Original Article

Bacterial Propagation in Municipal Water and Deep Tube-well Water in Kashipur Locality of Narayanganj City, Bangladesh

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ABSTRACT

Present study assessed the pathogenic prevalence in municipal water from 10 supply point and deep tube-well water from 10 deep tube-well point delivered across the Kashipur zone of Narayanganj city, Bangladesh along with their drug resistant pattern through conventional, biochemical and disk diffusion methods. The physico-chemical properties of the deep tube-well water were in satisfactory level, only the samples from location 1, 3, 5 and 9 were exceed the marginal limit for dissolved oxygen however most of the samples of supplied water cross the marginal limit of all parameter (dissolved oxygen, temperature, pH, electrical conductivity, total dissolved solid and turbidity). Pathogenic bacteria including *Escherichia coli* and *Staphylococcus* spp. were found in both supplied water and sewerage authority water (10^2 – 10^6 cfu/ml) and deep tube-well water (10^2 – 10^4 cfu/ml). Additionally, fecal coliforms, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Pseudomonas* spp. was monitored only among the supplied water. Both of the samples were found to be contaminated with heterotrophic bacteria up to 10^8 cfu/ml. Most of the bacteria were found to be resistant against more than one drug. Hence, the municipal water of the study area was microbiologically unsafe. The propagation of drug resistant strains was assumed to escalate the public health threat. A survey on public opinions were also conducted to know their daily life style and their concern on water treatment and diseases outbreaks caused by contaminated water.

Keywords: water pollution, water microbiology, water borne diseases, water purification, drug resistant

INTRODUCTION

For being a highly populated country, Bangladesh always has been considered as the extreme susceptible country in case of rapid transmission of diseases causing microorganisms from one person to another person by contaminated air, water and food [1]. As we know, consuming safe drinking water is the first and foremost requirement in our daily life for maintaining a healthy and fresh life. Bangladeshi peoples are fully dependent on the Water and Sewerage Authority (WASA) for their daily uses water including drinking. Unfortunately, this water may have huge possibility for

becoming contaminated when pass through the dirty and leakage water supply pipe by pathogenic microbes those are responsible for waterborne diseases including diarrhoea, dysentery, typhoid, salmonellosis, listeriosis, parasitic worm infestations, and viral infections [2]. An analytical survey of Global Vaccine Research Program in Bangladesh exposed that about 2.5 million children are dying every year due to diarrheal disease which is suspected to cause by pathogens from contaminated water.

Several reports have described previously about the existence of some enteric bacteria such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and others in the wa-

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ter body, which have the ability to cause infectious diseases [1,3,4]. The most significant reason of such contamination in water might be the water transport system by which water is transmitting from the supply point to the different consumer points [1]. Now-a-days consumers are more concerned about the supply of safe water at the consumer points from where they collect water for drinking and other purposes [5]. In this advanced technologies era, most of the developing countries are still now using some common methods which is increasing the microbial contamination of water as well as the rate of waterborne disease [6,7]. To identify the disease causing microorganisms within the water supply system, cultural and biochemical tests can be done as a confirmative test but for further confirmation, molecular techniques and specific antigen detection need to be applied which is so difficult due to the limited number of molecular tools or commercial kit [8,9]. Though, some of the microorganisms are culturable so those can be detected by some conventional methods but in terms of viable but non-culturable (VBNC) molecular approaches have no alternative [10,11].

Results of molecular studies have provided evidence that the pathogenic strains in the supply water body may contain varieties of virulence genes among which most common are *ctx*, *rtxA*, *tdh*, *elt*, *est*, *stx1* and 2, *invA*, *uidA*, etc., and these genes might be responsible for waterborne disease outbreaks [4,12–14]. Another major issue has been reported that contaminated water may also contain antibiotic resistant genes and which can be easily transmitted to the pathogens in water body making them multi-drug resistance which is now a very alarming issue not only in Bangladesh but also in worldwide [15–18].

Based on the above evidences, we planned to gather information of the pathogenic isolates collected from different water samples. We investigated the water quality of Kashipur Locality of Narayanganj City, Bangladesh which is supplied by the WASA in terms of detailed pathogenic dominance. A questionnaire survey was also conducted focusing on the respondents' problems, complains and mode of uses of the municipal water.

MATERIALS AND METHODS

Study area and sampling

The water supply systems in Dhaka city operated by Dhaka Water Supply and Sewerage Authority (DWASA), our experimental spot was the MODS zone-1, which situated at Kashipur locality of Narayanganj city. The present study was carried out to assess the quality of municipal water supplied

by DWASA as well as deep tube well water (400 ft. to 500 ft. depth) of the area. Water samples were collected from 20 different locations in October, 2018. Among them, 10 were from selected supplied pump stations (supplied water: SW) and another 10 were from the household deep tube-well points (deep tube-well water: DTW). The sample location mentioned in the map: the red circle represented as supply water point and the black square showed the deep tube-well water point (**Fig. 1**). Samples were collected aseptically in sterile screw capped bottles kept in a thermal stabilizing box maintained at 25°C, transported to the laboratory within one hour, and immediately subjected to microbiological analysis [1,19].

Physico-chemical status of the water samples

The physico-chemical properties of all the samples such as dissolved oxygen (DO), temperature, pH, electrical conductivity (EC), salinity, total dissolve solid (TDS) and turbidity were measured following the standard protocols and methods of American Public Health Association (APHA) [20] and American Society for Testing and Materials using different calibrated standard instruments. The pH of the water samples was measured by using a pH meter (HI 98130, Hanna Instruments, Woonsocket, USA).

The conductivity of the samples was measured using a conductivity meter (HI 98130, Hanna Instruments). The turbidity of the water samples was measured using a turbidity meter (2100P Turbidimeter, Hach Company, Loveland, USA). After achieving the reading stability, the value was recorded. TDS in water samples were carried out according to the standard methods of APHA [20,21] by the filtration process. The temperature of the water samples were measured in lab to avoid change of temperature with time using a thermometer. The Winkler method, also known as the iodometric technique was employed. The chemistry of the analysis is based on the addition of manganese II tetraoxosulphate vi solution followed by a strong alkali-iodide solution [22].

Isolation and enumeration of microorganisms Heterotrophic plate count (HPC) and fecal coliform count (FCC)

100 ml of each sample was filtered through a 0.45 µm membrane filter (Pall Corporation, Port Washington, USA) and placed on the Nutrient agar and MFC agar plates for the estimation of HPC and FCC, respectively. For heterotrophic bacterial assay, plates were incubated at 37°C for 24 hours while for estimating the fecal coliforms, incubation at 44.5°C for 24 hours was carried out [1].

Fig. 1 A location map of sampling point.

E. coli, *Klebsiella* spp., *Staphylococcus* spp. and *Pseudomonas* spp.

0.1 ml of each sample was spread on to MacConkey agar, annitol salt agar (MSA), and cetrimide agar for the isolation of *E. coli*, *Klebsiella* spp., *S. aureus*, and *Pseudomonas* spp., respectively. Afterward, all the plates were incubated at 37°C for 24 hours. Eosin methylene blue (EMB) agar media were further used for the observation of the production of green metallic sheen (if any) characteristic of *E. coli* strains [19,23].

Salmonella spp., *Shigella* spp. and *Vibrio* spp.

Initially, the membrane filtration method was used for the assessment of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. using *Salmonella Shigella* agar and the Thiosulfate Citrate Bile Salt Sucrose agar media, respectively. Due to the viable but non-culturable state of *Salmonella* spp., *Shigella* spp., and *V. cholerae* we used enrichment media; alkaline

peptone water for *V. cholerae* and selenite cystine broth for *Salmonella* and *Shigella* spp [24,25]. For the final identification of all isolates, several biochemical tests were performed including the triple sugar iron test, motility indole urease test, methyl red test, Voges Proskauer test, indole utilization test and the oxidase test [1].

Determination of antimicrobial susceptibility

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 laboratories inc, Franklin Lake, USA) with antibiotic discs (Neo-Sensitabs, Rosco Diagnostica, taastrup, Denmark) according to the modified Kirby-Bauer method [26]. 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

Table 1 Community surveillance parameter ($n = 1000$).

Parameters	($n = 1000$)
<i>Sex</i>	
Male	600 (60%)
Female	400 (40%)
<i>Age</i>	10–70 years
Length of stay	
1–5 years	300 (30%)
6–10 years	390 (39%)
Around 20 years	310 (31%)
<i>Marital status</i>	
Married	640 (64%)
Unmarried	360 (36%)
<i>Numbers of family members</i>	
1–3 members	360 (36%)
4–6 members	548 (54.8%)
> 6 members	92 (9.2%)
<i>Education</i>	
Graduates	420 (42%)
Bachelors	340 (34%)
Class 9 to 12 passed	190 (19%)
Primary level	50 (5%)
<i>Residence means</i>	
Rented house	670 (67%)
Own house	210 (21%)
Own flat	120 (12%)
<i>Financial background</i>	
Economically solvent	470 (47%)
Middle-income	430 (43%)
Bellow middle-income	70 (7%)
Extremely poor	30 (3%)

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 30 µg, nalidixic acid 30 µg, imipenem 10 µg, tetracycline 30 µg, erythromycin 15 µg, kanamycin 30 µg, chloramphenicol 30 µg, azithromycin 15 µg, trimethoprim-sulfomethoxazole 25 µg, gentamycin 10 µg, penicillin 10 µg, vancomycin 30 µg, 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 resistant [27].

Questionnaire Survey

Among the all citizen, a questionnaire review was conducted (**Table 1** and **Fig. 1**) to know their opinion about their disease outbreak, water purification methods, water quality etc. At the same time, the laboratory experiment was conducted for observing the actual scenario of water to make a correlation with the public opinion. Total 1,000 people of different ages were live in that specific area. During this survey, we provided a survey form to the residents with some basic questions such as their sex, age, length of stay, marital status, number of family members, education, residence type and financial background.

RESULT AND DISCUSSION

Physico-chemical status of the water samples (both supplied and deep tube-well water)

In case of supplied water, the DO was estimated within the range of 4.8 to 7.4 mg/l, the pH value of 10 SW samples varied from 8.6 to 10.4, the EC content of 10 SW samples varied from 280 to 540 µs/cm, the salinity was recorded within the range of 0.14 to 0.27 ppt, the TDS content of 10 SW samples varied from 132 to 251 ppm, the turbidity was recorded within the range of 0.25 to 2.09 NTU and the temperature of the 10 SW samples varied from 26 to 27. Subsequently, the pH value of 10 DTW samples varied from 6.5 to 6.9, the DO of 10 DTW samples varied from 4.3 to 7.4 mg/l, the EC content of 10 DTW samples varied from 286 to 314 µs/cm, the salinity was recorded within the range of 0.12 to 0.19 ppt, the TDS content of 10 DTW samples varied from 117 to 163 ppm, the turbidity was recorded within the range of 0.43 to 2.54 NTU and the temperature of the 10 DTW samples were constant at 26°C. According to our findings, most of the samples of supplied water exceeded the marginal limit of all parameters (DO, Temperature, pH, EC, Salinity, TDS and Turbidity) (**Table 2**).

Heterotrophic bacteria and Pathogenic prevalence in the municipal water

High number of pathogenic bacteria was observed in supplied water and then further biochemically identified (**Tables 3 and 4**). Fecal coliforms ($1.0 \times 10^2 - 3.1 \times 10^3$ cfu/ml), *Klebsiella* spp. ($1.4 \times 10^2 - 2.0 \times 10^3$ cfu/ml), and *E. coli* ($1.8 \times 10^2 - 2.0 \times 10^6$ cfu/ml) were found only in samples from consumer points where *Klebsiella* and Fecal coliform were completely absent in the deep tube-well water. *Salmonella* spp. and *Shigella* spp. were present within the range of $10^2 - 10^3$ cfu/ml but no growth of *Salmonella* spp. was observed

Table 2 Physical parameters of the water samples.

	Sample	DO (mg/l)	Temperature (°C)	pH	EC (µs/cm)	Salinity (ppt)	TDS (ppm)	Turbidity (NTU)
Supplied Water								
	SW-1	7.4	27	9.8	298	0.14	132	0.72
	SW-2	7.2	26	9.6	466	0.22	201	0.53
	SW-3	5.9	26	9.6	540	0.27	251	2.09
	SW-4	4.9	26	10.0	392	0.16	174	0.72
	SW-5	4.8	26	10.4	372	0.22	189	0.75
	SW-6	5.8	26	9.8	452	0.23	199	0.54
	SW-7	4.7	26	8.7	360	0.18	159	0.80
	SW-9	5.0	26	8.6	440	0.22	197	0.71
	SW-10	5.0	26	8.7	380	0.19	169	0.25
Deep tube-well water								
	DTW-1	4.3	26	6.9	304	0.12	134	0.93
	DTW-2	6.0	26	6.8	286	0.13	125	1.00
	DTW-3	7.0	26	6.6	300	0.14	127	0.53
	DTW-4	4.7	26	6.7	290	0.14	126	0.49
	DTW-5	6.0	26	6.7	302	0.15	135	1.57
	DTW-6	4.8	26	6.9	298	0.14	130	1.18
	DTW-7	4.5	26	6.8	314	0.15	139	1.34
	DTW-8	4.5	26	6.6	284	0.13	124	2.54
	DTW-9	7.8	26	6.5	376	0.19	163	1.40
	DTW-10	5.5	26	6.5	274	0.13	117	0.43

SW: supplied water

DTW: deep tube-well water

in sample 1, 6, 9 and 10 and sample 4, 8 and 10 showed no growth of *Shigella* spp. The growth of *Pseudomonas* spp. was noticed up to 10^5 cfu/ml excluding sample 1, 2, 3, 4 and 10 likewise the presence of *Staphylococcus* spp. was found up to 10^3 cfu/ml in samples except 4, 5, 8, 9 and 10. On the other hand most of the samples were found to be free from *vibrio* spp. Only sample 6 showed growth (**Table 3**).

In case of deep tube-well water, total viable bacteria was found in all the samples up to 10^7 cfu/ml, *E. coli* was present in sample 1, 6 and 9 up to 10^2 cfu/ml and *Staphylococcus* spp. was found in sample 1 and 5 (**Table 3**). Most importantly, the existence of heterotrophic bacteria was in very high range (up to 10^8 cfu/ml) in both supplied water and deep tube-well water.

Biochemistry of the isolates

Total eight types of biochemical test showed their result for the confirmation of *E. coli* and *Klebsiella* spp. (**Table 4**). The growth of *Escherichia coli* and *Klebsiella* spp. on Mac-Conkey agar plates were further transferred on to the EMB media and 6 samples among 11 were found as *E. coli* by

observing green metallic sheen and 4 were *Klebsiella* spp.

Finally, our study reported that some of the processed drinking water samples were not recommended for drinking because of the presence of indicator bacteria *E. coli* and *Klebsiella* spp., as coliform which also indicated the possible presence of other pathogenic bacteria. Several factors such as environmental contamination, inadequate processing and improper handling might be responsible for contamination of drinking water. Besides, the presence of drug resistance traits in the identified isolates might be able to make eradication the water associated diseases.

Community surveillance study: Public opinion and onset of diseases

The public opinion on the DWASA supplied water revealed a substandard quality of the water distributed across the Narayanganj area and exhibited the onset of enteric diseases among the community people. Regarding the general opinion on safety, 77.4% respondents reported that the supplied water was completely unsuitable for drinking without any treatment; while 22.6% respondents found that the supplied

Table 3 Microbiological analysis of the water samples.

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									
SW-01	2.6×10^7	2.3×10^3	2×10^6	2×10^2	Nil	2×10^2	Nil	Nil	6.2×10^2
SW-02	5.8×10^6	Nil	Nil	Nil	5.9×10^2	4.8×10^2	Nil	Nil	1.3×10^3
SW-03	7.5×10^8	Nil	Nil	Nil	1.1×10^2	4.4×10^3	Nil	Nil	6.6×10^2
SW-04	3.7×10^6	3.1×10^3	1.8×10^2	1.4×10^2	3.3×10^3	Nil	Nil	Nil	Nil
SW-05	5.6×10^5	Nil	Nil	Nil	4.8×10^2	3×10^2	Nil	7×10^2	Nil
SW-06	1.7×10^4	5.3×10^2	Nil	2×10^3	Nil	9.3×10^2	2.9×10^2	5×10^5	9.5×10^2
SW-07	9.9×10^2	Nil	Nil	Nil	1.7×10^2	4.5×10^3	Nil	4.5×10^3	2×10^2
SW-08	7.7×10^3	1×10^2	7.8×10^4	2×10^2	4.5×10^2	Nil	Nil	8.8×10^2	Nil
SW-09	2.0×10^5	Nil	Nil	Nil	Nil	8.4×10^2	Nil	6×10^5	Nil
SW-10	1.7×10^8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Deep tube-well water									
DTW-01	2.5×10^6	Nil	1.4×10^2	Nil	Nil	Nil	Nil	Nil	5.8×10^2
DTW-02	5.8×10^5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
DTW-03	7.5×10^7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
DTW-04	3.7×10^5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
DTW-05	5.6×10^4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	6.8×10^4
DTW-06	1.8×10^4	Nil	2.5×10^2	Nil	Nil	Nil	Nil	Nil	Nil
DTW-07	2.9×10^2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
DTW-08	5.7×10^3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
DTW-09	2.0×10^5	Nil	1.9×10^2	Nil	Nil	Nil	Nil	Nil	Nil
DTW-10	1.9×10^8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Nil: no bacterial growth

SW: supplied water

DTW: deep tube-well water

¹ Bacterial load after enrichment (Prior to enrichment, the recovery was nil). HPC: Heterotrophic plate count; FCC: Fecal coliform count

Table 4 Biochemical tests of different pathogens.

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

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

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

water might be used only for domestic purposes but not for drinking. The gross acceptability according to the physical properties of the supplied water among the community people was not that satisfactory. Among the 100% population only 50% respondents were found to drink supplied water after treatment by boiling and 20% were habituated with filtration methods. Rest of the 30% users was found to drink supplied water without any treatment because of their financial inability and lack of awareness (**Fig. 2**).

During the questionnaire review, we tried to figure out the actual condition of responder's daily life style especially regarding their basic knowledge on water purification and water borne diseases. In our target location, they were not familiar with the disinfectant procedure like chlorination, iron free aluminum sulfate purification, muriatic acid etc. Among the community, boiling method was more convenient and easy to perform. Meanwhile, according to the public opinion of Narayananj area the rate of diarrheal diseases were most frequent about 60% among the total population while dysentery cases were 40% as suspected due to the subsequent unawareness about water purification (**Fig. 3**).

Prevalence of drug resistant pathogenic bacteria in the municipal 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 5**). On the other hand, most of the isolates were found to retain higher sensitivity against imipenem and azithromycin.

Present study employed both microbiological approach and a community surveillance assay to decipher the level of health risk in the specific community due to the pathogenic proliferation among the municipal water from supply point

and deep tub-well water.

Higher prevalence rates of both heterotrophic bacteria and pathogenic water born bacteria were recorded in supplied water than the deep tube-well water. Notably, the microbiological quality of the municipal water was found unsafe which was observed through the existence of fecal coliforms, *E. coli* and *Klebsiella* spp. Overall, our data indicated that the microbial contamination of water mainly took place in the distribution system and/or domestic tanks or reservoirs as revealed through the high load of fecal coliforms among the supplied water samples but not in deep tube-well water. In case of deep tube-well water, it is not possible for pathogens to multiply in the deep aquifer layer of ground water. This might occur presumptively due to the development of bacterial biofilm, leakage in the pipeline, bacterial settlement inside the pipe, end point defect, improper water collection means, and most importantly the sewage line (adjacent or parallel to water distribution pipes) leakage. Existence of pathogens in the supply points also indicate that water supplied from the treatment plants might not properly meet the standard microbiological criteria. Therefore, more consideration should be needed in pathogenic reduction to an acceptable level from water before entering the distribution system usually by proper application of the treatment.

Many studies revealed the significant correlation of microbiological quality of water with risks of acquiring gastrointestinal disease [6]. As projected from the surveillance assay in the current study, the disease endemics (**Figure 3**) might be a result of the proliferation of several pathogenic bacteria among the supplied water as we identified (**Table 2**). It is deciphered that diarrhea could be caused mainly by *E. coli*, *Klebsiella* spp., typhoid by *Salmonella* spp., cholera by *Vibrio* spp. and dysentery by *Shigella* spp. Thus, the onset of the enteric diseases projected from the community surveillance

Fig. 2 Different purification methods applied by people to reduce the load of diseases causing microorganisms and other poisons.

Fig. 3 Diseases caused by contaminated water suspected by the users of that area.

Table 5 Antimicrobial susceptibility pattern of isolated pathogens.

Organisms Antibiotics	<i>E. coli</i>		<i>Klebsiella</i> spp.		<i>Shigella</i> spp.		<i>Salmonella</i> spp.		<i>Vibrio</i> spp.		<i>Pseudomonas</i> spp.		<i>Staphylococcus</i> spp.	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
AMP (10µg)	20%	80%	80%	20%	79%	21%	80%	20%	80%	20%	100%	0%	99%	1%
CIP (5µg)	67%	33%	98%	2%	10%	90%	80%	20%	10%	90%	70%	30%	ND	ND
PIP (10µg)	33%	67%	96%	4%	ND	ND	ND	ND	ND	ND	20%	80%	90%	10%
CEF (30µg)	67%	33%	100%	0%	2%	98%	20%	80%	70%	30%	34%	66%	ND	ND
AMO (10µg)	33%	67%	87%	12%	17%	83%	4%	96%	ND	ND	80%	20%	100%	1%
IPM (30µg)	0%	100%	0%	100%	9%	91%	16%	84%	70%	30%	20%	80%	ND	ND
PEN (10µg)	ND	ND	ND	ND	ND	ND	80%	20%	ND	ND	ND	ND	99%	1%
CHL (10µg)	45%	55%	24%	76%	52%	48%	48%	52%	40%	60%	66%	34%	ND	ND
TMP-SUL (25µg)	20%	80%	18%	82%	1%	99%	20%	80%	60%	40%	20%	80%	30%	70%
GEN (10µg)	ND	ND	ND	ND	0%	100%	20%	80%	ND	ND	34%	66%	34%	66%
AZI (15µg)	ND	ND	ND	ND	ND	ND	20%	80%	ND	ND	ND	ND	ND	ND
KNA (30µg)	ND	ND	ND	ND	ND	ND	20%	80%	ND	ND	ND	ND	ND	ND
NALI (30µg)	80%	20%	75%	25%	99%	1%	20%	80%	60%	40%	40%	60%	ND	ND
VAN (30µg)	ND	ND	ND	ND	100%	0%	ND	ND	ND	ND	ND	ND	70%	30%
ERY (15 µg)	ND	ND	ND	ND	ND	ND	ND	ND	10%	80%	ND	ND	25%	75%
OFL (5µg)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22%	78%

All the experiments have been done three times and the results were reproducible. One representative data have been shown. AMP = Ampicillin, AMO = Amoxicillin, CIP = Ciprofloxacin, CEF = Ceftriazone, NALI = Nalidixic acid, IPM = Imipenem, ERY = Erythromycin, KNA = Kanamycin, CHL = Chloramphenicol, AZI = Azithromycin, TMP/SUL = Trimethoprim-sulfomethoxazole, GEN = Gentamycin, PEN = Penicillin, VAN = vancomycin, OFL = Ofloxacin, PIP = Piperacilene ND = Not done, N = Number of isolates, R = resistance, S = Sensitive

could be correlated with our microbiological study. However, the pathogens might cause diseases by secreting several virulence enzymes or toxins which remained unidentified in this study. Also, a minor but significant fraction of diseases were undefined which might be due to the lack of correct information provided by the community people during the questionnaire survey.

Furthermore, the observed prevalence of *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. provides evidence for their presence in supply water in VBNC state which is alarming from a public health point of view; since these pathogens can turn over to active infectious state after entering into a host [24,25,28,29].

Contamination with trace levels of antibiotics and antibiotic resistant bacteria in surface water and finished drinking water may also greatly affect public health [30]. Showed that the quantities of most antibiotic resistant genes were higher in the tap water than those in the surface water, thereby suggesting that the water treatment could increase the antibiotic resistance of the surviving bacteria and/or induce the transfer of resistance genes among certain bacterial populations. Though we could not perform the specific detection of

antibiotic resistance genes due to poor settings and logistics; however, our data on the antibiotic resistance would be sufficient to reveal that the water distribution systems might serve as a reservoir for growth and spread of certain resistant antibiotic populations. We assume that through waterways, these resistant bacteria end up in agricultural fields, food animals and fisheries and ultimately enter into the food chain as has been described by Bester and Essack [31].

A limitation of our study could be put through its experimental approach only by the cultural and biochemical tests. Culture methods have over the years shown to lead to either misidentification or underestimation of microorganisms since only 10% of them are known to be culturable [25,32]. However, the enrichment of certain bacteria in the study might overrule such detection problems [25,33]. Further molecular and serological studies would minimize the possible false negative results.

CONCLUSIONS

The present investigation involving both 1) the primary laboratory data on the quantification of pathogens across

the water supply and 2) the community surveillance on the usage of that very water as well as on the subsequent disease outbreak would provide a guideline towards the other developing countries for a better public health management. This study was also emphasized on the cross contamination with the reason especially for the supply water which would provide sufficient information towards the water supply authority as well as municipal waste management authority to take maintainable action. This finding would also recommend many microbiologist, water analyst and environmental scientist to sort out the cost limiting way to purify and reduce the water pollution in both microbiologically and chemically. Finally, the detection of the drug-resistant pathogens in the municipal water supply points would further aid in the effective treatment during water borne disease outbreaks as well as may be included in the supply water monitoring curriculum at regular intervals.

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