

Full length research paper

# Bacteriological quality of tap water samples obtained from different sources in and around Mymensingh city of Bangladesh with particular focus on antimicrobial resistance of *Escherichia coli*

Shabnam Sharmin<sup>1</sup>, S. M. Lutful Kabir<sup>1\*</sup>, M. Enamul Hoque Kayesh<sup>2</sup>, A.K.M. Ziaul Haque<sup>3</sup> and M. Mufizur Rahman<sup>1</sup>

<sup>1</sup>Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

<sup>2</sup>Department of Microbiology, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Babugonj, Barisal, Bangladesh.

<sup>3</sup>Kazi Farms Poultry Laboratory, Holding no-8/1, Floor no-A3 & A4, Padma plaza (opposite of Gazipur Commerce College), Chandana - Chowrasta, Gazipur-1704, Bangladesh.

Accepted 6 September, 2013

The objective of this study was to assess the bacteriological quality of tap water samples obtained from different sources in and around Mymensingh city of Bangladesh. A total of 30 tap water samples were collected from August to October 2012 for this purpose. For achieving the above mentioned objective, methods of heterotrophic plate count (HPC) and total coliform count (TCC) were applied. Moreover, isolated *E. coli* from tap water samples were characterized by using biochemical and antimicrobial susceptibility tests. HPC and TCC were encountered highest in municipal tap water. In respect to antimicrobial susceptibility testing, most of the *Escherichia coli* isolates were resistant to erythromycin, ampicillin, tetracycline, streptomycin and ciprofloxacin. Furthermore, a few *E. coli* isolates were intermediate resistant to chloramphenicol and norfloxacin. However, most of the *E. coli* isolates were susceptible to azithromycin and gentamicin. Moreover, out of 12 *E. coli* isolates 11 (91.66%) isolates were detected as multidrug resistant. This study indicated the presence of multidrug resistant *E. coli* isolates in tap water in Mymensingh that warrants particular attention.

**Key words:** *Escherichia coli*, bacteriological quality, tap water, antimicrobial susceptibility, multidrug resistance.

## INTRODUCTION

The World Health Organization has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water or unavailability of water. It was estimated that nearly 1.5 billion people lack safe drinking water and that at least 5 million deaths per year can be attributed to water-borne disease. Health effects associated with water supplies in developing countries are evaluated to be based on four bacterial indicators of tropical drinking-water quality (faecal coliforms, *E. coli*, *Enterococci* and faecal *Streptococci*) and

their relationship to the prevalence of diarrhea disease in Cebu, Philippines (Moe *et al.*, 1991). The contaminated water or inadequate supply of safe drinking water causes various gastrointestinal diseases like diarrhea, dysentery and water-borne diseases like cholera, typhoid. It is now evident that most of the enteric diseases of human and animals are transmitted through contaminated food and water (Johnson *et al.*, 2003). So to get rid from suspended biological agents and to ensure the supply of pure drinking water, water must need prior treatment or purified before consumption.

From this view point of public health, it is highly imperative that potable water supply system should be safe. Water may be polluted at its sources by excreta or sewage, which is almost certain to have pathogenic micro-

\*Corresponding author. E-mail: [lkabir79@gmail.com](mailto:lkabir79@gmail.com)  
Tel: +88-091-67401-6 (ext. 2394).

organisms. Potable water system can become polluted with coliform and pathogenic bacteria due to lack of hygiene and sanitation. As a result, microbiological examination of water should routinely be carried out to monitor and control the quality and safety of drinking water. Although the concept of safe water is under consideration in Bangladesh, unfortunately science-based little information is available. Therefore, the present study was conducted to determine the bacteriological quality of tap water samples available in and around Mymensingh city and find out possible association of faecal contaminants as indicator and their interrelationship with resistance to antibiotics.

## **MATERIALS AND METHODS**

### **Collection of tap water samples**

A total of 30 tap water samples were collected in sterile conical flask from the Mymensingh city along with the surrounding localities in a view to prevent extraneous contamination of the water during the period of August to October 2012. After collection of water samples in a conical flask the mouth and neck of flask was covered with aluminum foil and taken to the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh for detail microbiological investigation.

### **Heterotrophic plate count (HPC)**

For the determination of heterotrophic plate count, 100 micro liters of serial tenfold dilution of tap water from original samples were transferred and spread on nutrient agar media using micro pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of plate with a sterile glass spreader. One sterile glass spreader was used for each plate. The plates were then taken in an incubator at 37°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the heterotrophic plate count. The heterotrophic plate count was calculated according to ISO (1995). The result of total bacterial count was expressed as the number of organism or colony forming units per milliliter (CFU/ml) of water samples.

### **Total coliform count (TCC)**

For the determination of total coliform count, 100 micro liters of serial tenfold dilution of tap water from original samples were transferred and spread on MacConkey agar media using micro pipette for each dilution. The dilu-

ted samples were spread as quickly as possible on the surface of plate with a sterile glass spreader. One sterile glass spreader was used for each plate. The plates were then taken in an incubator at 37°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total coliform count. The total coliform count was calculated according to ISO (1995). The result of total bacterial count was expressed as the number of organism or colony forming units per milliliter (CFU/ ml) of water samples.

### **Microscopic study for identification of *E. coli* suspected colonies by Gram's staining method**

Gram's staining was performed to determine the size, shape and arrangement of bacteria. Gram staining reaction was performed in accordance with the methods described by Merchant and Packer (1967). The organism if *E. coli* revealed gram-negative, pink color, large rod shape appearance and arranged in single or paired.

### **Biochemical studies for the identification of *E. coli* isolates**

Several biochemical tests were performed for the confirmation of the isolates as *E. coli*. The biochemical tests performed were sugar fermentation tests, indole test, methyl red (MR) test, Voges-Proskauer (VP) test and Citrate test as described by Cheesbrough, 2000. *E. coli* were characterized by their ability to ferment glucose, lactose (for some strains it is negative), maltose and produce gas (CO<sub>2</sub>), positive for indole test and MR test and negative for VP and Citrate utilization test.

### **Motility tests of *E. coli* isolates**

The motility test was performed in accordance with the method described by Cowan (1985) to differentiate motile bacteria from the non-motile one. Before performing the test, a pure culture of the organism was allowed to grow in nutrient broth. One drop of cultured broth was placed on the cover slip and was placed invertedly over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the concave depression of the hanging drop slide for better attachment of the cover slip to prevent air current and evaporation of the fluid. The hanging drop slide was then examined carefully under 100-power objective of a compound microscope using immersion oil. The motile and non-motile organisms were identified by observing motility in contrasting with to and fro movement of bacteria.

### **Most Probable Number (MPN)**

The most probable number (MPN) test for water examination

**Table 1.** Interpretive standards for disc diffusion susceptibility testing.

Name of antibiotic disc	Disc concentration	Diameter of zone of Inhibition(mm)		
		Sensitive	Intermediate	Resistant
Ampicillin (AMP)	10 µg	≥ 17	14-16	≤ 13
Chloramphenicol (C)	30 µg	≥ 18	13-17	≤ 12
Tetracycline (TE)	30 µg	≥ 15	12-14	≤ 11
Erythromycin (E)	15 µg	≥ 23	14-22	≤ 13
Azithromycin (AZM)	15 µg	≥ 18	14-17	≤ 13
Streptomycin (S)	10 µg	≥ 15	12-14	≤ 11
Gentamicin (CN)	10 µg	≥ 15	13-14	≤ 12
Nalidixic acid (NA)	30 µg	≥ 19	14-18	≤ 13
Ciprofloxacin (CIP)	5 µg	≥ 21	16-20	≤ 15
Norfloxacin (NOR)	10 µg	≥ 17	13-16	≤ 12

**Table 2.** Determination of heterotrophic plate count in municipal tap water.

Type of water	Sample code	HPC (cfu/100 ml)	Geometric mean of HPC (cfu/100 ml)
Municipal tap water	TW <sub>1</sub>	3.5x10 <sup>6</sup>	5.2x10 <sup>6</sup>
	TW <sub>2</sub>	2.5x10 <sup>6</sup>	
	TW <sub>3</sub>	2.3x10 <sup>6</sup>	
	TW <sub>4</sub>	5.7x10 <sup>6</sup>	
	TW <sub>5</sub>	4.8x10 <sup>6</sup>	
	TW <sub>6</sub>	7.8x10 <sup>6</sup>	
	TW <sub>7</sub>	6.7x10 <sup>6</sup>	
	TW <sub>8</sub>	8.6x10 <sup>6</sup>	
	TW <sub>9</sub>	5.8x10 <sup>6</sup>	
	TW <sub>10</sub>	6.4x10 <sup>6</sup>	
	TW <sub>11</sub>	2.7x10 <sup>6</sup>	
	TW <sub>12</sub>	5.5x10 <sup>6</sup>	

HPC = Heterotrophic Plate Count.

for the presence of coliforms was performed in accordance with the procedures described by Harley and Prescott (2002). An estimate of the number of coliforms (Most Probable Number) can also be done in the presumptive test. In this procedure, 15 lactose broth tubes were inoculated with the water samples. Five tubes received 10 ml of water, 5 tubes received 1ml of water and 5 tubes received 0.1ml of water. A count of the number of tubes showing gas production was then made, and the figure was compared to a table developed by American Public Health Association. The number was the most probable number (MPN) of coliforms per 100 ml of

the water sample.

### Antimicrobial susceptibility test

Susceptibility and resistance of different antibiotics was measured in vitro by employing the Kirby-Bauer method (Bauer et al., 1966). This method allowed for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that resulted from diffusion of the agent into the medium surrounding the disc. A suspension of test organism was prepared in nutri-

**Table 3.** Determination of heterotrophic plate count in tap water of BAU campus.

Type of water (Tap water in BAU campus)	Sample code	HPC (cfu/100 ml)	Geometric mean of HPC (cfu/100 ml)	Total geometric mean of HPC (cfu/100 ml)
Taposhi Rabeya Hall	TR1	1.1x10 <sup>5</sup>	1.3x10 <sup>5</sup>	
	TR2	1.5x10 <sup>5</sup>		
	TR3	1.3x10 <sup>5</sup>		
Sultana Razia Hall	SR1	1.2x10 <sup>5</sup>	1.5x10 <sup>5</sup>	
	SR2	1.9x10 <sup>5</sup>		
	SR3	1.4x10 <sup>5</sup>		
Fazilatunnesa Mujib Hall	FM1	1.0x10 <sup>5</sup>	1.2x10 <sup>5</sup>	1.6x10 <sup>5</sup>
	FM2	1.5x10 <sup>5</sup>		
	FM3	1.1x10 <sup>5</sup>		
Bangabandhu Hall	BN1	1.4x10 <sup>5</sup>	2.4x10 <sup>5</sup>	
	BN2	2.7x10 <sup>5</sup>		
	BN3	3.1x10 <sup>5</sup>		
Isha Khan Hall	IK1	1.2x10 <sup>5</sup>	1.9x10 <sup>5</sup>	
	IK2	2.7x10 <sup>5</sup>		
	IK3	1.8x10 <sup>5</sup>		
Microbiology Lab.	ML1	1.4x10 <sup>5</sup>	1.8x10 <sup>5</sup>	
	ML2	1.2x10 <sup>5</sup>		
	ML3	2.8x10 <sup>5</sup>		

HPC = Heterotrophic Plate Count.

ient broth by overnight culture for 24 hours at 37 °C. The broth were streaked using by sterile glass spreader homogenously on the medium. Antibiotic disc were applied aseptically to the surface of the inoculated plates at an appropriate special arrangement with the help of a sterile forceps on Mueller-Hinton agar plates. The plates were then inverted and incubated at 37 °C for 24 hours. The diffusion discs with antimicrobial drugs were placed on the plates and incubated for 24 hours at 37 °C. The antibiotics discs (Oxoid, Basingstoke, Hampshire, England) used were: ampicillin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), erythromycin (15µg), azithromycin (15 µg), streptomycin (10 µg), gentamicin (10 µg), nalidixic acid (30µg), ciprofloxacin (5 µg), norfloxacin (10 µg). Sterile glass spreader was used to spread the culture homogenously on the medium.

Antibiotic disc were applied aseptically to the surface of the inoculated plates at an appropriate special arrangement with the help of a sterile forceps. The plates were then inverted and incubated at 37 °C for 24 hours. After incubation, the plates were examined and the diameters of the zone of complete inhibition were observed. Isolates were classified as susceptible, intermediate and resistant categories based on the standard interpretation table (Table 1) updated according to the Clinical and Laboratory Standards Institution (CLSI, 2011).

#### DATA ANALYSIS

For data processing, the software Microsoft Excel 2007 and SPSS16 were used.

**Table 4.** Determination of total coliform count & MPN in municipal tap water.

Type of water	Sample code	TCC (cfu/100 ml)	Geometric mean of TCC (cfu/100 ml)	MPN/100ml
Municipal tap water	TW <sub>1</sub>	2.5x10 <sup>3</sup>	3.4x10 <sup>3</sup>	350
	TW <sub>2</sub>	2.9x10 <sup>3</sup>		220
	TW <sub>3</sub>	2.1x10 <sup>3</sup>		350
	TW <sub>4</sub>	3.5x10 <sup>3</sup>		170
	TW <sub>5</sub>	3.2x10 <sup>3</sup>		49
	TW <sub>6</sub>	5.0x10 <sup>3</sup>		220
	TW <sub>7</sub>	3.7x10 <sup>3</sup>		63
	TW <sub>8</sub>	4.3x10 <sup>3</sup>		49
	TW <sub>9</sub>	3.8x10 <sup>3</sup>		70
	TW <sub>10</sub>	5.5x10 <sup>3</sup>		170
	TW <sub>11</sub>	1.7x10 <sup>3</sup>		350
	TW <sub>12</sub>	2.4x10 <sup>3</sup>		79

TCC = Total Coliform Count, MPN = Most Probable Number.

## RESULTS

Heterotrophic plate count of municipal tap water was summarized in Table 2. The HPC of tap water sample code TW<sub>1</sub>, TW<sub>2</sub>, TW<sub>3</sub>, TW<sub>4</sub>, TW<sub>5</sub>, TW<sub>6</sub>, TW<sub>7</sub>, TW<sub>8</sub>, TW<sub>9</sub>, TW<sub>10</sub>, TW<sub>11</sub>, TW<sub>12</sub> were 3.5x10<sup>6</sup>, 2.5x10<sup>6</sup>, 2.3x10<sup>6</sup>, 5.7x10<sup>6</sup>, 4.8x10<sup>6</sup>, 7.8x10<sup>6</sup>, 6.7x10<sup>6</sup>, 8.6x10<sup>6</sup>, 5.8x10<sup>6</sup>, 6.4x10<sup>6</sup>, 2.7x10<sup>6</sup> and 5.5x10<sup>6</sup> cfu/100 ml respectively. The geometric mean of HPC of municipal tap water was 5.2x10<sup>6</sup> cfu / 100 ml. Heterotrophic plate count of tap water of BAU campus was presented in Table 3. The geometric mean of HPC of Taposhi Rabeya Hall, Sultana Razia Hall, Fazilatunnesa Mujib Hall, Bangabandhu Hall, Isha Khan Hall and Microbiology Laboratory were 1.3x10<sup>5</sup>, 1.5x10<sup>5</sup>, 1.2x10<sup>5</sup>, 2.4x10<sup>5</sup>, 1.9x10<sup>5</sup> and 1.8x10<sup>5</sup> cfu / 100 ml respectively. However, total geometric mean of HPC of BAU campus water was 1.6x10<sup>5</sup> cfu / 100 ml.

The summary of total coliform count and MPN values for municipal tap water represented in Table 4. The geometric mean of TCC of municipal tap water was 3.4x10<sup>3</sup> cfu / 100 ml and the MPN of the sample TW<sub>1</sub>, TW<sub>2</sub>, TW<sub>3</sub>, TW<sub>4</sub>, TW<sub>5</sub>, TW<sub>6</sub>, TW<sub>7</sub>, TW<sub>8</sub>, TW<sub>9</sub>, TW<sub>10</sub>, TW<sub>11</sub>, TW<sub>12</sub> were 350, 220, 350, 170, 49, 220, 63, 49, 70, 170, 350 and 79/100 ml respectively. The summary of total coliform count and MPN values of tap water of BAU campus was presented in Table 5. The geometric mean of TCC of Taposhi Rabeya Hall, Sultana Razia Hall, Fazilatunnesa Mujib Hall, Bangabandhu Hall, Isha Khan Hall and Microbiology Laboratory was 2.8x10<sup>2</sup>, 3.2x10<sup>2</sup>, 2.7x10<sup>2</sup>, 4.2x10<sup>2</sup>, 5.1x10<sup>2</sup> and 3.8x10<sup>2</sup> cfu/100ml respectively. The total geometric mean of TCC of BAU Campus water was 3.6x10<sup>3</sup> cfu/100ml and the MPN value of Taposhi Rabeya Hall, Sultana Razia Hall, Fazilatunnesa Mujib Hall, Bangabandhu Hall, Isha Khan

Hall and Microbiology Laboratory were 130, 110, 63, 49, 63 and 70 /100ml respectively.

A total of 12 *E. coli* strains were isolated from 30 tap water samples by using cultural and biochemical techniques. These 12 *E. coli* strains were subjected to antimicrobial susceptibility testing by disc diffusion method with 10 chosen antimicrobial agents as mentioned in Table 1.

The results of the antimicrobial susceptibility testing by disc diffusion method with 10 chosen antimicrobial agents are presented in Table 6. Out of 12 *E. coli* isolates, 11 (91.66%) were resistant to erythromycin and 7 (58.33%) were resistant to ampicillin, tetracycline, and ciprofloxacin. Furthermore, 3 (25%) were intermediate resistant to chloramphenicol and norfloxacin. On the otherhand, 9(75%) were susceptible to azithromycin and gentamicin. The results of antimicrobial resistance pattern of *E. coli* isolates are summarized in Table 7. Out of 12 *E. coli* isolates, 1 (8.33%) were resistant to each of 7 antibiotics, in where 1 (8.33%) were resistant to each of 6 antibiotics, 1(8.33%) were resistant to each of 5 antibiotics, 1(8.33%) were resistant to each of 4 antibiotics, 1(8.33%) were resistant to each of 3 antibiotics, 1 (8.33%) were resistant to each of 2 antibiotics. From this analysis it was evident that 11 (91.7%) *E. coli* isolates were multidrug resistant when considered resistant to 2 or more drugs (Table 7).

## DISCUSSION

The prime objective of this study was to assess the bacteriological quality of tap water samples collected from different sources in and around Mymensingh city of

**Table 5.** Determination of total coliform count & MPN in tap water of BAU campus.

Type of water (Tap water in BAU campus)	Sample code	TCC (cfu/100 ml)	Geometric mean of TCC (cfu/100 ml)	Total geometric mean of TCC (cfu/100 ml)	MPN/100ml
Taposhi Rabeya Hall	TR1	1.9x10 <sup>2</sup>	2.8x10 <sup>2</sup>	3.6x10 <sup>2</sup>	130
	TR2	2.6x10 <sup>2</sup>			
	TR3	3.9x10 <sup>2</sup>			
Sultana Razia Hall	SR1	2.8x10 <sup>2</sup>	3.2x10 <sup>2</sup>	3.6x10 <sup>2</sup>	110
	SR2	3.7x10 <sup>2</sup>			
	SR3	3.1x10 <sup>2</sup>			
Fazilatunnesa Mujib Hall	FM1	3.5x10 <sup>2</sup>	2.7x10 <sup>2</sup>	3.6x10 <sup>2</sup>	63
	FM2	2.9x10 <sup>2</sup>			
	FM3	1.7x10 <sup>2</sup>			
Bangabandhu Hall	BN1	3.3x10 <sup>2</sup>	4.2x10 <sup>2</sup>	3.6x10 <sup>2</sup>	49
	BN2	4.7x10 <sup>2</sup>			
	BN3	4.6x10 <sup>2</sup>			
Isha Khan Hall	IK1	3.8x10 <sup>2</sup>	5.1x10 <sup>2</sup>	3.6x10 <sup>2</sup>	63
	IK2	6.1x10 <sup>2</sup>			
	IK3	5.4x10 <sup>2</sup>			
Microbiology Lab.	ML1	2.9x10 <sup>2</sup>	3.8x10 <sup>2</sup>	3.6x10 <sup>2</sup>	70
	ML2	3.3x10 <sup>2</sup>			
	ML3	5.2x10 <sup>2</sup>			

TCC = Total Coliform Count, MPN = Most Probable Number.

**Table 6.** Results of Antimicrobial susceptibility of the isolated *E. coli* from tap water.

Name of isolates	No. (%)	AMP	C	TE	E	AZM	S	CN	NA	CIP	NOR
<i>E. coli</i> (n=12)											
Susceptibility	4(33.33)	5(41.66)	5(41.66)	0(0)	9(75)	3(25)	9(75)	7(58.33)	3(25)	5(41.66)	
Intermediate	1(8.33)	3(25)	0(0)	1(8.33)	1(8.33)	2(16.66)	2(16.66)	0(0)	2(16.66)	3(25)	
Resistant	7(58.33)	4(33.33)	7(58.33)	11(91.66)	2(16.66)	7(58.33)	1(8.33)	5(41.66)	7(58.33)	4(33.33)	

[Ampicillin (AMP) , Chloramphenicol (C) , Tetracycline (TE) , Erythromycin (E) , Azithromycin (AZM) , Streptomycin (S) , Gentamycin (CN) , Nalidixic Acid (NA) , Ciprofloxacin (CIP) , Norfloxacin (NOR) ]

Bangladesh. The geometric mean of HPC of municipal tap water was 5.2x10<sup>6</sup> cfu / 100 ml in this study. In addition, the geometric mean of HPC of BAU campus water was 1.6x10<sup>5</sup> cfu / 100 ml. On the other hand, the geometric mean of TCC of municipal tap water was 3.4x10<sup>3</sup> cfu / 100 ml and the MPN values of the sample TW<sub>1</sub> , TW<sub>2</sub> , TW<sub>3</sub> , TW<sub>4</sub> , TW<sub>5</sub> , TW<sub>6</sub> , TW<sub>7</sub> , TW<sub>8</sub> , TW<sub>9</sub> , TW<sub>10</sub> , TW<sub>11</sub> and TW<sub>12</sub> were 350 , 220 , 350 , 170 , 49 , 220 , 63 , 49 , 70 , 170 , 350 and 79 /100 ml respectively.

In addition, the total geometric mean of TCC of BAU Campus water was 3.6x10<sup>3</sup> cfu/100ml and the MPN values of Taposhi Rabeya Hall, Sultana Razia Hall, Fazilatunnesa Mujib Hall, Bangabandhu Sheik Mujib Hall, Isha Khan Hall and Microbiology Laboratory were 130, 110, 63, 49, 63 and 70/100ml respectively. In this study, it was found that HPC and TCC were highest in municipal tap water compare to bottled water collected from Mymensingh city. According to the world health report

**Table 7.** Results of antimicrobial resistance pattern of *E. coli* isolates.

Isolates	Resistance profiles	No. of isolates (%)
	No resistance demonstrated	-
	Resistant to 1 agent (E)	1 (8.33)
	Resistant to 2 agent (E-S)	1 (8.33)
	a. Resistant to 3 agent (AMP-E-S)	1 (8.33)
	b. Resistant to 3 agent (AMP-NOR-S)	1 (8.33)
	a. Resistant to 4 agent (TE-E-CIP-C)	1 (8.33)
	b. Resistant to 4 agent (TE-E-CIP-NOR)	1 (8.33)
<i>E. coli</i> (n=12)	c. Resistant to 4 agent (TE-E-NA-CIP)	1 (8.33)
	Resistant to 5 agent (AMP-E-AZM-CIP-S)	1 (8.33)
	Resistant to 6 agent (AMP-TE-E-NA-S-C)	1 (8.33)
	a. Resistant to 7 agent (AMP-TE-E-NA-NOR-S-C)	1 (8.33)
	b. Resistant to 7 agent (AMP-TE-CN-E-AZM-NA-CIP)	1 (8.33)
	Resistant to 8 agent (AMP-TE-E-NA-CIP-NOR-S-C)	1 (8.33)
	Resistant isolates	n=12 (100%)

[Ampicilin (AMP) , Chloramphenicol (C) , Tetracycline (TE) , Erythromycin (E) , Azithromycin (AZM) , Streptomycin (S) , Gentamycin (CN) , Nalidixic Acid (NA), Ciprofloxacin (CIP) , Norfloxacin (NOR)].

(2002), drinking water quality specifications world-wide recommend HPC limits from 100 to 500 cfu/ml in tap water. In this study, HPC was too high in case all types of tap water and high HPC measurements might be due to availability of favorable conditions for the bacterial growth in pipe system. The present study also revealed that tap water from different sources were contaminated with *E. coli* and other unidentified bacteria. A number of factors might be involved for such contamination. In Mymensingh the pipe system is very old and most of the pipes are poor in condition. There are leakage and breakage through which contaminants from outside the pipe might enter and get mixed with the supplied water. Due to lack of adequate water these pipes are often out of pressure. There is also an illegal practice of drawing water from pipes by suction. As a result, the pressure in the water main becomes less than the atmospheric pressure. Both of these phenomena might cause easier entrance of contaminants into pipelines. Moreover, due to improper layout of water supply lines and sewer lines there might be crossing between them. This might cause faecal contamination. Thus, it is very much possible that even if the water, while entering the pipes, satisfy the specification, it might no longer potable and palatable at the user's end. The findings of the present study correlate with the findings of Islam *et al.* (2010). However, the average heterotrophic bacteria for the tap water and USP water samples was 170 CFU/ml and less than 10 CFU/ml respectively according to Zam *et al.* (2010).

A total of 12 isolates were identified as *E. coli* on the basis of cultural and biochemical characteristics from tap water samples used in this study. The colony

characteristics of the isolated *E. coli* in different media resemble the colony characteristics of *E. coli* as stated by Escherich (1885) and Ali *et al.* (1998). The fermentation reaction by the isolates of *E. coli* in five basic sugars (dextrose, sucrose, fructose, maltose, and mannitol) was positive. Moreover, MR reaction and catalase tests were also positive for *E. coli*. The organism was able to ferment lactose, dextrose and mannitol, sucrose and maltose completely. The result of sugar fermentation tests agreed with the findings of Beutin *et al.*, (1997) and Sandhu *et al.*, (1996). These respective authors reported that although *E. coli* ferments all 5 basic sugars but it partially fermented sucrose and maltose. Variation of the results might be due to genetic factors and nature of inhabitant of the organisms. Malaney and Weiser (1962) isolated *E. coli* from pond water. Dragas and Tratnik (1975) stated that 21.5% of water were contained *E. coli*. Lin *et al.*, (1974) and Mieres and Bastardo (1975) isolated *E. coli* from river water. Johnson *et al.* (2003) detected *E. coli* and *Salmonella* in surface water. Abdel-Magid (1997) concluded that if the total coliform count becomes too numerous in water it should warrant more attention. Kravitz *et al.*, (1999) found coliforms in all unimproved and semi-improved water sources and they considered these types of water as non potable. They however found that *E. coli* was absent in majority of the improved water sources. Likewise in the present study *E. coli* was not detected and found absent in bottled water. The findings of the present study obviously demonstrated that protection of water sources is very important and the avoidance of contamination can promote hygienic quality of water supplies, where disinfection is not possible.

Nogueira *et al.* (2003) and Shelton *et al.* (2006) found faecal pollution of water samples. The findings of the present study correlate with the findings of Nogueira *et al.*, (2003) who found highest load of coliform organism in tap water samples. Analogously Opara (2005) found coliform organisms in two rural communities and the quality of rural water supplied was found to be bacteriologically unsatisfactory. Recent studies of Shayo *et al.*, (2007) obtained high coliform count in a rural district and overall, water supplies in the village. Campos *et al.*, (2002) analyzed the microbiological quality of water samples collected from selected houses and could not detect coliforms. On the other hand, Vollaard *et al.*, (2005) reported that one third of the households, were significantly associated with water contaminated with >100 fecal coliforms /100 ml. They did not however found any association with water source or any environment was encountered. Campos *et al.* (2002) analyzed the microbiological quality of water samples collected from selected houses and found that total coliform count was absent.

*E. coli* is able to acquire resistance easily; therefore it is a good bioindicator model for surveillance studies of antimicrobial resistance. Antimicrobial resistance testing was performed by disc diffusion method using 10 different antibiotics. In antimicrobial susceptibility testing, out of 12 *E. coli* isolates, 11 (91.66%) were resistant to enrofloxacin, 7 (58.33%) were resistant to ampicillin, tetracycline, streptomycin and ciprofloxacin. These findings are in partial agreement with Islam *et al.*, (2010). Furthermore, 3 (25%) were intermediate resistant to chloramphenicol and norfloxacin. On the contrary, 9 (75%) were susceptible to azithromycin and gentamicin, 7 (58.33%) were susceptible to nalidixic acid. These findings are in partial agreement with Islam *et al.*, (2010). Out of 12 *E. coli* isolates, 11(91.7%) were multidrug resistant. These findings are in partial agreement with Nazir *et al.*, (2005). Such high incidence of multidrug resistant might be due to indiscriminate use of antibiotics, which may eventually supercede the drug resistant microorganisms from antibiotic saturated environment. In Bangladesh, for many years antibiotic is randomly used for treatment purposes. People are not aware about the schedule use of antibiotics. Thus, resistant strains might be emerged by genetic recombination against one or more antimicrobial agent(s).

The major limitation of this study was time limit; seasonal variation was also not included. Secondly, the study was conducted with only limited number of samples. So, the study should also be performed at different location of Mymensingh city along with the consideration of total suspended solid (TSS), turbidity, pH, seasonal and temperature variation.

## ACKNOWLEDGEMENTS

The authors thank their scientific colleagues for their valuable comments on the manuscript. This study was performed in partial fulfillment of the requirements of a M.S. thesis for Shabnam Sharmin from the Department of Microbiology and Hygiene, Bangladesh Agricultural

University, Mymensingh, Bangladesh. Shabnam Sharmin was a recipient of NST fellowship from the Ministry of Science and Technology, Government of the People's Republic of Bangladesh.

## REFERENCES

- Abdel-Magid HM (1997). Assessment of drinking water quality in the Al-Gassim Region of Saudi Arabia. *Environment International*, 23: 247-251.
- Ali MY, Rahman MT, Islam MA, Choudhury KA, Rahman MA (1998). Characteristics of *E. coli* isolates of human and animal origin. *Progressive Agriculturist*, 9: 221-224.
- Bauer AW, Kirby WM, Sherris, JC (1966). Antibiotic susceptibility testing by a standard single disc method. *Am. J. Clin. Pathol.* 45: 493-496.
- Beutin L, Geier D, Zimmermann S, Aleksic S, Gillespie Campos JA, Farache FA, Faria JB (2002). Sanitary quality of water distributed to human consumption through the public supply system of Araraquara-Sao Paulo State. *Alimentos-e-Nutricao*, 13: 117-129.
- Cheesbrough M (2000). *District laboratory practice in tropical countries (part 2)*. Cambridge University Press, UK. 62-70.
- Clinical and Laboratory Standards Institute (2011). Performance standards for antimicrobial susceptibility testing; 21th informational supplement. CLSI document M100-S21. Clinical and Laboratory standards Institute. Wayne,pa.
- Cowan ST (1985). *Cowan and Steels manual for identification of bacteria (2<sup>nd</sup> ed)*. Cambridge University press, UK.158-160.
- Dragas AZ, Tratnik M (1975). On the value of examination of drinking water and swimming pools for the presence of enteropathogenic *E. coli*. *Microbial. Abst.* 10: 10878.
- Escherich T (1885). "Die Darmbakterien des Neugeborenen und Säuglinge". *Fortschr Med.* 3: 515-522.
- HA, Whittam TS (1997). Epidemiological relatedness and clonal types of natural populations of *E. coli* strains producing shiga toxins in separate population of cattle of sheep. *Applied. Environ. Microbiol.* 63: 2175-2180.
- Harley JP, Prescott LM (2002). *Laboratory exercises in microbiology*. Fifth Edition, The McGraw-Hill Companies. 285-288.
- Islam S, Begum HA, Nili NY (2010). Bacteriological safety assessment of municipal tap water and quality of bottle water in Dhaka City: health hazard analysis. *Bangladesh J. Med. Microbiol.* 4: 9-13.
- ISO (1995). Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/TC-36/Sc-6. The Netherlands.10-18.
- Johnson JYM, Thomas JE, Graham TA, Townshend I, Byrne J, Selinger LB, Gannon VPJ (2003). Prevalence of *E. coli* O157:H7 *Salmonella* spp. in surface waters of southern Alberta and its relation to manure sources. *Canadian J. Microbiol.* 49: 326-335.
- Kravitz JD, Nyaphisi M, Mandel R, Petersen E (1999). Quantitative bacterial examination of domestic water

- supplies in the Lesotho highlands: water quality, sanitation and village health. *Bull World Health Organ*, 77: 829-36.
- Lin S, Evans RL, Beuscher DB (1974). Bacteriological assessment of spoon river water quality. *Appl. Microbiol.* 28: 288-297.
- Malaney GW, Weiser HH (1962). Coliform, enterococci, thermotolerants, thermophiles and psychrophiles in untreated farm pond waters. *Appl. Microbiol.* 10: 44-51.
- Merchant IA, Packer RA (1967). *Veterinary bacteriology and virology*, 7th edn. The Iowa University Press, Ames, Iowa, USA. 286-306.
- Mieres RL, Bastardo JW (1975). Enterobacteria in the waters of the river Manzanares at Cumana (Venezuela). *Microbiol. Abstr.* 10: 11822.
- Moe CL, Sobsey MD, Samsa GP, Mesolo V (1991). Bacterial indicators of risk of diarrhoeal disease from drinking water in the Philippines. *Bull World Health Organ*, 69: 305-317.
- Nazir KHMNH, Rahman MB, Nasiruddin KM, Akhtar F, Khan MFR, Islam MS (2005). Antibiotic sensitivity of *E. coli* isolated from water and its relation with plasmid profile analysis. *Pak. J. Biol. Sci.* 8: 1610-1613.
- Nogueira G, Nakamura CV, Tognim MCB, Abreu-Filho BA, Dias-Filho BP (2003). Microbiological quality of drinking water of urban and rural communities, Brazil. *Rev. Saude Publica*, 37: 232-236.
- Opara AA (2005). Water supplies in some communities around Calabar, Cross River State, Nigeria: bacteriology of drinking waters. *Southeast Asian J. Trop. Med. Public. Health.* 36: 1025-1027.
- Sandhu KS, Clarke RC, McFadden K, Brouwer A, Louie M, Wilson J, Lior H, Gyles CL (1996). Prevalence of the *eaA* gene in verotoxigenic *E. coli* strain in dairy cattle in South-West Ontario. *Epidemiol. Infect.* 116: 1-7.
- Shayo NB, Chove BE, Gidamis AB, Ngoma OB (2007). The quality of water in small community supplies of Kingolwira village, Morogoro, Tanzania. *Tanzan Health. Res. Bull.* 9: 56-60.
- Shelton DR, Karns JS, Higgins JA, Kessel VJS, Perdue ML, Belt KT, Anelli RJ, Debroy C (2006). Impact of microbial diversity on rapid detection of enterohemorrhagic *E. coli* in surface water. *Microbiology Letters*, 261: 95-101.
- The World Health Report (2002). Geneva: World Health Organization, 2001.
- Vollard AM, Ali S, Smet J, van Asten H, Widjaja S, Visser LG, Surjadi C, van Diessel JT (2005). A survey of the supply and bacteriological quality of drinking water and sanitation in Jakarta, Indonesia. *Southeast Asian J. Trop. Med. Public Health.* 36: 1552-1561.
- Zam AS, Marino M, Khameesan A (2010). A random study of the microbiological quality of bottled drinking water in Canada. *Proceeding of the 110 th American Society for Microbiology (ASM) Conference*, Paper No. 4016.