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Prevalence of Multidrug-Resistant (MDR) and Extended-Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* in Household Drinking Water from Rural Areas of Gurugram, Haryana

Rajeev Kumar Jha[1], Manisha Khandait[2], Moumita Sardar[2], Mukesh Sharma[2], Ranjay Kumar Choudhary[3], Gowhar Rashid[3]

[1]Department of Medical Microbiology, S.G.T. University, Budhera, Gurugram, Haryana, India

[2]Faculty of Medicine & Health Sciences, S.G.T. University, Budhera, Gurugram, Haryana, India

[3]Amity Medical School, Amity University, Gurugram, Haryana, India

Address for correspondence: Dr. Manisha Khandait

Email: manisha_fmhs@sgtuniversity.org

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KEYWORDS
MDR, *E.coli*, ESBL, Groundwater, Municipality Supply water

ABSTRACT:

This study investigates the prevalence of multidrug-resistant (MDR) and Extended-Spectrum Beta-Lactamase (ESBL) Producing 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. The antibiotic susceptibility testing revealed significant resistance against Cefuroxime (58.33%), Amoxicillin/Clavulanic Acid (54.17%), and Ciprofloxacin (50%). Notably, Carbapenems (Ertapenem, Imipenem, Meropenem) each showed a resistance rate of 4.17%. All isolates were fully susceptible to Amikacin, with 0% resistance, suggesting that these antibiotics remain largely effective. Additionally, low resistance rates were observed with Piperacillin/Tazobactam (25%), Cefoparazone/Sulbactam (12.50%), Gentamicin (12.50%), and Tigecycline and Fosfomycin (8.33%) respectively. Further study revealed that out of 24 Escherichia coli isolates from rural Gurugram, Haryana, 14 were multidrug-resistant (MDR), with the highest prevalence in public taps (25%), followed by the bathroom (20.83%) and kitchen taps (12.50%). No MDR E. coli was found in borewell water. Additionally, 10 isolates were identified as ESBL-producing, most commonly from public taps (16.66%), with equal distribution in bathroom and kitchen taps (12.50%). Borewell water samples showed no ESBL-producing E. coli. The results underscore the growing challenge of antibiotic resistance in rural water sources, highlighting the need for ongoing surveillance and improved water quality management to mitigate the spread of resistant E. coli strains.

1. Introduction

Water is essential for the survival of all living organisms. However, the reduction of clean water sources and declining water quality are major global public health challenges. Contaminated water accounts for 4% of human deaths and 5% of global health problems [1]. Around 350 million people come into contact with various water bodies annually[2], increasing health risks when the water is contaminated, leading to waterborne diseases. These diseases, caused by viruses, bacteria, or parasites, pose significant health risks, particularly for vulnerable populations like infants, children, pregnant women, and those with compromised immune systems [3]. Diarrhoea, caused

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by water contamination, leads to about 2 billion cases annually and is the second leading cause of death in children under 5, with 1.9 million fatalities [1].

The main contributor to water contamination is the infiltration of faecal matter from humans and animals into water sources. The Bureau of Indian Standards (BIS) set drinking water specifications in 1983, revising them in 2010 and 2012 to align with international guidelines, requiring no detectable thermotolerant *E. coli* in 100 mL of water [4]. While *E. coli* has traditionally served as a key indicator of faecal pollution in water microbiology, it poses a more serious public health risk when these *E. coli* strains are multidrug-resistant pathogens [5]. Before the development of antibiotics, infections and diseases caused by pathogenic *E. coli* and similar bacteria frequently resulted in prolonged illness and higher mortality rates. The development of antibiotics and enhancements in their use for treating *E. coli* infections have substantially decreased the mortality rates associated with bacterial infections [6]. Regrettably, bacteria such as *E. coli* have swiftly developed resistance to numerous vital antibiotics that were previously very effective in treating bacterial infections and diseases over time.[7] Globally, approximately 700,000 deaths occur each year due to infections that are resistant to treatment[8]. If current trends persist, antimicrobial resistance could drive more than 24 million people into extreme poverty by 2030 and is projected to cause around 10 million deaths annually by 2050 [9]. The rising resistance of *E. coli* to vital antibiotics is an alarming global health problem. Multidrug-resistant strains of *E. coli* have now been found across a range of environmental and community settings [10, 11]. Identifying ESBL-producing *E. coli* in drinking water is crucial for evaluating the risk of antimicrobial resistance (AMR) and gastrointestinal infections. Plasmids frequently facilitate the transfer of ESBL-encoding genes among bacteria. [12], and aquatic environments are particularly favourable for the horizontal spread of AMR genes through various mobile genetic elements[13]. Therefore, studying the prevalence and traits of ESBL-producing *E. coli* in drinking water, particularly in densely populated regions with inadequate access to safe water and poor sanitation, is essential for mitigating the high risk of waterborne diseases. This study aims to examine the prevalence of multidrug-resistant (MDR) and ESBL-producing *E. coli* in Gurugram, Haryana, India.

2. MATERIAL & METHODS

2.1 Study area

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

2.2 Study duration

The research was conducted between March 2022 and February 2024 in the Department of Microbiology at 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 Sites for Sample Collection

After conducting multiple preliminary visits to different communities in the districts, 40 sampling locations were selected. These included kitchen taps, bathroom taps, public taps, and village tube wells. The samples were taken from areas that accurately represented the water sources and distribution networks serving the local population. The selection process mainly considered factors such as population size and water usage levels.

2.6 Samples size

A total of 120 water samples were collected for the study from kitchen taps, bathroom taps, public taps, and tube wells.

2.7 Inclusion Criteria:

All households utilizing groundwater and/or municipality supply water for drinking without filtration/boiling will be included in this study.

2.8 Exclusion Criteria:

Those houses using RO water or boiling water will be excluded in this study.

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2.9 Collection and transportation of samples

The sample collection process at each source followed WHO guidelines for drinking water quality assessment. Heat-sterilized 500 ml screw-capped bottles, containing a sufficient amount of sodium thiosulphate (0.1 ml of a 1.8% fresh aqueous solution per 100 ml of water), were used for collecting the samples. To sterilize the tap from which the water was drawn, cotton wool soaked in 70% ethanol was applied, and the tap was then left running for two minutes. Extreme care was taken to prevent accidental contamination during the collection process. Sterile glass bottles were carefully uncapped, filled with the water sample, and then recapped. The time of collection, site name, and water source were clearly labeled on each sample bottle. All samples were stored in cold boxes, transported to the microbiology laboratory within four hours, and kept at a temperature of 4-8ºC until testing [14, 15]

2.10 **Isolation and identification of *E. coli***

2.10.1 Membrane filtration technique

A measured 100 ml volume of each water sample was filtered using a 47 mm cellulose nitrate membrane filter (Sartorius Stedium Biotech GmbH, Göttingen, Germany) with a 0.45 µm pore size, utilizing a vacuum filtration system [16].

2.10.1.1 Isolation on Selective Media

After filtration, each membrane filter was placed onto a Chromogenic selective agar plate (Hi-Crome E. coli agar, Hi-Media Laboratories Pvt. Ltd, Mumbai, India)[16]. The agar plates were first incubated at 37°C for 4 hours, then further incubated for 16–22 hours at 44°C. After incubation, blue colonies indicated the presence of *E. coli*. Typical *E. coli* colonies were then selected and purified on MacConkey Agar [17].

2.10.2 **Presumptive identification of *E. coli***

Colony morphology

Small colonies measuring 2–3 mm in diameter, with a circular shape, regular edges, flat surface, smooth texture, lactose-fermenting characteristics, and a translucent appearance. **Gram's staining**

All *E. coli* isolates were processed using the standard Gram staining protocol, which involved applying crystal violet as the primary stain, utilizing Gram's iodine as a mordant, employing 95% ethanol as a decolourizer, and using safranin as a counterstain to confirm the isolates as Gram-negative bacilli.[18]

Motility test

The motility of the isolates was tested by the hanging drop method using nutrient

broth cultures. Rapid to and fro or forward movement by cells was considered as positive

motility.[19]

Biochemical confirmation:

The presumptive *E. coli* colonies, which appear blue or bluish-green, were transferred to nutrient agar (Oxoid, UK) and incubated at 37°C for 24 hours. Bacterial identification was performed using the automated Vitek-2 compact system, following the manufacturer's instructions. The Gram-negative (GN) card was employed for the identification of the bacteria. [20]

2.10.3 **Phenotypic screening for drug resistance pattern of *E. coli.***

Testing for antimicrobial susceptibility

The AST-N405 card was employed for antimicrobial susceptibility testing (AST) using the Vitek 2 compact system and the results were interpreted following Clinical and Laboratory Standards Institute (CLSI) guidelines [20, 21].

Criteria for multidrug resistance

Isolates exhibiting resistance to at least one agent across three or more distinct drug classes will be classified as multidrug-resistant (MDR)[22, 23]

2.10.4 Evaluating *E. coli* for ESBL Production

Phenotypic Disc Confirmatory Test (PDCT)

A phenotypic disc confirmatory test was performed by suspending bacterial isolates in sterile saline (0.9% NaCl) and adjusting the suspension to 0.5 McFarland standards. Sterile cotton swabs were dipped into the inoculum and used to uniformly streak the surface of the medium.

- A Ceftazidime (CAZ) disc with a concentration of 30 µg and a Ceftazidime + Clavulanic acid (CAC) disc with a concentration of 30 + 10 µg were positioned 30 mm apart from center to center.

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- A Cefotaxime (CTX) disc with a concentration of 30 µg and a Cefotaxime + Clavulanic acid (CEC) disc with a concentration of 30 + 10 µg were similarly placed 30 mm apart from center to center.

The plates were incubated overnight at 37ºC, and readings were taken. Isolates were classified as ESBL producers if the zone of inhibition difference between the antibiotic with the inhibitor and the antibiotic alone was 5 mm or less [24], [25]

2.11 Statistical Analysis

Data was graphically represented using STATA software, version 16 (STATA 16 Corp., College Station, TX), and GraphPad Prism version 8. The Chi-square test was employed to evaluate the association between the two variables.

3. Results

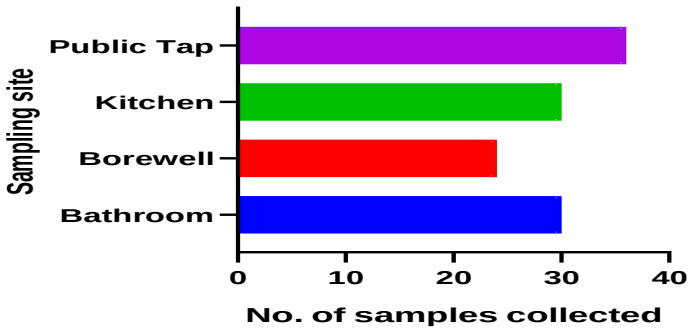
Throughout the study period, a total of 120 samples were collected, with 40 samples obtained in each season: Monsoon (July-September), Winter (October-February), and Summer (March-June), as shown in Table 1.

Table 1: Number of samples collected in different seasons

Season	n (n%)
Monsoon (July-Sep)	40(33.33)
Winter (Oct-Feb)	40 (33.33)
Summer (March-June)	40(33.33)
Total	120(100)

Figure 1 presents the distribution of samples collected from different sites. In total, 120 samples were gathered, with 36 samples (30%) sourced from public taps, 30 samples (25%) from kitchen taps, 30 samples (25%) from bathroom taps, and 24 samples (20%) from borewell water.

Figure 1: Number of samples collected in different sites



3.1. Distribution of *E. coli* in Water Samples

3.1.1. 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 (Table 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).

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

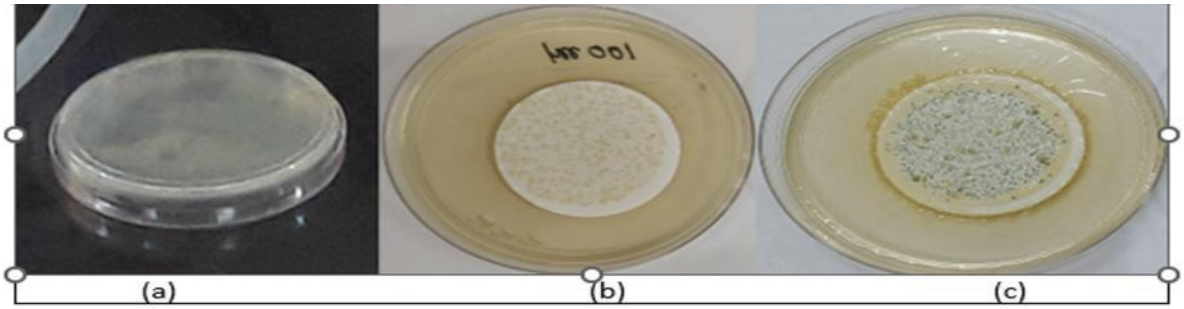

Figure 3: (a) Uninoculated culture media (b) Growth of microorganisms other than *E. coli*

(c) Bluish colonies indicating the presence of *E. coli*

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)

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)	

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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)

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3.1.2. 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)
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)

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

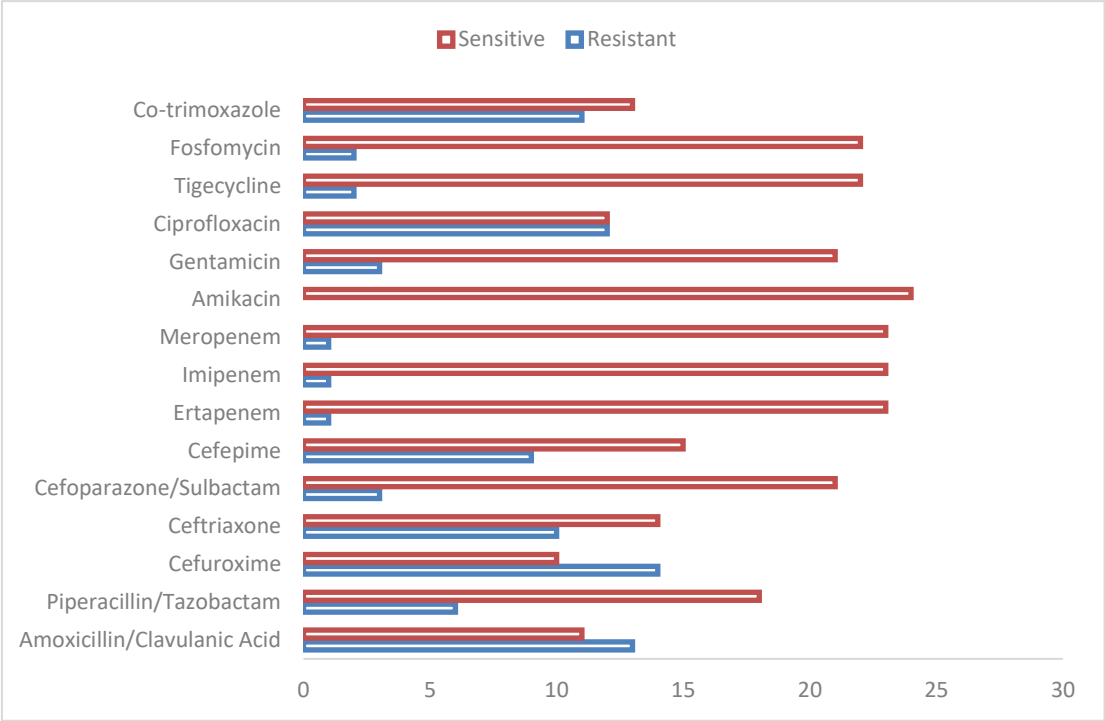

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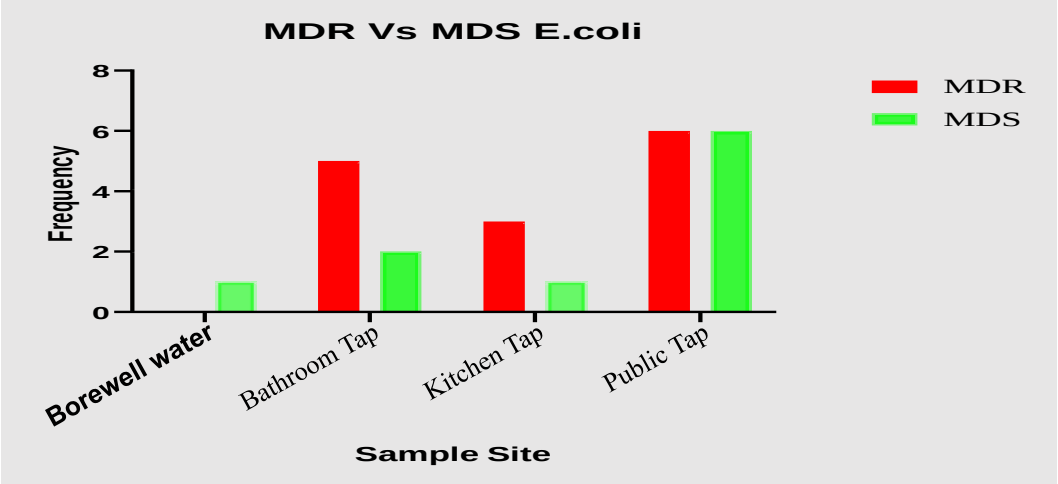
Table 5: Distribution of multidrug-resistant (MDR) *Escherichia* coli across different seasons

***E. coli* isolates (24)**			
Season	**MDR (n%)**	**MDS (n%)**	**P-Value**
Winter (Oct-Feb)	**3(12.5)**	**3(12.5)**	**0.890**
Summer (March-June	**3(12.5)**	**2(8.33)**	
Monsoon (July-Sep)	**8(33.33)**	**5(20.83)**	

The prevalence of multidrug-resistant (MDR) *E. coli* was evaluated across various seasons, as detailed in Table 5. The monsoon season (July-September) had the highest occurrence, with MDR *E. coli* comprising 33.33% of the isolates. However, no statistically significant difference was found between the prevalence of MDR and multidrug-sensitive (MDS) *E. coli* ($p > 0.05$).

Figure 5: Distribution of Multidrug-resistant (MDR) and Multidrug Susceptible (MDS) *Escherichia* coli from different sites



Likewise, no MDR *E. coli* was detected in borewell water, with only one MDS *E. coli* present. Bathroom taps had 20.83% MDR and 8.33% MDS *E. coli*, while kitchen taps showed 12.50% MDR and 4.17% MDS *E. coli*. Public taps exhibited an equal distribution of 25% MDR and 25% MDS *E. coli*.

Figure 6: Phenotypic Demonstration of Extended-Spectrum Beta-Lactamase (ESBL) Production in *Escherichia coli* Using Disc Tests

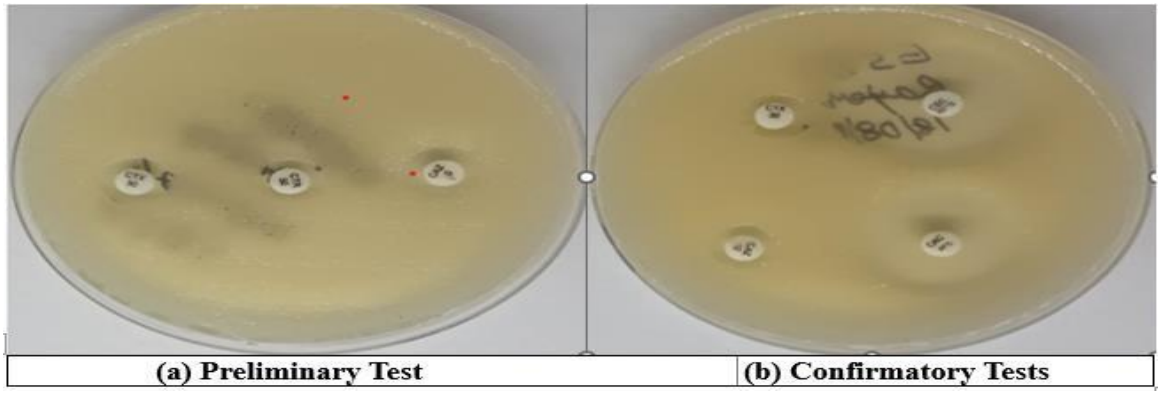


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Table 6: Distribution of Extended spectrum of Beta lactamases (ESBL)producing *Escherichia coli* across different seasons

***E. coli isolates* (24)**			
Season	**ESBL (n%)**	**Non-ESBL (n%)**	**P-Value**
Winter (Oct-Feb)	1(4.16)	5(20.83)	0.310
Summer (March-June)	2(8.33)	3(12.5)	
Monsoon (July-Sep)	7(29.16)	6(25)	

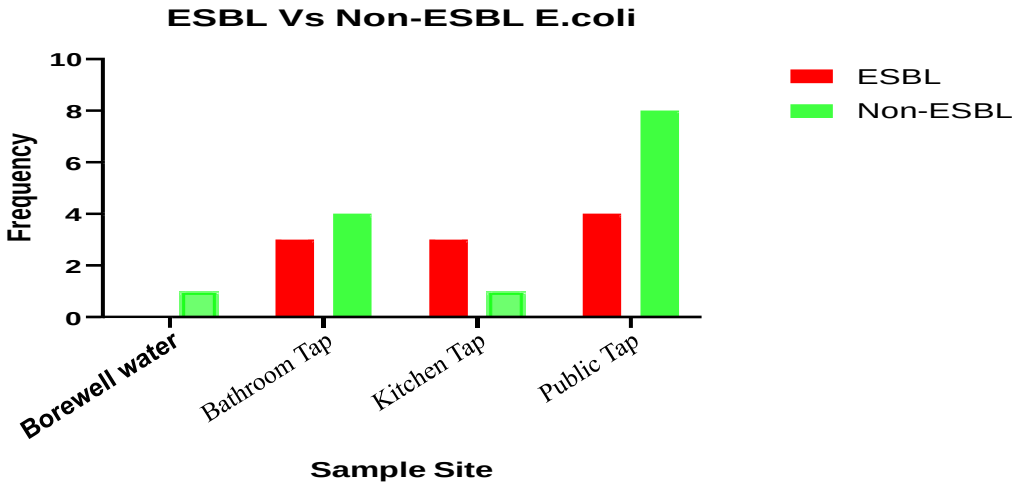
The occurrence of Extended Spectrum Beta-Lactamase (ESBL)-producing *E. coli* was also analysed across seasons, with 10 isolates identified in total. The highest incidence of ESBL-producing *E. coli* occurred during the monsoon season (July-September). However, no statistically significant difference was found in the prevalence of ESBL-producing and non-ESBL-producing *E. coli* across the seasons ($p > 0.05$), as outlined in Table 6.

Table 7: Prevalence of MDR, and ESBL, producing *E. coli* in groundwater and Municipality supply water

Sources	MDR(n%)	MDS(n%)	P-Value	ESBL(n%)	Non-ESBL(n%)	P-value
Ground Water	0(0.00)	1(4.17)	0.227	0(0.00)	1(4.17)	0.388
Municipality	14(58.33)	9 (37.5)		10(41.66)	13(54.17)	

Out of the 24 *E. coli* isolates, municipal supply water showed the highest prevalence of MDR *E. coli* at 58.33%. Both ESBL-producing and non-ESBL-producing *E. coli* were detected in the municipal supply. In contrast, no MDR or ESBL-producing *E. coli* was found in groundwater. Additionally, there was no statistically significant difference in the occurrence of MDR and ESBL-producing *E. coli* (Table 7).

Figure 7: Distribution of ESBL and non-ESBL-producing *Escherichia coli* across different sites.



Of the 24 *E. coli* isolates, borewell water contained no ESBL-producing strains and only one non-ESBL isolate ($p = 0.409$). In bathroom taps, 12.50% were ESBL-producing, while 16.66% were non-ESBL. Kitchen taps had 12.50% ESBL and 4.17% non-ESBL. Public taps showed 16.66% ESBL-producing

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and 33.33% non-ESBL *E. coli*. There was no statistically significant difference between ESBL and non-ESBL isolates across the different collection sites ($p > 0.05$), as illustrated in Figure 7.

4. Discussion

The present study aimed to evaluate the MDR and ESBL-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 MDR and ESBL 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, 23.96% 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 faecal contamination due to potential infrastructural deficiencies or shortcomings in water treatment processes.

Globally, various studies have highlighted the prevalence of *Escherichia coli (E. coli*) contamination in household drinking water, emphasizing its role as a significant contributor to waterborne diseases, thereby supporting the findings of our study. In a study in Ghana conducted by Osumanu et al, as per that study E. coli was detected in 79.6% of household drinking water samples, primarily due to poor sanitation practices [26]. Similarly, Balaraman et al. reported E. coli contamination in 94.1% (16 out of 17) of unsatisfactory water samples [27]. In Bangladesh, research conducted in Rohingya camps found *E. coli* in 10.5% of tubewell water and 34.7% of point-of-use (POU) supply water samples [28]. Ahmed's study in a different region of Bangladesh revealed a lower contamination rate of 9.23%, highlighting variability in contamination levels based on groundwater source management and geographical location [29].

Several studies conducted across different regions of India have reported significant *E. coli* contamination in drinking water sources. Sharma isolated E. coli from 16 out of 60 groundwater samples (26.67%) in the Brij region of Uttar Pradesh. Similarly, Batabyal et al. found E. coli in 24.3% of coliform-positive tube well samples, 19% of stored water samples, and 13.7% of tap water samples in West Bengal [30]. These findings underscore the widespread prevalence of *E. coli* in various water sources in India.

In our study, the public water supply exhibited the highest level of contamination, followed by water from submersible pumps. Srivastava et al. similarly reported that public water supplies exhibited higher levels of contamination, followed by water from submersible pumps [31]

During the monsoon season, our study identified the highest rate of *E. coli* contamination at 32.5%, likely due to heavy rainfall and flooding, which elevate the risk of waterborne diseases. Contamination rates were significantly lower in winter (15%) and summer (12.5%), highlighting the impact of seasonal variations on water quality. These findings align with previous research indicating higher microbial contamination during the rainy season, emphasizing the need for enhanced water monitoring and treatment. A study by Prasenjit Batabyal et al. in West Bengal analysed *E. coli* isolation data from water samples collected in winter, summer, and monsoon. In winter, 29 samples showed no contamination in tap or stored water, while 1 of 4 tube well samples tested positive, resulting in a 25% isolation rate and an overall contamination of 3.44%. In summer, among 184 samples, tap water had an 8% contamination rate, tube well water was 12.19%, and stored water was the highest at 25.80%, leading to a total of 17.93% positive samples. During the monsoon, 176 samples revealed a 22.44% contamination rate in tap water, 41.37% in tube well water, and 15.30% in stored water, with an overall positivity rate of 21.59%. [30]. These results underscore the seasonal trends in water contamination.

Our study revealed that out of *24 Escherichia coli isolates*, 14 were multidrug-resistant (MDR), with no MDR strains found in borewell water. In contrast, 20.83% of bathroom tap isolates and 12.50% of kitchen tap isolates were MDR, while public taps had an equal distribution of MDR and multidrug-sensitive

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(MDS) *E. coli* at 25% each. These findings suggest that *E. coli* contamination and antibiotic resistance vary by water source, with public and household taps showing higher MDR rates, likely due to environmental and human factors. This highlights the need for improved water management and sanitation to reduce the spread of resistant strains. Similarly, a study in China found that 49.5% *of E. coli* isolates from untreated drinking water were resistant to at least one antibiotic, and 24.0% exhibited multiple resistance to the 18 antibiotics tested [17]. These results underscore the global challenge of antibiotic-resistant waterborne pathogens and the importance of enhanced water treatment and monitoring.

In our study, we compared ESBL-producing and non-ESBL-producing *E. coli* isolates across different seasons. Of the 24 isolates, during winter (October to February), 4.16% were ESBL producers, while 20.83% were non-ESBL, with a p-value of 0.310, indicating no significant difference. In the summer (March to June), 8.33% were ESBL producers, and 12.5% were non-ESBL. The monsoon season (July to September) showed the highest percentage of ESBL isolates at 29.16%, with 25% being non-ESBL. Despite these seasonal variations, no significant difference was observed between ESBL and non-ESBL isolates during the winter.

In a study conducted in Tamil Nadu, 161 drinking water samples were analyzed, *E. coli* was the most frequently isolated organism (41 isolates, 25.4%), of which 23 were ESBL producers [32]. A study in Bangladesh found that 17.2% of the E. coli isolates were identified as ESBL producers, and 71% of these were categorized as multidrug-resistant (MDR)[33]. These findings highlight the growing prevalence of ESBL-producing *E. coli* strains in various regions, underscoring the need for effective strategies to combat antibiotic resistance in waterborne pathogens.

5. Conclusion

In conclusion, the higher prevalence of multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing *E. coli* strains underscores the urgent need for enhanced water quality monitoring and effective antimicrobial management strategies. Implementing strict public health measures is essential to ensure safe drinking water and mitigate the risks of waterborne diseases and antibiotic resistance.

Limitations of the study

One major limitation of this study is the relatively small sample size. A larger sample would have offered a more thorough understanding of the prevalence and distribution of Multidrug-Resistant (MDR) and Extended-Spectrum Beta-Lactamase (ESBL) *E. coli* in the area, potentially leading to stronger and more widely applicable results.

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