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Prevalence of Carbapenem-Resistant E. coli in Drinking Water from Rural Areas of Gurugram, Haryana

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ABSTRACT:
This study investigates the prevalence of carbapenem-resistant Escherichia coli in drinking water from rural areas of Gurugram, Haryana, India. Over 120 water samples were collected from various sources (public taps, kitchen taps, bathroom taps, and borewells) across three seasons (monsoon, winter, and summer). The results indicated only 4.17% of groundwater samples showed contamination. While as in the municipal tap water samples from different sources reveal varying levels of E. coli contamination. Public taps exhibited the highest contamination, with 33.33% of samples testing positive for E. coli, while 66.67% showed no microbial growth. Bathroom taps had a lower contamination rate, with 23.33% of samples positive for E. coli and 76.67% showing no growth. Kitchen taps were the least contaminated, with 13.33% of samples positive for E. coli and 86.67% showing no growth. These results indicate that public taps are more prone to E. coli contamination compared to bathroom and kitchen taps. Antibiotic susceptibility testing revealed significant resistance to Cefuroxime (58.33%), followed by Amoxicillin/Clavulanic Acid (54.17%), Ciprofloxacin (50%), and Co-trimoxazole (45.83%). Notably, all isolates were fully susceptible to Amikacin (0% resistance). The lowest resistance rates were recorded against Ertapenem, Imipenem, and Meropenem, each at 4.17%. These results indicate that only one of the 24 isolates exhibited Carbapenem resistance, which was further confirmed by PCR analysis revealing the presence of carbapenemase genes, blaOXA-48 and blaNDM. The findings highlight the potential health risks associated with the municipal water supply and underscore the need for improved water quality monitoring and management in the region.

1. Introduction

Water is a fundamental resource for life [1]. Ensuring a reliable, safe, and easily accessible water supply is crucial for all individuals. The World Health Organization (WHO) has focused on improving access to safe drinking water through assessment, effective management, strategic communication, and continuous monitoring. These efforts have led to significant health improvements and support the fundamental human right to clean water [2]. The Global Burden of Disease report highlighted that waterborne diseases were the second leading cause of mortality in 1990, but by 2020, they had dropped to the ninth leading cause[3]. (WHO (2015) noted that improving water supply, sanitation, and hygiene could mitigate nearly 4% of the global disease burden [4]. Evaluating water quality is essential to determine its cleanliness and suitability for various uses.

In India, which is home to approximately 17% of the global population, a significant portion of people lacks access to clean water[5]. The World Resources Institute reports that around 70% of India's water supply is contaminated with sewage discharge. Consequently, India ranks 120th out of 122 countries in terms of water quality[6] . Previous research by [7] found that 21% of communicable diseases in India are associated with the use of unsafe water.

Between 2010 and 2014, polluted water caused 13,000 deaths in India, with Uttar Pradesh experiencing the highest number of deaths (3,382), followed by West Bengal, Andhra Pradesh, and Odisha [8]. From 2013 to 2017, waterborne diseases like cholera, diarrhoea, typhoid, and viral hepatitis resulted in 10,738 fatalities, over 69.14 million illness episodes, and 73 million lost working days. During this period, bacterial infections

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were predominant, accounting for approximately 68.51 million cases and 8,595 deaths [9].

In rural India, deficiencies in knowledge, attitudes, and practices regarding water handling, sanitation, and defecation significantly contribute to the prevalence of waterborne diseases [10, 11]. The decline in water quality presents serious public health and ecological challenges [12, 13]. Accessible water sources, such as surface and groundwater, are vulnerable to contamination from various sources, including sewage discharges, faecal contamination, faulty drains, rainwater runoff, municipal waste, and industrial effluents[14].

This contamination contributes to the proliferation of Gram-negative bacteria that pose severe clinical consequences and a potential public health crisis worldwide [15]. Among these, carbapenem-resistant Enterobacteriaceae (CRE) are particularly concerning due to their extensive drug resistance. Reports indicate that CRE causes approximately 9,000 infections and 600 deaths annually in the United States [16, 17]. The rapid rise in the prevalence and clinical significance of infections caused by carbapenemase-producing Gram-negative bacteria (CP-GNB) has emerged as a worldwide health concern, with invasive infections associated with elevated mortality rates[18, 19].

Given the increasing prevalence of carbapenem-resistant *E. coli*, which contributes significantly to the burden of antibiotic-resistant infections, this study aims to assess the prevalence of carbapenem-resistant *E. coli* in Gurugram, Haryana, India.

2. MATERIAL & METHODS

2.1 Study area

The research was carried out at the Department of Microbiology, SGT Medical College Hospital and Research Institute in Budhera, Gurugram.

2.2 Study duration

The study was conducted from March 2022 to February 2024 at the Department of Microbiology, SGT Medical College Hospital and Research Institute, Budhera, Gurugram.

Ethical Considerations: The study was conducted after the Institutional Ethics Committee of SGT University (IEC/FMHS/PhD/S/2022-14).

2.3 Study Design: Community-based prospective study.

2.4 Sampling frequency and periodicity

Samples were collected thrice a year during: Monsoon - July-September, Winter - October-February, Summer - March-June. The same household was monitored for one year. Sampling was done one time from each house during each season.

2.5 Sample Collection Sites

Following several initial visits to various communities within the districts, 40 sampling sites were chosen, including kitchen taps, bathroom taps, public taps, and village tube wells. Samples were collected from locations that were representative of the water sources and distribution networks supplying water to the residents. The selection was primarily based on factors such as population and usage levels.

2.6 Site Observation Details

Before collecting water samples, significant observations were noted at the sampling locations. These included assessing the sanitary conditions and identifying potential sources of contamination that could affect the water quality.

2.7 Collection and transportation of samples

The sample collection method at each source adhered to WHO guidelines for drinking water quality assessment. Water samples were collected in heat-sterilized 500 ml screw-capped bottles containing an appropriate amount of sodium thiosulfate (0.1 ml of a 1.8% fresh aqueous solution per 100 ml of water sample). The mouth of the tap used for sampling was sterilized with cotton wool soaked in 70% ethanol, and the tap was allowed to run for two minutes. Extreme care was taken to prevent accidental contamination during collection. Sterile glass bottles were carefully uncapped, filled with water, and then recapped. The time of collection, site name, and source were recorded on the sample bottle. All samples were preserved in cold boxes, transported to the microbiology laboratory within 4 hours, and maintained at 4-8ºC until use [20, 21].

2.8 Isolation and identification of *E. coli*

2.8.1 Membrane filtration technique

A 100 ml volume of each water sample was filtered through a 47 mm membrane filter (Cellulose Nitrate, Sartorius Stedium Biotech GmbH, Göttingen, Germany) with a 0.45 µm nominal pore size using a vacuum filtration system [22].

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2.8.1.1 Isolation on selective media

After filtration, each membrane filter was placed on a Chromogenic selective agar plate (Hi-Crome E. coli agar, Hi-Media Laboratories Pvt. Ltd, Mumbai, India) [22]. The plates were initially incubated at 37°C for 4 hours, followed by an additional incubation at 44°C for 16–22 hours. Post-incubation, blue colonies indicated the presence of E. coli. Typical E. coli colonies were isolated and purified on MacConkey Agar [23]. Biochemical confirmation was then performed using the automated Vitek-2 system, bioMérieux, France, following the manufacturer's instructions. The Gram-negative (GN) card was used for bacterial identification[24].

2.8.1.2 Antimicrobial susceptibility testing

The AST-N405 card was utilized for antimicrobial susceptibility testing (AST) using the Vitek 2 compact system. Selected E. coli isolates were tested against 15 antibiotics, including Amoxicillin/Clavulanic Acid, Piperacillin/Tazobactam, Cefuroxime, Ceftriaxone, Cefoperazone/Sulbactam, Cefepime, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline, Fosfomycin, and Co-trimoxazole. The AST-N405 card was automatically filled by a vacuum device, sealed, inserted into the Vitek 2 reader-incubator module (incubation temperature: 35.5°C), and subjected to kinetic fluorescence measurement for identification. Turbidity was monitored every 15 minutes to assess susceptibility. After the incubation cycle, bacterial isolates were identified, and minimum inhibitory concentration (MIC) values were determined for each antibiotic on the card. E. coli ATCC 25922 was used as a positive control, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines[24, 25]

2.9 Phenotypic expression of carbapenemase production by Rapidec® carba NP test bioMerrieux

The Carba NP test, a confirmatory biochemical assay, was employed for the rapid detection (within 2 hours or less) of carbapenemase production.

2.10 Carbapenemase-producing genes of *E.coli* by molecular technique.

The Xpert Carba-R Assay was used for the detection and differentiation of the *bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48, and *bla*IMP gene sequences associated with carbapenem-non-susceptibility. The test utilizes automated real-time polymerase chain reaction (PCR) [26].

2.11 Statistical Analysis

Using STATA software, version 16 (STATA 16 Corp., College Station, TX) and Graphpad Prism version 8, the data was depicted graphically and the Chi-square test was used to assess the association between the two variables.

3. Results

A total of 120 samples were collected throughout the study period, with 40 samples collected in each season: Monsoon (July-September), Winter (October-February), and Summer (March-June), as illustrated in Figure 1.

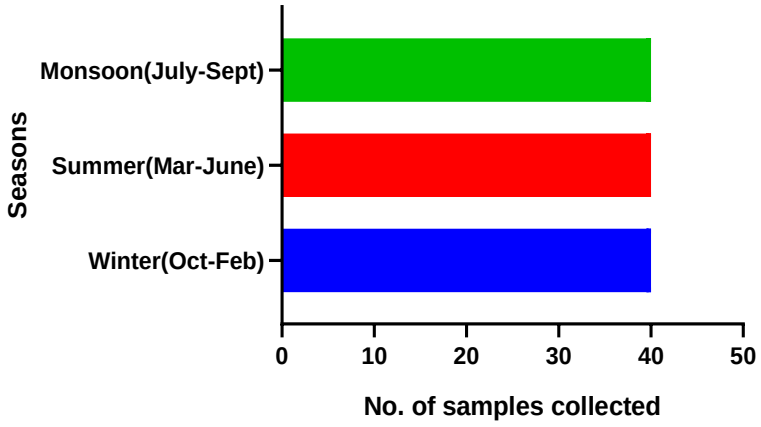

Figure 1: Number of samples collected in different seasons

Journal of Chemical Health Risks
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Table 1 shows the distribution of samples collected from different sites. A total of 120 samples were collected, with 36 samples (30%) from public taps, 30 samples (25%) from kitchen taps, 30 samples (25%) from bathroom taps, and 24 samples (20%) from borewell water

Table 1: Number of samples collected in different sites

Site	n(n%)
Public Tap	36(30)
Kitchen Tap	30(25)
Bathroom Tap	30(25)
Borewell water	24(20)
Total	120(100)

Distribution of *E. coli* in Water Samples

Membrane Filtration Technique

We employed the Membrane Filtration technique to isolate *E. coli*, as depicted in Figure 2 & 3. A total of 120 water samples were collected, comprising both groundwater and municipality-supplied tap water. Of these, 24 samples (20%) were from groundwater, and 96 samples (80%) were from municipality tap water, as shown in Figure 2. After applying the membrane filtration technique, *E. coli* was detected in 1 (4.17%) of the 24 groundwater samples, with no growth observed in the remaining 23 samples (95.83%). In contrast, E. coli was present in 23 (24%) of the 96 municipality tap water samples (Table 2).

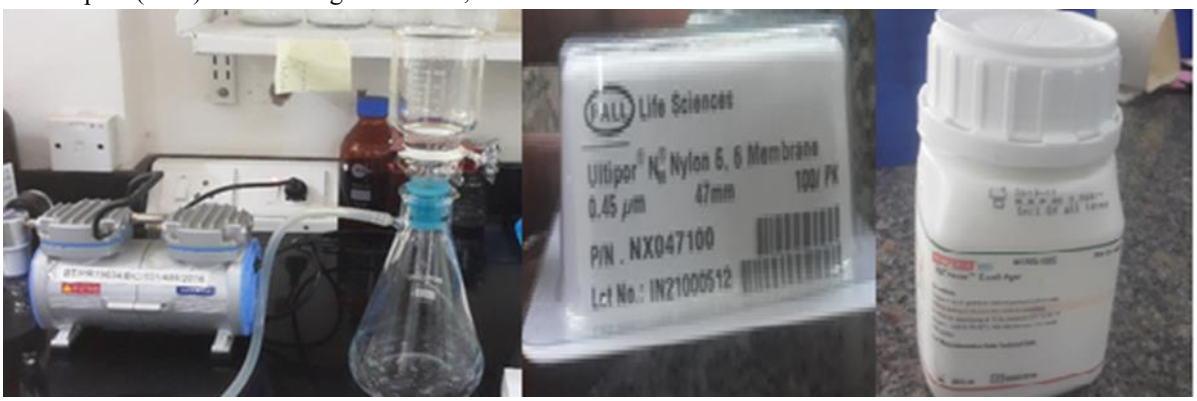
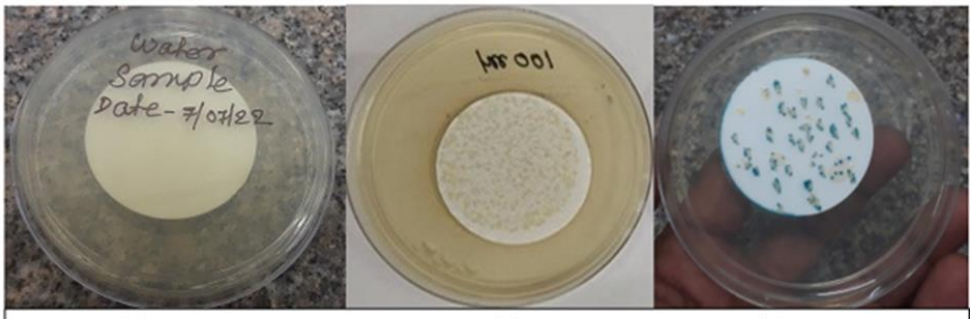


Figure 2: Equipment and Culture media technique in Membrane filtration method



(a) (b) (c)

Figure 3: (a) Culture media with no microbial growth (b) Growth of microorganisms other than E.coli, (c) Bluish colonies indicating the presence of E.coli

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Table 2: Overall distribution of *E. coli* in ground and municipality supply water

Sources of water sample	Sample collected (n%)	No growth (n%)	E. coli (%)
Ground			
Borewell	24(20)	23(95.83)	01(4.17)
Municipality			
Tap water	96(80)	73(76.04)	23(24)
Total	120 (100)	96(80)	24(20)

There was a statistically significant difference between groundwater and municipality-supply water (P<0.05), with municipality-supply water showing a higher contamination rate of *E. coli* (23.96%) compared to groundwater (4.17%). Furthermore, samples collected during the monsoon season exhibited an increased *E. coli* contamination rate of 32.50%, which was statistically significant. Additionally, the contamination rate was highest in public tap water, at 33.33% (Table 3).

Table 3: E. coli distribution in water samples based on origin, season, and sites

Parameter	E. coli (n%)	No growth (n%)	P-value
Origin of water			
Ground	1(4.17)	23(95.83)	0.030
Municipality	23(23.96)	73(76.04	
Season			
winter	6(15)	34(85)	0.049
Summer	5(12.50)	35(87.50)	
Monsoon	13(32.50)	27(67.50)	
Different sites of water			
Borewell	1(4.17)	23(95.83)	0.032
Bathroom Tap	7(23.33)	23(76.67)	
Kitchen Tap	4(13.33)	26(86.67)	
Public Tap	12(33.33)	24(66.67)	

Antibiotic Resistance and Susceptibility in *E. coli* Isolates

The antibiotic susceptibility testing of *E. coli* isolates, as presented in Table 4 and Figure 4, revealed that the highest resistance was observed against Cefuroxime (58.33%), followed by Amoxicillin/Clavulanic Acid (54.17%), Ciprofloxacin (50%), and Co-trimoxazole (45.83%). Notably, all isolates were fully susceptible to Amikacin (0% resistance). The lowest resistance rates were recorded against Ertapenem, Imipenem, and Meropenem, each at 4.17%. These results indicate that only one isolate out of 24 was resistant to Carbapenem.

Table 4: Antibiotic Resistance and Susceptibility in *24 E. coli* Isolates

Antibiotics	Resistance(n%) of *E. coli*	Sensitive(n%) of *E. coli*
Amoxicillin/Clavulanic Acid	13(54.17)	11(45.83)
Piperacillin/Tazobactam	6(25)	18 (75)
Cefuroxime	14(58.33)	10(41.67)
Ceftriaxone	10(41.66)	14(58.33)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(5), 396-405 | ISSN:2251-6727

Cefoparazone/Sulbactam	3 (12.50)	21(87.50)
Cefepime	9(37.50)	15(62.50)
Ertapenem	1(4.17)	23(95.83)
Imipenem	1(4.17)	23(95.83)
Meropenem	1(4.17)	23(95.83)
Amikacin	0(0.00)	24(100)
Gentamicin	3(12.50)	21(87.50)
Ciprofloxacin	12(50)	12(50)
Tigecycline	2(8.33)	22(91.67)
Fosfomycin	2(8.33)	22(91.67)
Co-trimoxazole	11(45.83)	13(54.17)

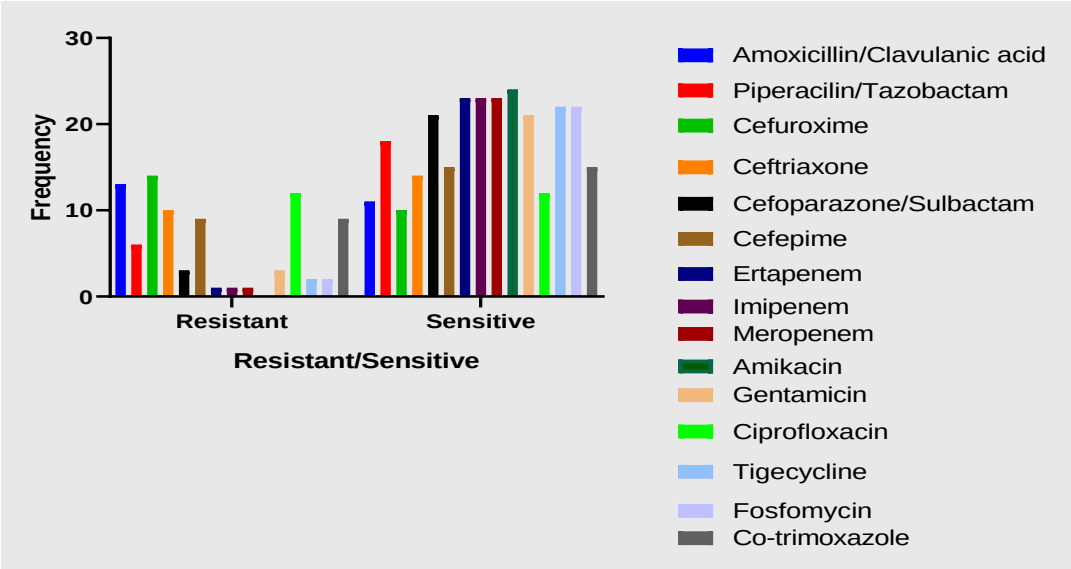

Figure 4: Antibiotic Resistance/sensitivity VS Frequency of *E. coli*

Distribution of carbapenemase-producing *E. coli*

Among the 24 *E. coli* isolates, only one carbapenemase-producing strain was found in the municipal water supply, with this result not being statistically significant ($p = 0.831$). Interestingly, this carbapenem-resistant *E. coli* was detected during the summer season (March-June), but the prevalence did not significantly differ from other seasons ($p = 0.138$). Additionally, there was no significant difference in the prevalence of carbapenemase-producing *E. coli* across various water collection sites ($p = 0.791$)

Phenotypic Analysis: Confirmatory Biochemical Rapidec® Carba NP test

The only one isolates carbapenem-resistant *E.coli* was again tested for carbapenemase production activity, revealing similar results obtained by the Antibiotic susceptibility test , that also validates our results, revealing that only one carbapenemase-producing *E. coli* was detected in public municipal taps during the summer season. Figure 5 shows the Rapidec® Carba NP test.

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Figure 5: The Rapidec Carba NP test indicates the presence of carbapenemase-producing *E. coli*.

Genotypic Analysis: Detection of Carbapenem-Resistant Genes.

PCR analysis identified two carbapenem-resistant genes: oxacillinase-48 (*bla*OXA-48) and New Delhi metallo-beta-lactamase (*bla*NDM). These results are illustrated in Figures 6.

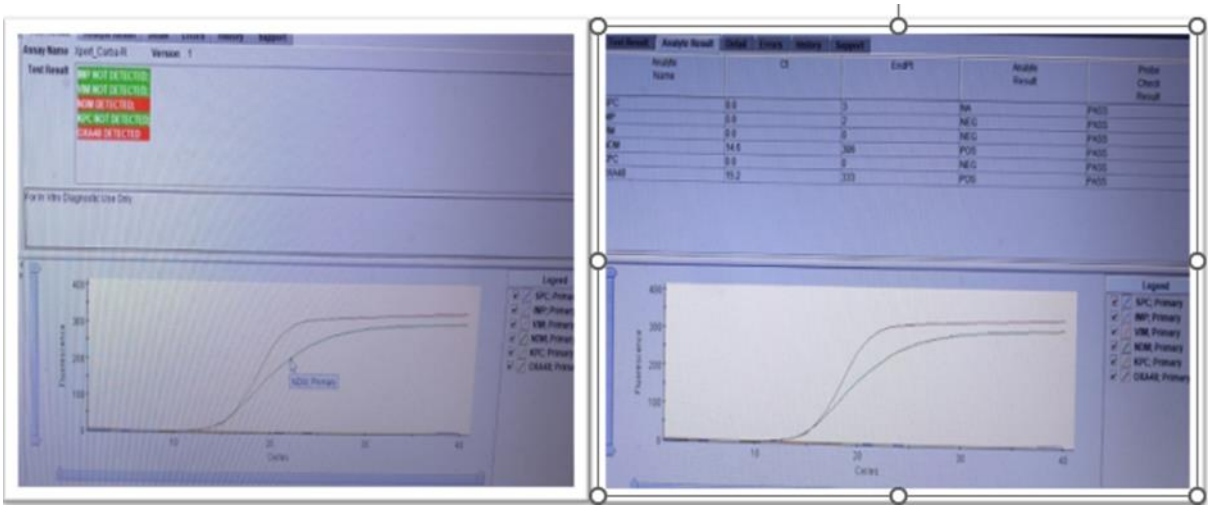

Figure 6: PCR Amplification Curves for blaNDM and blaOXA-48 Genes. The curves illustrate the detection of blaNDM and blaOXA-48 genes in the samples, showing distinct amplification patterns and threshold cycle (Ct) values for each gene.

4. Discussion

The present study aimed to evaluate the Carpanemese-producing *Escherichia coli* (*E. coli)* in water sources, as well as the antibiotic resistance. A total of 120 water samples were collected from various sources, including public taps, kitchen taps, bathroom taps, and borewell water, across three distinct seasons—monsoon, winter, and summer. The study findings revealed several critical insights into the prevalence of Carbapenem-resistant *E. coli* contamination and its resistance patterns in the drinking water of Gurugram Haryana.

The significant difference in *E. coli* contamination levels between municipality-supplied tap water and groundwater highlights concerns regarding the safety and reliability of municipal water systems. In this study, 24% of the municipality-supplied tap water samples tested positive for *E. coli*, while only 4.17% of the groundwater samples showed similar contamination. This suggests that municipality-supplied water may be more vulnerable to fecal contamination due to potential infrastructural deficiencies or shortcomings in water treatment processes.

Water is known to be an efficient vehicle for the spread of *E. coli* and has been linked to numerous outbreaks globally [27-29]. Contaminated groundwater, while less prevalent in this case, is still a notable concern. In fact, in a related study, 26.7% of 60 groundwater samples contained E. coli, reflecting significant fecal contamination in the region. Singh reported a similar trend, identifying *E. coli* in 25% of bore well water samples in Mathura [30]. On the other hand, Ahmed found a lower contamination rate of 9.23%, suggesting variability in contamination levels depending on the

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JCHR (2024) 14(5), 396-405 | ISSN:2251-6727

location and management of groundwater sources [31]. The findings emphasize the need for stringent water quality monitoring and enhanced treatment protocols, especially in municipal systems, to mitigate health risks associated with *E. coli* contamination.

Seasonally, the monsoon period exhibited the highest rate of *E. coli* contamination (32.50%), which is consistent with the increased risk of waterborne diseases during this time due to heavy rainfall and possible flooding. The contamination rates were significantly lower in the winter (15%) and summer (12.50%) seasons, highlighting the impact of seasonal variations on water quality. The observed patterns align with previous studies that have reported elevated microbial contamination during the rainy season, emphasizing the need for enhanced monitoring and treatment of water during these periods. Among the different sites, public tap water showed the highest contamination rate (33.33%), consistent with a study conducted in Hangzhou City by [23], followed by bathroom taps (23.33%) and kitchen taps (13.33%). Borewell water, often regarded as a relatively safer source, exhibited the lowest contamination rate of 4.17%. The elevated contamination rates in public taps could be attributed to the higher likelihood of exposure to external contaminants, lack of proper maintenance, or potential cross-contamination from other sources. These findings underscore the necessity for stringent water quality surveillance, particularly in public water distribution systems.

The antibiotic susceptibility testing revealed a concerning level of resistance among *E. coli* isolates, particularly to beta-lactam antibiotics such as Cefuroxime (58.33%). This finding of *E.coli* sensitivity towards various antibiotics were similar to the study conducted in Canada by [32]. The high resistance to these antibiotics poses a significant public health risk, as beta-lactams are commonly used in the treatment of bacterial infections. Conversely, the isolates were fully susceptible to Amikacin, and resistance to carbapenem antibiotics Ertapenem, Imipenem, and Meropenem—was relatively low (4.17% each), with only one isolate demonstrating carbapenem resistance. This suggests that while carbapenem resistance remains low, the potential for its increase warrants close attention, particularly in light of the global rise in carbapenem-resistant Enterobacteriaceae.

Interestingly, the study identified only one carbapenemase-producing *E. coli* isolate, which was found in municipal tap water during the summer season. These findings align with a study conducted on drinking water samples in Bareilly, India, where no carbapenem-resistant *E. coli* was detected[15]. The low prevalence of carbapenemase production, as confirmed by both phenotypic (Rapidec® Carba NP test) and genotypic (PCR detection of *bla*OXA-48 and *bla*NDM genes) methods, indicates that while the occurrence of such resistant strains is currently limited, ongoing surveillance is crucial to prevent potential outbreaks. The absence of a statistically significant difference in carbapenemase production across different water sources and seasons suggests that the risk of widespread dissemination remains contained, but vigilance is necessary.

5. Conclusion

The findings from this study highlight the significant seasonal and site-specific variations in *E. coli* contamination of water sources, with higher contamination rates observed during the monsoon season and in public taps. The detection of antibiotic resistance, particularly to beta-lactams, and the presence of a carbapenemase-producing isolate underscore the pressing need for robust water quality monitoring and the implementation of effective antimicrobial stewardship programs. As waterborne pathogens continue to pose a major public health challenge, especially in resource-limited settings, these results provide critical insights for guiding public health interventions and policies aimed at ensuring safe drinking water and mitigating the spread of antibiotic-resistant bacteria.

Limitations of the study

One of the primary limitations of this study is the relatively small sample size. A larger number of samples would have provided a more comprehensive understanding of the prevalence and distribution of carbapenem-resistant *E. coli* in the region, potentially yielding more robust and generalizable findings.

Conflict **of Interest:** The authors declare no competing interests

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Journal of Chemical Health Risks
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