Research Article

A study of prevalence of pathogenic bacteria, particularly, fecal coliforms and their antibiotic resistance pattern in environmental water samples of a tertiary-care hospital, Ahmedabad

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Abstract

Background: The spread of antibiotic resistance in environmental bacteria is a known phenomenon. *Escherichia coli* are a part of the normal flora of human and animal fecal matters, which may contaminate the soil and water. By far, no study is done in our region to know the prevalence of antibiotic resistance in environmental bacteria.

Objective: To know the prevalence of pathogenic bacteria, particularly, fecal coliforms along with their antibiotic resistance profile in environmental water body in our hospital.

Materials and Methods: Fifty water samples consisting of drinking water, water from the drainage pipes, and water from the leaking pipes of General Hospital, Sola, Ahmedabad, India, were studied for the determination of the prevalence of antibiotic-resistant coliforms. Water samples were collected for analysis of fecal contamination and to detect the most probable number (MPN) of fecal coliforms by multiple-tube fermentation technique. Antibiotic resistance of the *E. coli* isolates and *Klebsiella* were determined by Kirby–Bauer disc diffusion method using Clinical and Laboratory Standards Institute (CLSI) guidelines.

Result: Of the 50 samples collected from the civil hospital, 22 samples (44%) showed contamination with fecal coliform. Of the 22 positive samples, *E. coli* was isolated from 12 water samples; *Klebsiella* was isolated from 10 samples. Majority of the isolates from the drinking water were sensitive to cotrimoxazole, quinolones, chloramphenicol, aminoglycosides, third-generation cephalosporins, and tetracyclins. However, all the *E. coli* isolated from drainage water were resistant to cotrimoxazole, third-generation cephalosporins, and gentamicin. About 50% isolates from the drainage supplies showed resistance to quinolones and tetracyclins. Isolates from tap water and leaking pipe lines were sensitive to majority of the drugs.

Conclusion: A comparison of isolates from drinking water with drainage water revealed that antibiotic resistance is widespread in environmental bacteria. A very higher proportion of antibiotic resistance in drainage water isolates can be correlated with an extensive and sometimes over use of antibiotics, along with the use of higher antibiotics only, even for the treatment of mild infections in patients and spillage of resistant bacteria from patients to the environment. Resistance to higher antibiotics such as third-generation cephalosporins and aminoglycosides, which are hope for severe infections is alarming, as the replacement of sensitive bacterial flora with resistant florae will invite serious infections and difficult treatment situations. This study alarms for an appropriate and judicious antibiotic usage.

KEY WORDS: Antibiotic-resistant bacteria, environmental bacteria, thermotolerant *E. coli*

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Introduction

The antibiotic-resistant bacteria (ARB) are creating a major public health issue, globally, owing to their presence and spread, especially in aquatic ecosystems that are known for the ARB and antibiotic resistance genes (ARGs). Although several studies have detected ARB in drinking water systems, most of the earlier studies had focused on cultivable bacteria and/or indicator organisms. There is a scarce knowledge regarding the effects of ARGs in the drinking water bodies, and, recently, they were found to be the emerging contaminants. The emergence of antimicrobial-resistant strains of pathogenic bacteria has become a great threat to the public health.[1] However, in our region, the study of antibiotic resistance of bacteria from environment such as soil, water, or from fish is scanty.

E. coli is a part of the normal flora of human and animal fecal matters, which may contaminate the soil and water.[2] *E. coli* in water sources is accountable for disease outbreaks[3–5] and mortality worldwide in recent years. Infection with these bacteria may be transmitted through an accidental ingestion or deliberate consumption and direct contact with *E. coli*-contaminated water. Although most *E. coli* strains are harmless, some are pathogenic and can cause diseases[6] such as diarrhea, urinary tract infections, respiratory problems, and even life-threatening bloodstream illnesses[7] among others, as new pathogenic strains of these bacteria have been emerging. The increased resistance shown by *E. coli* till date[8] has become a major issue in the treatment of infections by causing difficulties in the mode of treatment through severe infections, which in turn affects the efficiency of the presently followed treatments.[9] Water bodies act as a source of these drug-resistant *E. coli* strains,[10] because they contain the ARB and ARGs, which spread the resistance to opportunistic pathogens,[11] such as *E. coli* bacteria. This study aims to know the prevalence of pathogenic bacteria in environmental water in our hospital and their antibiotic resistance pattern.

Materials and Methods

This prospective study that was done to determine the prevalence of antibiotic resistance in environmental bacteria from water samples of different areas of General Hospital, Sola, Ahmedabad, India, in the Department of Microbiology, GMERS Medical College, Sola, Ahmedabad, from December 2013 to March 2014. Total 50 water samples were analyzed for the determination of the prevalence of antibiotic-resistant coliforms. All samples were collected from different sites [Table 1].

Samples from RO system, tapwater, and leakage pipe line were treated as drinking water. Before the collection of water sample from the tap, first of all, it was sterilized by spirit lamp and then collected in a dry, clean, autoclaved, leak-proof, and sterile glass container. Each sample was labeled with the name of the area, and the date of collection. All the samples were stored in refrigerator at a temperature of 4°C for further use. Water samples were collected for the analysis of fecal contamination and to detect the most probable number (MPN) of fecal coliform by multiple-tube fermentation technique. Water sample was added in MacConkey broth [with indicator bromocresol purple (BCP)]; double strength as 50 mL of sample was inoculated into a bottle containing 50 mL of double strength broth; 10 mL of sample was inoculated into each of the five bottles containing 10 mL of double strength broth, and 1 mL of sample was inoculated into each of the five tubes containing 5 mL of single strength (SS) broth. The tubes and bottles were incubated at 44°C for 24 to 48 h in incubator. At the end of the 24-h incubation period, each tube and bottle were examined for the presence of turbidity, color change to yellow, and gas. If present, gas can be seen in the Durham tube. Negative tubes and bottles were reincubated for further 24-h period. At the end of this period, the tubes and bottles were checked again for turbidity, color change, and gas production. The MPN was found from the test result by referring to MPN table.[12–15]

Table 1: Water collection sites

No.	Water collection sites	Number of samples collected
1	Reverse osmosis (RO) plants	25
2	Tap water from washroom	17
3	Leakage pipeline	5
4	Drainage (sewage) pipeline	3

Inoculation Procedure for Presumptive Fecal Coliform Count from Drainage (Sewage) Water Sample

Before inoculation of the water sample into SS MacConkey's broth, a serial dilution of the sample was done using phosphate buffer saline. Serial dilution up to 10^{-5}, 10^{-6}, and 10^{-7} were prepared.

1. One milliliter of undiluted sample was inoculated into the tube containing 5 mL of SS broth.
2. One milliliter of diluted sample (each 10^{-5}, 10^{-6}, and 10^{-7}) was inoculated into each of the five tubes containing 5 mL of SS broth.

Determination of MPN for Drainage (Sewage) Water

After the incubation, the number of tubes in which lactose fermentation with acid and gas production occurred was counted. The MPN was found from the test result by referring to the MPN table for the MPN of fecal coliforms, bacteria isolated from drainage (sewage) water (also called McRady's table) MPN procedure for irrigation water, EPA, 2002, as mentioned below.

- When calculating the coliform concentration for drainage water, the MPN index (3 × 5 tubes) from the table was first found. The calculation of the coliform concentration uses the following formula: (MPN index number/lowest dilution) × 100.
- For example, if we had used 10^{-2}, 10^{-3}, and 10^{-4} dilutions and our MPN index was 2 ($2/10^{-2}$) × 100 = 2.0×10^4.

Antibiotic sensitivity test (AST) was done by Kirby–Bauer Disc diffusion method on Mueller–Hinton agar for 16–24 h incubation. All *E. coli* that were isolated were subjected for AST, which was performed by disc diffusion test, according to NCCLS guideline.[14] Gram-negative panel consisted of ampicillin + sulbactum (20/10), cotrimoxazole (30 μg),

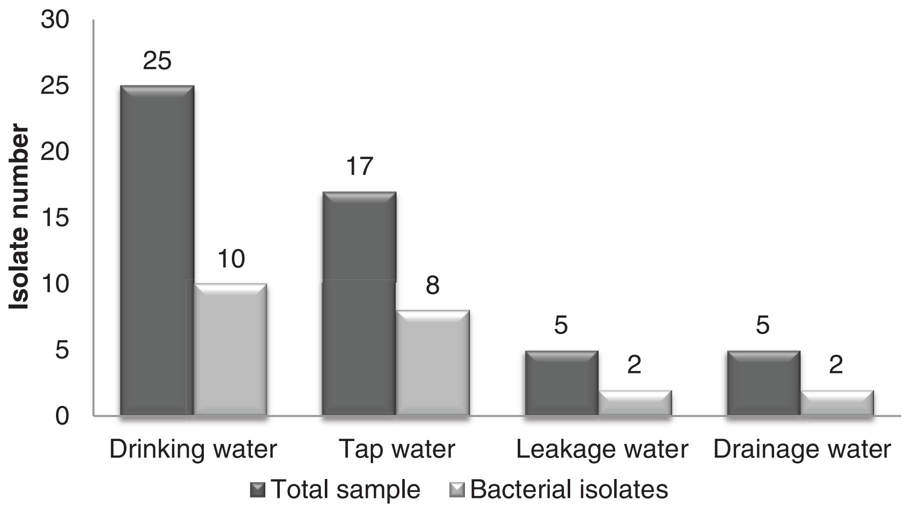

Figure 1: Bacterial isolates from different sources.

Table 2: Comparison of the number of *E. coli* and *Klebsiella* in the different sources of water

	Drinking water	Tap water	Leakage water	Drainage water
Escherichia coli	5	3	2	2
Klebsiella spp.	5	5	0	0

chloramphenicol (30 *μ*g), ciprofloxacin (5 *μ*g), tetracycline (30 *μ*g), ofloxacin (5 *μ*g), amikacin (30 *μ*g), gentamicin (10 *μ*g), cefoxitin (30 *μ*g), cefotaxime (30 *μ*g), and ceftazidime (30 *μ*g).

Result

In this study, of the 50 samples collected from civil hospital, 22 samples (44%) showed contamination with fecal coliform. Of the 22 positive samples, 12 samples showed contamination with *E. coli* and 10 samples with *Klebsiella* spp.

Of the 22 positive samples, 10 were from drinking water, 8 from tap water, 2 from drainage water, and 2 from leakage water. So, in our study, we observed more positivity rate in drainage water [Figure 1].

In our study, a total of 12 *E. coli* were observed in different sources; their numbers shown in different sources were as follows: in drinking water (five), tap water (three), leakage water (two), and drainage water (two). In this study, thermotolerant *E. coli* were observed in the highest number when compared with *Klebsiella* [Table 2].

All the bacteria isolated from drinking water (100%) were sensitive to cotrimoxazole (BA), ciprofloxacin (RC), ofloxacin (ZN), chloramphenicol (CH), gentamicin (GN), amiacin (AK), and nitrofurantoin (NI). *E. coli* from drainage water (100%) were resistant to cotrimoxazole (BA), cefotaxime (CF), piperacillin (PC), nitrofurantoin (NI), and gentamicin (GM) [Table 3].

All the Isolated bacteria (100%) were sensitive to amikacin, whereas *Klebsiella* spp. was 100% sensitive to cotrimoxazole (BA), cefotaxime (CF), ciprofloxacin (RC), ofloxacin (ZN), gentamicin (GM), and amikacin (AK). *Klebsiella* species, on the other hand, showed high resistance frequency to ampicillin (60%); only 10% of the *Klebsiella* strain was resistant to chloramphenicol (CH), tetracycline (TE) [Table 4]. The highest sensitivity frequency of *E. coli* was observed with amikacin (100%), ofloxacin (91.66%), and ciprofloxacin (91.66%). *E. coli* were 50% sensitive and showed resistance to ceftizoxime (CI).

Discussion

The widespread emergence of antibiotic resistance, particularly, multidrug resistance, among bacterial pathogens has become one of the most serious challenges in clinical therapy. Environment containing antibiotic residues exert selection pressure and contribute to the appearance of resistant bacteria. In light of the potential health risks, many studies have focused on antibiotic-resistant bacteria from various ecosystems.[16]

In this study, 12 thermotolerant *E. coli* and 10 *Klebsiella* spp. isolates that were isolated from different water sources (drinking water, tap water, leakage water, and drainage water) were further analyzed for the prevalence of antibiotic-resistant members. The result of the tests revealed that all of the isolates of *E. coli* (100%) were sensitive to amikacin, whereas isolates of *Klebsiella* (100%) were sensitive to cotrimoxazole (BA), cefotaxime (CF), ciprofloxacin (RC), ofloxacin (ZN), gentamicin (GM), and amikacin(AK).

Table 3: Sensitivity (%) *of E. coli* and *Klebsiella* spp. against 13 selected antibiotics in different sources

Drug	*Escherichia coli*								*Klebsiella spp.*			
	Drinking water		Tap water		Leakage water		Drainage water		Drinking water		Tap water	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
AS	80	20	66.6	33.3	50	50	50	50	20	80	40	60
BA	100	0	100	0	100	0	0	100	100	0	100	0
CF	40	60	100	0	100	0	0	100	100	0	100	0
PC	40	60	66.6	33.3	100	0	0	100	80	20	80	20
CH	100	0	66.6	33.3	50	50	100	0	100	0	60	40
RC	100	0	100	0	100	0	100	0	100	0	100	0
CI	80	20	100	0	50	50	50	50	80	20	80	20
TE	40	60	66.6	33.3	100	0	50	50	100	0	40	60
ZN	100	0	100	0	100	0	50	50	100	0	100	0
GM	100	0	100	0	100	0	0	100	100	0	100	0
AK	100	0	100	0	100	0	100	0	100	0	100	0
NI	100	0	100	0	100	0	0	100	100	0	100	0

Table 4: Comparison of resistance in *Escherichia coli* in this study with another study

Antibiotics	*Escherichia coli*		*Escherichia coli*: Ref. [21]	
	S (%)	R (%)	S (%)	R (%)
Cotrimoxazole (BA)	83.33	16.67	88	4
Ciprofloxacin (RC)	91.66	8.34	96	4
Tetracycline (TE)	66.66	33.34	42	42

Table 5: Comparison of the multidrug-resistant bacteria in this study with other studies

	Antibiotics	Resistance (%)
This study	Two to eight antibiotics	66.66
Debmandal et al.[16]	Three or more antibiotics	57
Bahiru et al.[21]	Three or more antibiotics	96.30

Overall resistance was most frequently observed to ampicillin (16.6%), chloramphenicol (16.6%), tetracycline (33.3%), cotrimoxazole (16.6%), piperacillin (41.66%), and ciprofloxacin (8.34%). Most of these antibiotics have been widely used for therapeutic purposes against bacterial infections in humans and animals and as growth promoters in agriculture and aquaculture.[17–19]

The results are also in accordance with other reports. A similar study made in the water supplies of rural Venda communities, South Africa, also showed that more than 95% and 75% of *E. coli* isolates were sensitive to ciprofloxacin and cotrimoxazole, respectively. Similarly, TNAS[20] and Biyela and Bezuidenhout[21] isolated *E. coli* from river water and demonstrated that none of the isolates were resistant to ciprofloxacin.

The results of the study also showed that nearly 40% (10 out of 25) of the drinking water sources were contaminated. The majority of the isolates showed remarkable resistance for cefotaxime (CF) and piperacillin (PC) than resistance in any other source. Antibiotic usage and public awareness creation activities are required to improve the observed efficacy of these two antibiotics in the study area.

Multidrug-resistant bacteria were also observed from water samples collected at General Hospital. Of the 12 *E. coli* isolates tested for antibiotic resistance, eight of them (66.66%) showed multiple resistances for two to eight antibiotics.

This study is compared with other studies as shown in the Table 5

The presence of a large number of coliforms of environmental source in the absence of bacterial pathogens is of no consequence, because these organisms are usually considered as harmless. However, this is not necessarily true if the bacteria in question possess transferable drug resistance. Once these organisms enter the gastrointestinal tract of humans, they may colonize the human gut themselves and transfer their resistance to already colonized bacteria to the sensitive pathogens with which their host may become infected.[26]

Conclusion

Among 50 (100%) water samples of hospitalized area tested, 22 (44%) were positive for the presence of fecal coliform. On the basis of the results of our study, it has been recommended that of the 22 samples, 12 (24%) were positive for thermotolerant *E. coli* contamination, whereas 10 (20%) were positive for *Klebsiella* spp. contamination. In our study, the numbers of bacteria from the sources were drinking water (10), tap water (eight), leakage water (two), and drainage (sewage) water (two). Of the10 drinking water samples, five were positive for thermotolerant *E. coli* and five were positive for *Klebsiella*. Among the total isolates, 13 showed multiple resistance to two to eight antibiotics.

This study revealed that amikacin, ofloxacin, and ciprofloxacin are the best antibiotics to treat *E. coli* infection. Antibiotic susceptibility studies revealed that water from hospital contain antibiotic-resistant *E. coli* strain, which may serve as a reservoir for ARGs in water environment.

This study will provide us a baseline reference for ARB spread in environment and community and help us in devising preventive tools for the emergence of resistant bacteria in hospitals, which will help the hospital administration in implementing guidelines for reducing the emergence of ARBs and the morbidity and mortality arising out of them.

Awareness creation on water quality and sanitation, together with the construction of protected water sources, should be encouraged to reduce the risk of water-borne diseases in the area and to monitor the widespread antibiotic resistance among the environmental bacterial isolates.

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