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Chlorine resistant bacteria isolated from drinking water treatment plants in West Bengal

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ABSTRACT

Resistance to different concentrations of chlorine have become a major problem in some areas of West Bengal. The study involves surveillance of different drinking water treatment plants located in West Bengal for the isolation of chlorine resistant bacteria as contaminants. The risk assessment studies involved extensive microbiological work based on isolation and enumeration of pathogens through membrane filter technique, identification of isolates based on their morphological characteristics and enumeration of chlorine resistant bacteria from drinking water treatment plants. The chlorine content of water was measured at the time of plating. All the water systems maintained more or less 1 mg/ L residual free chlorine despite of that many species like *Staphylococcus aureus*, *Klebsiella*, *Pseudomonas* and *Clostrdial* were isolated from these water systems. The experimental result states that the Gram positive bacteria were more resistant to chlorination than Gram negatives. WTP-IV was more at risk where *E. coli* was isolated at a sub-optimal dosage of chlorine, 0.5 mg/L. All the isolates survived except *E. coli* and *Serratia* at 2 mg/L chlorine for 30 min. The results suggests emergence of chlorine resistant pathogens from chlorinated water supply systems of West Bengal. The experimental results implies on employment of effective preventive measures keeping in view the emergence of chlorine resistant forms and demands formulation of new strategy in the upcoming revised Standard operating procedures for safe drinking water which must be adopted by water systems, most vulnerable to chlorine treatment.

Keywords: Water treatment plant; Chlorination; Resistance to chlorine; Emerging pathogen; Bacteriological studies; Quality management

1. Introduction

Bacterial contaminant of drinking water is a major health burden in the water treatment plants of West Bengal, India. Chlorination of water kills abundant pathogens but some species of bacteria shows persistent resistance towards it. Chlorine treatment using sodium hypochlorite 35% is considered the most reliable method of water treatment. However, there are reports of selection of chlorine resistant species in places where chlorination is a regular practice. Chlorine is a chemical agent that acts on the enzymes of the microorganisms, inactivates them alters the permeability of the membrane and affects several metabolic processes; killing most of the pathogens of water [1]. The most resistant one survives chlorination. A recent study mentioned isolation of *Actinomycetes* and *Aeromonas* from chlorine treated drinking water and untreated surface water by standard plate count technique, before and after contact with chlorine (1–2 mg/L) for 1 h. The study revealed that chlorination selects for gram-positive bacteria [2]. There are reports of most resistant microorganisms like *Actinomycetes* and some *Micrococci* that were able to survive a 2 min exposure to 10 mg residual free chlorine per liter. The gram-positive spore-forming bacilli were more resistant to chlorine but the most sensitive bacteria

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were readily killed by chlorine concentrations of 1.0 mg/L. The resistant species survived were mostly gram-positive *Micrococci*, *Corynebacterium*, *Arthrobacter*. Among, gram negatives *Klebsiella*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Moraxella*, and *Acinetobacter* were isolated [3]. Recently, an investigation of Khabur river water, the main source of drinking water in Zahko-Duhok city, Iraq showed the sensitivity of microorganisms towards chlorine. These isolates were also susceptible towards various antibiotics [4]. Bacteria isolated from post-chlorinated water samples are more resistant to chlorine disinfection than pre-chlorinated water. The microorganisms like *Staphylococcus aureus*, *Micrococcus varians* and *Aeromonas hydrophila* are fully resistant to chlorine at concentration as high as 2 mg/L. The most resistant bacterium of all isolates is *Staphylococci* [5]. There are many effective means of treating water apart from chlorination but none are as effective as chlorine. Chlorine treatment is one of the most effective methods of water disinfection. The large scale drinking water treatment plant still encourages use of chlorine as a standard process to treat large volume of water [6,7].

In this study the drinking water treatment plants I, II, III, IV and V failed to produce microbiologically safe drinking water. These treatment plants were monitored throughout the year. The study concerns mitigation of the problems associated with emerging chlorine resistant bacterial species. Treatment plant-I is installed with iron elimination plant, after which the water reaches chlorination chamber, finally the chlorinated water is stored in storage tank. Water treatment plant-II, III, IV and V, are designed sufficiently with pre-ozonation/UV systems (enabled after filtration units and before chlorination chamber). Despite of filtration and chlorination the treatment plants perpetually failed to produce microbiologically safe drinking water post chlorination and storage. These treatment plants were chosen for the study, as the previous surveillances suggest presence of bacterial contaminants. The water systems maintained an overall high level of residual free chlorine (1 mg/L), but could not meet the bacteriological standards of water quality. All the treatment plants sufficiently produced final water that complied with the physico-chemical parameters but more often failed to comply with the bacteriological quality.

2. Materials and methods

2.1. Collection of sample

More than 100 samples were tested from drinking water treatment plants (WTP-I, II, III, IV and V). Both raw and final water (treated chlorinated water sample collected after 30 min contact time with chlorine). Sampling was done after flaming the tap with 80% alcohol and running off for 5 min then stored in 600 ml sterile container. All the samples were immediately taken to laboratory for test. The water samples were immediately divided in two portions inside laminar air flow chamber. One portion contained 500 ml of water that was taken for microbiological analysis and other aliquot of 100 ml was kept for physico-chemical analysis.

2.2. Microbiological studies for isolation and identification of bacterial isolates

Microbiological test were conducted to find the presence of heterotrophs (by heterotrophic plate count method-HPC) using plate count agar (Hi-media). Selective isolation was also done using selective agar media-EMB (Levine) agar (Hi-media) for *E. coli*, Egg yolk mannitol sucrose agar for *S. Aureus*, Aspergine-proline for *Pseudomonas*, Differential Reinforced Clostridial broth (DRCB) for *Clostridia* were used as selective media. Differential media like Mac conkey and Chromocult agar were also used for isolation and differentiation of Fecal and Total coliform. Platting was done in a sterile condition by passing 100 ml of water via milipore membrane filter. For isolation of *Pseudomonas*, inoculation was done on four fold volume of Aspergine proline broth by inoculating one volume of sample in it. For isolating *Clostridia*, equal volume of sample 50 ml was inoculated aseptically to 50 ml of sterile DRCB broth. The HPC replica plates were kept at 22°C and 35°C. For *Pseudomonas*, *Clostrial sp*, fecal coliform, *E. coli* inoculants were incubated at 42°C as they are thermo-tolerant and the total coliforms plates were kept for incubation at 35°C. Identification to genus level was performed by studying the cultural characters, Gram's staining, morphological and biochemical characteristics.

2.3. Physico-chemical properties of sample

The microbiological quality of water is directly influenced by the physico-chemical nature of water. Hence, the chlorine content, pH, TDS and turbidity of the 100 ml aliquot of each water sample were analysed at the same time when bacteriological analysis were conducted following the standard protocol.

2.4. In-vitro study of chlorine resistance pattern

The bacterial isolates obtained were subjected to chlorination for contact time of 30 min. The concentration gradient was set to 0.5 mg/L and 2 mg/L. After chlorination for 30 min the reaction was stopped by using sodium thiosulphate and pour platting was done by transferring 1 ml of the suspension onto sterile plate count agar, after that incubation was done at 35°C, then the results were recorded.

3. Results and discussion

The results were tabulated after annually monitoring the sample water from each treatment plant. The results are graphically represented based on 10 observations (Figs. 1, 2). The physico-chemical parameters are tabulated in Table 1. Totally nine isolates were obtained, all of which were able to survive 0.5 mg/L residual chlorine. At 2.0 mg/L chlorine, all strains survived except for *E. coli*, *Serratia* (Tables 2, 3, and 4).

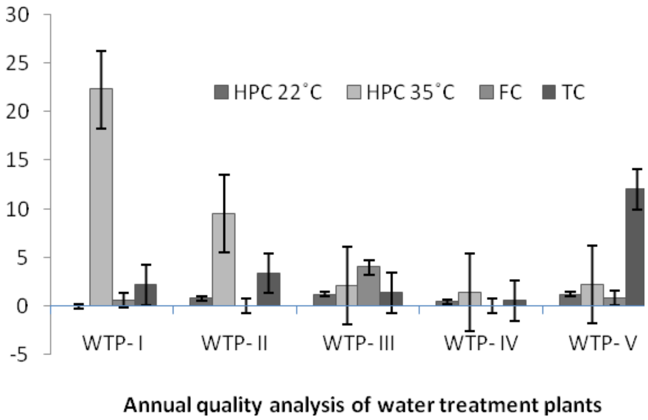

Fig. 1. Enumeration of HPC, FC and TC from Raw water of different treatment plants. *HPC – heterotrophic plate count, FC – fecal coliform, TC – total coliform and RFC mg/ L of raw water from different WTP-water treatment plant.

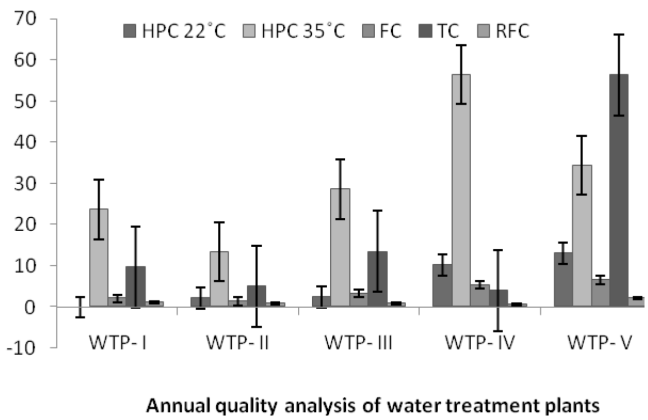

Fig. 2. Enumeration of HPC, FC and TC from post chlorinated water of different treatment plants. *HPC – heterotrophic plate count, FC – fecal coliform, TC – total coliform and RFC mg/ L of post-chlorinated water from different WTP-water treatment plants.

4. Conclusion

The experimental result states that the gram positive bacteria were more resistant to chlorination than gram negatives. This may be due to cellular constitution and differentiation between cell wall of gram positive and gram negative. The gram negatives like *Pseudomonas*, *Klebsiella* and *Enterobacter* were isolated imparting good resistance against chlorination. *E. coli* was isolated when a sub-optimal dosage of chlorine, 0.5 mg/L was maintained at water treatment plant-IV. All strains were resistant to chlorine concentration up to 0.5 mg/L. At 2 mg/L chlorine for 30 min, all the isolates survived except *E. coli* and *Serratia*. Observation of the result showed that the chlorination process reduced the total number of heterotrophic counts but greatly enhanced the proportion of chlorine resistant bacteria (Figs. 1 and 2). The treatment plants for drinking water system should have residual chlorine of less than 0.5–1 mg/L as per standard operating procedure of BIS (BIS report 1998) [18]. Despite of maintaining higher residual chlorine of 1.5–2 mg/L at water treatment plant – I and V, they failed to produce good quality drinking water. The maintenance of chlorine in water system for <2 mg/L is detrimental to public health. Hence, the treatment plants cannot maintain such high level of chlorine in final water (as per BIS: 14543:2004). Therefore, the final product fails to satisfy the bacteriological quality.

This study may confer importance of pilot scale water quality management in context to the disease transmission through water contamination. It provides insights on emergence of these pathogens that may cause water borne disease outbreaks throughout the region. The Indian Standard operating procedures (SOPs) for drinking water must employ effective preventive measures keeping in view the emergence of chlorine resistant forms and must provide a strategy to overcome such problems, failing which there would be a crisis for safe drinking water in West Bengal. The standard revised SOPs for safe drinking water must be adopted by those water systems which are mostly vulnerable to chlorine treatment.

Table 1
Physico-chemical analyses of samples from different water treatment plants

Location	Sample source	pH*	Turbidity (NTU)*	TDS (mg/L)*	RFC (mg/L)*
WTP-I	Raw	7.2 ± 2.4	2 ± 0.6	110	–
	Post-chlorination	7.4 ± 2.4	1 ± 0.3	102	1.05 ± 1.29
WTP-II	Raw	7.4 ± 2.4	2 ± 0.6	235	–
	Post-chlorination	7.8 ± 2.6	1 ± 0.3	74	0.94 ± 0.31
WTP-III	Raw	7.2 ± 2.4	2 ± 0.6	220	–
	Post-chlorination	7.4 ± 2.4	1 ± 0.3	53	0.81 ± 0.27
WTP-IV	Raw	7.2 ± 2.4	2 ± 0.6	72	–
	Post-chlorination	7.6 ± 2.5	1 ± 0.3	45	0.55 ± 0.18
WTP-V	Raw	7.4 ± 2.4	2 ± 0.6	395	–
	Post-chlorination	7.6 ± 2.5	1 ± 0.3	56	2.2 ± 0.73

*Arithmetic mean and standard deviation
** arithmetic mean of the values

Table 2
Isolation of chlorine resistant coliforms from water treatment plants

Location	Sample source	E.coli	Serratia	Proteus	Klebsiella	Pseudo monas	Entero bacter
WTP-I	Post-chlorination	–	+	+	–	–	+
WTP-II	Post-chlorination	–	–	+	+	+	+
WTP-III	Post-chlorination	–	–	+	+	+	+
WTP-IV	Post-chlorination	+	+	+	+	+	+
WTP-V	Post-chlorination	–	–	+	+	+	+

*WTP – Water treatment plant, * (+) for presence and (–) for absence of growth

Table 3
Isolation of chlorine resistant gram positives from water treatment plants

Location	Sample source	Micro coccus	S. aureus	Clostridia
WTP-I	Post-chlorination	–	+	–
WTP-II	Post-chlorination	+	+	+
WTP-III	Post-chlorination	+	+	+
WTP-IV	Post-chlorination	+	+	+
WTP-V	Post-chlorination	+	+	+

WTP – Water treatment plant, (+) for presence and (–) for absence of growth

Table 4
Chlorine resistant pattern of isolates in 2 mg/L of chlorine solution

	WTP-I	WTP-II	WTP-III	WTP-IV	WTP-V
E. coli	–	–	–	S	–
Enterobacter	I	I	I	S	I
Clostridia	–	I	I	S	I
K. pneumonia	R	I	I	I	I
Micrococcus	–	S	S	S	S
Pr. myxofaciens	R	I	I	S	R
Pr. mirabilis	R	S	I	S	R
P. aeruginosa	–	R	R	R	R
S. entomophila	R	–	–	–	I
S. marscecens	I	–	–	S	–
Staphylococcus aureus	R	R	R	R	R

*R – resistant, I – intermediate and S – sensitive

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