

Study on Microbiological Quality Analysis of Tap-Water and the Effects of Halo-tab Plus Tablet on Water Born Microorganisms.

Nafisa Tabassum¹, Mousumi Akter², Mrityunjoy Acharjee^{2,*}

¹School of Life Science (SLS), Independent University, Bangladesh (IUB), Plot16, Block B, Aftabuddin Ahmed Road, Bashundhara, Dhaka 1229.

²Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka 1217.

*Corresponding Author: Mrityunjoy Acharjee

Abstract: Present study attempted to focus on the existence of coliform, fecal contamination and others pathogenic microorganisms in tap water as well as the effects of halo-tab plus as disinfectant to reduce the growth of water born microorganisms. Total 11 tap water samples are collected from different household and restaurant in Dhaka city Bangladesh. Elevated number of fecal coliforms, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Pseudomonas* spp. was monitored among the tap water. The heterotrophic bacteria was present within the range of 10^2 to 10^8 cfu/ml. Most of the isolates were found to be resistant against ampicillin (10 µg), ciprofloxacin (5 µg), ceftriazone (30 µg), penicillin (10 µg), nalidixic acid (30 µg) and vancomycin (30 µg). All the pathogens including coliform and fecal coliform were found to be reduce 100% after the treatment of halotab-plus. Nevertheless, the municipal water of the study area was microbiologically unsafe, and the propagation of drug resistant strains was assumed to escalate the public health threat.

Key words: Water born microbes, Antibiotic resistance; Halotab-plus

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I. Introduction

Safe drinking water is the main aspect to ensure the public and environmental health safety, which means that it must be free from pathogenic bacteria^{1,2,3}. Several water born pathogenic bacteria are frequently disseminated in water sources, among them enteric pathogens are the striking issue for the water microbiologist as well as environmental scientist¹. As a consequence, sources of fecal pollution in waters devoted to human activity must be strictly controlled. Enteropathogens, such as *Escherichia coli* O157:H7, are generally present at very low concentrations in environmental waters within a diversified microflora. Complex methods are required to detect them, and these are extremely time-consuming^{1,4}. In most of the cases coliforms are present in huge array surrounded by the intestinal flora of humans and other warm-blooded animals, and are thus found in fecal wastes^{1,5}.

Therefore, coliforms, detected in higher concentrations than pathogenic bacteria, are used as an index of the potential presence of enteropathogens in water environments^{1,4}. The use of the coliform group, and more specifically *E. coli*, as an indicator of microbiological water quality dates from their first isolation from feces at the end of the 19th century⁶. Coliforms are also routinely found in diversified natural environments, as some of them are of telluric origin, but drinking water is not a natural environment for them⁶⁻⁹. Their presence in drinking water must at least be considered as a possible threat or indicative of microbiological water quality deterioration. Positive total coliform samples in a treated water which is usually coliform-free may indicate treatment ineffectiveness, loss of disinfectant, breakthrough¹⁰, intrusion of contaminated water into the potable water supply^{11,12} or regrowth problems¹³ in the distribution system, and, as a consequence, should not be tolerated. The use of the coliform group as an indicator of the possible presence of enteric pathogens in aquatic systems has been a subject of debate for many years. Many authors have reported waterborne disease outbreaks in water meeting the coliform regulations¹⁴⁻¹⁷. However, different methods are available for the purification of drinking water such as boiling, filtration, sieving and others^{1,3}. Considering all these fact, present study was designated to chalk out the existence of different microorganisms as well as detect the effects of halo-tab plus tablet to eliminate the growth of water borne pathogens.

II. Material And Methods

Study Area, Sampling

The study was designed to determine the quality of commercially available tap water collected from different house hold and restaurant in Dhaka city. Total 11 water samples were collected and transported in the laboratory^{18,19}.

Microbiological quality analysis of water samples

An aliquot of 0.1 ml of each sample was introduced on to nutrient agar, MacConkey agar and Membrane fecal coliform (mFC) agar through spread plate technique for the isolation of total viable bacteria (TVB), coliform (*E. coli*, *Klebsiella* spp.) and fecal coliform consecutively. Plates were incubated at 37 °C for 24 hours excluding mFC agar plates which were incubated at 44.5 °C. Presence of green metallic sheen on EMB agar media was further specified the presence of *E. coli*. Manitol Salt agar and TCBS agar were used to determine the *Staphylococcus* spp. and *Vibrio* spp. For the final identification all isolates were biochemically analyzed by following the standard methods^{18,19}.

Biochemical identification of other gram negative Coliform bacteria

Besides the presence of *E. coli* colony with green metallic sheen, there were also other colonies in the EMB medium which were identified by using standard biochemical methods to find a complete microbiological profile of the drinking water samples^{18,19}.

Determination of antibiotic susceptibility pattern of the isolates

All the isolates were tested for antibiotic susceptibility against 16 antibacterial drugs (including first, second and third generation drugs) by disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) with antibiotic discs (Neo-Sensitabs, Rosco, Denmark) according to the modified Kirby-Bauer method²⁰. A single colony of each isolate was inoculated into 2 ml of Mueller-Hinton broth, and incubated at 37 °C for 4 hours. The culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and spread evenly over the entire surface of Muller-Hinton agar. Antibiotic discs of appropriate concentrations (ampicillin 10 µg, amoxicillin 10µg, ciprofloxacin 5 µg, ceftriazone 30µg, nalidixic acid 30 µg, imipenem 10 µg, tetracycline 30 µg, erythromycin 15 µg, kanamycin 30 µg, chloramphenicol 30 µg, azithromycin 15µg, trimethoprim-sulfamethoxazole 25µg, gentamycin 10µg, penicillin 10µg, vancomycin 30µg, ofloxacin 5 µg and piperacillin 10 µg) were placed aseptically over the surface at appropriate spatial distance of 5 mm. Plates were then inverted and incubated at 37 °C. After 24 hours, plates were examined and the diameters of the zones of inhibition were measured and interpreted as susceptible, intermediate and resistant²¹.

Water treatment strategy

Water samples were treated with one very common disinfectants, e.g. Halotab (Halazone USP, 15 mg; Sonear Laboratories Ltd., Dhaka, Bangladesh). Halotab was used as per the instruction of the manufacturer, i.e. one tablet per 3 l of water for 30 min²².

III. Results

Propagation of coliform and fecal coliform in drinking water due to the poor sanitation and hygienic condition are the main causative agent of diarrhea and dysentery^{4,23}. Such diseases are very common in developing countries like Bangladesh in where the water distribution line and sewerage line are present in same way^{1,2,4}. However, peoples are more reliable on the filtration and boiling methods to reduce the growth of water born infectious pathogens¹. Considering the consumers' health safety present study attempted to focus on the treatment procedure like boiling and filtration along with alam potash, Zeoline₋₂₀₀, Halotab, Bleaching powder. Another important aspect of our study was to detect the resistant properties of all isolates found in the samples.

Pathogenic prevalence along the municipal water

High numbers of pathogenic bacteria were observed in supplied water which was further biochemically identified (Tables 1 & 2). The heterotrophic bacterial count was observed in all the samples within the range of 10²cfu/ml and 10⁸cfu/ml. The fecal contamination and *Klebsiella* were detected in samples 1,4,6&8 up to 10³cfu/ml while the *E. coli* was present in sample 1,4&8 up to 10⁸ cfu/ml. The growth of *Salmonella* spp. and *Shigella* spp. were present within the range of 10² – 10³ cfu/ml. The growth of *Salmonella* spp. was present in almost all samples except sample 1,6,9&10 likewise the *Shigella* spp. was only absent in samples 4,8&10. The growth of *Pseudomonas* spp. was noticed up to 10⁵cfu/ml in sample 5,6,7,8&9 likewise the presence of

Staphylococcus spp. was found up to 10³cfu/ml in sample 1,2,3,6&7. On the other hand most of the samples were free from *vibrio* spp. contamination only sample 6 showed the growth (table 1).

The presence of coliform and fecal coliform in drinking water indicates that the presence of other pathogens which may lead the various water borne diseases ^{1,5}.

Table 1: Microbiological assessment of tap water.

Sample Number	HPC (cfu/ml)	FCC (cfu/ml)	<i>E. coli</i> (cfu/ml)	<i>Klebsiella</i> spp. (cfu/ml)	<i>Salmonella</i> spp. (cfu/ml)	<i>Shigella</i> spp. (cfu/ml)	<i>Vibrio</i> spp. (cfu/ml)	<i>Pseudomonas</i> spp. (cfu/ml)	<i>Staphylococcus</i> spp. (cfu/ml)
Supplied water									
S01	2.6×10 ⁷	2.3×10 ³	2×10 ⁶	2×10 ²	0	2×10 ²	0	0	6.2×10 ²
S02	5.8×10 ⁶	0	0	0	5.9×10 ²	4.8×10 ²	0	0	1.3×10 ³
S03	7.5×10 ⁸	0	0	0	1.1×10 ²	4.4×10 ³	0	0	6.6×10 ²
S04	3.7×10 ⁶	3.1×10 ³	1.8×10 ²	1.4×10 ²	3.3×10 ³	0	0	0	0
S05	5.6×10 ⁵	0	0	0	4.8×10 ²	3×10 ²	0	7×10 ²	0
S06	1.7×10 ⁴	5.3×10 ²	0	2×10 ³	0	9.3×10 ²	2.9×10 ²	5×10 ⁵	9.5×10 ²
S07	9.9×10 ²	0	0	0	1.7×10 ²	4.5×10 ³	0	4.5×10 ³	2×10 ²
S08	7.7×10 ³	1×10 ²	7.8×10 ⁴	2×10 ²	4.5×10 ²	0	0	8.8×10 ²	0
S09	2.0×10 ⁵	0	0	0	0	8.4×10 ²	0	6×10 ⁵	0
S10	1.7×10 ⁸	0	0	0	0	0	0	0	0

HPC: Heterotrophic bacteria; FCC: Fecal coliform count

Biochemistry of the isolates.

Total eight types of biochemical test showed their result for the confirmation of *E. coli* and *Klebsiella* spp. (Table 2). The growth of *E. coli* and *Klebsiella* spp. on MacConkey agar plates were further transferred on to the EMB media and 4 samples among 11 were found as *E. coli* by observing green metallic sheen.

Table 2: Biochemical tests of different isolates

Assumed Pathogenic microorganisms	TSI				Motility	Indole Production	MR	VP	Citrate utilization	Catalase	Oxidase
	Slant	Butt	Gas	H ₂ S							
<i>E. coli</i>	Y	Y	+	-	+	+	+	-	-	+ve	-ve
<i>Klebsiella</i> spp.	Y	Y	+	-	+	-	-	-	+	+ve	-ve
<i>Vibrio</i> spp.	R	Y	-	-	+	-	+	-	-	+ve	+ve
<i>Staphylococcus</i> spp.	Y	Y	-	-	+	-	+	-	-	+ve	-ve
<i>Pseudomonas</i> spp.	R	Y	-	-	+	-	+	-	-	+ve	+ve
<i>Salmonella</i> spp.	R	Y	-	-	+	-	+	-	-	-ve	-ve
<i>Shigella</i> spp.	R	Y	+	-	-	+/-	+	-	-	-ve	-ve

All the experiments have been done three times and the results were reproducible. One representative data have been shown.

- TSI Triple Sugar Iron Test
- Y Yellow (Acid)
- R Red (Alkaline)
- MR Methyl red
- VP Voges-Proskauer

Prevalence of drug resistant bacteria in the supplied water

For most of the pathogenic isolates, higher rates of resistance were found against ampicillin (10 µg), ciprofloxacin (5 µg), ceftriazone (30 µg), penicillin (10 µg), nalidixic acid (30 µg), and vancomycin (30 µg) (Table 7). On the other hand, most of the isolates were found to retain higher sensitivity against imipenem, gentamycin and azithromycin.

Table 3. Antimicrobial susceptibility pattern of different pathogenic isolates found in the tap water.

Organisms	<i>E. coli</i> n=3		<i>Klebsiellasp.</i> N=4		<i>Shigellaspp.</i> N=7		<i>Salmonella spp.</i> N=6		<i>Vibrio spp.</i> N=1		<i>Pseudomonasp</i> p. N=5		<i>Staphylococcus spp.</i> N=5	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
AMP (10µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
CIP (5µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
CEF (30µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
IPM (30µg)	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
PEN (10µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
GEN(10µg)	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
AZI (15µg)	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
NALI (30µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
VAN(30µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
ERY (15 µg)	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%

Effects of halo-tab tablet to reduce the growth of water born micro-flora

As described previously, that the people are very much familiar with several water treatment procedures in their daily life such as boiling, filtration and sieving. Additionally they also use chlorine, alum-sulfate and halo-tab^{1,3,22}. In our study, the effect of halo-tab tablet was very remarkable (table 4). Most of the pathogens were found to be eliminated 100% after the treatment of halo-tab plus²². Only the growth of heterotrophic bacteria was presence but the load was recorded at least 2 log lower than the previous count (table 4).

Finally, our study reported that the some of the processed drinking water samples were not recommended for drinking because of the presence of indicator bacteria *E. coli* and *Klebseillaspp*, as coliform, which also indicated the possible presence of other pathogenic bacteria. However, the use of halotab-plus can be a good water treatment tools if the users can apply it in proper doges. There are several factors such as environmental contamination, inadequate processing and improper handlings are accountable behind the contamination of drinking water. Besides, the presence drug resistance traits in the identified isolates might be responsible to make serious obstacle to eradicate the water associated diseases.

Table 4:Microbiological assessment of tap water after the treatment with halotab-plus tablet

Sample Number	HPC (cfu/ml)	FCC (cfu/ml)	<i>E. coli</i> (cfu/ml)	<i>Klebsiella spp.</i> (cfu/ml)	<i>Salmonella spp.</i> (cfu/ml)	<i>Shigella spp.</i> (cfu/ml)	<i>Vibrio spp.</i> (cfu/ml)	<i>Pseudomonas spp.</i> (cfu/ml)	<i>Staphylococcus spp.</i> (cfu/ml)
Supplied water									
S-01	2.6×10 ⁵	0	0	0	0	0	0	0	0
S-02	5.8×10 ⁴	0	0	0	0	0	0	0	0
S-03	7.5×10 ⁶	0	0	0	0	0	0	0	0
S-04	3.7×10 ⁴	0	0	0	0	0	0	0	0
S-05	5.6×10 ³	0	0	0	0	0	0	0	0
S-06	1.7×10 ²	0	0	0	0	0	0	0	0
S-07	9.9×10 ²	0	0	0	0	0	0	0	0
S-08	7.7×10 ²	0	0	0	0	0	0	0	0
S-09	2.0×10 ³	0	0	0	0	0	0	0	0
S-10	1.7×10 ⁶	0	0	0	0	0	0	0	0

IV. Conclusion

Polluted water associated diseases are the major problem for the communities of developing countries due to the lack of knowledge on sanitation, unhygienic management of environment and water bodies, lack of education and training, and the extraordinary burden of wastes saturated with pathogenic microorganisms. In current research, we detected *E. coli* and *Klebsiella spp.* as coliform which indicate the presence of other

pathogenic bacteria which is very alarming for the consumers. However, the important aspect of this study is to evaluate the effects of halotab-plus tablet to ensure the quality of tap water. This solution was able to kill the pathogens successfully from the tap water. Therefore, the consumers and others company can implement this methods to eliminate the existence of water microbes as well as water born disease.

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