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Quality Analysis of Dhaka WASA Drinking Water: Detection and Biochemical Characterization of the Isolates

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Abstract
The aim of the study was to assess the microbiological quality of Dhaka WASA drinking water. A total of 45 samples were collected from different outlets of WASA water supply chain. Among the these samples 29 samples were collected from house tap, 5 samples from street pipe line tap and 11 samples from WASA source pump. The results of the Total Viable Count (TVC) showed that 62 % samples of house tap water, 60 % pipeline water and 45.45 % WASA pump water were exceeded the BDS standard (1240:2001) and WHO Guideline for drinking. The highest count was 2×10^6 cfu/ml in the house tap water of Gandaria. Total coliform and *E. coli* count ranged from <1.8 (MPN) /100 ml to >1600 (MPN)/100 ml. Among all the tested samples, 57.78 % water samples were positive for coliform and 51.11 % samples were positive for *E. coli* bacteria. Out of twenty three *E. coli* isolates, 8 isolates were subjected to biochemical and microscopic examination for confirmation. All 8 isolates were detected as *E. coli* based on biochemical parameters. The antibiotic sensitivity pattern of those isolates was determined. Most of them were found resistant to Ampicillin, Amoxicillin, kanamycin, Penicillin, Sulphomethoxazole antibiotics. Nearly all of them were found sensitive to Gentamycin and Nalidixic acid. The samples collected from different house tap water and road side tap water were more contaminated than WASA source pump water. It may therefore be concluded that distribution lines of Dhaka WASA supply chain might be the main source of microbiological contamination of drinking water. In this regard further investigations with more representatively drawn samples are required.
Key words: Drinking Water, Microbial Contamination, Indicator microorganisms, Coliform, *E. coli*

Introduction

Dhaka, the capital city of Bangladesh, has become a megacity with a population of nearly 12.5 million, which is increasing at an annual rate of over 5% (Haq, 2006). In order to meet the ever increasing demand of safe drinking water, Dhaka Water Supply Authority (DWASA) of Bangladesh has installed a number of deep tube wells that tap the upper aquifers. However, in most parts of the city, the current groundwater abstraction exceeds the recharge rate, causing the ground water to be mined systematically and be depleted of its reserve. Thus, there is an urgent need to alleviate the demand on the upper aquifers and explore more sustainable sources to augment the present water supply. This implies a conjunctive use of groundwater and surface water in order to maintain the balance between anthropogenic demand and water's natural availability. However, the surface water along these peripheral rivers is known to be highly polluted due to municipal and industrial untreated wastewaters that are discharged (Subramanian, 2004; Kamal *et al*., 1999).

Water is essential to sustain life, and a quality (adequate, safe and accessible) supply must be ensured to all. Water is unsafe for human consumption when it contains pathogenic, or disease-causing microorganisms. The high prevalence of diseases such as diarrhoea, typhoid fever, cholera and bacillary dysentery among the populace has been traced to the consumption of unsafe water and unhygienic drinking water production practices (Mead *et al*., 1999). The most dangerous form of water pollution occurs when faecal contaminants enter the water supply. Pathogens such as *Salmonella* spp, *Shigella* spp, *Vibrio cholerae* and *E. coli* being shed in human and animal faeces ultimately find their way into water supply through seepage of improperly treated sewage into ground water (DiPaola, 1998). Inadequate sanitation and unhygienic practices account for the major source of microbial contamination of any potable water (Sahota, 2005).

Microbiological water analysis is a method of analysing water to estimate the numbers of microbes present and, if needed, to find out what sort of microbes they are. It is very expensive and time consuming process to test for all the possible microbial pathogens in water, so a single group of microorganisms that came from the same source as human pathogens is used to indicate the presence of pathogens. In 1914, the U.S. Public Health Service adopted the use of coliform bacteria as "indicator microorganisms" to indicate the presence of faecal contamination in water. Ideally, if indicator microorganisms are detected in a substance, it indicates the presence of faecal contamination and therefore possible presence of pathogenic microorganisms in the water. The coliform bacteria group consists of several genera of bacteria belonging to the family *Enterobacteriaceae.* These mostly harmless bacteria live in soil, water, and the digestive system of animals. Faecal coliform bacteria, which belong to this group, are present in large numbers in the faeces and intestinal tracts of humans and other warm-blooded animals, and can enter water bodies from human and animal waste. If a large number of faecal coliform bacteria (over 200 colonies/100 millilitres (ml) of water sample) are found in water,

*Correction note: The authors name has been revised.

it is possible that pathogenic (disease- or illness-causing) organisms are also present in the water.

Indicator microorganisms are tested for because they are easier and cheaper to test for all the possible pathogens that might be present. It is very important to note the presence of coliforms, faecal coliforms, or even *Escherichia coli* in water does not mean that pathogenic microorganisms are present. It only gives an indication that they might be present. Presence of coliform or faecal coliform bacteria does not determine whether a sample will make someone ill.

The current study was designed to resolve the microbiological quality of household water and source water of WASA collected from different location of Dhaka city which were mainly used in drinking and other household purpose. As the microbiological condition is very important for water quality, so one of the aim of this piece of research work was to find out the possible cause of faecal contamination that might cause severe waterborne fatal disease.

Materials and Methods

The whole experiment was carried out in Food microbiology section, Institute of Food Science & Technology (IFST), BCSIR, Dhaka.

Sample collection

Total 45 samples were collected from different WASA water pump, WASA distribution pipe line and different houses. Experiments were carried out within 1-8 hours after collecting the samples. As a negative control 20% blank sample (distilled water) was used in this investigation. All the samples were kept at 4°C until these were analyzed.

Enumeration of Total Viable Count (TVC) by pour plate method

Serial dilutions of water samples were made up to 10^{-4} dilution. Pour plate method using Plate Count Agar (PCA) was applied to enumerate total bacteria in water sample. The Plates having 25-250 colonies after incubation were selected for counting. The TVC was determined by multiplying colony numbers with reciprocal dilution factor and reported as CFU/ml and the results per dilution counted were recorded.

The Most Probable Number (MPN) method

The Most Probable Number (MPN) method is a statistical, multi-step assay consisting of presumptive, confirmed and completed phases. In the assay, serial dilutions of a sample were inoculated into broth media. Then the number of gas positive (fermentation of lactose) tubes were scored, from which the other 2 phases of the assay were performed and then the combinations of positive results were used to consult a statistical tables, to estimate the number of organisms present. Typically only the first 2 phases were performed in coliform analysis, while all 3 phases were done for *E. coli*.

Presumptive test for coliforms and *E. Coli*

For presumptive test of coliform and *E. coli* sterile LST broth were used. For each sample 10 ml was transferred in 5 LST tubes by sterilized pipette, 1ml was transferred in 5 tubes by sterilized micropipette, 0.1ml was transferred in 5 tubes. LST tubes were then incubated at 35°C tubes were examined and recorded reactions at 24 ± 2 h for gas. From each gassing LST tube, a loopful of suspension to a tube of BGLB broth was transfer, avoiding pellicle if present. BGLB tubes at 35°C Incubated and examined for gas production at 48 ± 2 h. most probable number (MPN) of coliforms was Calculated based on proportion of confirmed gassing LST tubes for 3 consecutive dilutions.

From each gassing LST tube from the Presumptive test, a loopful of each suspension was transferred to a tube of EC broth. EC tubes were Incubated 24 ± 2 h at 45.5 °C in a circulatory water bath and examined for gas production. Results of this test were used to calculate coliform MPN.

Completed test for *E. Coli*

To perform the Completed test for *E. coli*, each gassing EC tube was gently agitated and streaked for isolation, a loopful to a EMB agar plate and incubated for 18-24 h at 35°C. Plates for suspicious *E. coli* colonies, i.e., dark centered and flat, with or without metallic sheen was examined.

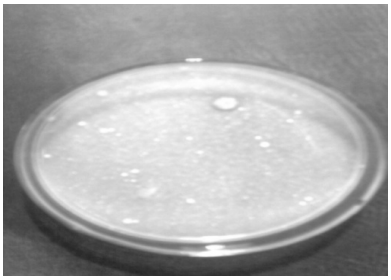

Fig. 1. Total viable bacteria on PCA plate

Fig. 2. LST broth

Fig. 3. BGBB broth

Fig. 4. EC broth Biochemical studies of the *E. coli* isolation

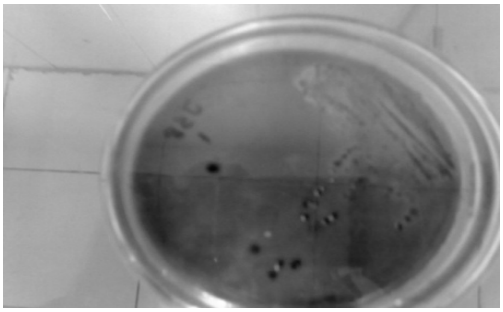

Fig. 5. EMB plate with green metallic sheen of *E.coli*

According to the Bergey's manual of systematic bacteriology, several biochemical tests were performed to study the biochemical characteristics of the *E.coli* bacteria found in EMB plate culture. Eight isolates were selected for the biochemical studies. The tests were -Urease production test, Oxidase test, Catalase reaction, Indole test, Citrate test, Methyl-red test, Voges-proskauer (V.P.) test , Motility test,

Fermentation test:
Arabinose, Rhamnose, Xylose,Hexose: Glucose, Sucrose, Lactose, Mannitol

Antimicrobial sensitivity testing
The antimicrobial sensitivity testing was done by the agar disk diffusion method as described by NCCLS (2000). The antimicrobial agent used were- ampicillin, (AMP), amoxicillin (AML), chloramphenicol (C), erythromycin, (E), tetracycline (T), gentamicin (G), sulphomethoxazole (S), kanamycin (K), penicillin (p) , nalidixic acid (N)

Results and Discussion

The results of enumeration of TVC and enumeration and detection of coliform and *E. coli* were documented in table 1.

TVC is not a direct indicator of faecal contamination; it does indicate variation in water quality and potential for pathogen survival and growth. It was recommended that the TVC should not exceed 500 per ml in tap water (LeChevalliar *et al.,* 1980). According to WHO (1996) and BDS (1240:2001) the standard for TVC is 1×10^3 cfu/mL. The present study showed that Total bacterial Count in water sample ranged from <1.8 cfu/ml to 2.2×10^6 cfu/ml. From the results of total viable count it was found that among 45 samples 25 samples are within BDS and WHO standard 26 samples are exceed the standard. 62% house tap water and 60% pipe line water and 45.45% pump water exceed the BDS and WHO standard. So most of contamination occur in pipe line and house tap water. Most of the pump water of WASA which use deep tube well is free of bacterial load.
In this study, among the 45 samples 57.78% samples exceed the BDS standard and WHO guideline for coliform bacteria and 51.11% for *E. coli* bacteria. Total Coliforml and *E.coli* Count in water samples ranged from <1.8 to >1600(MPN)/100 ml. These values for Total coliform and *E .coli* are unacceptable for drinking water (WHO, 1996). The highest amount was found in house tap water. So the source of contamination mainly is the distribution system of water.

Table 1. Result of total viable count and enumeration of coliform, *E. coli* of supplied water of Dhaka city

SL.	Sample ID	Site of location	TVC Cfu/ml	Coliform (MPN/100ml)	E.coli (MPN/ 100ml)	BDS standard and WHO guide line is $<10^3$/ml for TVC and 0 (MPN)/100 ml
01	VS1	WASA pump, deep tubewell	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
02	VS2	WASA pump, deep tubewell	0	<1.8	<1.8	Values are within BD Standard &WHO Guideline
03	VS3	House tap	65	33	11	Values exceed BDS Standard&WHO Guideline for coliform and E.coli
04	VS4	House tap	78	7.8	7.8	Values exceed BDS Standard &WHO Guideline for coliform And E.coli
05	VS5	House tap	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
06	VS6	House tap	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
07	VS7	House tap	1.8×10^4	<1.8	<1.8	Values exceed BDS Standard &WHO Guideline for TVC
08	VS8	House tap	2.2×10^4	7.8	4.5	Values exceed BDS Standard &WHO Guideline
09	LS1	Pipe line	2×10^3	23	23	Values exceed BDS Standard &WHO guideline
10	LS2	WASA pump, deep tubewell	1.8×10^3	49	49	Values exceed BDS Standard &WHO Guideline
11	LS3	House tap	2.0×10^3	>1600	>1600	Values exceed BDS Standard &WHO Guideline
12	LS4	WASA pump, deep tubewell	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
13	LS5	House tap	2.0×10^3	>1600	>1600	Values exceed BDS Standard &WHO Guideline
14	LS6	Tap water mixed connection	2.0×10^6	>1600	>1600	Values exceed BDS Standard &WHO Guideline
15	LS7	Road side tap mixed connection	2.2×10^3	>1600	54	Values exceed BDS standard & WHO guide line
16	LS8	Road site tap beside WASA pump	0	<1.8	<1.8	Values are within BDS standard & WHO guide line
17	B1	Blank sample		<1.8	<1.8	Values are within BDS standard & WHO guide line
18	B2	Blank sample	0	<1.8	<1.8	Values are within BDS standard & WHO guide line
19	ARD	Analytical research division, Dhaka lab	0	<1.8	<1.8	Values are within BDS standard & WHO guide line
20	H1	Tap water	25	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
21	H2	Tap water	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
22	H3	Tap water	2.5×10^4	150	9	Values exceed BDS Standard &WHO Guideline
23	KS1	House Tap	52	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
24	KS2	WASA Pump Deep tubewell	200	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
25	KS3	House Tap	25	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
26	KS4	House Tap	180	4.5	<1.8	Values exceed BDS Standard &WHO Guideline for coliform

27	KS5	Pipe line	160	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
28	KS6	WASA Pump Deep tubewell	43	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
29	KS7	Pipe line	2.0×10^5	3.6	<1.8	Values exceed BDS Standard & WHO Guideline for TVC & coliform
30	KS8	Hotel Tap	2.0×10^5	20	<1.8	Values exceed BDS Standard &WHO Guideline for TVC & coliform
31	KS9	Treated water Of Chandighat treatment plant	1.5×10^4	240	240	Values exceed BDS Standard &WHO Guideline
32	KS11	House Tap	2.4×10^3	920	920	Values exceed BDS Standard &WHO Guideline
33	KS12	Blank sample	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
34	JS1	House Tap	2.50×10^5	>1600	>1600	Values exceed BDS Standard &WHO Guideline
35	JS2	House Tap	2.4×10^5	>1600	28	Values exceed BDS Standard &WHO Guideline
36	JS3	House Tap	7.2×10^2	<1.8	<1.8	Values are within BDS Standard &WHO Guideline for TVC
37	JS4	WASA Pump Deep tubewell	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
38	JS5	House Tap	2.50×10^5	>1600	>1600	Values exceed BDS Standard &WHO Guideline
39	JS6	House Tap	1.24×10^3	250	>1600	Values exceed BDS Standard &WHO Guideline
40	JS7	House Tap	2.16×10^4	>1600	>1600	Values exceed BDS Standard &WHO Guideline
41	JS8	WASApump,Deep tubewell	2.50×10^3	<1.8	<1.8	Values are within BDS Standard &WHO Guideline for TVC
42	JS9	Blank sample	0	<1.8	<1.8	Values are within BDS Standard &WHOGuideline
43	MS1	House Tap	2.2×10^5	240	240	Values exceed BDS Standard &WHOGuideline
44	MS2	House Tap	1.8×10^5	920	540	Values exceed BDS Standard &WHO Guideline
45	MS3	House Tap	180	240	79	Values exceed BDS Standard &WHO Guideline
46	MS4	WASA pump, Deep tubewell	2.2×10^3	0	0	Values exceed BDS Standard &WHO Guideline for TVC
47	MS5	WASA pump, Deep tubewell	2.5×10^3	79	49	Values exceed BDS Standard &WHO Guideline
48	MS	House Tap	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline
49	MS7	House Tap	2.5×10^5	24 0	49	Values exceed BDS Standard &WHO Guideline
50	MS8	House Tap	4.8×10^3	240	23	Values exceed BDS Standard &WHO Guideline
51	MS9	Blank sample	0	<1.8	<1.8	Values are within BDS Standard &WHO Guideline

Note: blank samples were done for the quality control of the processes

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Generic percentage of water samples:

The percentage of bacteria found from three different sources exceeded BDS standard and WHO guideline (figure 8). It was found that house tap water had the highest prevalence of bacteria while the pump water had the lowest occurrence.

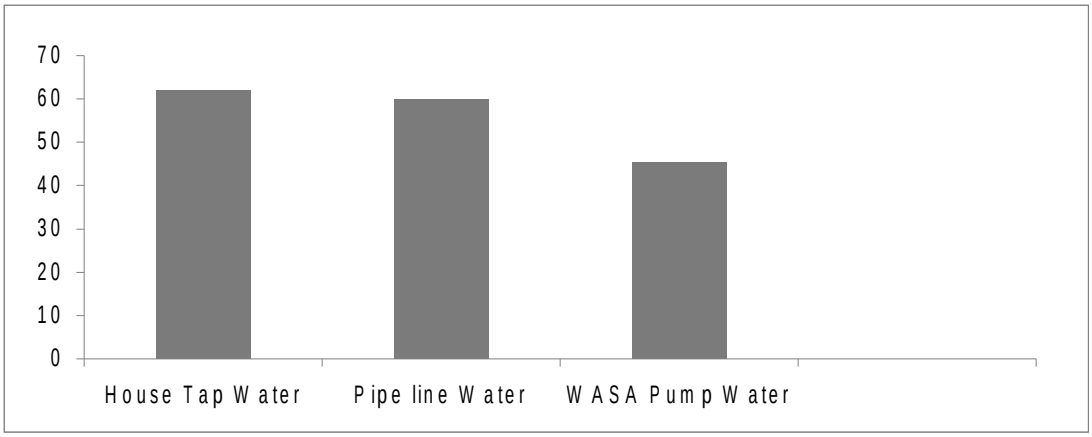


Fig. 6. Percentage of total bacteria in house tap water, pipe line water and WASA pump water

Biochemical studies of selected *E. coli* isolates

Out of 23 *E. coli* isolates from the confirmed test on EMB plates 8 were subjected to further biochemical assays. The result of different biochemical studies of isolated colonies were presented in table 2and table 3.

Table 2. Results of biochemical studies

Sample ID	catalase	oxidase	Indole	MR	VP	Motality	Urease	Citrate test	Suspected microorganism
S-1	+	-	-	+	-	+	-	+	*E. coli*
S-2	+	-	-	+	-	+	-	+	*E. coli*
S-3	+	-	+	+	-	+	-	-	*E. coli*
JS-8(1)	+	-	+	+	-	+	-	+	*E. coli*
JS-8(2)	+	-	+	+	-	+	-	-	*E .coli*
S-9	+	-	+	+	-	+	-	-	*E. coli*
S-10(1)	+	-	+	+	-	+	-	-	*E .coli*
S-10(2)	+	-	+	+	-	+	-	-	*E .coli*

Table 3. Result of Fermentation Test

Sample ID	Sucrose	Mannitol	Rhamnose	Lactose	Arabinose	Glucose	Xylose
S-1	+	+	+	+	+	+	+
S-2	+	+	+	+	+	+	+
S-3	+	+	+	+	+	+	+
S-9	+	+	+	+	+	+	+
S-10(1)	+	+	+	+	+	+	+
S-10(2)	+	+	+	+	+	+	+
JS-8(1)	+	+	+	+	+	+	+
JS-8(2)	+	+	+	+	+	+	+

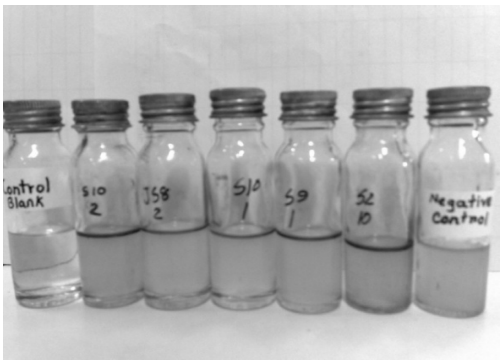


Fig. 7. Indole Test

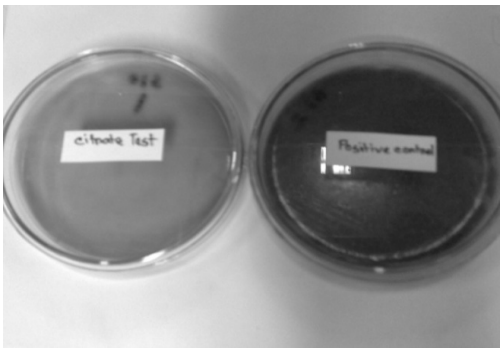


Fig. 8. Citratre utilization Test

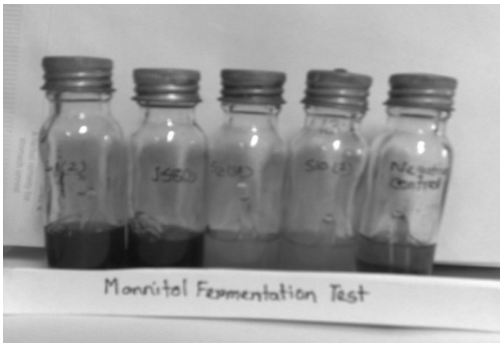


Fig. 9. Manitol Fermentation

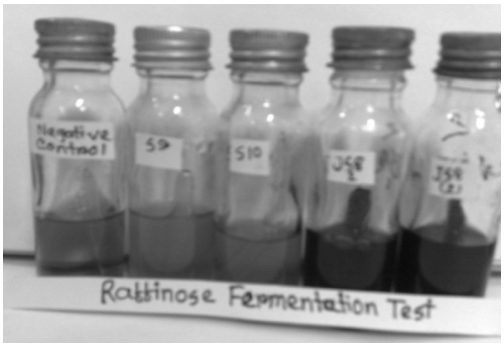


Fig. 10. Raffinose Ferment

Antibiogram of isolated coliform

Different organisms required different methods of susceptibility testing. Susceptibility of isolated *E. coli spp* to different antimicrobial agents was measured in vitro by following NCCLS (2000) methodology. Some isolates were found resistant to ampicillin, (AMP), amoxicillin(AML), chloramphenicol(C), erythromycin, (E), tetracycline (T), gentamicin (G), sulphomethoxazole (S), kanamycin (K), penicillin (p) , nalidixic acid (N), while some other were found sensitive to them. Antibiotic sensitivity pattern of different isolates were documented (table 4).

Table 4. Antibiotic sensitivity pattern of different isolates

Isolated *E.coli* strains	Resistant	Intermediate	Sensitive	No. of antibiotics to which bacteria are resistant	No. of antibiotics to which bacteria are sensitive
S-1	AML,AMP,T,K, N,P	C, S,	G, E	6	2
S-2	P, AMP, E, C, T, S	G, AML, K,	N	6	1
S-3	N, K, P, AML, S, AMP, T, C		G, E	8	2
JS-89(1)	N, K, AML, S, AMP, T,P	E, C	G	7	1
JS-8(2)	AML, P, E,AMP, S	G, N,	K, C,T	5	3
S-9		G, N,AML,P,	K, T,E,S,C, AMP	0	6
S-10(1)	AML,P,N,AMP,E,G	K	T,C,S	5	3
S-10(2)	G, K, P, E, S, C	AML, N	T, AMP	6	2

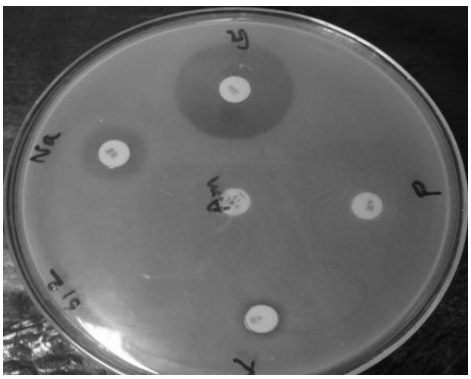
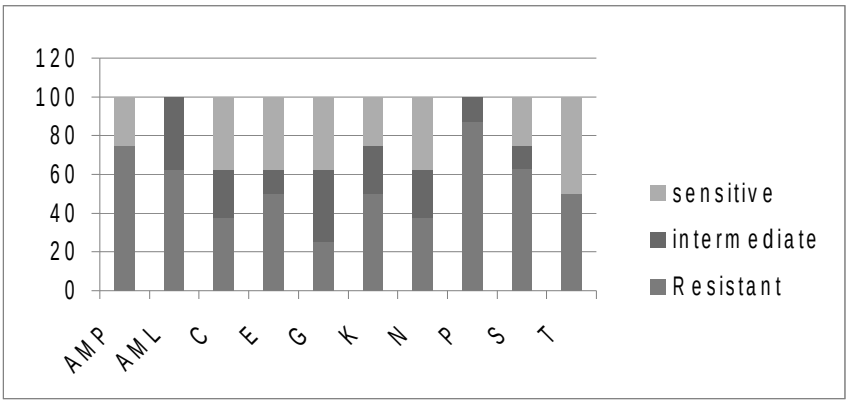


Fig.11. Antibiotic sensitivity test



[Amp (ampicillin),
AML (amoxicillin),
C (chloramphnecol),
E (erythromycin),
G (gentamycin),
K (kanamycin),
N (nalidixic acid),
P (penicillin),
S (sulphomethoxazol),
T (tetrecyclin)]

Fig.12. Antibiotic sensitivity pattern

Conclusion

It may be, concluded that the bacteriological quality of WASA source water was superior than pipeline and house tap water, so contamination of water take place in distribution system and/or domestic tanks or reservoirs. The results emphasize the importance of adopting appropriate routinely monitoring system in order to prevent or to diminish the chances of contamination in order to drinking water. In this regard, the the present study of microbiological quality of WASA water does not meet BDS 1240:2001 standards. For public health interest it is advisable that before drinking Dhaka WASA water should either be treated or boiled.

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