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Epidemiological Surveillance of Drinking Water in Ludhiana

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Abstract

The coliform group of bacteria has remained the corner stone of national drinking water regulation. Epidemiological surveillance of 180 samples in Ludhiana city included treated source water, raw water and treated piped water. A total of 119 (66%) of samples were non-potable. Pathogens isolated from non-potable water samples were *E. coli* (80%), *Enterobacter* (85%), *Pseudomonas* (40%), *Klebsiella pneumoniae* (80%), *Streptococcus faecalis* (70%), *Aeromonas hydrophila* (80%), *Yersinia enterocolitica* (40%) and *Proteus* (80%). *Aeromonas* spp. was the most prevalent bacterial species in chlorinated distribution water (10.6%) and the most common species in raw water (10.5%). The piped water tested potable by conventional indicator technique was positive for emerging pathogens so researchers have focused on safe drinking water regulation amendment. The emerging and environmental contaminants isolates were *Aeromonas hydrophila, Yersinia enterocolitica Proteus mirabilis* and *Pseudomonas.* These contaminants capable of growth in low nutrient condition (similar to water distribution system) should be proposed as indicators of distribution system integrity. The occurrence suggests of inadequate chlorination and potential biofilm formation in distribution pipes.

Key words : Drinking water, Coliforms, Non-potable, Pathogens, Distribution system.

Drinking water quality is of vital concern to mankind, since it is ranked as food and high standards are set for quality and safety. Many developing regions suffer from either chronic shortages of freshwater or the pollution of readily accessible water resources (1). Fecal pollution of drinking water causes waterborne diseases like typhoid, enteric fever, gastroenterities, dysentery, meningitis, hepatitis, shigellosis, cholera, diarrhoea, entamoebiasis and giardiasis. In developing countries each year, 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. Water quality is commonly measured by indicators such as heterotrophic plate count (HPC), total coliform count (TCC), fecal coliform count (FCC) and *E. coli*, based on their presence, concentration, sensitivity to disinfection, relative survival in water when compared to other waterborne pathogens of fecal origin and the efficiency of their detection processes. Each indicator has notable limitations given that they are proxy measures of water quality and to minimize the indicator-specific limitations in water quality monitoring, it is often advisable to use multiple indicators at a time. This is particularly important in view of reports that have suggested that the absence of an indicator organism does not mean the absence of other pathogenic organisms (2). In the recent year new or emerging pathogens like *Campylobacter jejuni, E. coli* 0157:H7, *Yersinia enterocolitica, Aeromonas* spp., *Mycobacterium* spp., *Pseudomonas aeruginosa, Proteus mirabilis,* new enteric viruses and microsporidia have arisen as a problem in drinking water production and distribution (3). Beyond microbial measurements of water quality, safe water also has physico-chemical qualities such as pH, salinity, hardness, dissolved oxygen and phosphates. These qualities are readily affected by climatic events and impact on the survival of microoganisms and efficiency of treatment processes. Therefore, the primary goal of water quality management from health perspective is to ensure that consumers are not exposed to pathogens that cause disease. Protection of water sources and treatment of water supplies have greatly reduced the incidence of these diseases in developed countries. Therefore, microbial analysis of the source water is necessary for risk assessment and risk management.

Methods

Water samples were collected from hand pumps,

Table 1. Microbiological survey of water samples for potability supplied by Municipal Corporation of Ludhiana district.

Source	No. of samples analyzed	Quality of water		Percentage of potable samples
		Potable	Not potable	
Haibowal	32	Nil	32	Nil
Bhai Randhir Singh Nagar	24	17	7	70.8
Raj Guru Nagar	20	14	6	70.0
Sarabha Nagar	21	15	6	71.4
Punjab Mata Nagar	24	5	19	20.8
Urban Vihar	28	4	24	14.3
Tajpur Road	16	3	13	18.7
Maharaj Nagar	15	3	12	20.0
Total	180	61	119	33.8

tubewells/submersible pumps and the finished drinking water of Municipal Corporation supply lines of Ludhiana city of Punjab. Water samples were collected in 4-liter pre-sterilized glass bottles and placed on ice stored in and transported to laboratory within 3 h after collection and analyzes were completed within 7 h after collection by APHA (4). Residual chlorine concentration was estimated using HiMedia Testing kit and sodium thiosulfate was added to neutralize any chlorine residual in the drinking water samples. Total coliforms were enumerated by most-probable-number (MPN) technique according to standard methods of APHA (4). Coliforms recovered by the MPN technique were carried through the completed test. HPC bacterial density was also enumerated by the pour plate technique. Fecal coliforms were enumerated on m-FC medium. *E. coli* was enumerated on EMB agar, *Klebsiella* on MacConkey agar-inositol-cabenicillin agar, *Enterobacter* and *Proteus* on UTI differential agar, *Salmonella* and *Shigella* on Raj-Hans Salmonella-Shigella (SS) agar, *Vibrio* on TCBS agar, *Aeromonas* on m-Aeromonas medium, *Citrobacter* on Simmons citrare agar, *Pseudomonas* on Pseudomonas agar, *Clostridium* on Robertson-cooked meat medium and *Staphylococcus aureus* on Baird parker agar. The plates were incubated at 37 C for 48—96 h. Preliminary identification was attempted using classical technique including physico-chemical and biochemical tests. All isolates were phenotypically identified. Acid production from carbohydrates was tested under anaerobic conditions with peptone water and phenol red (0.0018 g/liter) containing sugar at final concentration of 1%. Citrate utilization was determined in tubes of simmons' citrate agar, H_2S production was assayed in tubes of triple sugar iron agar medium (TSI, Difico). KB001 : HiMViC™ Biochemical kit and KB009 HiCarbohydrate™ kits (HiMedia) were used along with conventional tube or plate assay methods to prepare the biochemical profile of isolates.

Table 2. Enumeration on non-potable water samples of Ludhiana district by MPN index.

Source	MPN index/ 100 ml
Haibowal	>1500
Bhai Randhir Singh Nagar	3
Raj Guru Nagar	3
Sarabha Nagar	3
Punjab Mata Nagar	>1500
Urban Vihar	>1500
Tajpur Road	>1500
Maharaj Nagar	1100

Results and Discussion

Surveillance Study of Water Supply

The magnitude of human morbidity and mortality associated with waterborne infections diseases has led to the development of epidemiological surveillance studies. Microbiological analysis of 180 water samples in winter and summer season in different localities and suburban areas of Ludhiana were conducted for a period of 1 year (Table 1). Moreover, the source e.g. hand pumps, tubewells/submersible pumps and Municipal Corporation supply lines and location of drinking water resources also influence the microbiological quality of water. In this study, we included the indicators of pollution such as total and thermo-tolerant coliforms to determine and ascertain the hygienic quality of water sources in plumbing lines of urban and rural communities of Ludhiana.

Municipal Corporation Supply Lines

The monitoring of microbiological water safety was analyzed for limited number of well choosen parameters instead of screening for all the possible pathogens. A total number of 180 drinking water samples were examined out of which, 61 samples were potable (34%) and 119 were non-potable (66%). None

Table 3. Identification of HPC bacteria in distribution water, raw water and distribution water during a chlorine failure. *Number of strains identified for the entire period of 1 year.

Organism	Distribution water		Raw water		Distribution water during chlorine failure	
	*Total	Percent of total	Total	Percent of total	Total	Percent of total
Escherichia coli	4	3.8	6	6.3	2	6.2
Klebsiella paneumoniae	5	4.8	4	4.2	1	3.1
Enterococcus faecalis	3	2.8	5	5.2	2	6.2
Citrobacter freundii	4	3.8	6	6.3	2	6.2
Enterobacter	10	9.6	8	8.4	3	9.4
Pseudomonas aeruginosa	6	5.7	8	8.4	3	9.4
Salmonella enteritidis	5	4.8	3	3.1	0	0
Shigella flexneril	7	6.7	7	7.3	0	0
Aeromonas hydrophila	11	10.6	10	10.5	6	18.7
Yersinia enterocolitica	5	4.8	8	8.4	4	12.5
Proteus mirabilis	6	5.7	6	6.3	5	15.6
Vibrio parahaemolyticus	4	3.8	7	7.3	0	0
Clostridiun perfringens	1	0.96	1	1.0	0	0
Staphylococcus aureus	3	2.8	4	4.2	0	0
Unidentified	30	28.8	12	12.6	4	12.5
Total	104	99.5	95	99.5	32	99.8

of the samples from Haibowal and Tajpur was potable, followed by Urban Vihar (14.3%) and Punjab Mata Nagar (20.8%). A sewage effluent brooke named Buddha Nala flows through Haibowal and due to presence of slums their prevail unhygienic conditions, overcrowding, improper disposal of sewage and water, low sanitary conditions which has resulted in continuous endemic in this area, resulting in maximum non-potable samples. In Punjab Mata Nagar, inadequately laid plumbing lines, crossing over of sewage pipes with fresh water supply, non-chlorination of water reservoir and attitude of authorities were responsible for non-potable samples. The incidence of the resulting diseases increased during monsoon season because of land surface flooding and surface run off. People residing in these areas besides throwing garbage and litter on the roads, had taken illegal water and sewerage connections. As a result of wrong alignment of water pipeline, sewerage water starts intermixing with the drinking water (5).

The highest percentage of potable sample was reported from Bhai Randhir Singh Nagar (70.8%), Raj Guru Nagar (70.0%) and Sarabha nagar (71.4%). The MPN index/100 ml of different area like Haibowal, Tajpur Road, Maharaj Nagar and urban Vihar was >1500 coliforms/100 ml, Raj Guru Nagar, Bhai Randhir Singh Nagar, Sarabha Nagar MPN index/100 ml was <3 coliforms/100 ml (Table 2). Pathogens isolated from non-potable water samples were *E. coli* (80%), *Enterobacter* (85%), *Pseudomonas* (40%), *Klebsiella pneumoniae* (80%), *Streptococcus faecalis* (70%), *Aeromonas hydrophila* (80%), *Yersinia enterocolitica* (40%) and *Proteus* (80%).

HPC in Piped Potable Water Supply

The control of the aesthetic quality of potable water has also been attributed to the control of HPC bacteria in distribution lines. Turbidity, heterotrophic plate count and total coliform count indicated general microbial water quality, whereas *E. coli* indicated fecal pollution. Large densities of HPC bacteria have been reported to interfere with the detection of coliforms. Enumeration of HPC bacteria was a useful tool to indicate the presence of opportunistic pathogens, the potential for coliform suppression and drinking water quality deterioration in a distribution system. Identification of HPC bacteria in potable drinking water permitted an assessment of the bacterial diversity present, whereas enumeration of HPC bacteria provided a threshold evaluation of the water quality in a distribution system (6). Elevated HPC levels indicated a potential health risk posed by opportunistic pathogens. Increased HPC levels indicated

Table 4. Incidence of emerging pathogens in piped potable water supply of Ludhiana district.

Source	Percent of potable sample	Organism	Percent of isolates
Distribution water	23.5	*Aeromonas hydrophila*	81.0
		Yersinia enterocolitica	60.0
		Proteus mirabilis	84.0
Raw water	15.7	*Aeromonas hydrophila*	83.0
		Yersinia enterocolitica	55.0
		Proteus mirabilis	75.0
Distribution water during chlorine failure	28.8	*Aeromonas hydrophila*	75.0
		Yersinia enterocolitica	64.0
		Proteus mirabilis	82.0

deterioration of general water quality and inadequate disinfection. Several trends were apparent in the seasonal distribution and species diversity of the HPC population present in the distribution water. As is evident from results (Table 3), there was greater species diversity in the warmer summer period and in the fall after the first major precipitation than during the cold winter months. Of the 14 identifiable bacterial groups, an average of seven were detected in drinking water during November, December, January and March compared with an average of 12 groups detected in June, July, September and October. Some species and strains of *Pseudomonas, Flavobacterium, Proteus, Bacillus,* Actinomycetes and yeasts have been shown to suppress coliform detection. The significance of coliform suppression in municipal and rural water supplies has been reported (7) and it has been speculated that suppression can atleast partly be attributed to elevated HPC numbers. Pathogen occurrences have also been noted in the absence of detectable coliforms in samples with high HPC numbers. High levels of coliform counts indicate a contaminated source, inadequate treatment or post treatment deficiencies.

Representative genera of *E. coli, Klebsiella pneumoniae, Enterococcus faecalis, Citrobacter freundii, Enterobacter, Aeromonas hydrophila, Pseudomonas aeruginosa, Salmonella enteritidis, Shigella flexnerii, Vibrio parahaemolyticus* and *Clostridium perfringens* were isolated from drinking water distribution system (Table 3); where they appear to form biofilms and by sloughing of cell aggregates from the treatment filters or pipe walls coliform bacteria survive, thrive and are continuously dispersed off in drinking water supplies. Adhesion of biofilm coliforms occur mostly at the hydrophobic region of the hydrophilic-hydrophobic interface of distribution pipelines which resulted in sufficient growth and agglomeration into an organized structure (8). The growth of environmental and clinical coliform bacteria under conditions typical of drinking water distribution system reported that *E. coli* made uo to 80% of the total coliforms. The distribution network is susceptible to contaminate water supply as coliforms which have been isolated are well adapted as biofilms in potable water distribution systems. The surface of abraded pipeline with repeated use increased their ability to entrap bacteria which creates a harborage for bacterial growth and protection from cleaning and chlorination as capsulated bacteria are resistant to chlorine (9).

Aeromonas spp. was the most prevalent bacterial species in chlorinated distribution water (10.6%) and the most common species in raw water (10.5%) (Table 3). Opportunistic pathogens such as *Pseudomonas* spp. comprised some 15.3% of the bacterial population in distribution water. The changes in the HPC population in the distribution system were therefore not a simple function of bacterial quality in the run water. It can be hypothesized that interaction of water temperature, fall precipitation and disinfection efficiency all influenced the species diversity in drinking water derived from surface sources which are only chlorinated and not filtered.

Incidence of Emerging Pathogens in Piped Potable Water Supply

There is no regulation governing the presence

of *Aeromonas hydrophila, Yersinia enterocolitica* and *Proteus mirabilis* in India. Drinking water is not tested for these organisms directly, instead *E. coli* is used as an indicator of their presence. The piped water tested potable by conventional indicator technique were positive for emerging pathogens so researchers had to focus on safe drinking water regulation amendment. A total of 81% of potable water sample were positive for *Aeromonas hydrophila* in distribution water, 83% in raw water intake to the distribution system and 75% in distribution water during chlorine failure (Table 4).

Aeromonas is a causative agent of gastroenteritis and ubiquitous in water including chlorinated water (10). It can survive standard chlorination and thus recolonize the water distribution networks after the chlorination process (11). *A. hydrophila* is known to have a widespread distribution, being able to inhabit a variety of aquatic habitats. The presence of *A. hydrophila* in drinking water need public health appraisal and that further work should be undertaken to permit reevaluation of standards for the quality of drinking water. In water distribution system, *E. coli, Aeromonas* and *Pseudomonas aeruginosa* are able to proliferate at low temperature 4—10 C and in thermotolerant coliforms, *E. coli, Klebsiella pneumonia* and *Citrobacter freundii* have been detected in both chlorinated and non-chlorinated water supply and at higher temperature >40 C.

Since the present drinking water quality is based on the absence of coliforms, the opportunistic pathogens and secondary invaders comprising the HPC go unrecognized even though their numbers oftenfreatly exceed those of the coliform group. Elevated heterotophic plate count (HPC) count, total coliform count and thermotolerant coliform count were found throughout the distribution system regardless of water source, seasonal variation, chlorination / treatment in Municipal Croporation supply indicate the overall microbiological status of the system and not necessarily the possibility of risk to public health.

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