

ASSESSMENT OF MICROBIAL QUALITY OF CHLORINATED DRINKING TAP WATER AND SUSCEPTIBILITY OF GRAM NEGATIVE BACTERIAL ISOLATES TOWARDS CHLORINE

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ABSTRACT

Sixty (56.1%) water samples crossed the permissible limit of WHO guideline value in heterotrophic plate count and total coliform count each. Ten different genera of gram negative bacteria were recovered in which *E. coli* was predominant followed by *Citrobacter* spp., *Shigella* spp., *Enterobacter* spp., *Providencia* spp., *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., *Proteus* spp. and *Edwardsiella* spp. Higher the temperature of water sample, higher the bacterial growth was obtained ($p= 0.002$) and similarly higher level of free residual chlorine in water reduced the bacterial growth ($p= 0.037$) whereas increase or decrease of pH ($p= 0.454$), turbidity ($p= 0.164$) and conductivity ($p= 0.969$) didn't affect the microbial growth. A negative correlation ($r= -0.162$) between heterotrophic plate count and free residual chlorine was observed however without statistical significance ($p= 0.096$). Similarly, a negative correlation ($r= -0.383$) between total coliform count and free residual chlorine was observed with statistical significance ($p= 0.001$). In chlorine assay, all tested eight different genera of gram negative bacteria were found to be chlorine resistant at 0.2 mg/l for a contact time of 30 minutes. Average time required for $T_{99.9}$ (3-log) and $T_{99.99}$ (4-log) reduction of viable isolates from initial population of 2×10^6 cells/ml were found to be less than 30 minutes and greater than 60 minutes respectively. Log inactivation of various bacterial isolates with chlorine concentration of 0.2 mg/l for a contact time of 30 minutes were found to be ranged from 3 to 3.5-log. Emergence of chlorine resistant organisms in drinking water probably demands alternate disinfection or mitigation strategy.

Keywords: Chlorine Resistance, Drinking Water, Disinfection, Gram Negative Bacteria, Residual chlorine

INTRODUCTION

Around 1.2 billion people around the world lack access to safe drinking water and twice that many lack adequate sanitation. As a result, the World Health Organization estimates that 3.4 million people, mostly children, die every year from water-related diseases [27]. In Nepal, the total cases of Diarrhoea was 1,398,106 and among them 206 diarrhoeal death occurred in the fiscal year (FY) 2064/065 (2007/2008). Among top ten reasons for hospitalization, on the same FY, diarrhoeal and gastroenteritis of presumed infectious origin becomes the 2nd leading causes and accounts 5.40% of total patients hospitalized [6]. Many of the water borne diseases can be prevented with appropriate water treatment and proper sanitation and hygiene practices.

Disinfection is an important final step among different steps in drinking water treatment and is the principle barrier for preventing pathogen breakthrough into water supply systems. Chlorination is the most widely used method of disinfection for community water distribution systems and reservoirs; however there may be an increased resistance of bacterial strains to chlorine inactivation [8, 11, 21]. Detection of coliforms in high-quality treated waters is often

attributed to ineffective disinfection and/or bacterial regrowth in distribution systems [16]. The formation of sessile communities and their inherent resistances to disinfectants are potentially a significant cause of persistent and short term deterioration on microbiological water quality in drinking water distribution systems [18].

Bacteria from chlorinated systems were more resistant than those from the unchlorinated waters [7]. Free chlorine has been suggested to be the water quality parameter that reacts with the widest range of contaminants [26]. Chlorination when properly designed and operated, the process is well-developed, inexpensive and efficient. However several drawbacks of chlorine-based disinfection systems have been identified, including the formation of (potentially) hazardous disinfection by-products and the discovery of waterborne microbial pathogens that are relatively resistant to chlorine inactivation. These factors define the risks to be balanced in the design of disinfection systems, and dictate (new) optimization strategies for chlorination [23].

The efficiency of drinking water treatment against the gram negative bacterial isolates should be regularly monitored as water treatment plants couldn't eliminate all coliforms [17]. If chlorine-resistant microbial forms exist, they should be recovered from chlorinated water distribution systems [21]. Therefore this study was carried out to assess the physicochemical and bacterial quality of drinking water and to explore the chlorine resistant gram negative bacteria in the municipal drinking water supplies of Kathmandu Metropolitan City.

MATERIALS AND METHODS

This study was conducted at Environment and Climate Change Laboratory of Nepal Academy of Science and Technology from April 2009 - August 2010. A total of 107 water samples were randomly collected from Bansbari (52) and Mahankalchaur (55) treatment and distribution plant. The water treatment and distribution plant uses the chlorination process with the free residual chlorine of 0.1-0.2 ppm for a contact time of 30 minutes [19].

Physicochemical Analyses of Water Samples

Physicochemical analyses of water samples were performed according to APHA, 1998 like pH by pH meter, turbidity by nephelometer, conductivity by conductivity meter and temperature by thermometer within 4 hrs of collection in laboratory. Field determination of free, combined and total residual chlorine was determined by Chlorometer on spot [1].

Microbiological Sampling and Taxonomic Identification

The samples were collected aseptically from randomly selected tap water in a sterile bottle containing 3% (w/v) sodium thiosulfate solution. The samples were stored on ice and returned to the laboratory (within 4 hrs). Where appropriate portion (100 ml) were filtered through 0.45 μ m Membrane filters and placed in M-endo agar. Samples of 0.1 ml were also spread plated on to plate count agar. The plates were incubated at 35°C for 2 days at which time colonies were enumerated and transferred to PCA slants for cold storage (4°C) and subsequent taxonomic identification. Gram negative bacteria were identified according to characteristics described in Bergey's Manual [10].

DETERMINATION OF BACTERIAL CHLORINE RESISTANCE

Liquid Assay Procedure

Bacteria were grown overnight in tryptic soy broth in an orbital shaker at 23°C. The cells were harvested by centrifugation at 3,500×g for 15 minutes and were washed twice in sterile ice-cold 50mM monobasic potassium phosphate buffer (pH 7.0). After the final centrifugation, the bacteria were suspended in buffer to an optical density of 1.05 (measured at 580 nm), and 10µl of this suspension were transferred to each of 7 reaction tubes containing 10ml of the 50mM phosphate buffer. The final cell concentration in each tube was approximately 4.0×10⁶ per ml. Equi-volume of sample and double strength calcium hypochlorite solution were mixed to obtain the desired half concentration of both sample and calcium hypochlorite solution in next six reaction tubes. The last one tube served as an untreated control and received no chlorine but instead equi-volume of sample and sterile chlorine demand free distilled water was added. The applied calcium hypochlorite concentrations in the tubes were varied from 0 to 1.25 mg/l. After time (t) = 0, 15, 30, 45 and 60 minutes at 23°C, 100µl of sterile 1.0 M sodium thiosulfate was added to each tube. The tubes were chilled to 2°C, and portions were plated on Plate Count Agar to rescue surviving bacteria. The plates were incubated at 35°C for 48 hrs, at which time colonies were enumerated [21].

RESULTS AND DISCUSSION

Physicochemical and Microbiological analyses

The conductivity of all water samples was found within the WHO guideline value but not all of the samples for pH, turbidity and free residual chlorine was found within the WHO guideline value. The bacteriological analysis of water samples showed that 60 (56.1%) samples were above the WHO guideline value for total coliform and heterotrophic plate count each. A total of 134 bacteria of 10 different genera were isolated and identified, where *E. coli* was predominant followed by *Citrobacter* spp., *Shigella* spp., *Enterobacter* spp., *Providencia* spp., *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., *Proteus* spp. and *Edwardsiella* spp. There was a significant relation between the temperature (p= 0.002), free residual chlorine (p= 0.037) and the growth of the organisms but no significant relation between the conductivity (p=0.969), pH (p=0.454) and turbidity (p= 0.164) (Table 1). The correlation between heterotrophic plate count and free residual chlorine was found insignificant (p= 0.096) with negative correlation (r= -0.162). Likewise, the correlation between total coliform count and free residual chlorine was found significant (p= 0.001) with negative correlation (r= -0.383) (Figure 1 and 2).

Table 1 Difference of Conductivity, pH, Temperature and Turbidity between the growth of organism

Variables	Growth (N=71)	No growth (N=36)	p-value
Conductivity	58.28±24.499	58.47±24.754	0.969
pH	7.199±0.413	7.264±0.444	0.454
Temperature	16.596±3.962	14.328±1.885	0.002
Turbidity	1.797±4.870	0.656±0.350	0.164

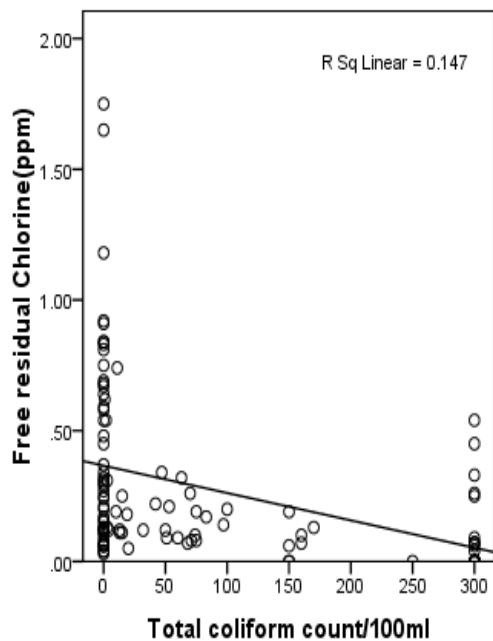


Figure 1 Relation between Free residual chlorine and Total coliform counts

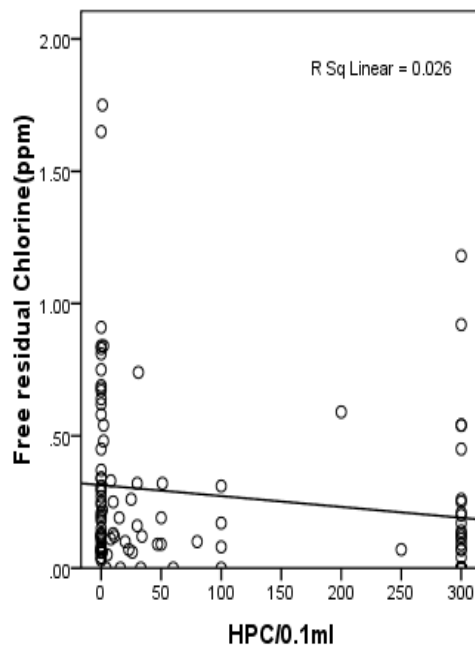


Figure 2 Relation between Free residual chlorine and Heterotrophic plate count

Microbial quality of drinking water was assessed by physicochemical and microbial parameters. The temperature of the analyzed water varied between 10.2 to 26.3°C with the average temperature 15.8 °C. High temperature has negative impact on water quality by enhancing microbial growth and may increase taste, odor, color and corrosion problem [25]. However, previously average temperature of drinking water of Kathmandu was slightly higher ranging from 18.7 - 19.2 °C [2, 24]. All water samples for conductivity was found to be within WHO guideline value ($\leq 1500 \mu\text{S}/\text{cm}$). In agreement to this study, it was reported all 111 water samples having conductivity within WHO guideline value. As for drinking water, it should remain constant; a sudden increase of conductivity indicates pollution from industrial and domestic waste water [8]. pH of 3.7% was found to be below the WHO guideline value (6.5-8.5). It was reported in agreement with this study that 6.66% samples were below WHO guideline value [12]. Previously, it was found that all tested water samples were within WHO guideline value [3, 9, 20]. pH of water gets changed with time due to exposure to air, biological activity and temperature changes [25]. Turbidity of 93.3% water samples was within WHO guideline value ($\leq 5 \text{ NTU}$). Similarly, it was revealed the turbidity varied upto 48 NTU [3]. Turbidity may result from insufficient filtration during water treatment of sediments, mineral precipitates or biomass within the distribution system [25]. The water samples collected were of high quality according to the current standards of Nepal except few samples which crossed the permissible limits.

The microbiological analyses of water revealed that 56.1% samples were above the WHO guideline value for the heterotrophic plate and total coliform count each. There was a decreasing trend of microbial contamination from 2007 to 2009 [9, 20, 24]. Coliform have been used extensively as the basis for regulating the microbial quality of drinking water. Good quality drinking water can suffer serious contamination in distribution systems because of breaches in the integrity of pipelines and storage reservoirs.

Physico-chemical parameters influence the growth of microorganism in water [14]. Increase in temperature increases the bacterial growth ($p= 0.002$) whereas increase or decrease of pH ($p= 0.454$), turbidity ($p= 0.164$) and conductivity ($p= 0.969$) didn't affect the microbial growth. The association between the growth of organisms and the FRC was found to be significant ($p= 0.037$) means microbial load decreases with the increase in FRC. The correlation between the FRC and HPC was found to be negatively correlated ($r= -0.162$) and insignificant ($p= 0.092$) means FRC and HPC didn't affect each other. But the correlation between the FRC and total coliform count (TCC) was found to be negatively correlated ($r= -0.383$) and significant ($p= 0.001$) means FRC and TCC affect each other. Similarly, it was reported that the correlation between FRC and HPC was negatively correlated and was found to be insignificant at ($p<0.05$) in Balaju reservoir but was found significant in Sundarighat reservoir. But the correlation between FRC and TCC was found to be negatively correlated and was significant in Sundarighat reservoir but was found insignificant in Balaju reservoir [9].

Bacterial Chlorine Resistance

Bacterial isolates of eight different genera namely *Escherichia coli*, *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp., *Shigella* spp., *Salmonella* spp., *Providencia* spp. and *Pseudomonas* spp. were tested and found to be chlorine resistant at concentration of 0.2 mg/l for a contact time of 30 minutes. Time required reducing the organisms by 3-log ($T_{99.9}$) and 4-log ($T_{99.99}$) at Nepal standard of 0.2 mg/l of residual chlorine concentration were found to be less than 30 minutes and greater than 60 minutes for all eight different types of organisms tested. Log inactivation of various organisms with the residual concentration at Nepal Standard was found to be the highest reduction of *Citrobacter* spp. (3.54-log) to the lowest of *Escherichia coli* (3.097-log) (Table 2).

Table 2 Log inactivation of various organisms with chlorine concentration of 0.2 mg/l for a contact time of 30 minutes

Organisms	Log inactivation
<i>Citrobacter</i> spp.	3.545
<i>Klebsiella</i> spp.	3.426
<i>Shigella</i> spp.	3.260
<i>Providencia</i> spp.	3.240
<i>Pseudomonas</i> spp.	3.240
<i>Enterobacter</i> spp.	3.190
<i>Salmonella</i> spp.	3.125
<i>Escherichia coli</i>	3.097

Eight different genera of bacterial water isolates tested by chlorine assay method were found to be chlorine resistant at concentration of 0.2 mg/l of residual chlorine for a contact time of 30 minutes (Nepal Standard). It was found that total coliform were present in those samples which were within guideline value for free residual chlorine [9, 22]. It was showed that the attachment of bacteria to surfaces provided the greatest increase in disinfection resistance. Other mechanisms which increased disinfection resistance included the age of the biofilm, bacterial encapsulation and previous growth condition which increased resistance to chlorine from 2 to 10-fold. These resistance mechanisms were multiplicative [15].

Survival of the opportunistic pathogenic microorganisms and its persistence in the water distribution systems may be due to their capability to resist chlorine. The probable mechanism include: modification of cell surface structures which may lead to increase aggregation or clumping of cells in situ, microbial adhesion to pipe surfaces or to suspended particulate matter such as detritus or clay particles, extrusion of protective extracellular capsular or slime layers and formation of resistant spores.

The fact that relatively high numbers of microorganism could be routinely isolated from the chlorinated distribution plants suggests that certain bacteria may possess mechanisms enabling them to survive in chlorinated environments. Addition studies should be conducted to determine which mechanism lead bacteria to be resistant to chlorine as our study showed all the eight different genera of bacterial isolates tested were resistant.

Gram negative bacterial isolates were found to be chlorine resistant at 0.2 mg/l for a contact of 30 minutes tested. Present Nepal guideline (0.2 mg/l) didn't seem to be sufficient to disinfect at a contact time of 30 minutes. However, overall quality of drinking water and effective chlorination can be generalized by investigating other concerned parameters.

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