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Bacteriological Quality Assessment of Groundwater and Surface Water in Chennai

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ABSTRACT

Water bodies have played a vital role throughout civilizations in the growth and continue to be a factor of importance in the modern economic growth of all the contemporary societies. Contamination of water sources with microbes can occur through rainfall overflow and agricultural inputs, mixing with sewage effluents. Water acts as a medium for the proliferation and dissemination of bacteria which cause human disease. Therefore, consumption of safe water is one of the most important requirements in public health. A total of 20 water samples were collected from both surface and groundwater from in and around Chennai. The MPN index of the surface water was found to be high when compared with groundwaters. Total coliform count of as many as 1100 MPN/100 mL and greater were obtained. A total of 11 different bacterial species were identified from both ground and surface water samples. The prevalence of bacterial isolates was of *Staphylococcus aureus, CoNS, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Vibrio cholera, Salmonella typhi, Aeromonas hydrophila* and *Citrobacter freundii*. These organisms were resistant to many classes of antibiotics. The facts on multiple antibiotic resistance profiles of bacteria isolated from water and the resistance patterns suggested that there has been an indiscriminate use of the antibiotics tested. High occurrence of these multiple antibiotic resistant organisms in the drinking water distribution system could potentially pose a threat to humans consuming this water.

INTRODUCTION

About 75% of the earth's surface is covered by water which is vital for all forms of life. Groundwater refers to water that occurs below the water-table in the zone of saturation. Groundwater is the only source of fresh water which is utilized in many parts of the world, whereas most of the times surface waters are either absent or polluted (Haniffa et al. 1993). Surface water is water that gets collected in a stream, river, lake, wetland etc. Many industries and municipalities use treated surface water due to high cost of drinking water. In the recent past there has been a steady increase in the pollution of river waters with deleterious microbes, including bacteria, viruses, parasites, as well as fungi (Niyogi 2005, Abraham et al. 2007). Majority of microbes in water are from faeces from human and other mammals. Pathogens can enter waters either from a point source, non-point sources or both. Rainwater surface run-offs, storm sewer spillages or overflow cause non-point microbial pollution of waters, while point-source pollution comes from discharge of untreated or partially treated effluents from wastewater treatment plants (Donovan et al. 2008, Musyoki et al. 2013).

When the water is contaminated with faecal material a wide range of pathogenic microorganisms can be transmitted to humans. Some of them include enteropathogenic agents such as Salmonella, Shigella, Enteroviruses, and multi cellular parasites as well as opportunistic pathogens, like *Pseudomonas aeruginosa*, *Klebsiella* sp., *Vibrio parahaemolyticus* and *Aeromonas hydrophila* (Hodgkiss 1988). The occurrence and spread of antibiotic-resistant bacteria (ARB) are grave public health problems worldwide. Now aquatic ecosystems are a recognized reservoir for ARB and antibiotic resistance genes. An emerging issue for the general public and the drinking water industry is the presence of trace levels of antibiotics and ARB in source water and finished drinking water which affects public health (Scott et al. 2000). The aim of the present study was to characterize the presence of coliforms from surface and groundwater bodies in and around Chennai and to determine the resistance pattern of the isolates towards commonly used antibiotics. This study was done keeping in view the recent Chennai floods and Vardah cyclones that caused devastations to both human lives and the environment.

MATERIALS AND METHODS

Study Area

A total of 20 water samples were collected, of which 10 were

from surface waters (Lakes) and 10 from groundwater (Well) in and around Chennai. Water samples were collected from North Jaganadhan Nagar, Thathan Kuppam, Villivakkam, Teynampet, Chrompet, Chitlapakkam, Pallavaram, Hasthinapuram, Velachery, Chembarabakkam, Chitlapakkam, Namangalam, Pallikaranai, Puzhal, Ambattur and Porur.

Collection of Samples

Water samples were aseptically collected in 1 litre sterile bottles. Prior to sampling, the bottles were rinsed thoroughly with the water of the same source from which the sample was to be collected and rinsed water was discarded. The bottle cap was aseptically removed, and the bottle was immersed into the lake to collect the water. The bottle was brought up to the surface and covered with the bottle cap.

In open wells, water is available from 1 meter below from the ground. A sterile sampler was introduced into the well with the help of a rope and the water was taken out. The sampling bottle was kept unopened until the moment at which it was required for filling. During sampling the stopper and neck of the bottle were protected from contamination. Samples were labelled and transported in ice packs to the laboratory. They were examined for precipitate, colour, pH and odour. They were further processed for microbiological examination of coliforms and other pathogens.

Processing of the Water Samples

The water samples were subjected to MPN (Most Probable Number) technique to detect the quality of water. MPN is most commonly applied for quality testing of water, i.e. to ensure whether the water is safe or not in terms of bacteria present in it. A group of bacteria commonly referred as faecal coliforms act as an indicator for faecal contamination of water. It was done by multiple tube fermentation test to determine the total coliform count as per the standard methods. (Bartram & Pedley 1996)

Detection of Selected Pathogenic Bacteria from Water Samples

One mL of each water sample was inoculated into 5 mL of nutrient broth, selenite F enrichment broth and alkaline peptone water and was incubated at 37°C for 6-8 hrs. They were further inoculated on to MSA (Mannitol salt agar) media, Cetrimide agar, DCA (Deoxycholate citrate agar) media and TCBS (Thiosulfate citrate bile salt agar) and the plates were incubated for about 24-48 hrs at 37°C, respectively.

Antibiotic Sensitivity Testing

Once the organism was identified, the antibiotic sensitivity testing was carried out as per the standard protocol by Kirby Bauer disc diffusion method (Bauer et al. 1966) towards the following antibiotics - Erythromycin (15 mcg), Nalidixic acid (30mcg), Rifampicin (30 mcg), Teicoplanin (30 mcg), Vancomycin (30 mcg), Amoxyclav (30 mcg), Amikacin (30 mcg), ceftazidime/clavulanic acid (30/10 mcg), ceftriaxone (30 mcg), ciprofloxacin (5 mcg), ceftazidime (30 mcg) and gentamicin (10 mcg).

RESULTS

Physical Properties of Water Samples

Water samples were tested for their physical properties like pH, colour, odour and precipitate. The pH of all the ground as well as the surface water was found to be neutral. The colour of the water samples ranged from being colourless to slight yellowish. Strong odour and precipitates were observed predominantly in surface waters when compared to groundwaters.

MPN Technique

The water samples were subjected to MPN technique to detect the quality of water. MPN is the most commonly applied method for quality testing of water to ensure whether the water is potable or not. The MPN index of the surface water was found to be high when compared with groundwater. Total coliforms of as many as 1100 MPN/100 mL and greater were enumerated in surface water. With respect to groundwater, MPN index of >1100 MPN/100 mL was observed at North Jaganadhan Nagar and the least MPN index of 28 MPN/100 mL was observed at Teynampet. All the sampling places of both ground and surface waters showed the presence of coliform bacteria. Hence, it can be concluded that the water samples were heavily contaminated with coliform bacteria and were found to be unsatisfactory for drinking purpose (Tables 1 & 2).

Prevalence of Bacterial Isolates from Water Samples

A total of 11 different bacterial species were identified from both ground and surface water samples. The prevalence of bacterial isolates was *Staphylococcus aureus, CoNS, Enterococcus faecalis, E. coli, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Aeromonas hydrophila* and *Citrobacter freundii* (Table 3).

Determination of MPN Index for Boiled Water

As a control study, one surface and one groundwater sample was boiled to check the efficiency of boiling in killing the coliforms present in them. MPN technique was done and it was found to be negative for the presence of coliforms.

Table 1: MPN Index of groundwater.

Sample Number	Area	MPN Index per 100 mL
1A	North Jaganadhan Nagar (3rd street)	>1100
2A	Thathan Kuppam	460
3A	Villivakkam	75
4A	North Jaganadhan Nagar	210
5A	Teynampet	28
6A	Chrompet	150
7A	Villivakkam	150
8A	Chitlapakkam	460
9A	Pallavaram	460
10A	Hasthinapuram	210

Table 2: MPN Index of surface water.

Sample Number	Area	MPN Index per 100 mL
1B	Velachery (100 feet road)	1100
2B	Chembarabakkam	1100
3B	Chitlapakkam	>1100
4B	Namangalam	460
5B	Pallikaranai	1100
6B	Puzhal	460
7B	Ambattur	1100
8B	Porur	>1100
9B	Pallavaram	>1100
10B	Hasthinapuram	>1100

Table 3: Prevalence of bacterial isolates from water samples. Groundwater (n = 74), Surface water (n = 52)

S. No	Name of the organisms	Percentage (Groundwater)	Percentage (Surface water)
1	*Staphylococcus aureus*	5(6%)	3(6%)
2	*CoNS*	3(4%)	1(2%)
3	*Enterococcus faecalis*	8(11%)	5(10%)
4	*E. coli*	10 (13%)	10(19%)
5	*Klebsiella pneumoniae*	10(13%)	10(19%)
6	*Enterobacter aerogenes*	10(13%)	10(19%)
7	*Pseudomonas aeruginosa*	6(8%)	2(4%)
8	*Vibrio cholerae*	7(9%)	3(5%)
9	*Salmonella typhi*	6(8%)	5(10%)
10	*Aeromonas hydrophila*	5(6%)	2(4%)
11	*Citrobacter freundii*	4(5%)	1(2%)

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing of the bacterial isolates was done as per Kirby Bauer's disc diffusion method. Gram positive organisms showed 100% resistance towards vancomycin, nalidixic acid, erythromycin, ciprofloxacin and teicoplanin. They were 100% sensitive towards rifampicin. Gram negative organisms exhibited 100% resistance towards ceftazidime/clavulanic acid, amoxyclav followed by gentamicin, ceftriaxone and ceftazidime. These isolates were sensitive towards amikacin and ciprofloxacin.

DISCUSSION

Potable water must be free from pathogenic microorganisms and chemical substances that are hazardous to health (Lamikanra 1999). Bacteria indicative of faecal pollution or pathogens should not be found in drinking water. A sensitive method of quality assessment of drinking water is the detection of faecal indicator bacteria as it is not possible to examine water for every possible pathogen that might be present (WHO 2004). There has been an imbalance between supply and demand and it is the main reason which has led to competition and thereby resulted in pollution and environmental degradation. Failure of disinfection methods of raw water at the treatment area or mixing of sewage through cross-connections, leakages could cause bacteriological pollution of drinking water supplies.

Main cause of water pollution is due to human impact (Palit 2012). By studying the physico-chemical and microbial characteristics, the significance of water as a potent ecological factor can be appreciated. In the present study, water samples were tested for their physical properties like pH, colour, odour and precipitate found. The pH of all the ground as well as the surface water was found to be neutral. The colour of the water samples ranged from being colourless to slight yellowish. Strong odour and precipitates were observed predominantly in surface waters when compared to groundwaters. The negative logarithm of hydrogen ion concentration is known as pH. It expresses the intensity of acidic or alkaline condition of a solution. pH is an extremely important variable because most microorganisms can survive within a narrow range of pH and it also helps in the solubility of most metals. pH is also an important factor with respect to chemical treatment of water including disinfection process.

Microbial contamination of drinking water is the most common and wide spread health risk and therefore its control must always be of supreme importance. Monitoring microbial presence, especially faecal coliform bacteria (FC) determines the quality of water. In this study, the MPN index was high for surface waters when compared to groundwater. The water analysed in this study has clearly shown that they are loaded with indicator organisms which are the indication of faecal pollution and thus the human interference.

In our study, a total of 11 different bacterial species were identified from both ground and surface water samples. The prevalence of bacterial isolates was *Staphylococcus aureus, CoNS, Enterococcus faecalis, E. coli, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Aeromonas hydrophila* and *Citrobacter freundii*. According to EPA, the presence of *E. coli* indicates recent sewage or animal waste contamination (EPA 2001). The temperature and the presence of nutrients in the tank favours the survival of enteric bacteria, like *Salmonella* spp. and *E. coli* (Leclerc et al. 2002). Enteric pathogens such as *Salmonella, Shigella* and *Vibrio* are principally transmitted through contaminated drinking water, as reported by Edberg et al. (2000).

P. aeruginosa is ubiquitous, free-living bacterium in the environment. It is often found in natural waters such as lakes and rivers in concentrations of 10/100 mL to >1,000/100 mL. Nevertheless, it is not often found in drinking water. *Pseudomonas* is usually found in 2% of samples, or less, and at concentrations up to 2,300 mL or more often at 3-4 CFU/mL. The occurrence of *Pseudomonas* in drinking water is due to its ability to form biofilms and colonize the plumbing fixtures (i.e., faucets, showerheads, taps, pipelines, etc.) than its presence in the water system or treated drinking water (Mena & Gerba 2009).

The occurrence and spread of antibiotic-resistant bacteria (ARB) globally are serious public health problems. Aquatic ecosystems are a designated reservoir for ARB and antibiotic resistance genes. The existence of trace levels of antibiotics and ARB in source water and finished drinking water may also greatly affect public health and is an emerging issue for the general public and the drinking water industry (Scott et al. 2000). In our study, antibiotic sensitivity testing of the bacterial isolates was done as per Kirby Bauer's disc diffusion method. Gram positive organisms showed 100% resistance towards vancomycin, nalidixic acid, erythromycin, ciprofloxacin and teicoplanin. They were 100% sensitive towards rifampicin. Gram negative organisms exhibited 100% resistance towards cefotaxime/clavulanic acid, and amoxyclav followed by gentamicin, ceftriaxone and ceftazidime. These isolates were sensitive to amikacin and ciprofloxacin.

CONCLUSION

An assessment of the bacteriological quality of drinking water in the present study confirmed the presence of various bacterial species including opportunistic pathogens such as

Aeromonas and Pseudomonas spp. This drastic effect in the increase of microorganisms may be because of the recent Chennai floods followed by Vardah cyclone. This has caused the mix up of sewage with the water bodies resulting in water pollution. This could have been the reason for all the water samples showing the presence of coliforms and other organisms. The isolated bacteria were found to be resistant to several classes of antibiotics. The data on multiple antibiotic resistance (MAR) profiles of bacterial isolates from water and the resistance patterns of organisms in drinking water suggested that there has been an indiscriminate use of the antibiotics tested. The high occurrence of multi-drug resistant organisms in the drinking water system may possibly pose a risk to humans consuming this water.

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