

Full Length Research Paper

Epidemiological surveillance, virulence and public health significance of *Listeria* spp. from drinking water

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Epidemiological surveillance of drinking water from Punjab, India reported occurrence of *Listeria* spp. in 58.67% of Municipal Corporation (MC), 51.38% submersible pump and 12.5% hand pumps and *Escherichia coli* in 53.71% of MC, 29.16% submersible pump and none of samples from hand pumps. There was no positive correlation between the simultaneous occurrence of *Listeria* spp. and *E. coli* ($P < 0.005$; $R^2 = 0.89$). Isolates were identified serologically and confirmed by polymerase chain reaction (PCR) amplification using specific primers targeting a 1200 bp fragment of the 16S rRNA gene. All isolates of *Listeria* spp. were haemolytic on 5% sheep blood agar and positive for congo dye uptake and showed multiple drug resistance, multiple antibiotic resistance (MAR) indices of 0.86 (> 0.2). One isolate of *Listeria* spp. was molecularly identified by sequencing of 16S rRNA gene, and its gene sequence was submitted to National Center for Biotechnology Information (NCBI) under accession no. JF798637. Using this isolate, histopathological studies were carried out and it caused significant histopathological and ultrastructural alterations in experimentally infected BALB/c mice. The conventional methods cannot predict the presence of these potentially enteropathogenic microorganisms in drinking water and hence represents a serious public health concern.

Key words: Epidemiological, *Listeria* spp., haemolytic, serologically histopathological, multiple antibiotic resistance (MAR).

INTRODUCTION

Safe water supplies and environmental sanitation are vital for protecting the environment, improving health and alleviating poverty. Every effort should be made to achieve a drinking-water quality as safe as practicable (WHO, 2004). The World Bank estimates that 21% of communicable diseases in India are water related. Diarrhoeal diseases kill an estimated 1.8 million people each year. The highest mortality from diarrhoea is in children

under the age of five, highlighting an urgent need for focused interventions to prevent diarrhoeal disease in this age group. The provision of clean drinking water has been given priority in the Constitution of India, with Article 47 conferring the duty of providing 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 (www.wateraid.org).

The spectrum of water-borne diseases is expanding, and majority of diseases once believed to be conquered are on the rise. Zoonoses are of increasing concern for human health; next to pathogens with human-to-human transmission as they pose the greatest challenges to

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Abbreviations: MC, Municipal Corporation; PCR, polymerase chain reaction; SEM, scanning electron microscope.

ensure the safety of drinking water. *Listeria* spp. is an ubiquitous bacteria widely distributed in nature, lives in plant-soil environments and has been isolated from about 42 species of domestic and wild mammals and 22 species of birds, as well as fish, crustaceans, insects, sewage, water, feedstuffs, milk, cheese, meconium, feces and soil (Kirkan et al., 2006). The possible reason for occurrence of *Listeria* spp. in drinking water utilities may be fecal wastes from domestic animals, which may enter water system by direct contamination of the water or through seepage or surface runoff. Domestic and wild animals contaminate water by defecation in unprotected surface water, through runoff and as a result of seepage of water through soil result in contamination of ground water and may result in the incident of *Listeria* spp. in ground water.

The clinical syndromes of the disease caused by pathogenic species of *Listeria* in human include invasive listeriosis, non-invasive gastrointestinal disease, as well as local skin and eye symptoms (Maijala et al., 2001). Invasive listeriosis causes meningoencephalitis, encephalitis, sepsis, and abortions, and has a high mortality rate (20 to 30%). On the other hand, non-invasive listeriosis causes fever, diarrhea, muscle pain, headache, nausea, vomiting and abdominal pain in healthy adults (Lunden et al., 2004). *Listeria* species were generally reported to be susceptible to most antibiotics (Li et al., 2007) but more recently, reports of antibiotic resistance in *Listeria* spp. have been published (Arslan and Ozdemir, 2008; Conter et al., 2009). Thus, more information is required on the pattern of dispersion of antibiotic resistance among *Listeria* spp.

Most study on the isolation, virulence and antimicrobial susceptibility profiles of *Listeria* species focus almost exclusively on clinical and/or food isolates, with little information on water borne listeriosis. Keeping all this in view, the present research was proposed with the objective to study epidemiology, virulence and antibiotic susceptibility of *Listeria* spp. isolates 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 accommodates 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 with migratory population. The samples were treated with sodium thiosulfate to inactivate any residual halogen compound present in the sample ($\text{Na}_2\text{S}_2\text{O}_3$ 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). The samples were analyzed within 24 h 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 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) (Sahota et al., 2010), developed in the 'Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India for total coliforms, faecal coliforms and *Listeria* spp..

Isolation of *Listeria* spp.

Aliquots of samples from positive Bacteriological water testing kit (BWTK) (Sahota et al., 2010) were streaked on *Listeria* selective agar (Ceftazidime 2.0 mg/500 ml and Amphotericin B 2.5 mg/500 ml) (HiMedia Laboratories Pvt. Limited, Mumbai, India) and incubated at 37°C for 24 to 48 h. Observed for typical colonies were blue with a yellow background (Rhamnose positive) or blue without a yellow background (Rhamnose negative).

Biochemical confirmation

Colonies suspected to be *Listeria* were characterized using Gram stain, catalase reaction, umbrella-shaped motility pattern using motility test medium, fermentation of mannitol, rhamnose and xylose performed according to Bergey's manual of systematic bacteriology (Seeliger and Jones, 1987).

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, 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), scanning electron microscope.

Serological identification of *Listeria* spp.

HiListeria Latex test kit (HiMedia, Mumbai, India) was used for rapid identification of *Listeria* spp. from solid agar. The sensitised latex particles in solution/suspension containing antigen was used, against which the anti-serum was placed and observed for agglutination of the particles and compared with positive and negative control.

Nucleic acid isolation

Nucleic acid were isolated by lysis of bacteria in a solution containing 0.5% sodium dodecyl sulfate and 5 mg of lysozyme per ml, followed by extraction with phenol-chloroform (Hirono et al., 1992). Nucleic acid samples were precipitated with ethanol and

dissolved in Tris-EDTA (TE) buffer (10 mM Tris chloride, 1 mM EDTA [pH 8.0]). The nucleic acid content was quantified by determining the optical density (OD) at 260 nm (OD_{260}) and was adjusted to give a final concentration of 200 µg/ml in TE buffer. Template DNA for PCR was prepared by further dilution in distilled water to a concentration of 2 µg/ml.

Identification of *Listeria* spp. by polymerase chain reaction (PCR)

A 1.20 kb portion of the regulatory region of 16S rRNA was amplified with the primers (forward primer 5'-GGACCGGGGCTAATACCGAATGATAA-3') and reverse primer 5'-TTCATGTAGGCGAGTTGCAGCCTA-3') (Wiedmann et al., 1993).

Determination of virulence markers

The isolates were tested for β-hemolytic activity on agar base supplemented with 5% sheep erythrocytes (Gerhardt et al., 1981) and Congo red dye uptake (Paniagua et al., 1990).

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 30 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 ≥ 0.10 D 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 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 isolates from environments where antibiotics are seldom or never used (Krumperman, 1985).

Molecular characterization

DNA was isolated from overnight grown bacterial cells in nutrient broth according to the standard methods (Sambrook et al., 2001). For amplification of 16S rRNA gene, the following primers were used: forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-ACGGGCGGTGTGTC-3' (Weisberg et al., 1991). DNA amplification was performed with Genamp PCR system (Applied Biosystem, USA). 16S rDNA amplicon was gel eluted using GenElute gel extraction kit (Sigma-Aldrich, USA) and was ligated into the pTZ57R/T vector as per manufacturer's instruction (Fermentas, USA). Ligated plasmid was used to transform chemically competent *Escherichia coli* DH5α by heat shock method. After screening of the positive clone, the sequence was generated by chain termination method using an Applied Biosystem automated sequencer (Delhi University South Campus, Delhi, India). The 16S rRNA gene sequence was compared with Ribosomal Database Project-II (RDP-II) (Cole et al., 2003) and with those from GenBank using the BLASTN program (Altschul et al., 1997). The sequences were aligned using the CLUSTALW program (Thompson et al., 1997). The evolutionary distance was calculated by Kimura 2 para-

meter, a phylogenetic tree was constructed by the neighbor-joining method. Bootstrap analysis was based on 1,000 replicates. The MEGA 4.0 package (Tamura et al., 2007) was used for all analyses. The 16S rRNA gene sequence determined in this study was deposited in GenBank of NCBI data library.

Virulence studies and histopathology

A group of five mice each 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 cells/ml, for 24 h of *Listeria* spp. The inocula were then withdrawn and the animals served with clean water after 24 h period. Those in control (five mice) 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.

RESULTS AND DISCUSSION

A total of 418 drinking water samples from three different water utilities Municipal Corporation (242), submersible pump (144) and hand pump (32) were analyzed for occurrence of *Listeria* spp. The positive BWTK was used for the isolation of *Listeria* spp. from drinking water. Out of 418 drinking water samples, 52.63% samples were positive for presence of *Listeria* spp. The growth of *Listeria* spp. was observed on *Listeria* selective supplement medium, colonies were blue with a yellow background. In nutrient broth, *Listeria* spp. produced uniform turbidity. The isolates observed under microscope were gram positive and rod shaped. The morphology was further confirmed by electron microscopy, which confirmed it to be rod shaped with round edges and approximate 1.31 to 2.28 µm in size (Figure 1).

The isolates of *Listeria* spp. were further confirmed by HiListeria Latex test kit (HiMedia, Mumbai, India). Latex agglutination test uses the technique of absorbing anti-serum for a specific antigen onto latex particles of uniform diameter, 0.8 microns. When the sensitised latex particles are placed in solution/suspension containing antigen against which the anti-serum is directed, agglutination of the particles occurs and is visible on inspection. Polyvalent antisera prepared against purified flagellin proteins from *Listeria monocytogenes* and *Listeria grayi*, was used to coat latex particles. When mixed with a suspension containing *Listeria species*, the latex particles rapidly agglutinate to form visible clumps (Figure 2).

These 220 selected *Listeria* isolates were identified using PCR. The PCR primers for the specific detection of *Listeria* spp. was tested successfully under the reaction conditions described, that is, PCR products of desired size (1200 bp for *Listeria* spp.) was only obtained in reactions containing genomic DNA of the targeted serologically identified isolates (Figure 3). No product was detected when genomic DNA from other than the target organism was used. The PCR assay provides a more rapid means of accurate identification of *Listeria*

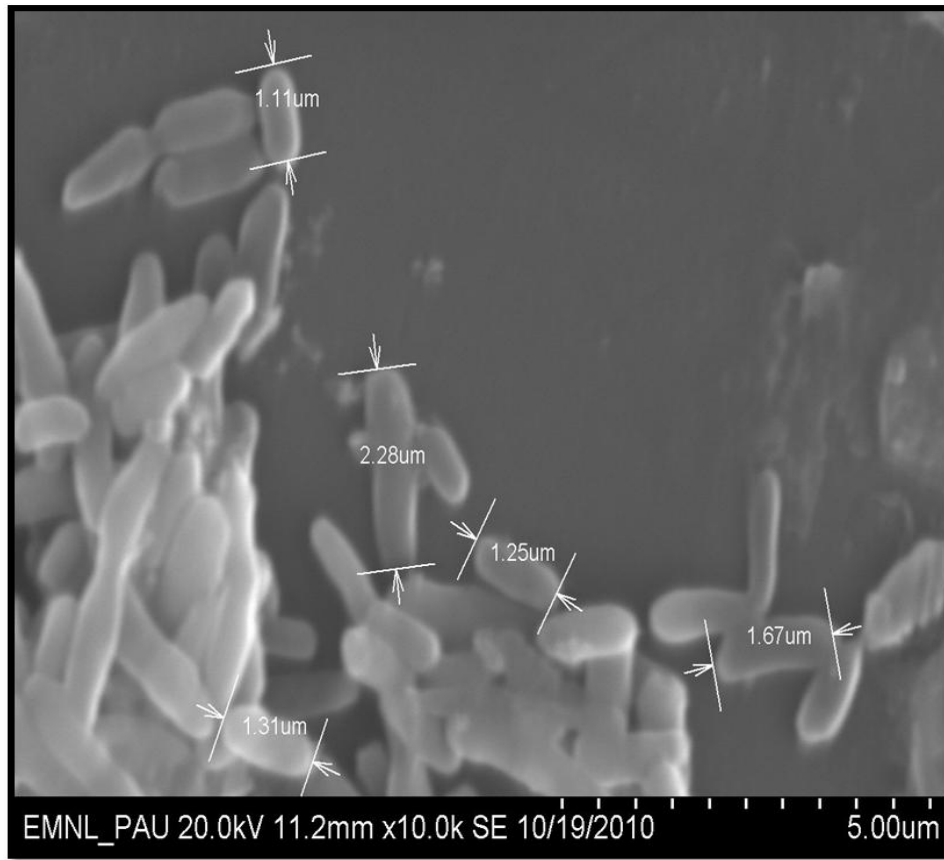


Figure 1. SEM images of *Listeria* spp. Length: 1.31 to 2.28 μM , width: 0.60 μM .

spp. than present standard methods of biotyping which are time-consuming and laborious. Use of the PCR would significantly reduce the time required to identify *Listeria* *spp.* isolates and can be used directly after primary selective culture of this organism. The entire test could be completed in 6 h, including the DNA extraction step. The reason for occurrence of *Listeria* *spp.* in drinking water utilities may be poorly processed sewage effluents, malfunctioning of septic tanks, seepage from sanitary landfills, and failure of the disinfection and infiltration of contaminated water through cross connection in distribution system.

Correlation between occurrence of *E. coli* and *Listeria* *spp.*

Listeria *spp.* occurred in 58.67% of MC drinking water samples, 51.38% submersible pump drinking water samples and 12.5% of samples from hand pumps drinking water samples whereas, *E. coli* occurred in 53.71% of 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 *Listeria* *spp.* and *E. coli* ($P < 0.005$; $R^2 = 0.89$). It is suggested that the dependence on classical

pollution indicators like *E. coli* and culturable total and faecal coliforms may be misleading, as some of these indicators are reported to be more susceptible to disinfection than more resistant bacteria like *Listeria*. The implication of this is that a water supply may be adjudged fit and safe for human consumption based on the *E. coli* and coliform standards, whereas in actual sense, it may contain more deadly pathogens like *Listeria*. In addition, due to emphasis on the monitoring of classical pollution indicators as stated earlier, not much is being done with regards to the survival and molecular epidemiology of resistant isolates of *Listeria* species in drinking water, either as free or attached cells.

Thus the overall study revealed that, *E. coli* do not adequately reflect the occurrence of pathogens in drinking water, may be due to their relatively high susceptibility to disinfection. Thus, public health is not protected by using these common indicators.

Determination of virulence marker of *Listeria* *spp.*

Haemolysis is the key character to distinguish the pathogenic and non-pathogenic species of *Listeria* *spp.* It is used to distinguish the two species most frequently isolated that is, *Listeria monocytogenes* (haemolytic) and *Lis-*

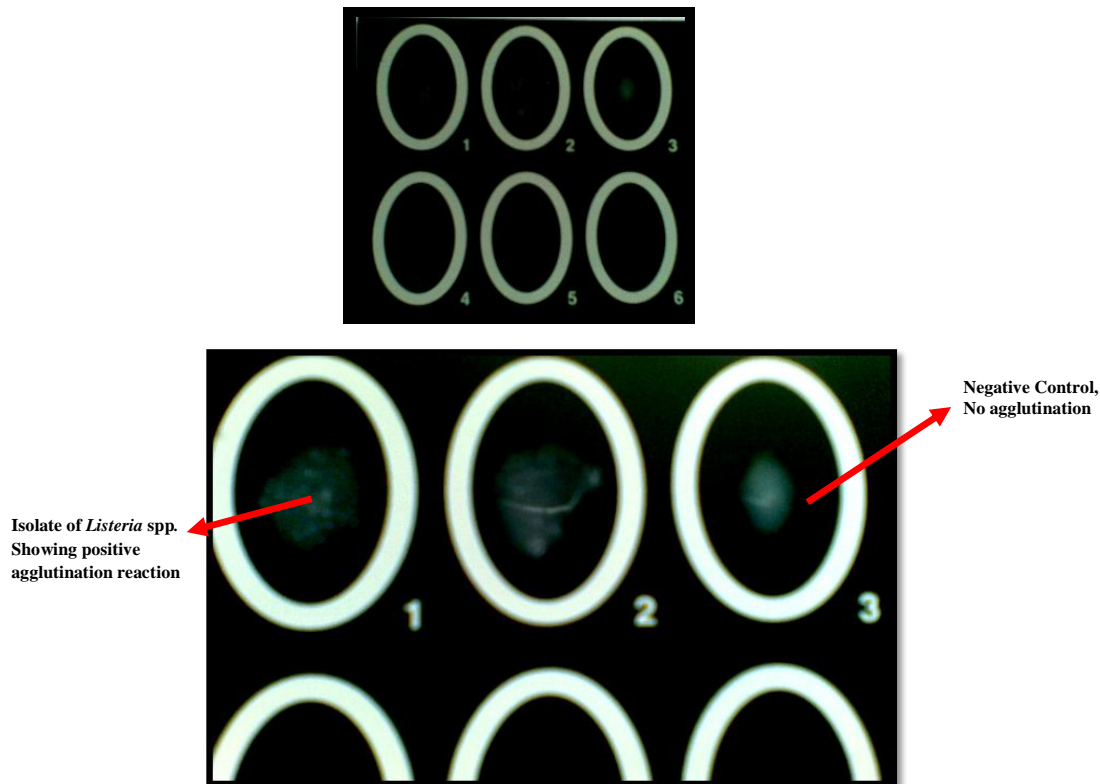


Figure 2. Serological Identification of *Listeria* spp.

terea innocua (non-haemolytic). Also, hemolysin has been associated with lecithinase production. In the study, it was found that all the serologically identified isolates of *Listeria* spp. were haemolytic on 5% sheep blood agar. These results highlight the pathogenic potential of *Listeria* spp. which poses a public health concern. It is also important to comment that the presence of any *Listeria* spp. may be indicative of poor hygiene and cross contamination scenarios which could favour the persistence of *L. monocytogenes* (Azevedo et al., 2005).

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 and Kapperud, 1986), serum resistance (Pai and DeStephano, 1982), hydrophobicity (Lachica and 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. The ability to take up dye is associated with the presence of a virulence plasmid. Uptake of Congo red dye has been shown to be a marker for virulence in several enteropathogenic

and nonenteropathogenic bacteria. All the isolates of *Listeria* spp. were positive for Congo dye uptake; this finding suggests that all were potential enteric pathogens.

Nunes and Hofer (1994) also analyzed a total of 130 *Listeria* isolates in order to evaluate lecithinase production and capacity for Congo red adsorption as markers of pathogenicity. *L. monocytogenes* cultures present 51.8 and 88.8% positivity rates for Congo red adsorption and lecithinase production, respectively, whereas 80.8 and 100% of *L. innocua* cultures were negative for the two tests, respectively.

Antibiotic susceptibility of *Listeria* spp

A total of 220 isolates of *Listeria* spp. were isolated from 418 drinking water samples from three different water utilities, 142/242 Municipal Corporation, 74/144 submersible pump and 4/32 hand pumps. These isolates were phenotyped, using antimicrobial susceptibility test against panel of 30 antibiotics. A total of 79.09% isolates were resistant to bacteriostatic antibiotic (chloramphenicol C/25 mcg and fusidic acid Fc/10 mcg), lincosamides (lincomycin L/2 mcg and clindamycin Cd/2 mcg), glycopeptide antibiotics (vancomycin Va/30/20 mcg and teicoplanin Te/30 mcg), macrolides (erythromycin E/15 mcg), oxazolidinone (Linezolid Lz/30 mcg), 73.18% Listerial isolates were resistant to quinolones (Co-trimoxazole Co/25 mcg ciprofloxacin Cf/5 mcg) aminoglycosides (streptomycin

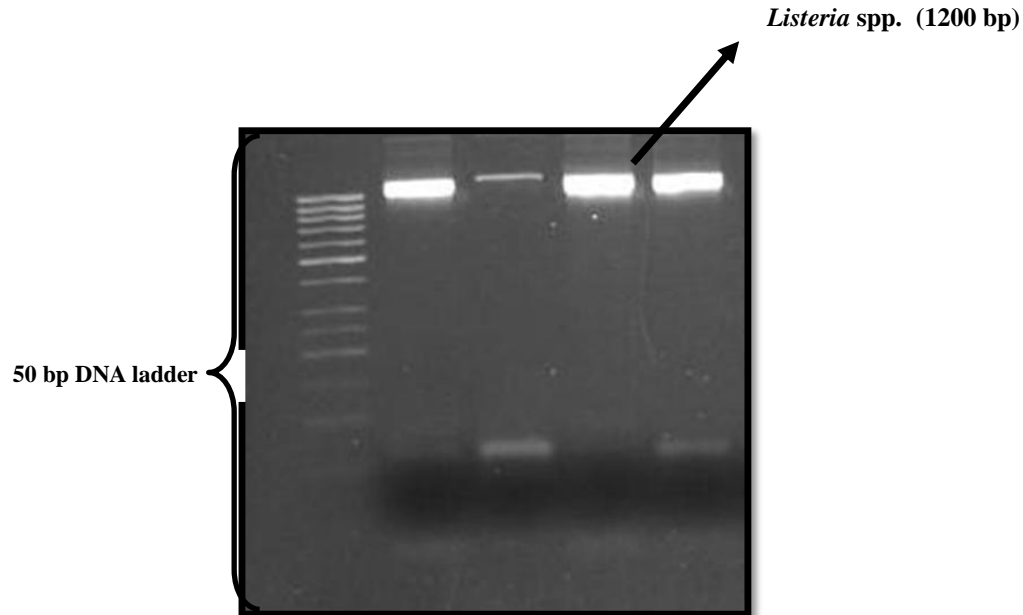


Figure 3. PCR with primers specific to 16S rRNA gene of *Listeria* spp., PCR products were electrophoresed and visualized with ethidium bromide.

S/10 mcg), monobactams (amoxyclav Ac/30 mcg), narrow spectrum beta-lactam antibiotic (ovacillin), penicillins (Penicillin, G (P) 10 units; methicillin, M/5 mcg and ampicillin, A/10/10 mcg); tetracyclines (tetracycline, T/30 mcg) and 69.55% isolates were resistant to cephalosporins (cefepime Cpm /30 mcg, ceftazidime, Ca/30 mcg; cefuroxime, Cu/30 mcg; ceoxacillin, Cx/1 mcg; cephalothin, Ch/30 mcg; cephotaxime, Ce/30 mcg; cephoxitin, Cn/30 mcg).

Sensitivity was only observed against vancomycin, Va/30 mcg; erythromycin, E/15 mcg; ofloxacin, Of/30 mcg and gentamicin, G/10 mcg by 81.36% of total *Listerial* isolates tested. The most of bacterial isolate from drinking water was resistant to four out of thirty 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. MAR index for *Listerial* isolates is 0.86 (> 0.2).

The possible reason for high MAR may be contamination of drinking water by wastewater effluents which normally contain high concentration of antibiotics after treatment, thereby raising chances of antibiotic contaminants perturbing the microbial ecology; increasing proliferation of antibiotic resistant pathogens; and posing threats to human health as well as create challenges for the water industry on issues of water reuse and water resource planning (Daughton and Ternes, 1999). Multiple drug resistance in *Listeria* species have also been attributed to antimicrobial selective pressure and gene transfer mechanism between and amongst *Listeria* species and close relatives of the bacteria such as *Enterococcus*, *Strepto-*

coccus and *Staphylococcus* species (Safdar and Armstrong, 2003). Donlan and Costerton (2002) also reported the acquisition of inherent resistance to antimicrobial agents by attached bacterial species.

The widespread use of antimicrobials in human and veterinary medicine, as well as in animal production, has accelerated the development of drug resistance in a variety of pathogenic bacteria. *L. monocytogenes* are important Gram-positive pathogens of public health concern. Antibiotic resistance in *L. monocytogenes* is mainly due to acquisition of three types of movable genetic elements: self-transferable plasmids, mobilizable plasmids, and conjugative transposons (Charpentier and Courvalin, 1999). *Enterococci* and *Streptococci*, in particular, represent a reservoir of resistance genes for *L. monocytogenes*. The gastrointestinal tract of humans is considered the most probable site where the acquisition by *Listeria* spp., of conjugative plasmids and transposons from *Enterococcus-Streptococcus* takes place (Doucet-Populaire et al., 1991).

Molecular characterization

One biochemically characterized isolate of *Listeria* spp., GP3 from drinking water was further subjected to 16S rRNA gene sequencing. The 16S rRNA gene sequence of GP3 *Listeria* spp. was compared with 16S rRNA gene sequence of DQ065845 *Listeria seeligeri*, FJ557241 *Listeria rocourtiae*, FJ774256 *L. monocytogenes*, FJ774247 *L. innocua*, DQ065843 *L. seeligeri*, X98529 *Listeria ivanovii*, FJ774232 *L. innocua*. On the basis of nucleotide homology and phylogenetic analysis, the isolate

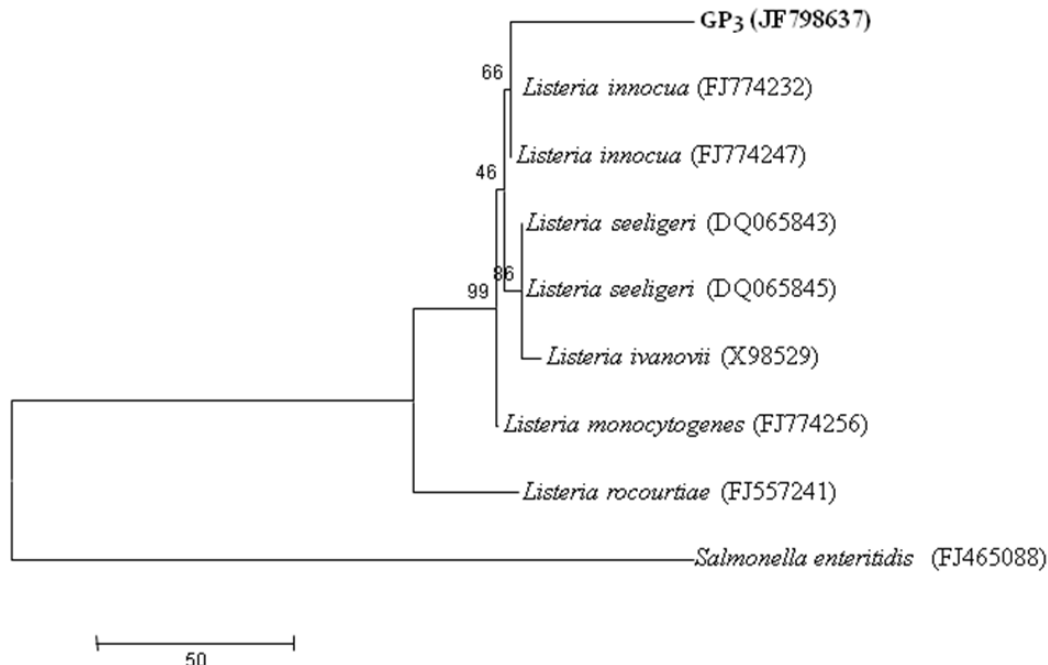


Figure 4. The phylogenetic dendrogram of *Listeria* spp. GP3 and related strains based on the 16S rRNA sequence.

GP3 showed some percentage of homology with all the species of *Listeria* available in gene bank but Drancourt et al. (2000) recommended $\geq 99\%$ sequence similarity of 16S rRNA gene sequences to the sequence deposited in DNA databases for a valid species designation, based on a large collection of environmental and clinical unidentifiable bacterial isolates. Finally, Stackebrandt and Ebers (2006) revised the former recommendations and suggested a higher 16S rRNA gene sequence similarity threshold range of 98.7 to 99% as the point at which DNA-DNA reassociation experiments should be mandatory for testing the genomic uniqueness of novel isolate(s). So, the isolate of *Listeria* spp. GP3 from drinking water could be new Listerial species, as it showed only some percentage of similarity with other Listerial isolates and hence grouped as *Listeria* spp. and, submitted to NCBI under an accession number JF798637 (Figure 4).

Virulence studies and histopathology

Listeriosis can manifest itself as sepsis, meningitis, encephalitis, intrauterine infections and spontaneous abortions in pregnant women. The onsets of these diseases are usually preceded by persistent fever or gastrointestinal symptoms such as nausea, vomiting and diarrhoea. *L. monocytogenes* is more prone to affect target populations such as pregnant women, newborn infants, immunocompromised individuals on corticosteroids, patients with cancer and other chronic diseases and the elderly.

In the present study, pathogenic potential of *Listeria* spp. from drinking water was investigated in the mice

model. One biochemically and molecularly characterized isolate of *Listeria* spp. (GP 3) with accession number JF798637, isolated from drinking water was selected to study the course of infection in mice (five). Animals (mice) were deprived of drinking water for 24 h and then allowed to drink from aqueous bacterial suspension containing 1×10^8 cells/ml of the *Listeria* spp., GP 3 for 24 h. It was found that on the 10th day after inoculation of animals with the bacterial broth culture, signs of depression and isolation was observed. The faecal samples showed the presence of 1×10^{15} cells/g of the *Listeria* spp., (GP 3). The experimental mice died on the 12th (one), 15th (three), and 22nd (one) days, 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 revealed vacuolar and granular degeneration of hepatocytes, focal areas of mononuclear cell infiltration in periportal area and necrosis of focal area (Figure 5). The intestine showed sloughing of mucosal lining epithelium, infiltration of mononuclear cell in lamina propria and hypertrophy of villi (Figure 6). The lungs revealed oedema, haemorrhage and congestion (Figure 7).

The kidney showed coagulated type of necrosis, mild granular degeneration of tubular epithelium, increase in cellularity in the glomeruli and in interstitium, decrease in urinary space, and swollen glomeruli (Figure 8). The bacterial colonies in myocardium, focal infiltration of mononuclear cells, degeneration and necrosis of myocardium, valvular endocarditis and congestion was observed in

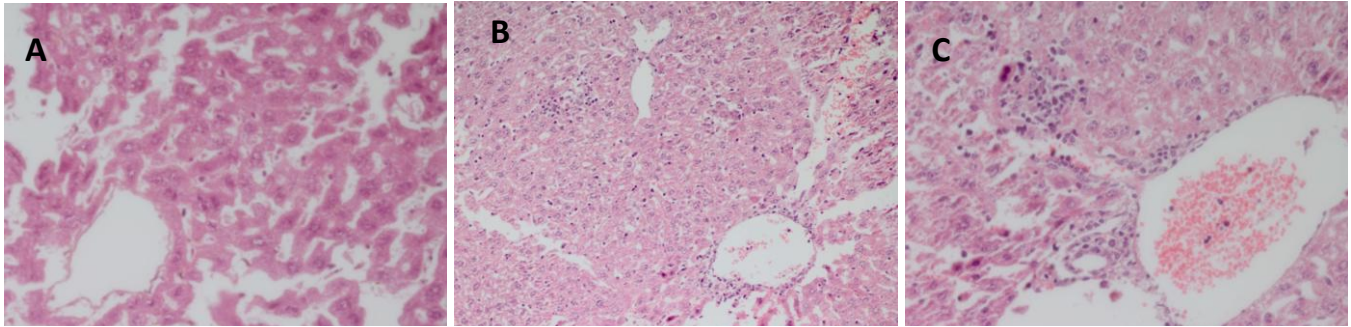


Figure 5. **A.** Histological tissue of mice (liver) infected with *Listeria* spp. showed vacuolar and granular degeneration of hepatocytes (40x) (H and E stain). **B.** Histological tissue of mice (liver) infected with *Listeria* spp. showed granular degeneration of hepatocytes, focal areas of mononuclear cell infiltration in periportal area and necrosis of focal area (20x) (H and E stain). **C.** Histological tissue of mice (liver) infected with *Listeria* spp. showed granular degeneration of hepatocytes, focal areas of mononuclear cell infiltration in periportal area and necrosis of focal area (20x) (H and E stain).

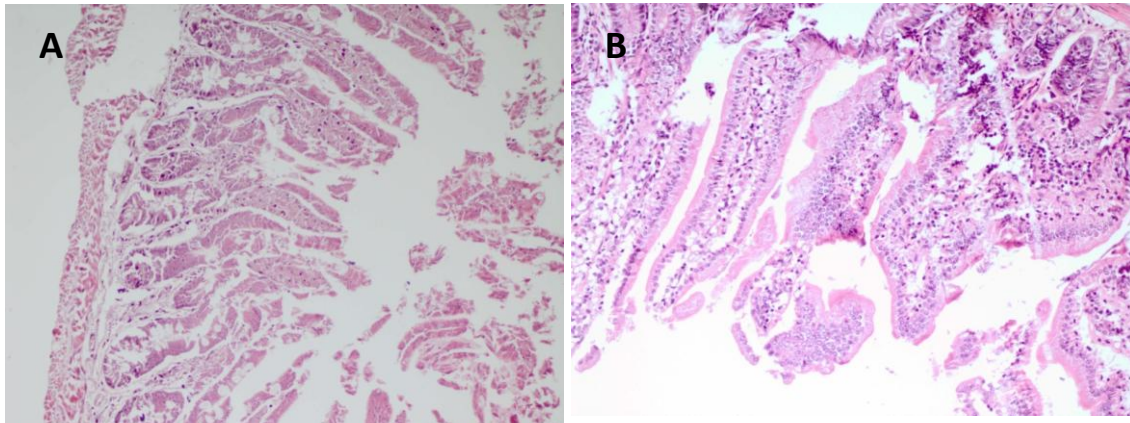


Figure 6. **A.** Histological tissue of mice (intestine) infected with *Listeria* spp. showed sloughing of mucosal lining epithelium, infiltration of mononuclear cell in lamina propria and hypertrophy of villi (20x) (H and E stain). **B.** Histological tissue of mice (intestine) infected with *Listeria* spp. showed hypertrophy of villi (20x) (H and E stain).

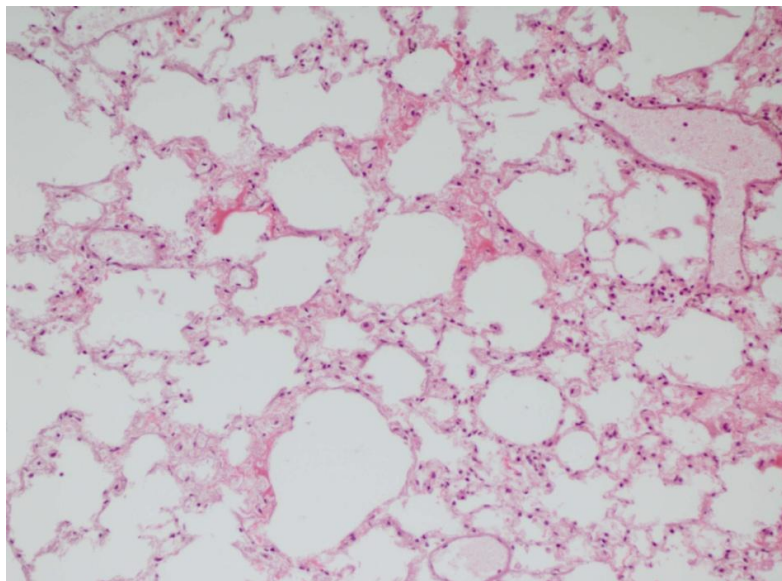


Figure 7. Histological tissue of mice (lungs) infected with *Listeria* spp. showed oedema, haemorrhage and congestion (20x) (H and E stain).

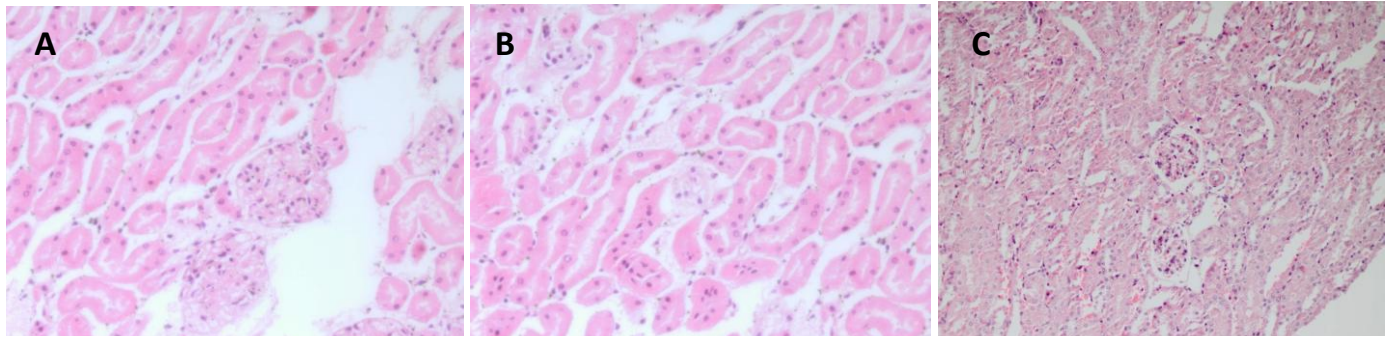


Figure 8. **A.** Histological tissue of mice (kidney) infected with *Listeria* spp. showed increase in cellularity in the glomeruli, decrease in urinary space and glomeruli swollen (40x) (H and E stain). **B.** Histological tissue of mice (kidney) infected with *Listeria* spp. showed coagulated type of necrosis (40x) (H and E stain). **C.** Histological tissue of mice (kidney) infected with *Listeria* spp. showed mild granular degeneration of tubular epithelium, increase in cellularity in interstitium and glomeruli.(20x) (H and E stain).

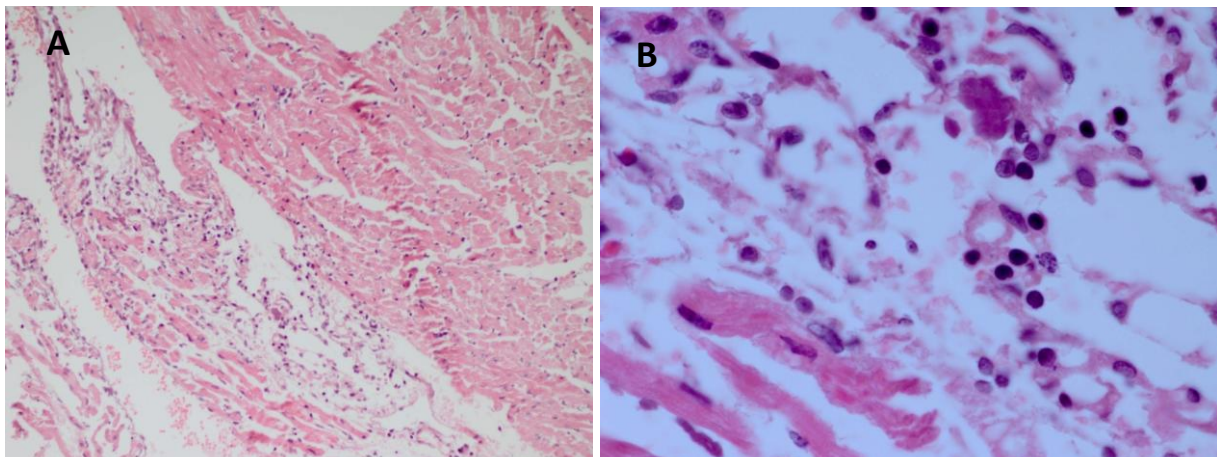


Figure 9. **A.** Histological tissue of mice (Heart) infected with *Listeria* spp. showed bacterial colonies in myocardium, focal infiltration of mononuclear cells, degeneration and necrosis of myocardium, valvular endocarditis and congestion (20x) (H and E stain). **B.** Histological tissue of mice heart infected with *Listeria* spp. showed revealed bacterial colonies in heart (100x) (H and E stain).

heart (Figure 9).

Moshtaghi et al. (2006) also reported pathological changes in the liver, spleen, brain, kidneys and intestine. Histopathological examination of the liver revealed congestion and pyogranulomas consisting of necrosed hepatic cells, macrophages and neutrophils. In kidneys, histopathological changes were characterized by the presence of focal interstitial nephritis, focal areas of neutrophils and lymphocytes in interstitial tissue and atrophy of tubules. Similarly, Fowler et al. (1988) compared virulence of the five species of *Listeria* spp. in C57BL/6 and BALB/c mice in terms of histological changes in mouse livers. *L. seeligeri* AB2 produced mild PMN infiltration, *L. monocytogenes* SLCC 2540 produced micro-abscess and hepatocyte degeneration, *L. ivanovii* W 5379 produced micro-abscess and hepatocyte degeneration and *L. ivanovii* W 5379 produced residual granuloma in the liver.

The incubation period for listeriosis is variable, and ranges from three to 70 days, with the median incubation

period being three weeks. It can be observed from this work that the strain of *Listeria* spp. isolated from drinking water has the same incubation period in the laboratory animals studied (12 to 22 days). This could be due to their near similar physiologic disposition as in man.

Conclusion

This is the first study conducted in Ludhiana city, Punjab, India showing the occurrence of *Listeria* spp. in 52.63% of drinking water samples. Discrepancies have been observed in terms of the occurrence of faecal indicators and emerging pathogens. The use of faecal indicator (*E. coli*) raises questions regarding their reliability in assessing the bacteriological quality of water, particularly because of their poor correlation with pathogenic microorganisms. Update and new culture methods should be included in standard methods for monitoring, sampling and analysis of these emerging pathogens. Ensuring

the appropriate level of chlorination can guarantee the water quality.

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