

Tap Water Microbial Contamination in Prayagraj: Isolation, Purification, and Characterization of Pathogenic Bacteria and their Antibiotic Susceptibility Patterns

AANCHAL EUSEBIUS¹, M.P. RAJU¹, KARTIKEYA SHUKLA¹ AND PRIYANKA SINGH^{2,3,*}

¹Amity Institute of Environmental Sciences, Amity University, Uttar Pradesh, 201303, India

²Bioquest Research Solution, Narayan Enterprises, Prayagraj, Uttar Pradesh 211002, India

³Centre of Food Technology, University of Allahabad, Prayagraj, Uttar Pradesh 211002, India

E-mail: aanchaleusebius@gmail.com, prmade@amity.edu, kshukla@amity.edu, priyankasingh29@gmail.com

*Corresponding author

ABSTRACT

Since water is necessary for life, it is crucial for preserving human health and biological processes. However, serious health dangers are associated with drinking water contamination, especially in areas where access to clean water is scarce. The purpose of this study was to assess the physicochemical and microbiological properties of tap water from 10 distinct locations in Prayagraj, Uttar Pradesh, India. Water samples were collected and analyzed to isolate and identify harmful bacteria. The study revealed the presence of several gram-negative bacteria, including *Leminorella grimontii*, *Morganella morganii*, and *Citrobacter braakii*, among others. Temperature and pH levels varied throughout the sampling sites and according to physicochemical analysis some samples had levels of alkalinity over the WHO-recommended safe limits. Antibiotic susceptibility tests indicated that the isolated bacterial strains displayed varying levels of resistance to different antibiotics, with higher resistance to Gentamicin than to Ampicillin. These findings highlight the significance of ongoing water quality monitoring and improvement to reduce the risk of waterborne illnesses, particularly in areas with poor access to clean water and sanitation. This study provides valuable insights into the microbiological ecology of Prayagraj tap water and emphasizes the need for effective water management strategies to protect human health and welfare.

Key words: Tap water, Biochemical analysis, Physicochemical analysis, Resistance, Ampicillin, Gentamicin

INTRODUCTION

Water is essential to all cells and is necessary for life on Earth as we know it. As a universal solvent, water transports nutrients, maintains macromolecular structure, stabilizes the plasma membrane, and dissolves salts, inorganic and organic chemicals, and gases involved in metabolic reactions (Frenkel-Pinter et al. 2021). It also helps maintain hemostasis and body volume/weight. Clean drinking water is a basic human right and can have negative health effects on users if it is tainted with opportunistic pathogenic germs (Levallois and Villanueva 2019). Therefore, it is crucial to protect human health by keeping drinking water free of microbiological contamination (Kristanti et al. 2022). Most aquatic systems are dominated by bacteria and fungi, and microorganisms play specific roles in recycling materials and purifying water in natural habitats (Sehna et al. 2021). Fecal contamination of water bodies can lead to the presence of coliform bacteria

(Bhumbla 2020).

Many individuals face challenges in accessing clean water. While it may be standard practice for every household to have access to a clean, treated water supply, waterborne diseases are widespread in developing nations where access to sanitary facilities and clean water is scarce (Gomathi 2018). According to the WHO, approximately 5 million people die each year from water-related diseases, with cholera being the most common intestinal microbial illness and accounting for nearly 50% of cases (Shayo et al. 2023). Furthermore, 1.5 million children die annually from diarrheal illnesses, and 2.5 billion people lack access to improved sanitation (Mokomane et al. 2018).

Drinking water contaminated with human or animal waste poses significant microbiological risks (Yates 2018). The primary source of fecal bacteria, including pathogens, is wastewater discharged into coastal seawaters and freshwaters (Gonçalves et al. 2023). When water quality deteriorates from an

ecological or public health perspective, it is referred to as water pollution (Lin et al. 2022). Any biological, physical, or chemical material that is present in detectable excess and is known to be harmful to other desired living organisms is pollution (Du Plessis 2022). Heavy metals, sediments, some radioactive isotopes, phosphate, nitrogen, sodium, arsenic, heat, fecal coliform bacteria, other pathogenic bacteria, viruses, and protozoan pathogens are among the contaminants that can be found in water (Sojka and Jasku³a 2022). The contamination of city water supplies by human and animal sources is the main danger to the general public's health in developing nations (Khan et al. 2022). Excreta from animals or human sources, which can act as a carrier or a vector of infectious diseases in active cases, contaminate water (Fong 2017).

The present study has been undertaken to check the microbial quality of tap water in the urban area of Prayagraj. An effort was made to isolate and identify the pathogenic bacteria discovered in the tap water collected from Ten distinct locations of Prayagraj. Additionally, physiochemical properties like pH, and Temperature, offer solutions for enhancing the water's quality so that it becomes potable for Human consumption. The study also analyzed the antibiotic susceptibility of the isolates.

The results, of this study are crucial for public health, as the presence of pathogenic bacteria in tap water can lead to serious illnesses such as gastroenteritis, cholera, and other waterborne diseases. Moreover, understanding the antibiotic resistance patterns of these isolates can help in managing potential outbreaks and guiding appropriate treatment strategies.

MATERIALS AND METHODS

Study area

Prayagraj city is located in the Southeastern part of Uttar Pradesh (98 m asml) (Gabril 2019). It covers an area of about 5482 km². According to the 2011 Indian Census, the population of Prayagraj city was approximately 595439. Tap water from ten sampling sites viz., Allahpur (S1), Beli (S2), Civil Lines (S3), Katra (S4), Muirabad (S5), Mumfordganj (S6), Mutthiganj (S7), Naini (S8), Teliarganj (S9), Rajapur (S10) were collected during January 2024 to April 2024 (Fig. 1). Samples of tap water were collected in sterile bottles and kept cool until they could be taken to the laboratory and analyzed for physicochemical parameters like temperature and pH.

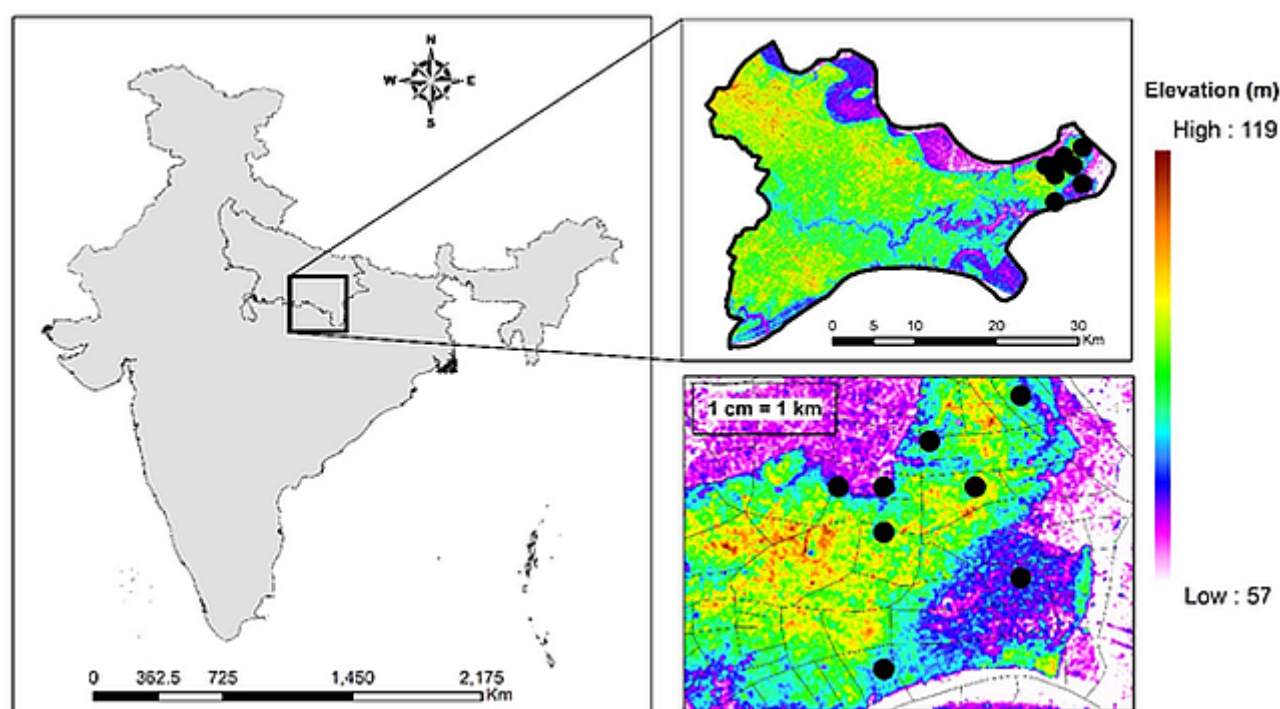


Figure 1. Map of sampling location at Prayagraj

Isolation and identification of pathogenic bacteria

The tap water samples (1 ml) were serially diluted in buffered peptone water (9 ml). Diluted samples were pour-plated onto nutrient agar and MaConkey agar, used for isolating pathogenic bacteria. These plates are incubated at 37°C for 24 hrs. Colonies were selected based on their morphological characteristics. Using the streaking plate approach, colonies were sub-cultured twice to purify them. Gram staining was performed on young cultures, and all isolates were found to be Gram-negative. Biochemical identification tests, both primary and secondary, were performed on each of the Gram-negative isolates (Mulamattathil et al. 2014).

Identification of the isolates was performed according to their morphological, cultural, and biochemical characteristics (Singh et al. 2016). The morphological characteristics of the isolates such as gram reaction, color, and type of colony elevation and opacity were studied. The isolates were characterized based on motility test, catalase test, Methyl reduction and Voges-Proskauer test, citrate utilization, indole production in tryptone broth, and L-arginine hydrolysis test as described in Bergy's Manual of Systematic Bacteriology (Boone and Castenholz 2021).

RESULTS AND DISCUSSION

Physicochemical characters of tap water

The temperature of water varied slightly in different sampling points during the current study. Allahpur (S1) which is close to river Ganga and is surrounded by various unorganized commercial establishments like small restaurants, hotels, and cottage industries recorded the highest temperature (21.5°C), and the lowest was at Mutthiganj (S6) the lowest (20.5°C) (Fig. 2). Bonacina et al. (2022) state that a variety of factors, including climatic fluctuations and geographic features can cause considerable variations in water temperature across different places. Jones and Brown (2020) pointed out that such variance can be due to local climate changes on the regional level. This suggests that the differences in temperature among the sampled locations may be attributable to localized climatic shifts, reflecting the dynamic interplay between environmental factors and geographical settings (Coelho et al. 2023).

In the present study, the highest values of pH (9.37) were found at Rajapur (S9) and the minimum (9.16) at Muirabad (S3) (Fig. 3). These variations in pH was due to chemical treatment done before supplying the water which increases the microbial load of the water samples making it alkaline and unsafe for human health as it can cause various health problems like, cholera, diarrhea, heart problems, etc. The WHO limit recommended for pH is 6.5-8.5 for potable water. All values are above the permissible limit of BIS showing the high level of alkalinity in the water samples. According to Kamal and Hashmi (2021) pH of the water distribution network of 6 locations in Pakistan found in the range of 7.33-7.79 within the permissible limit by WHO. This variability in tap water pH can likely be attributed to fluctuations

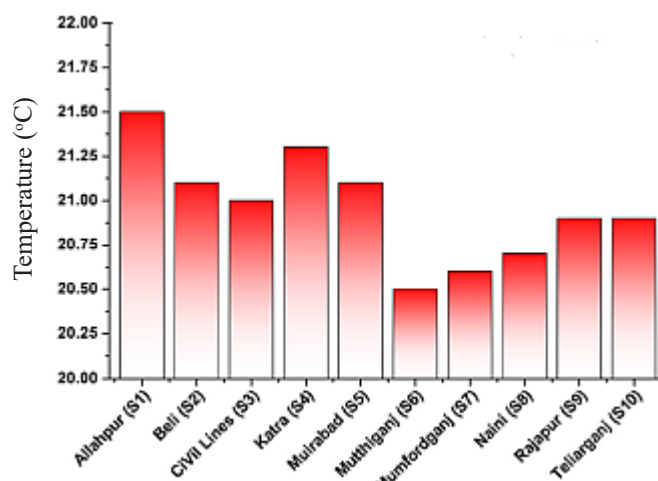


Figure 2. Variations in the temperature of the tap water samples

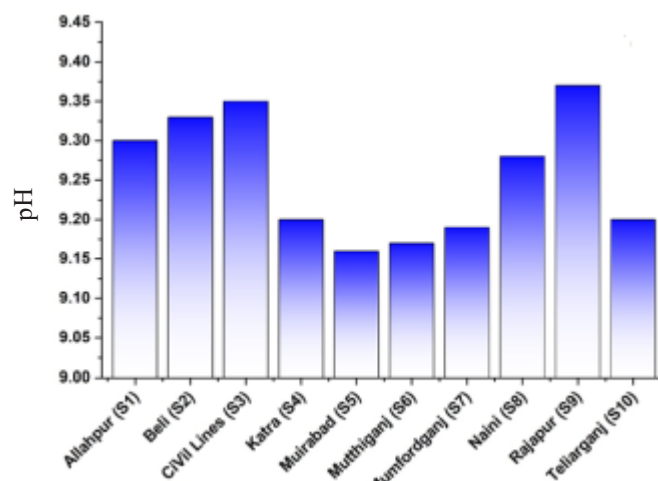


Figure 3. Variations in the pH of the tap water samples

in temperature and microbial activity within the water distribution system (Zhang et al. 2021).

Isolation and identification of bacterial species

Based on the morphology of the colonies and gram staining, 69 isolates were identified, pure-cultured, and preserved in soft agar plates (Table 1). The colony morphology of the strains varied, exhibiting variations in color, size margin, shape, gram response, and opacity (Tripathi et al. 2023). The isolates from tapwater samples were gram-negative, varied in size, and had a glossy, raised-elevation look and a translucent, shiny appearance with a convex elevation (Table 2). Colonies with smooth, creamy, or off-white appearance, displaying various

characteristics such as entire, smooth, even, or irregular margins, and slightly raised with translucent to opaque features, were identified as *Citrobacter braakii*, comprising 17% of the isolates (Fig. 4). It exhibited negative response for Arginine Hydrolysis, Motility, Voges-Proskauer test, Methyl Red, and Catalase Activity, while showing positive response for Citrate Utilization, Indole Test, and Triple Sugar Iron Test. *Leminorella grimontii* (13%) exhibited smooth, convex colonies with a creamy white to pale yellow color, entire margins, and raised or convex elevation with an opaque or slightly translucent appearance. It displayed positive response only for Citrate Utilization and Triple Sugar Iron Test. *Morganella morganii* (14%) displayed round,

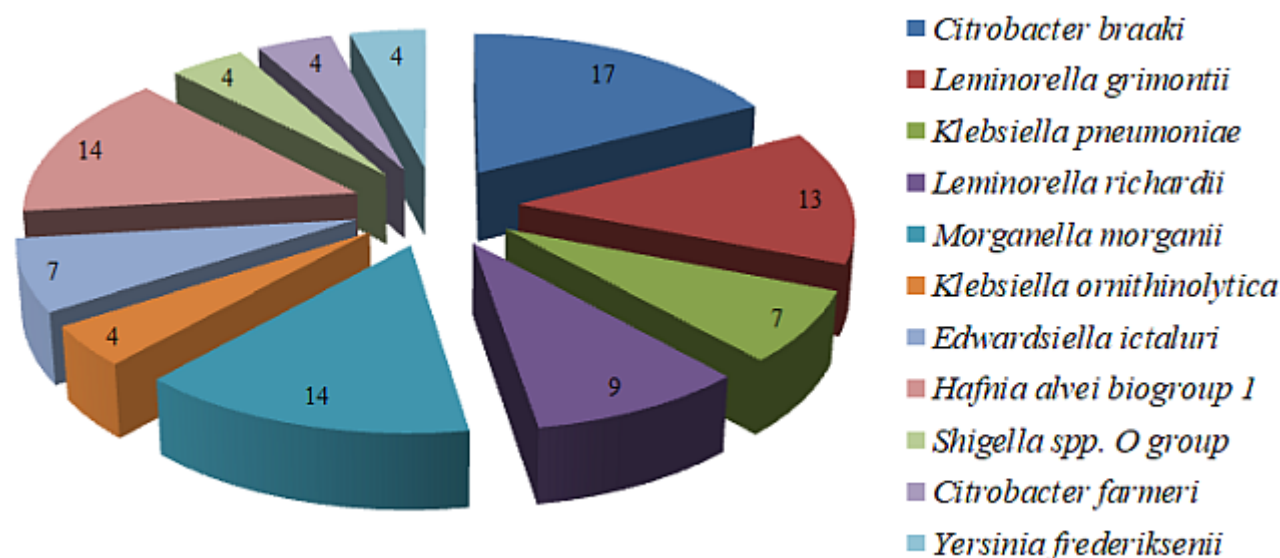


Figure 4. Prevalence of bacterial strains

Table 1. Isolation of bacteria from water samples

Sampling sites	Number of samples	Number of isolates	Total plate count	Coliform count
Allahpur (S1)	1	10	1.16*10 ⁵ cfu/ml	4.8*10 ³ cfu/ml
Beli (S2)	1	8	1.1*10 ⁴ cfu/ml	Nil
Civil Lines (S3)	1	7	2.8*10 ⁴ cfu/ml	3*10 ² cfu/ml
Katra (S4)	1	8	3.03*10 ⁴ cfu/ml	Nil
Muirabad (S5)	1	5	1.6*10 ⁷ cfu/ml	4.9*10 ³ cfu/ml
Mutthiganj (S6)	1	6	1.33*10 ⁵ cfu/ml	3*10 ² cfu/ml
Mumfordganj (S7)	1	8	4.0*10 ⁷ cfu/ml	3.25*10 ⁴ cfu/ml
Naini (S8)	1	6	1.1*10 ⁴ cfu/ml	Nil
Rajapur (S9)	1	7	2.7*10 ⁷ cfu/ml	5.1*10 ³ cfu/ml
Teliarganj (S10)	1	4	8.6*10 ⁴ cfu/ml	3*10 ² cfu/ml

Table 2. Morphological characteristics of cultures isolated

Isolates	Isolates (%)	Gram reaction	Type of colony	Color	Margin	Elevation	Opacity
<i>Citrobacter braakii</i>	17%	negative	Smooth, creamy or off-white	shades of white or cream	entire, smooth, even or irregular	slightly raised	translucent to opaque
<i>Leminorella grimonitii</i>	13%	negative	Smooth, convex colonies	creamy white to pale yellow	entire, smooth, well-defined edges	raised or convex	opaque or slightly translucent
<i>Klebsiella pneumoniae</i>	7%	negative	Mucoid	cream to light yellow	smooth or slightly irregular	convex or raised	opaque or slightly translucent
<i>Leminorella richardii</i>	9%	negative	circular or irregular	white, cream, or pale yellow	Smooth or slightly undulate	raised & slightly doomed	opaque or slightly translucent
<i>Morganella morgani</i>	14%	negative	round, convex & smooth	yellowish, grayish, or slightly pink	entire or slightly undulated	raised or convex	translucent to opaque
<i>Klebsiella ornithinolytica</i>	4%	negative	Smooth, mucoid & convex	cream to light yellow	entire, smooth, or slightly raised	convex or raised	mucoid or opaque
<i>Edwardsiella ictaluri</i>	7%	negative	smooth, opaque	creamy or white	smooth or well-defined	raised or convex	opaque or translucent
<i>Hafnia alvei</i> biogroup 1	14%	negative	circular, irregular, filamentous	creamy, white, yellow, or slightly pink	entire, smooth, undulate, lobate	flat, raised, convex, umbonate	opaque, translucent or transparent
<i>Shigella</i> spp. O group	4%	negative	smooth, opaque	white to creamy yellow	smooth & even edge	convex, raised or doomed	opaque
<i>Citrobacter farmeri</i>	4%	negative	smooth, circular & convex	creamy or light yellow	entire	raised or convex	opaque
<i>Yersinia frederiksenii</i>	4%	negative	smooth, convex & entire	translucent or whitish-gray	smooth	raised or convex	translucent to opaque

convex, and smooth colonies with yellowish, grayish, or slightly pink coloration, along with entire or slightly undulated margins and raised or convex elevation, showing a translucent to opaque appearance. It demonstrated positive response for Citrate Utilization and Indole Test. *Hafnia alvei* biogroup 1 (14%) exhibited circular, irregular, and filamentous colonies with creamy, white, yellow, or slightly pink coloration, entire, smooth, undulated, or lobate margins, and flat, raised, convex, or umbonate elevation, displaying opaque, translucent, or transparent appearance. These isolates showed positive response only for the Methyl Red Test. These characteristics delineate the fundamental traits of the *Citrobacter*, *Leminorella*, *Morganella*, and *Hafnia* groups. However, different strains showed variable response (Table 3) in terms of Arginine Hydrolysis, Motility, Voges-Proskauer test, Methyl Red, and Catalase Activity, Indole Test, Triple Sugar Iron Test, and Citrate Utilisation as also reported by Chauhan et al. (2020).

Antibiotic susceptibility of isolates

All the isolates showed greater susceptibility to Gentamicin (a second-generation antibiotic) compared to Ampicillin (a first-generation antibiotic) (Table 4). This was evidenced by larger zones of inhibition observed with Gentamicin. Lee et al. (2019) indicated that only a small percentage (9.5%) of *Citrobacter* species were susceptible to Ampicillin. Similarly, Blekher et al. (2000) found that while a few isolates of *Leminorella* species were susceptible to Ampicillin, more than half were resistant to Gentamicin. Conversely, Zaric et al. (2021) noted high susceptibility of *M. morgani* to Gentamicin. Abbott et al. (2011) observed that the majority of *H. alvei* strains were less susceptible to Ampicillin but uniformly susceptible to Gentamicin. The increased susceptibility

Table 3. Biochemical characteristics of isolates

Isolates	Catalase test	Citrate utilization	Methyl red test	Voges proskauer	Indole test	Arginine hydrolysis	Motility test	Triple Sugar iron test	Probable identified organism (with %)
1S1,3S1,5S1,9S1,4S2,2S3,6S3,7S3,3S5,3S8,1S9,6S9	-	+	-	-	+	-	-	+	<i>Citrobacter braakii</i> (17%)
6S1,8S1,5S2,8S2,1S6,3S7,6S7,2S10,4S10	-	+	-	-	-	-	-	+	<i>Leminorella grimontii</i> (13%)
2S1,3S3,10S1,4S6,6S6	-	+	-	-	-	-	-	-	<i>Klebsiella pneumoniae</i> (7%)
7S1,1S2,3S2,5S3,6S4,1S7	-	-	-	-	-	-	-	+	<i>Leminorella richardii</i> (9%)
4S1,2S2,4S3,1S4,2S4,2S5,4S5,5S7,2S8,5S8	-	+	-	-	+	-	-	-	<i>Morganella morganii</i> (14%)
6S2,2S7,1S8	-	-	-	-	+	-	-	+	<i>Klebsiella ornithinolytica</i> (4%)
7S2,7S4,8S4,5S5,6S8	-	-	-	-	-	-	-	-	<i>Edwardsiella ictalurid</i> (7%)
1S3,3S4,1S5,2S6,5S6,7S7,8S7,1S9,4S9,7S9	-	-	+	-	-	-	-	-	<i>Hafnia alvei</i> biogroup 1 (14%)
4S4,3S6,3S9	-	-	-	+	+	+	-	-	<i>Shigella</i> spp. O group (4%)
5S4,4S8,5S9	-	-	+	-	+	+	-	-	<i>Citobacter farmeri</i> (4%)
4S7,2S9,3S10	-	-	-	+	+	-	-	-	<i>Yersinia frederiksenii</i> (4%)

+ = Positive reaction; - = Negative reaction or no reaction

of *C. braakii*, *L. grimontii*, *M. morganii*, and *H. alvei* biogroup 1 to Gentamicin over Ampicillin stems from multiple factors (Lee et al. 2019). Gentamicin's protein synthesis disruption, distinct from Ampicillin's cell wall targeting, plays a role in inhibiting bacterial growth and survival (Igwebuike et al. 2020). These bacteria may harbor resistance mechanisms against Ampicillin, like α -lactamase production, but not against Gentamicin (Varela et al. 2021). Gentamicin's molecular structure potentially enhances cell wall penetration, and variations in bacterial membrane properties also contribute to its differential efficacy against various bacterial strains (Athauda 2023). These findings align with previous research, indicating a trend where most tested bacterial species exhibit higher susceptibility to Gentamicin than Ampicillin, and if first-generation and second-generation antibiotics can treat the tap water at the initial stage before supplying then we don't need fifth-generation antibiotics like Ceftaroline and Ceftobiprole to treat the water. Even after this if the person gets affected after consuming treated tap water he/she can be further treated with lower doses of antibiotics. This suggests the potential effectiveness of Gentamicin as a broad-spectrum antibiotic against these prevalent isolates.

CONCLUSION

The efficiency of the Water Works Department becomes questionable when people get adversely affected using tap water as it should be a potable means, and the physicochemical and microbial properties should be well within limits. Another chance is of leakages in the pipelines under the drains or below the municipal waste dump yards where the chances of piped/tap water getting contaminated are increased. So, a comprehensive analysis of tap water samples was done which revealed multifaceted insights into water quality and microbial content. Variations in temperature and pH levels, influenced by climatic fluctuations and geographical features, underscore the dynamic nature of water systems. The observed alkalinity exceeding permissible limits indicates potential contamination, possibly attributed to microbial activity and environmental factors.

Table 4. Antibiotic susceptibility of isolates

Isolates	Ampicillin (µg)			Gentamicin (µg)		
	5	10	25	5	10	25
<i>Citrobacter braakii</i>	8 mm	12 mm	14 mm	10 mm	15 mm	17 mm
<i>Leminorella grimontii</i>	7 mm	11.5 mm	14 mm	9 mm	13 mm	16 mm
<i>Morganella morganii</i>	7.5 mm	10.5 mm	13 mm	11 mm	16 mm	18 mm
<i>Hafnia alvei</i> biogroup 1	9.5 mm	13.5 mm	15 mm	12 mm	18 mm	24 mm

Examination of bacterial colonies unveiled a diverse array of gram-negative strains, each exhibiting unique morphological and biochemical characteristics. Predominant isolates, such as *Citrobacter braakii*, *Leminorella grimontii*, *Morganella morganii*, and *Hafnia alvei* biogroup 1, showcased distinct traits delineating their respective groups. However, variability in biochemical reactions among strains underscores the complexity of microbial populations in tap water. Furthermore, antibiotic susceptibility testing highlighted a higher susceptibility to Gentamicin over Ampicillin among prevalent isolates, aligning with previous findings. These findings collectively emphasize the necessity for ongoing monitoring and management strategies to ensure the safety and quality of tap water for public health. To guarantee that treatment procedures are successful in lowering the risk of waterborne illnesses and to give a thorough understanding of the water quality across various sources, future research should also compare results with samples of groundwater and river water.

ACKNOWLEDGMENT

We thank Bioquest Research Solution, Narayan Enterprises, Prayagraj for facilities. We gratefully acknowledge the cooperation of all the individuals who participated in the study.

Authors' contributions: All authors contributed equally.

Conflict of interest: Authors declare no conflict of interest.

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Received: 27th May 2024

Accepted: 29th June 2024