Biomonitoring of indicator and emerging pathogens in piped 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 110 samples in Ludhiana city by IS-10500-1991 BIS and Bacteriological water tesing kit included treated source water, treated piped water and treated piped filtered water. A total of 73 (66%) of samples were bacteriological non-potable. The piped water tested bacteriologically potable by conventional indicator technique IS-10500-1991 were reported positive for presence of emerging pathogens by Bacteriological water tesing kit, so researchers have focused on safe drinking water regulation amendment. The emerging and environmental contaminants isolates phenotypically characterized and molecular characterized were *Aeromonas hydrophila*, *Yersinia enterocolitica* , only phenotypically characterized were *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 is suggestive of inadequate chlorination and potential biofilm formation in pipes. [Report and Opinion. 2010;2(1):14-21]. (ISSN: 1553-9873).

Key words: coliform; non potable; emerging pathogens; standards of drinking water

INTRODUCTION

Water borne diseases affect one third of World's population. 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 3 million people in developing regions of the world die each year, primarily children aged <5 years (WHO 2006). Water demand at present exceeds the available renewable water [1]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. There is need to upgrade our laboratories to international standards to support surveillance, monitoring, and curbing the spread of infectious agent in general and exotic zoonotic infections in particular.

In the recent year new or emerging pathogens have arisen as problem in drinking water production and distribution. These include newly recognized pathogens from faecal sources like *Campylobacter jejuni*, pathogenic *Escherichia coli*, *Yersinia enterocolitica*, new enteric viruses and microsporidia. Some new environmental pathogenic species of bacteria able to grow in water distribution systems, as *Aeromonas spp*., *Mycobacterium* sp and *Pseudomonas aeruginosa*, *Proteus mirabilis* (Szewzyk et al 2000). Most pathogens in drinking water are generally faecal in origin (Ashbolt, 2004).

Drinking water monitoring based on test for coliform bacteria as indicator of faecal contamination originated 100 years ago, but today water is treated and piped through elaborate distribution system. Monitoring water for indicator and emerging pathogens is very important for protecting the public health. Environmental pathogenic contaminant *Pseudomonas* spp., *Proteus mirabilis*, *Aeromonas* spp are capable of growth in low nutrient conditions similar to water distribution system, should be proposed as indicators of distribution system integrity. The occurrence in water suggests inadequate chlorination and potential biofilm formation. All indicators must be monitored frequently for appraisal of the distribution system network integrity. The present investigation was carried out with following objective of epidemiological surveillance studies for the evaluation of quality of treated, piped and membrane filtered water for indicator and emerging pathogens.

MATERIALS AND METHODS

Collection of water sample: Water samples were collected from Municipal Corporation supply lines of Ludhiana city of Punjab. These were stored in presterilized bottles and transported to laboratory and analyzed immediately. The samples of drinking water were tested by IS-10500-1991 BIS and Bacteriological water tesing kit in months of July and August.

Culture conditions and characteristics of the bacteria: Bacteriological potable and non-potable water sample after pre enrichment in selective broth

were streaked on differential media (EMB, MacConkey, UTI differential agar, CIN, TSI ,Baird parker agar and DCA).The plates were incubated at 37 ^{o} C for 48-96 hrs. Preliminary identification was attempted using classical technique including physiochemical and biochemical tests. Oxidase and catalase production were tested using oxidase discs respectively. Acid production from carbohydrates was tested under anaerobic conditions with peptone water and phenol red (0.0018g/L) 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.

Elemental analysis of water samples: Elemental analysis of water samples was done using ICAP-AES, PAU, Ludhiana .

RESULTS AND DISCUSSION

SURVEILLANCE STUDY OF WATER SUPPLY

The magnitude of human morbidity and mortality associated with waterborne infectious diseases has led to the development of epidemiological surveillance studies. The presence of any indicator (total or feacal coliforms) in finished water suggests break in treatment barriers, indicating risk to public health.Biofilms are formed in water distribution system, in inner walls of pipes and taps which consists primarily of viable and nonviable microorganisms embedded in polyanionic extracellular polymeric substance anchored to a surface

Municipal Corporation Supply

The monitoring of microbiological water safety is analyzed for limited number of well chosen parameters instead of screening for all the possible pathogens.

Microbiological analysis of water in different localities of Ludhiana was conducted for a period of 1 year (Table 1).A total number of 110 drinking water samples were examined out of which, 37 samples were bacteriological potable (34%) and 73 were non potable (66%). None of the samples from Haibowal and Taj Pur was potable, followed Urban Vihar (9) and Punjab Mata Nagar (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 results in continuous endemic in this area, resulting in maximum non potable samples. In Punjab Mata Nagar, crossing over of sewage pipes with fresh water supply, non chlorination of water reservoir and lackadaisical attitude of Municipal Corporation authorities were responsible for non potable samples. The incidence of the gastroenteritis 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 wrong alignment and inadequately laid plumbing lines results in inter mixing of seepage of sewerage ..

The highest percentage of potable sample were reported from posh area,Bhai Randhir Singh Nagar (70%) Raj Guru Nagar (70%) and Sarabha nagar (70%). The MPN index/100ml of undeveloped colonies like Haibowal , Taj pur Road, Maharaj Nagar and Urban Vihar was >1500 coliforms/100ml,where as in developed colonies, Raj Guru Nagar, Bhai Randhir Singh Nagar ,Sarabha Nagar MPN index/100ml was <500 coliforms/100ml (Table 2). Eight different types of pathogenic bacteria were isolated from 37 bacteriologically non-potable water samples supplied by Municipal Corporation. *E. coli* was isolated from 80 % of samples, *Enterobacter* 85% samples, *Pseudomonas* 40% samples, *Klebsiella* 80% samples, *Streptococcus faecalis* 70% samples and *Aeromonas* 80% samples, *Yersinia* 40%and *Proteus* 80% samples (Fig. 1).

Membrane Filters

Water samples from 40 filters were drawn and tested for bacteriological potability (Table3). A total of 80% of samples were non-potable after 96 hrs of incubation. Indicator bacteria in filtered water exposed to numerous physical and chemical factors are injured so require prolonged incubation period of 96 hrs. These injured and stressed bacteria fail to grow under selective condition commonly used for their detection.

Incidence of emerging pathogen 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 . The piped water tested potable by conventional indicator technique were positive for emerging pathogens so researchers have to focus on safe drinking water regulation amendment. A total of 81% of bacteriologicaly potable water sample were positive for *Aeromonas hydrophila*, 60% were positive for *Yersinia enterocolitica* and 84% were positive for *Proteus mirabilis* (Table4).

Aeromonas hydrophila

Aeromonas is a causative agent of gastroenteritis and ubiquitous in water including chlorinated water (Gavriel et al 1998). Some strains of *Aeromonas* isolated from water possess virulence traits, such as adhesions,hemolysins and cytotoxic enterotoxins presumably involved with human pathogenicity (Schubert, 2000). All the isolates morphologically and biochemically and molecularly characterized were identical and showed the typical characteristics of *Aeromonas hydrophila*. Gram negative ,motile, occurring in pairs ,colonies on TSA agar plates were creamy-white, circular and convex with an entire margin, developing with in 48 hrs at 22 ^{o} .All the isolates were catalase and oxidase positive, carbohydrate utilized include arabinose ,saccharose , trehalose and glucose. Carbohydrates like xylose , melibiose , adonitol rhamnose ,cellobiose ,raffinose were not utilized (Table 5).

Aeromonas can survive standard chlorination and thus recolonized in the water distribution networks after the chlorination process (Van der Kooij ,1991). *Aeromonas hydrophila* (Amjab et al 2006) inhibited the growth of *Staphylococcus aureus, E. coli ,Klebsiella pneumoniae and Candida albicans*. The presence of A. *hydrophila* in drinking water needs strong public health appraisal and further work be undertaken to reevaluate the standards for the quality of drinking water.

Yersinia enterocolitica

Yersinia enterocolitica is emerging enteric pathogen responsible for a wide spectrum of clinical manifestation including acute gastroenteritis (Okwori et al 2007).Out of 37 potable water sample screened for *Y. enterocolitica* 22 (60%) were positive for *Y. enterocolitica*. Water samples were streaked on Cefsulodin-irgasan-novobiocin (CIN) incubated at 25^{o}C, after 18-24 hrs of incubation presumptive *Y. enterocolitica* produce characteristic bull eye morphology with deep red center and clear colourless periphery (Table 5). All the isolates showed oxidase negative and catalase positive. Indole and Methyl red positive, Voges Proskauer's negative. All the isolates had ornithine decarboxylase activity but no lysine decarboxylase activity. H_2S was not produced by most of the isolates. Carbohydrates utilized were arabinose, xylose, saccharose, trehalose, cellobiose and glucose.

Proteus mirabilis

A total of 84% of potable water sample, were positive for *P.mirabilis*. All isolates were subjected to biochemical characterization by using HiMedia biochemical kit (Table 5). Isolates were oxidase negative, catalase and methyl red positive. Voges proskauer's and indole negative , capable of utilizing citrate as a sole carbon source , show lysine decaroxylase and ornithine decaroxylase activity. The isolates were able to utilize malonate and carbohydrates utilized include xylose, saccharose, trehalose and glucose. *Proteus mirabilis* after initial colonization ascends the ureters and initiates an interaction with epithelial cells of the renal pelvis, which allows colonization of the kidney leading to pylonephritis and ascending urinary tract infection.

ELEMENTAL ANALYSIS OF WATER SAMPLES

The elements analyzed are required by human body in trace amounts for carrying out metabolic pathways and by coenzymes and are supplied in water and food. Heavy metals even at low concentrations can cause toxicity to human and other forms of life. The toxicity of heavy metal ion is owing to their ability to bind with protein molecules and prevent replication of DNA and thus subsequent cell division

To avoid health hazards it is essential that these metal should be present within the permissible limits as recommend by BIS or WHO. The results depict that the concentration of all the 20 elements were below the permissible limits in all the samples studied (Table 13). In The essential elements AI, Na, Ca, B, Fe, K, Mg and S were present below the permissible limits in all the samples. Elements like Fe and Mn causes corrosion or encrustation of pipeline walls or clog the pipelines resulting in accumulation of organic matter and biofilm formation.

Table 1. Bacteriological* analysis of Municipal Corporation water samples

Sr. no.	Source	Number of samples analyzed	Quality of water	
	Municipal corporation		Potable	Non-potable
1	Dugri	10	1	9
2	Haibowal	10	0	10
3	Model town	10	4	6
4	Bhai Randhir Singh Nagar	10	7	3
5	Karnail Singh Nagar	10	5	5
6	Punjab Mata Nagar	10	2	8
7	Sarabha Nagar	10	7	3
8	Raj Guru Nagar	10	7	3
9	Urban Vihar	10	1	9
10	Tarapur Road	10	0	10
11	Maharaj Nagar	10	3	7
		110	37	73

*IS-10500-1991 BIS and Bacteriological water tesing kit

Table 2. Enumeration of coliforms by MPN* method at 37° C

MPN index/100ml	Area
<500	Raj Guru Nagar Bhai Randhir Singh Nagar Sarabha Nagar
500-1000	Karnail Singh Nagar Punjab Mata Nagar
1000-1500	Model Town Dugri Haibowal
>1500	Tarapur Road Maharaj Nagar Urban Vihar

*IS-10500-1991 BIS

Table 3. Microbiological* survey of piped tap water and filtered water samples

Sr. no.	Source	% of non-potable sample	Quality of water
1	Membrane filter	80%	Non-potable after 96 hrs of incubation
2	Tap water	100%	Non-potable after 48 hrs of incubation

*IS-10500-1991 BIS and Bacteriological water tesing kit

Table 4. Incidence of emerging pathogens* in potable piped water sample

Emerging microorganisms	Presence in potable water sample
Aeromonas hydrophila	81%
Yersinia enterocolitica	60%
Proteus mirabilis	84%

* Bacteriological water tesing kit

Table 5. Biochemical characteristics of emerging pathogens

Biochemical properties	*Aeromonas hydrophila*	*Yersinia enterocolitica*	*Proteus mirabilis*
ONPG	+	+	-
Lysine	+	-	-
Ornithine	-	+	+
Urease	-	+	+
TDA	-	-	+
Nitrate	+	+	+
H2S	-	-	+
Citrate utilization	-	-	+
Voges Proskauer's	+	-	-
Methyl red	+	+	+
Indole	+	+	-
Malonate	-	-	-
Esculin hydrolysis	+	-	-
Arabinose	+	+	-
Xylose	-	+	+
Adonitol	-	-	-
Rhamnose	-	-	-
Cellobiose	-	+	-
Melibiose	-	-	-
Saccharose	+	+	+
Raffinose	-	-	-
Trehalose	+	+	+
Glucose	+	+	+
Lactose	-	-	-
Oxidase	+	-	-

Table 6(a). ELEMENTAL ANALYSIS OF WATER SAMPLES

Sr.no	B PPM (1)	Ca PPM (75)	Cu PPM (0.05)	Fe PPM (0.3)	K PPM
1.	0.125	17.17	0.003	0.048	2.38
2.	0.140	66.58	0.004	nd	1.36
3.	0.182	40.49	0.008	nd	7.13
4.	0.284	83.42	0.040	0.011	5.19
5.	0.110	75.74	0.005	nd	6.47
6.	0.257	71.45	0.006	nd	4.43
7.	0.081	64.61	0.008	0.045	3.93
8.	0.318	75.34	0.028	0.127	5.66
9.	0.123	102.82	0.047	0.016	4.04
10.	0.088	74.50	0.015	0.017	3.82
13.	0.144	35.04	0.004	nd	4.55
11.	0.080	89.09	0.059	0.014	1.12
14.	0.050	74.91	0.011	0.035	6.30
12.	0.061	78.45	0.006	nd	1.77

15.	**0.123**	**47.73**	**0.002**	**0.004**	**6.48**
16.	**0.202**	**55.42**	**0.004**	**0.082**	**8.04**
17.	**0.100**	**55.02**	**0.069**	**0.002**	**2.83**
18.	**0.184**	**37.60**	**0.003**	**nd**	**3.18**
19.	**0.118**	**30.16**	**0.009**	**0.014**	**2.17**
20.	**0.223**	**42.78**	**0.002**	**nd**	**3.18**
21.	**0.306**	**64.32**	**0.013**	**0.155**	**8.20**
22.	**0.256**	**51.00**	**0.002**	**0.042**	**9.20**
23.	**0.492**	**56.35**	**0.008**	**0.080**	**7.66**
24.	**0.277**	**56.16**	**0.013**	**0.115**	**7.07**

Table 6(b). ELEMENTAL ANALYSIS OF WATER SAMPLES

Address	**B PPM (1)**	**Ca PPM (75)**	**Cu PPM (0.05)**	**Fe PPM (0.3)**	**K PPM**
1	0.125	17.17	0.003	0.048	2.38
2	0.140	66.58	0.004	nd	1.36
3	0.182	40.49	0.008	nd	7.13
4	0.284	**83.42**	0.040	0.011	5.19
5	0.110	75.74	0.005	nd	6.47
6	0.257	71.45	0.006	nd	4.43
7	0.081	64.61	0.008	0.045	3.93
8	0.318	75.34	0.028	0.127	5.66
9	0.123	**102.82**	0.047	0.016	4.04
10	0.088	74.50	0.015	0.017	3.82
11	0.080	**89.09**	0.059	0.014	1.12
12	0.061	78.45	0.006	nd	1.77
13	0.144	35.04	0.004	nd	4.55
14	0.050	74.91	0.011	0.035	6.30
15	0.123	47.73	0.002	0.004	6.48
16	0.202	55.42	0.004	0.082	8.04
17	0.100	55.02	0.069	0.002	2.83
18	0.184	37.60	0.003	nd	3.18
19	0.118	30.16	0.009	0.014	2.17
20	0.223	42.78	0.002	nd	3.18
21	0.306	64.32	0.013	0.155	8.20
22	0.256	51.00	0.002	0.042	9.20
23	0.492	56.35	0.008	0.080	7.66
24	0.277	56.16	0.013	0.115	7.07

Table 6(c). ELEMENTAL ANALYSIS OF WATER SAMPLES

Address	**Mg PPM (30)**	**Mn PPM (0.05)**	**P PPM**	**S PPM**	**Zn PPM (5)**	**Result potable)**	**(Potable/not**
1	16.73	0.011	0.073	3.24	0.019	Potable	
2	29.64	0.003	0.070	11.66	0.013	Potable	
3	**31.93**	0.079	0.073	5.12	0.146	Potable	

4	**31.22**	0.014	0.066	10.40	0.559	Potable
5	27.11	0.155	0.077	6.99	0.876	Not Potable
6	27.73	0.002	0.059	9.08	0.143	Not Potable
7	23.94	0.031	0.070	3.37	0.286	Not Potable
8	**35.13**	0.133	0.077	4.49	2.301	Potable
9	25.05	0.032	0.056	15.75	0.452	Potable
10	19.40	0.234	0.063	20.97	2.090	Potable
11	14.30	0.84	0.073	8.79	0.640	Potable
12	11.03	0.296	0.045	11.35	0.120	Potable
13	17.65	0.006	0.087	5.61	0.110	Not Potable
14	15.97	0.259	0.052	11.29	0.464	Potable
15	14.12	0.005	0.052	7.18	0.040	Potable
16	23.64	0.138	0.049	5.74	0.077	Not Potable
17	24.08	0.008	0.063	2.23	1.196	Potable
18	20.84	0.012	0.035	0.77	0.043	Potable
19	7.37	0.034	0.042	6.73	0.261	Potable
20	21.80	0.010	0.045	2.16	0.428	Potable
21	**36.99**	0.115	0.066	31.10	0.563	Potable
22	37.98	0.035	0.073	44.07	0.591	Potable
23	28.83	0.076	0.066	44.15	0.595	Potable
24	24.30	0.092	0.066	6.76	0.421	Potable

CONCLUSION

1) The assessment of coliform and faecal indicator bacteria has significant limitation with regard to emerging microbial bacteria in potable water supply with respect to public health.
2) Occurrence of coliform and emerging pathogens in finished water in the absence of known breaches of treatment, or barrier continue to be a major problem in distribution pipes and drinking water industry.
3) To accurately assess and manage risks from waterborne diseases, it is necessary to understand distribution of pathogens, impact of plumbing lines, disinfectants and survival strategies of pathogens with in water
4) There is need to improve out moded risk assessment protocols improve dissemination of information on water testing so as to coordinate surveillance system for the early detection, tracking and evaluation of the emerging waterborne pathogens.

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