SHORT COMMUNICATION

Prevalence of Multidrug-Resistant *Escherichia coli* in Drinking-Water in and around Ayodhya (U.P.), India

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Abstract

Thirty five *Escherichia coli* isolates were recovered from 122 drinking-water samples collected from different sources comprising hand pumps (64), university campus water supplies (23), municipal water supplies (20) and domestic water purifiers (15) from different areas in Ayodhya and Sultanpur districts of Uttar Pradesh. *Escherichia coli* was isolated, identified, subjected to antimicrobial susceptibility and determination of Multiple Antibiotic Resistance (MAR) index. All *Escherichia coli* isolates (100%) exhibited resistance against Tetracycline and Furazolidone followed by 94.29, 82.86, 82.86, 71.43, 68.57, 65.71, 51.43, 42.86, 5.71, 5.71, 5.71 and 2.86% isolates against Chloramphenicol, Ampicillin, Amoxicillin/Clavulanic acid, Kanamycin, Nalidixic acid, Trimethoprim, Sulphadiazine, Polymyxin B, Gentamicin, Norfloxacin, Co-trimoxazole, and Streptomycin, respectively. The MAR index of *Escherichia coli* was 0.55. The presence of *Escherichia coli* in drinking-water indicates conclusive evidence of recent faecal pollution.

Key words: Antimicrobial susceptibility, Drinking-water, *Escherichia coli*, Multi-drug resistant.

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Introduction

Presumably, water sources contaminated with antimicrobial-resistant bacteria is one of the major contributing factors for the higher prevalence of antibiotic resistant bacteria from humans living in developing countries (Okeke *et al.,* 1999). Aquatic environment provides a convenient platform for the acquisition and dissemination of antibiotic resistance, since it is the main receptacle for pollution from agriculture, industry or domestic life (Pereira *et al.,* 2013). Antimicrobial-resistant *Escherichia coli* can be ingested *via* food, water and direct contact with fecal contamination (Lyimo *et al.,* 2016). Antimicrobial-resistant bacteria are ubiquitous in environmental waters (Sharma *et al*., 2017).

Multidrug-resistant (MDR) isolate refers to an isolate that is resistant to at least one antibiotic in three or more drug classes (Anonymous, 2019). Antibiotic resistance in pathogenic bacteria is a serious public health issue. Infectious diseases control in recent years is a major health concern globally due to high increase in number of microorganisms that are resistant to conventional antimicrobial agents (Udoh *et al.,* 2020). The emergence and spread of antimicrobial resistant pathogens that have acquired new resistance mechanisms, leading to antimicrobial resistance, continues to threaten the ability to treat common infections (Anonymous, 2021). Multiple antibiotic resistance (MAR) index is the ratio of number of antibiotics to which organism is resistant to the total number of antibiotics to which organism is exposed. MAR index or profile provides useful information for the evaluation of a health risk (Krumperman, 1983). The present study was undertaken to isolate and identify *Escherichia coli* from different sources of drinking-water, and determination of antimicrobial susceptibility test and MAR index to assess the microbiological quality of drinking-water.

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Materials and Methods

Sample Collection

Total 122 samples of drinking-water from different sources comprising hand pumps (64), university campus water supplies (23), municipal water supplies (20) and domestic water purifiers (15) were collected between July, 2018 and August, 2021 from different areas in Ayodhya and Sultanpur districts of Uttar Pradesh. All the samples were collected aseptically transported to the laboratory under ice and kept under refrigeration in the laboratory until analysis.

Isolation and Identification of *Escherichia coli*

Escherichia coli were isolated and identified from drinking-water samples as per method described by Thani *et al.,* (2016).-

Antimicrobial Susceptibility Testing

All isolates of *Escherichia coli* were also subjected to *in-vitro* antimicrobial susceptibility test by disc diffusion methods (Bauer *et al*., 1966). A 0.5 McFarland turbidity standard was prepared by adding 0.05 mL of 1% solution of anhydrous barium chloride in a tube containing 9.95 mL of 1% cold solution of pure sulphuric acid. The tube was sealed and kept in the refrigerator. This turbidity standard corresponds the bacterial concentration of 150 million/mL in the liquid culture. Inoculum turbidity was compared with that of 0.5 McFarland standards. Inoculums were diluted or incubated further as necessary to attain comparative turbidity.

In the present study, fourteen anti-microbial agents were selected on the basis of their use in the treatment of *Escherichia coli* infections in recent years. The anti-microbial discs used were Amoxicillin/clavulanic acid (20/10 µg), Ampicillin (10 µg), Chloramphenicol (10 µg), Co-trimoxazole (25 µg), Furazolidone (50 µg), Gentamicin (10 µg), Kanamycin (10 µg), Nalidixic acid (30 µg), Norfloxacin (10 µg), Polymyxin B (300 µg), Streptomycin, tetracycline (30 µg), Sulphadiazine (100 µg), and Trimethoprim (30 µg). The culture of *Escherichia coli* isolates were inoculated on Muller-Hinton agar (Hi-Media) plates, anti-microbial discs applied on inoculated plates and incubated immediately at 37°C and examined after 24 h. After incubation, the diameter of the zones showing complete inhibition (including the diameter of the disc) was measured using zone scale PW096, of dimension 370 x 65 mm (Hi-Media) and recorded in millimetres. The isolates were interpreted as sensitive, intermediate or resistant as per the interpretation chart provided by the manufacturer.

Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index was determined for each isolate by using the formula as per Krumperman (1983). MAR index = a/ (b * c), where 'a' is the aggregate antibiotic resistance score of all isolates from the sample, 'b' is the number of antibiotics, and 'c' is the number of isolates from the sample.

Results and Discussion

A total of 35 *Escherichia coli* isolates were recovered from 122 drinking-water samples from different sources. All isolates of *Escherichia coli* were subjected to *in-vitro* anti-microbial susceptibility test to assess their sensitivity/resistance status. As all 35 isolates of *Escherichia coli* were found multidrug resistant, and shows a very high degree of multiple drug resistance in *Escherichia coli* isolates from different drinking-water sources.

In the present study, all isolates of *Escherichia coli* (100%) exhibited resistance against each of tetracycline and furazolidone, followed by 94.29%, 82.86%, 82.86%, 71.43%, 68.57%, 65.71%, 51.43%, 42.86%, 5.71%, 5.71%, 5.71% and 2.86% isolates against Chloramphenicol, Ampicillin, Amoxicillin/clavulanic acid, Kanamycin, Nalidixic acid, Trimethoprim, Sulphadiazine, Polymyxin B, Gentamicin, Norfloxacin, Co-trimoxazole, and Streptomycin, respectively (Table 1). Three isolates exhibited resistance against 10 antimicrobial agents. A very high level of antimicrobial resistance against *Escherichia coli* isolates was observed in the present study which might be due to frequent and injudicious

Table 1. Antimicrobial susceptibility of *Escherichia coli* isolates against different Antimicrobials.

Sr. No.	Antimicrobials	No. of sensitive isolate (%)	No. of intermediate isolate (%)	No. of resistant isolate (%)
1	**Ampicillin**	4 (11.43)	2 (5.71)	29 (82.86)
2	Amoxicillin/clavulanic acid	4 (11.43)	2 (5.71)	29 (82.86)
3	Gentamicin	32 (91.43)	1 (2.86)	2 (5.71)
4	Norfloxacin	33 (94.29)	0 (0.00)	2 (5.71)
5	Streptomycin	34 (97.14)	0 (0.00)	1 (2.86)
6	Kanamycin	10 (28.57)	0 (0.00)	25 (71.43)
7	Tetracycline	0 (00)	0 (0.00)	35 (100.00)
8	Chloramphenicol	2 (5.71)	0 (0.00)	33 (94.29)
9	Polymyxin B	16 (45.71)	4 (11.43)	15 (42.86)
10	Co-Trimoxazole	31 (88.58)	2 (5.71)	2 (5.71)
11	Sulphadiazine	1 (2.86)	16 (45.71)	18 (51.43)
12	**Trimethoprim**	6 (17.14)	6 (17.14)	23 (65.71)
13	Nalidixic acid	6 (17.14)	5 (14.29)	24 (68.57)
14	Furazolidone	0 (0.00)	0 (0.00)	35 (100.00)

Table 2. Multiple Antibiotic Resistance (MAR) index of *Escherichia coli* isolates (n = 35).

Sr.No.	No. of isolate	No. of antimicrobials against which the isolate was resistant	Total No. of antimicrobials against which the isolate was resistant
1	1	3	3
2	9	6	54
3	5	7	35
4	5	8	40
5	12	9	108
6	3	10	30
	Total No. of isolate (c) = 35	Total No. of antimicrobials used against each isolate (b) = 14	Aggregate antibiotic resistance score (a) = 270

MAR Index = a/ (b* c) = 270/ (14 x 35) = 270/490 = 0.55
a= aggregate antibiotic resistance score of all isolates from the sample
b= number of antibiotics
c= number of isolates from the sample

use of these antimicrobials for prevention and control of infections in animals and human beings, which is a serious public health problem. Similar to present findings, Baruah *et al*. (2005) reported 95.62% sensitivity of *Escherichia coli* isolates against gentamicin. Conversely, Odonkor and Addo (2018) reported lower resistance in *Escherichia coli* isolates against tetracycline (21.45%).

In the present study, multi-drug resistant *Escherichia coli* was detected in large number of drinking-water samples, which indicates intensive use of antimicrobial agents in food animal production as well as injudicious use of antimicrobial agents for the treatment of *Escherichia coli* infections in humans.

The Multiple Antibiotic Resistance (MAR) index of *Escherichia coli* was determined (Table 2) and found as 0.55. This high MAR index indicates injudicious and frequent use of several antimicrobial agents for preventive as well as therapeutic purposes. The findings of the present study was almost similar to the observations of Jaulkar *et al*. (2011). MAR index greater than 0.2 indicates high risk source of contamination where antibiotics are often used (Osundiya *et al*., 2013).

Conclusion

In the present study, the high prevalence of multidrug-resistant *Escherichia coli* in the drinking-water with high MAR index indicates high level of risk of contamination of the sampled water sources.

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