterocolitica* could survive the low-pH environment of the stomach due to the expression of a urease. The bacteria adsorbed to intestinal epithelium, eventually colonizing the underlying lymphoid tissues of the small intestine,once in the Peyer's patches (PP), and the mesenteric lymph nodes (MLN), the bacteria are able to replicate and survive. At the same time, the host mounts an inflammatory response characterized by an influx of neutrophils and macrophages. Subsequent disseminated infection results in neutrophil and macrophage infiltration, bacterial microcolony formation, microabscesses, and granuloma lesion formation occurring primarily in the MLN, liver, and spleen (Pepe et al., 1995).

Antibiotic susceptibility of *Y. enterocolitica*

A total of 291 isolates of *Y. enterocolitica* were isolated from 418 drinking water samples from three different water utilities, 189/242 Municipal Corporation, 85/144 Submersible pump and 17/32 Hand pumps, were phenotyped, using antimicrobial susceptibility test against panel of 24 antibiotics.

Y. enterocolitica isolates were resistant to two out of three penicillins, Ampicillin/sulbactum 10/10 mcg and Ampicillin 10 mcg whereas 100% isolates were sensitive to Piperacillin 100 mcg. For quinolones, 99% isolates were resistant to Co-Trimoxazole 25 mcg and 100% isolates were resistant to Nalidixic acid 30 mcg and 100% were sensitive to Ofloxacin 5 mcg, Ceprofloxacin 5 mcg and Levofloxacin 5 mcg.

All the isolates of *Y. enterocolitica* were sensitive to all the tested aminoglycosides (Amikacin 30 mcg, Tobramycin 10 mcg and Gentamicin 10 mcg).Similarly all the isolates showed sensitivity to Netillin 30 mcg and Aztreonam 30 mcg and resistance to Amoxyclave 30 mcg and Augmentin 30 mcg (monolactams) and showed sensitivity to β-lactum: carbapenem (Imipenem 10 mcg). All β-lactams antibiotics interfere with the synthesis of bacterial cell wall.

L-Lactam resistance in *Y. enterocolitica* is largely due to the expression of two chromosomally encoded L-lactamases called BlaA (a class A enzyme) and BlaB (a class C (AmpC) L-lactamase), (Cornelis and Abraham, 1975), the differences in L-lactam susceptibility are predominantly due to differences in BlaA and BlaB

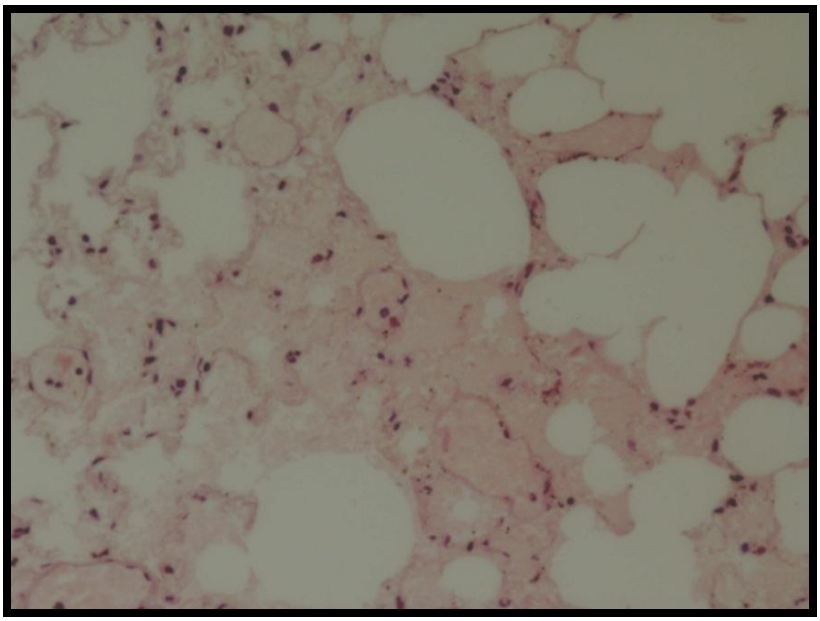

Plate 3. Histological tissue of mice (Lung) infected with *Yersinia enterocolitica* showing congestion and oedema (20×) (haematoxylin and eosin stain).

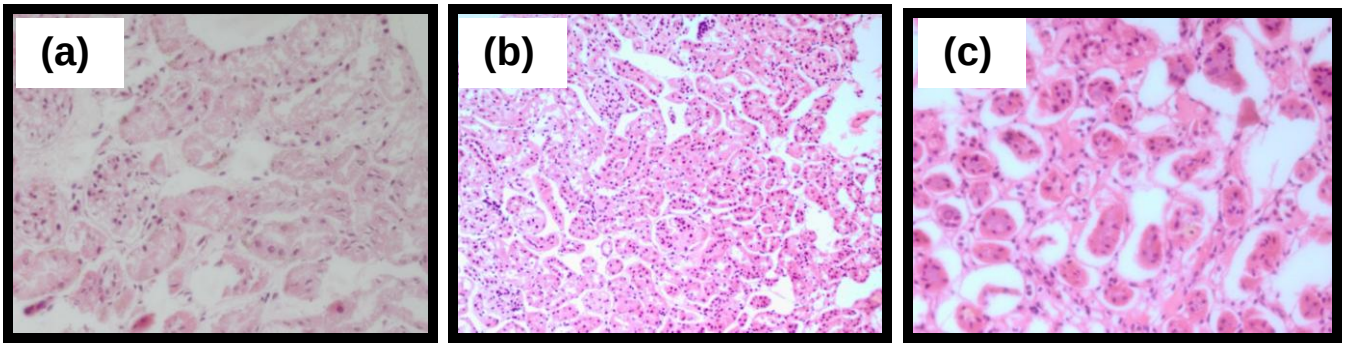

Plate 4. Histological tissue of mice (kidney) infected with *Yersinia enterocolitica* showing (a) degeneration and necrosis of tubular epithelium (b) mild granular and vacuolar degeneration, desquamation of tubular epithelium and focal area of interstitial nephritis and increase in cellularity and (c) mild granular and vacuolar degeneration (40×) (haematoxylin and eosin stain).

expression which depend on the biovar and -within some biovars - on the individual strain. The A-type latamase hydrolyses a variety of penicillins and cephalosporins. Beta-lactamase of B-type show stronge cephalosporinase activity (Pham et al., 2000). The isolates of *Y. enterocolitica* showed resistance to 60% cephalosporins i.e. isolates were sensitive to Cephotaxime 30 mcg, Ceftazidime 30 mcg and showed resistance to Cefuroxime 30 mcg, Cephoxitin 30 mcg and Cephalothin 30 mcg.

Similarly *Y. enterocolitica* isolates were found to be resistant to both the Tetracyclines (Tetracycline 30 mcg and Doxicycline 30 mcg). Tetracycline resistance is thought to originate from the use of antibiotic in animal production (Schroeder et al., 2002). Further all the isolates showed resistance to other antibiotics like Nitrofurantoin 300 mcg.

A high incidence of resistant bacteria has particularly been reported from developing countries, where antibiotics are freely available and their use is not subjected to any regulation. The administration of antimicrobial agents for the treatment of bacterial infections in both veterinary and human medicine poses a potential risk because it leads to the selection of strains resistant to antibiotics. The main reason for the increase in resistant bacteria population is apparently in the application of antibiotics as prophylaxis and growth stimulants in animals. For that reason, the use of antibiotics was gradually restricted, and in 2006, their use as growth stimulants was completely banned in the

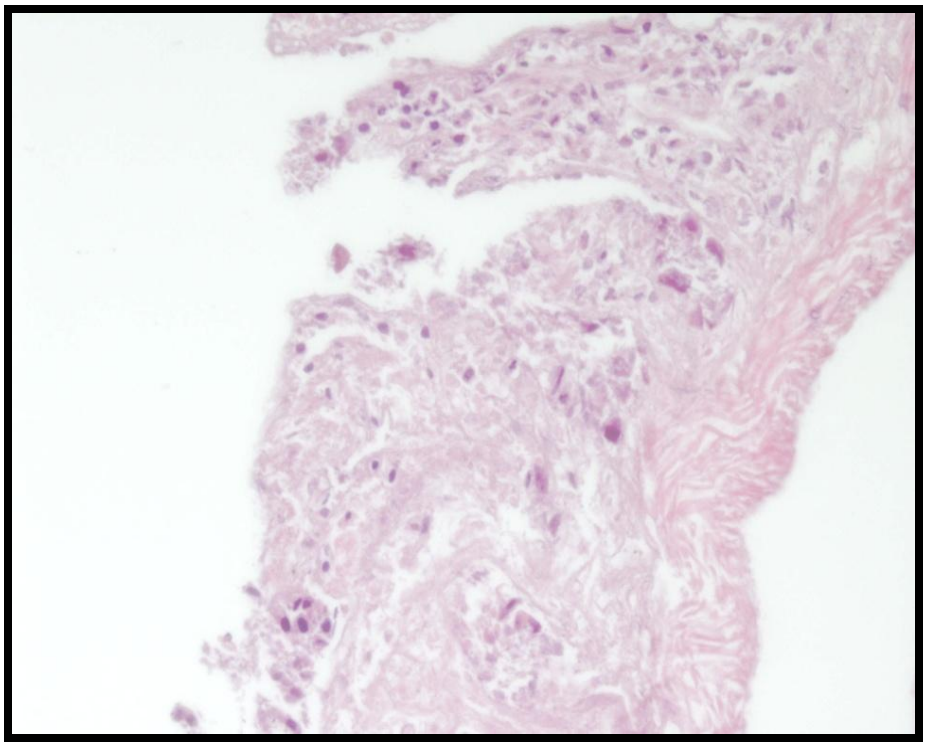

Plate 5. Histological tissue of mice (Intestine) infected with *Yersinia enterocolitica* showing necrotic enteritis and infiltration of mononuclear cells in lamina propria (40×) (haematoxylin and eosin stain).

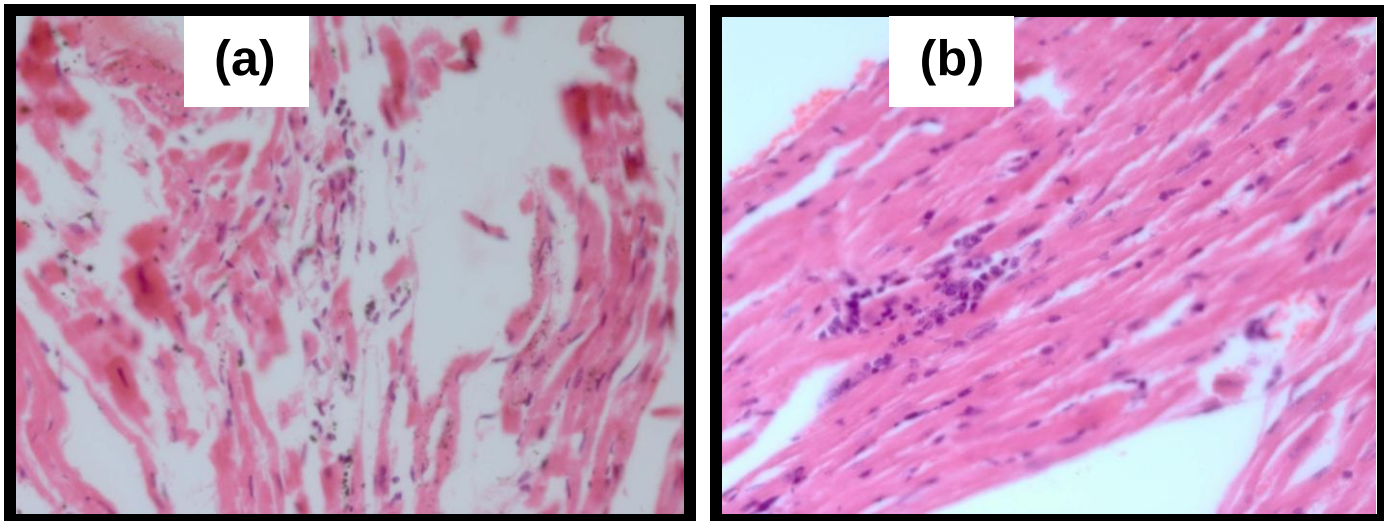

Plate 6a. Histological tissue of mice (Heart) infected with *Yersinia enterocolitica* showing (a) myocardial degeneration, epicarditis and infiltration of mononuclear cells and (b) myocarditis, congestion and focal infiltration of mononuclear (40×) (haematoxylin and eosin stain).

European Union.

Conclusion

This is the first study conducted in District Ludhiana, Punjab State that investigated the occurrence of *Y. enterocolitica* in drinking water, which is a major issue worldwide. The reliability of faecal indicator bacteria raises questions in assessing the bacteriological quality of water, particularly because of their poor correlation with *Y. enterocolitica. E. coli* was reported in 41.14% of

drinking water samples, whereas emerging pathogens *Y. enterocolitica* was in 69.61%. The appropriate level of treatment during storage and distribution can guarantee the water quality.

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