Journal of Applied and Natural Science 3 (2): 298-302 (2011)

JANS

Seasonal variation in bacterial contamination of water sources with antibiotic resistant faecal coliforms in relation to pollution

Kshama Tripathi and A. K. Sharma*

Department of Zoology, University of Lucknow, Lucknow-226007, INDIA

*Corresponding author. E-mail: sharma_ajayk@lkouniv.ac.in

Abstract: Water sample were collected from piped supplies, surface water and ground water sources in different locations of Lucknow city during summer, monsoon and winter season. Bacteriological quality of samples was determined by enumerating coliform isolated were subject to antibiotic susceptibility test with disc diffusion method. Maximum coliform and faecal coliform contamination were recorded during summer (67% and 75%) and monsoon (67% and 58.3%) while minimum during winter (50% and 50%). All the test isolates exhibited resistance (for nine antibiotics) was shown by river isolates. Antibiotic resistance index (ARI) ranged from 0.050 to 0.150 exhibiting the broad spectrum resistance for 3 to 9 out of 10 antibiotics tested. Occurrence of faecal pollution indicator organisms and multiple antibiotics resistance bacterial population in drinking water is alarming and a sign of potential health with therapeutic problems.

Keywords: Coliform, Antibiotic resistance, Water, Season and pollution

INTRODUCTION

Water is of paramount for health and environment. Safe drinking water sources are the prime requirement for human health. Microbial contamination of drinking water is major cause of several water-borne infectious diseases among consumers. Since last few decades most of surface water sources like rivers, dams, lakes, pounds, tanks etc. are being contaminated and are not safe for human consumption. The condition of water has been highly deteriorated. The ground water was once considered to be safe has also turned out to be miserable. These problems are due to indiscriminate and rapid urbanization industrialization, agrochemical uses with improper waste management and ignorance of mankind towards the natural water sources and environment. Water pollution is the root cause of most of the water borne diseases. As per world health organization (WHO) estimates about 80% disease are globally associated with contamination of water. Theses can be prevented or minimized to a great extent through provision of safe drinking water supply and sanitation.

Faecal contamination of natural drinking water source is the most serious threat to human health by causing various water borne infectious diseases. Coliform and faecal coliforms are established indicator organism is reliable and a very sensitive method for detection of faecal contamination in water due to sewage disposal or through other means. These microorganisms are widely distributed in nature; and their presence and diversity may be used as also a test for suitability of water (Okpokwasili and Akuugobi, 1996). Therefore, these indicator organisms have been recommended by regulatory agencies for water quality monitoring. According to WHO guidelines for drinking water, there should be <10 coliform and no faecal coliforms or faecal streptococci in 100 ml of any potable water sample (WHO, 2004).

Since last few decades the increased and indiscriminate use of antibiotic therapy for infectious diseases, promotion growth in animal husbandry and food preservation has caused emergence of resistance against many important and even new generation plasmid (an extra chromosomal cell organelle) mediated and transferable in nature resulting into its spread among the aquatic including coliforms.

In present study, an attempt has been made to determine the variation in density and distribution of coliforms as well as faecal coliforms in various water sources like municipal, surface on ground water during summer, monsoon and winter seasons from different areas of Lucknow city along with antibiotic resistance among the isolated faecal coliforms.

MATERIALS AND METHODS

Water samples were from municipal water supply, four surface water sources and four ground water sources, selected on the basis of their availability, socio-economic condition, location of the source, level of pollution and population status. Samples of municipal water from old

ISSN : 0974-9411 (Print), 2231-5209 (Online)

as well as new residential areas, surface water from river, pond and ground water from wells and hand pumps were considered. All the water samples were collected in borosilicate heat resistant sterilized glass bottles during summer, monsoon and winter season of the year. Water samples were analyzed for their bacteriological quality by determining the most probable number (MPN) of total coliforms and fecal coliforms with multiple tube fermentation method according to standard protocol (APHA, 1998).

The fecal coliforms isolated from different stations were subject to antibiotic susceptibility test by Disc diffusion method (Bauer *et al.,* 1966). Discs of ten antibiotics (µg) viz; Amoxicillin (10), Chloramphenicol (30), Ciprofloxacin (10), Kanamycin (30), Gentamycin (10), Nalidixic acid (30), Norfloxacin (10), Streptomycin (10), Tetracycline (30) and Trimethoprim (05) were used in this study. Fresh culture of all the test strains was prepared in nutrient broth cultures were showed separated by sterile cotton swab on surface of Mueller Hinton agar plates. Discs impregnated with appropriate concentration of each antibiotic, were dispensing on the surface of sowed agar plates (5 discs per plate) which were incubated overnight at 37^{o}C. Antibiotic resistance was estimated measuring their respective zones of inhibition around each antibiotic disc as per standard (NCCLS, 2002). Antibiotic resistance index (ARI) of each test strain was also calculated by using the formula: ARI= y/n x, where y=total number of resistance strains; n= total number of test strains and x=total number of antibiotics tested (Hinton and Linton, 1983).

All the dehydrated culture media and antibiotic discs were obtained from Hi Media Laboratories Pvt. Ltd. Mumbai, India.

RESULTS AND DISCUSSION

There is a variable effect of seasons and climatic conditions on occurrence of indicator bacteria. In surface water sources like rivers, ponds and streams, the bacterial density is also according of municipal sewage and industrial effluent as well as dilution with rain water. So different water sources have been examined regularly because pollution is often intermittent and may not be detected if examination is limited.

The surface water samples from river and ponds were observed to be most contaminated with >1100/100 ml coliform and faecal coliform during all the three seasons. The coliform count was >1100/100 ml during monsoon and winter in samples from upstream (Gaughat) as well as downstream (Pipraghat) of the river Gomti and Hussainabad pond. But fecal coliform count was little less in upstream (1100/100 ml) during monsoon and winter than downstream. It has been observed that, as the river precedes towards downstream the bacterial load

Table 1. Antibiotic resistant fecal coliform isolates of water samples collected from Lucknow City.

Water Sources	Amoxy-cillin Am-10µg	Chlora-phenicol Cp-30µg	Ciprofl-oxacin Cf-10µg	Kanam-ycin Km-30µg	Gentam-ycin Gm-10µg	Nalidixi-n acid Na-30µg	Norflox-acin Nx-10µg	Strepto-mycin Sm-10µg	Tetracy-cline Tc-30µg	Trimeth-oprim Tm-5µg	ARI
River-US	R	R	R	R	R	R			R	R	0.133
River-DS	R	R	R	R	R	R		R	R	R	0.150
Pond	R	R			R						0.050
Well(Chowk)	R					R			R		0.050
Well(Gomtinagar)	R			R		R			R		0.066
Hand Pump(Chowk)	R			R		R	R		R		0.083

ARI = Antibiotic resistance index, R=Resistant; US=Up stream; DS=Down stream

Table 2. Seasonal variation in bacteriological quality of water samples collected from Lucknow city showing coliform contamination.

Sl.No	Sampling sources	Sampling sites	Coliform/100 ml*		
			Summer	Monsoon	Winter
1	Municipal Supply	Chowk	9	8	<3
2	-- Do --	Daliganj	43	43	<3
3	-- Do --	Gomtinagar	15	4	<3
4	-- Do --	Indiranagar	<3	<3	<3
5	Gomti River	Upstream(Gaughat)	>1100	>1100	>1100
6	-- Do --	Downstream (Pipraghat)	>1100	>1100	>1100
7	Pond	Hussainabad	>1100	>1100	>1100
8	-- Do --	La-Martinere	>1100	460	4
9	Open Well	Chowk	>1100	>1100	1100
10	-- Do --	Gomtinagar	>1100	>1100	240
11	Hand Pump	Indiranagar	<3	<3	<3
12	-- Do --	Chowk	9	43	43

Bureau of Indian Standard (BIS) : Drinking water specification (1996); ID 10500:1991, <10 Coliforms/100ml

increases which indicate higher contamination (Rajurkar *et al.,* 2003). La-Martinere pond was least contaminated only with 4 coliform and < 3 fecal coliform per 100ml during winter season. This may be attributed to absence of sewage discharge or other sources of faecal contamination in vicinity of these ponds (Tables 2 and 3).

The increase in riverine pollution in downstream was apparent from observations of bacteriological analysis of river water. Coliform contamination in upstream is more than its faecal coliform, especially during monsoon and winter season because there are no major sources of faecal contamination in this stretch which indicates that samples were contaminated with some non-faecal sources like agricultural runoff, decaying vegetation and /or domestic wastes (Duncan and Raxxell,1972). Downstream is highly contaminated with coliform as well as fecal coliform because till then river has received all the municipal sewage of the city. Increased level of coliform in downstream of these rivers has also been observed by Pathak (1991) which may be attributed to municipal sewage contamination.

Coliform contamination in ground water from wells was >100/100ml in summer and monsoon seasons but it was less contaminated in winter with 1100 and 240/100ml. Gomtinagar well water was most contaminated with faecal coliform 1100, >1100 and 23/100 ml in all the three seasons. This may be due to sewage in Gomti river, flowing through this area. Well in Chowk was less contaminated with faecal coliforms in all the three seasons with 120, 93 and 23/100

Table 3. Seasonal variation in bacteriological quality of water samples collected from Lucknow city showing faecal coliform contamination.

Sl.No	Sampling sources	Sampling sites	Faecal coliform/100 ml*		
			Summer	Monsoon	Winter
1	Municipal Supply	Chowk	4	<3	<3
2	-- Do --	Daliganj	4	<3	<3
3	-- Do --	Gomtinagar	<3	<3	<3
4	-- Do --	Indiranagar	<3	<3	<3
5	Gomti River	Upstream(Gaughat)	>1100	1100	>1100
6	-- Do --	Downstream (Pipraghat)	>1100	1100	>1100
7	Pond	Hussainabad	>1100	>1100	>1100
8	-- Do --	La-Martinere	240	460	>3
9	Open Well	Chowk	120	93	23
10	-- Do --	Gomtinagar	>1100	>1100	23
11	Hand Pump	Indiranagar	<3	<3	<3
12	-- Do --	Chowk	4	23	43

Bureau of Indian Standard (BIS) : Drinking water specification (1996); IS 10500:1991. No (<1) Coliforms/100ml (absent).

ml during summer, monsoon and winter respectively. Hand pump water from Indiranagar was found to be bacteriologically safe without coliform and faecal coliforms but hand pump water from Chowk was contaminated with coliform and faecal coliform during all the seasons, as soak pits have been created in this area. Municipal water samples were observed to be bacteriologically safe with no coliform and faecal coliform during winter but were contaminated with higher number of coliforms during summer and monsoon seasons. These samples were free from faecal coliform during monsoon and winter seasons. Coliform count in samples from Chowk, Daliganj and Gomtinagar was 9, 43 and 15/100 ml in summer and 4, 43 and 4/100ml in monsoon seasons respectively. Thus, maximum contamination was observed during summer followed by monsoon while it was minimum during winter. Faecal coliform in samples from Chowk and Daliganj may be due to sewage drains running parallel to pipe supplies which may be damaged and /or with loose joints. Pathogenic microorganism like *Escherichia coli* and *Salmonella sp.* can grow and persist in biofilms formed due to organic as well as inorganic nutrient on inner face (Paymint and Robertson, 2004). Instead, Gomtinagar and Indiranagar, comparatively sparsely populated areas have proper water supply and sewage system so there are less chances of sewage contamination.

The antibiotic susceptibility test revealed that all the isolates were resistant to Amoxycillin and sensitive to Norfloxacin and streptomycin except isolates from samples of hand pump in Chowk and the Gomtinagar (Pipraghat), respectively. Isolates from all the samples except from Hussainabad pond were resistant to Nalidixic acid and Tetracycline. Earlier studies have revealed that environmental isolates of bacteria have developed resistance for wide spectrum including the new generation antibiotics. Gomti river had developed resistance for most of the antibiotic tested. Isolate from upstream was sensitive to Norfloxacin and Streptomycin, while that from downstream were sensitive to Norfloxacin only. Isolates from all the station have exhibited multiple antibiotic resistance (MAR). Antibiotic resistance for three to nine out of ten antibiotics tested (Table 1).

Pathak *et al.* (1993) also reported multiple antibiotic resistant (MAR) *E. coli* from urban drinking water. MAR patterns have been reported among the aquatic bacterial population in various surface waters (Jones *et al*., 1986; Baghel *et al*., 2003), particularly the coliform bacteria (Ramateke *et al*., 1991). Kaspar *et al.* (1990) noticed that indexing the antibiotic resistance among E. coli may be useful to identify the sources of faecal contamination in water. Recently, Vantarakis *et al*., (2006) have observed that MAR analysis is useful tool in differentiating the faecal *E.coli* from human being and animals.

The high occurrence of amoxicillin resistance may be due to production of β-lactamase enzyme by the bacteria neutralizing the antimicrobial activity by β-lactam antibiotics (Amyes *et al*., 1992). The β-lactam group of antibiotics such as amoxicillin and carbenicillin has more pronounced effect of antibiotic resistant bactetial profile in the primary water sources than antibiotics used as food additives (Mulamattathil *et al.,* 2000).

Previous researches have shown that the cell membrane protein component of such bacteria change and this change may be limited to enhanced resistance for antimicrobial substances (Brown, 1977; Harakeh *et al.,* 1985). Bacterial resistance may also be acquired as a result of mutation in the bacterial chromosomes. Such mutation appears spontaneously as a result of damage to DNA and the antibiotic provide the selection pressure for these drug resistant mutants (Wary, 1986). Therefore, such case studies on water quality surveillance are significant source of information of universal use in identifying outbreak etiology, assessing water borne public health risk and drinking water quality management (Percival *et al.,* 2004).

Thus, it is concluded that microbial contamination in different water sources is significantly affected with seasonal variations, waste disposal and various socio-biological activities like bathing, cloth washing, defaecations and domestic and as well as industrial garbage dumping effluents from various sources, like municipal sewage, industries, agriculture lands and others, affect the microbial quality of drinking water sources in various ways. Occurrence of multiple antibiotic resistant bacterial populations further aggravate the problem of safe drinking water sources in urban areas

ACKNOWLEDGEMENTS

The authors are grateful to The Director, Indian Institute of Toxicology, Research Lucknow for all necessary assistance in performing this study. Thanks are also to Head, Department of Zoology, University of Lucknow, Lucknow for the assistance in conducting the study.

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