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Seasonal assessment of antibiotic-resistant bacteria and pathogenicity in rural tap water

ABSTRACT

Ordinary people lack constant drinking water and rely on tap water for their daily needs. Among them, approximately 20% of families used a water purifier, and 80% used tap water directly. In rural areas, this water is carried to houses through pipelines, which pass through the drainage and are contaminated due to pipeline leakage. Mostly, these waters contain antibiotic-resistant bacteria, and direct water use causes a high risk of waterborne disease in humans. In this study, an assessment of antibiotic-resistant bacteria was done during the winter and summer seasons of the tap water. The pathogenicity analysis by bile esculin, D-mannitol test, and their identification by biochemical reactions with the help of Advanced Bacterial Identification Software (ABIS) was carried out. Bacterial colonies were isolated from agar plates through Total Viable Counts (TVC) to determine the specificity of the microbes present in a water sample. Isolates of bacteria were identified based on their morphological, physiological, and biochemical appearances. Susceptibility patterns of isolates were resistant to ofloxacin, cefotaxime, ornidazole, sparfloxacin, co-trimoxazole, cefixime, metronidazole, and norfloxacin through zone of inhibition analysis. This determined the resistance pattern in isolated bacterial strains as per Clinical and Laboratory Standards Institute, 2020, in petriplates after 24 hours. This study is designed to determine the presence of antibiotic-resistant bacteria and their pathogenicity in the collected drinking water, which is alarming for people who insist on demanding additional treatment from different water sources.

Keywords: *Antibiotic-resistant bacteria, Tap water, Antibiotic susceptibility*

1. INTRODUCTION

Antibiotic-resistant bacteria derived from different human activities and atmospheric pressure are the cause of the deaths of human beings worldwide, as stated by the World Health Organization (WHO) [1, 2]. Bacteria are prevalent in surface water, soil, and plants and constitute the colonization of humans [3] and food products [4] [5], contaminated rural environments [6] [7], environmental pollutants [8] [9]**,** livestock [10][11]**,** and pet animals [12]**.** These bacteria can adapt to environmental conditions and exchange their genetic material with other microorganisms by horizontal gene transfer [13, 14]**.**

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This adaptation of resistance increases the failure of clinical treatment and the risk of infecting human beings [15]**.**All genes are directly and indirectly responsible for clinical and environmental antibiotic resistance; it is not restricted to hospitals [16]**.** Hence, detecting antibiotic-resistant bacteria in drinking water is necessary to identify the high risk of gastrointestinal diseases caused by the transmission of antimicrobial resistance (AMR) [3]**.** The gastroenteritis outbreaks due to diarrheagenic bacteria are also caused by contaminated drinking water; they are responsible for several waterborne diseases [17]**.** Water systems interconnect people, agronomy, and animal farming, originating from various surroundings [18]**.** These environments are connected with microbes, antibiotic-resistant bacteria (ARE), phages, plasmids, antibiotic resistance genes, and different living beings [19]**.** An environmental factor of an ecosystem varies, such as the richness of organic matter, as well as temperature, redox condition, and level of

antibiotics, which also affect the antibiotic resistance of bacteria [20]. It has been reported that the resistance of bacteria to cephalosporins is because of point mutations that have their origin in different forms of extended-spectrum beta-lactamase (ESBL) genes like blaSHV, blaTEM, and blaCTX-M [21]**.** Carbapenem antibiotics, including meropenem, imipenem, and ertapenem, treat ESBL bacteria [22]**.** It has been found that the transmission of environment-isolated ESBL-producing bacteria is a significant issue for urban water bodies [23, 24]**.** There is an observation that antibiotic resistance is increasing daily because of human activities that are prone to the environment [15]**.** As per the WHO reports, 4.6% of global deaths are due to adverse water quality, and millions of people are killed by waterborne diseases all over the world [25]. However, contaminated drinking water causes 50% of global diseases**.** Contamination of drinking water is the reason for the spread of skin, gastrointestinal, cancer, and other infectious diseases [26]**.** The spread of pathogens primarily in water comes from excretory products of humans and animals through the oral means of living organisms. Drinking water analysis is necessary to determine its quality and protect human beings from diseases caused by microorganisms [27]. The contamination of groundwater is caused by the improper operation of septic tank systems and their inadequate maintenance; consequently, there is the direct injection of surface application of wastewater [28]. Treating diseased humans and animals frequently takes antibiotics to protect them [29]**.** It has been noticed that inappropriate usage of antibiotics carries resistance in microorganisms, which requires much effort for their treatment [30]**.** Microorganisms can survive in adverse conditions or inhibit their growth [31]. Resistance to antibiotics is a universal issue, whereas medicines used as antibiotics are ineffective and are specifically customized for patients. There is an alert from the World Health Organization about the careful use of antibiotics. Recently, many drug-resistant bacteria have shown their inability to treat the emerging infectious disease [32]**.** The mishandling of antibiotics has resulted in new resistance to antibiotic-resistant infections. WHO has reported that the highest menace humans face is the fear of antimicrobial resistance [33]**.**

Recently, antibiotics have assisted in saving many living beings, which has minimized the disease in numerous human beings worldwide [34]**.** However, it is significant to know that antibiotics reduce the death rates of emerging drug-resistant bacterial strains [35]. Infections caused by antibiotic-resistant bacteria are estimated to affect 10 million humans annually by 2050, and are estimated to cost the global economy about $100 trillion [36]. Overuse of antibiotic drugs leads to financial burdens for underdeveloped countries due to their poor health care systems. They need advanced tools to identify the many infectious diseases [37]. In rural areas, water passes through the pipelines to the respective houses, and these pipelines are connected through the drainage system, which is contaminated due to faecal, agricultural waste, rainwater, cattle infection, and the disposal of other polluted materials in the dam. Hence, people have always had discomfort with their health, like dysentery, stomach upset, typhoid, jaundice, food poisoning, tuberculosis, etc. So far, the polluted water is treated with chlorine at frequent intervals; however, the chlorination process is unsuitable for treating all types of microorganisms. At this point, the water qualities were checked using the chemical method to evaluate the presence of chemical composition and its concentration**.** However, a report on the microbiological analysis of water in these rural areas needs to be conducted. It is necessary to analyze the presence of microorganisms to know the water quality for public health and hygiene benefits.

Present investigations state that bacterial strains were isolated in two seasons, winter and summer, to determine the specificity of microbes in the tap water. In contrast, these strains were analyzed and aimed for antibiotic resistance patterns. Objectives are also included to find the presence of antibiotic-resistant bacteria and their pathogenicity in the drinking water to understand the threats to the usage of drinking water and the challenges of its treatment.

2. MATERIAL AND METHODS

2.1 Sample collection:

A total of 48 samples of drinking water were collected during the winter (December 2023) and summer (May 2024) seasons in sterile, non-contaminated bottles in triplicate [38].

2.2 Isolation of bacteria

To identify bacterial resistance in the collected drinking water samples, bacteria were isolated under sterile conditions. The water sample was serially diluted, and 0.1 ml of the respective dilution was plated on nutrient agar. All plates were incubated at 37°C for 24 hours. After incubation, bacterial density was estimated as colony-forming units (CFU)/ml [39]. Presumptive colonies of different bacteria were then selected based on morphological features from each culture plate and sub-cultured in the nutrient broth to preserve the pure culture of the bacterial colonies.

2.2.1 Antibiotic susceptibility analysis:

The Kirby–Bauer disk diffusion method was used to analyze the antibiotic susceptibility of pure cultures of bacterial isolates on Mueller-Hinton agar, which was solidified in Petri dishes. Bacterial cultures were then swabbed onto the agar plates. The susceptibility patterns of the isolates were determined against Ofloxacin (OF, 5 µg/disc), Cefotaxime (CTX, 30 µg/disc), Ornidazole (ORD, 30 µg/disc), Sparfloxacin (SPX, 5 µg/disc), Co-trimoxazole (COT, 25 µg/disc), Cefixime (CFM, 5 µg/disc), Metronidazole (20 µg/disc), and Norfloxacin (NX, 10 µg/disc). After 24 hours, the zone of inhibition was measured in the Petri dishes, and the resistance pattern in the isolated bacterial strains was determined [40].

2.2.2 Pathogenicity analysis:

Pathogenicity analysis was performed to detect the presence of specific pathogens in collected water samples. Bile esculin agar and mannitol tests confirmed the presence of pathogenic bacteria and their ability to hydrolyze esculin in the presence of bile.

2.2.3 Biochemical analysis:

A biochemical test was performed to identify bacterial species based on their morphological, biochemical, and metabolic characteristics in the collected water samples [41]. Tests for dextrose production, lactose fermentation, glucose production, glycerol production, sucrose production, Maltose production, D-Mannitol, Gelatine production, indole production, methyl red, V.P test, citrate utilization, oxidase activity, urease activity, pigment production, nitrate reduction, starch hydrolysis, casein hydrolysis, growth at 45^0C, growth at 65^0C, growth at 7% NaCl, growth with lysozyme, H_2S production, motility Test, bile esculin testwere conducted to assess the metabolic activities of the bacteria and identify the microbes [42].

The morphological and biochemical characteristics of the isolated strains were compared with standard values from the ABIS database to establish an identification scheme. The accuracy and similarity percentage with species from the database were determined using the ABIS encyclopedia. A total of 25 tests were conducted for this process. Detailed information on the isolated strains, including the species' morphological-biochemical characteristics, cultural traits, ecology, and pathogenicity, was quickly retrieved from the ABIS encyclopedia, including the species' morphological-biochemical characteristics, cultural traits, ecology, and pathogenicity.

3. RESULTS

3.1 Results of Isolation of Bacteria

A total viable count was performed to isolate bacterial colonies from a selected sample of drinking tap water. Temperature variation is a key factor that affects bacterial growth, as it is directly influenced by microbial activity [43]. Bacterial community composition also depends on geographical location and weather conditions [44]. Furthermore, the maximum number of bacterial colonies was detected in winter, compared to summer. A lower number of bacterial colonies was observed in summer due to higher temperatures [45, 46]. Samples 1 and 2 showed more bacterial growth in three different tap water (TW) samplesTW1, TW2, and TW3 (Table 1). However, fewer bacterial colonies were found in sample 8in TW3. Compared to the winter season, the summer season exhibited less microbial activity in all selected drinking water regions. It was noted that sample5 showed the least bacterial growth (0.6×10^3) in TW3.

A total of 99 colonies were isolated based on their morphological features, which were then used to prepare pure cultures for further analysis. The sample codes are referred to as S1-S10 based on the number of isolated bacteria per season and area (Table 2). Colonies with similar characteristics were selected: 6, 7, 7, 5, 10, 7,6, and 7 in the winter season based on their shape, surface texture, color, margin, height, and opacity. Similarly, 5, 5, 6, 5, 5, 7, 5, and 6 bacterial colonies were isolated in the summer season based on their morphological characteristics. Bacterial isolates were found to have smooth, moist, or dry surfaces. In addition, most colonies were visually selected based on their differences in white, yellow, or cream color. Some bacterial colony margins were observed to be entire, swarming, or undulating. The elevation of the colonies ranged from convex and flat to slightly raised. In addition to flat and umbellate borders, most bacterial isolates had convex colonies. The opacity of the colonies was primarily either opaque or translucent.

Gram staining analysis of the isolated colonies revealed that 35 of the 40 strains were rod-shaped (Fig. 1). Among them, 80% were gram-negative rods, 7.5% were gram-positive rods, and 12.5% were gram-positive cocci**.**

Table 1 Total Viable Count of different drinking tap water (TW) samples

	Winter season			**Summer season**		
Area	**TW1 (cfu/ ml)**	**TW2 (cfu/ ml)**	**TW3 (cfu/ ml)**	**TW1 (cfu/ ml)**	**TW2 (cfu/ ml)**	**TW3 (cfu/ ml)**
Sample 1	4.9×10^3	5.6×10^3	4.2×10^3	2.0×10^3	1.5×10^3	1.3×10^3
Sample2	6.6×10^3	5.2×10^3	6.2×10^3	3.4×10^3	3.6×10^3	2.0×10^3
Sample 3	1.8×10^3	1.6×10^3	1.5×10^3	1.0×10^3	1.6×10^3	1.2×10^3
Sample 4	4.2×10^3	3.6×10^3	4.8×10^3	2.5×10^3	2.4×10^3	2.1×10^3
Sample 5	1.2×10^3	1.8×10^3	1.6×10^3	0.8×10^3	1.0×10^3	1.0×10^3
Sample 6	3.4×10^3	3.2×10^3	4.9×10^3	4.3×10^3	3.3×10^3	2.3×10^3
Sample 7	4.9×10^3	2.8×10^3	4.3×10^3	3.4×10^3	2.1×10^3	3.1×10^3
Sample 8	6.0×10^3	3.9×10^3	0.6×10^3	2.3×10^3	1.7×10^3	1.2×10^3

TW= tape water from different tap i.e. 1, 2, 3

Table 2: Total number of bacteria samples isolated from water samples

Area	**No. of bacteria isolated in the winter season**	**The number of bacterial colonies isolated in the summer season**	**Total no. of bacteria.**
Sample 1	6 (S1-S6)	5 (S1-S5)	11
Sample 2	7 (S1-S7)	5 (S1-S5)	12
Sample 3	7 (S1-S7)	6 (S1-S6)	13
Sample 4	5 (S1-S5)	5 (S1-S5)	10
Sample 5	10 (S1-S10)	5 (S1-S5)	15
Sample 6	7 (S1-S7)	7 (S1-S7)	14
Sample 7	6 (S1-S6)	5 (S1-S5)	11
Sample 8	7 (S1-S7)	6 (S1-S6)	13
Total no. of bacteria.	55	44	99

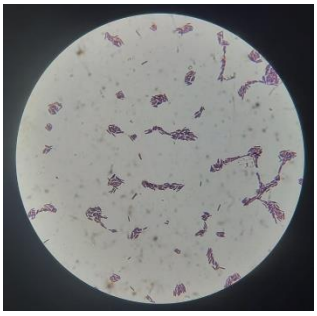

Figure 1. Rod-shaped gram-positive bacteria

3.2 Results of Antibiotic Susceptibility Analysis

Antibiotic susceptibility analysis was performed on 99 isolated bacteria against eight antibiotics**.** Resistance and sensitivity analyses were conducted for 55 isolates in the winter season and 44 isolates in the summer season **(Fig. 2).** In the winter season, the isolates showed the highest resistance to metronidazole (96.36%), followed by ornidazole (96.36%), cefixime (74.54%), and ofloxacin (72.73%). Co-trimoxazole (23.64%) and cefotaxime (20%) exhibited the least resistance, with sparfloxacin (5.45%) and norfloxacin (3.64%) showing the lowest resistance. Sensitivity analysis indicated the effectiveness of the antibiotics in inhibiting bacterial growth [47]. Sparfloxacin (94.55%) and norfloxacin (96.36%) were the most effective, followed by cefotaxime 80%, co-trimoxazole (76.36%), ofloxacin (27.27%), cefixime (25.46%), ornidazole (3.64%), and metronidazole (3.64%) during the winter season. During the summer season, the resistance pattern was similar, with metronidazole (98%), ornidazole (91%), ofloxacin (85%), cefixime (84%), co-trimoxazole (33%), and cefotaxime (18.2%). Both sparfloxacin and norfloxacin showed no resistance (0%). The sensitivity pattern in the summer season was as follows: sparfloxacin and norfloxacin (100%), followed by cefotaxime (81.8%), co-trimoxazole (77%), cefixime (16%), ofloxacin (15%), ornidazole (9%), and metronidazole (2%) **(**Fig. 2).

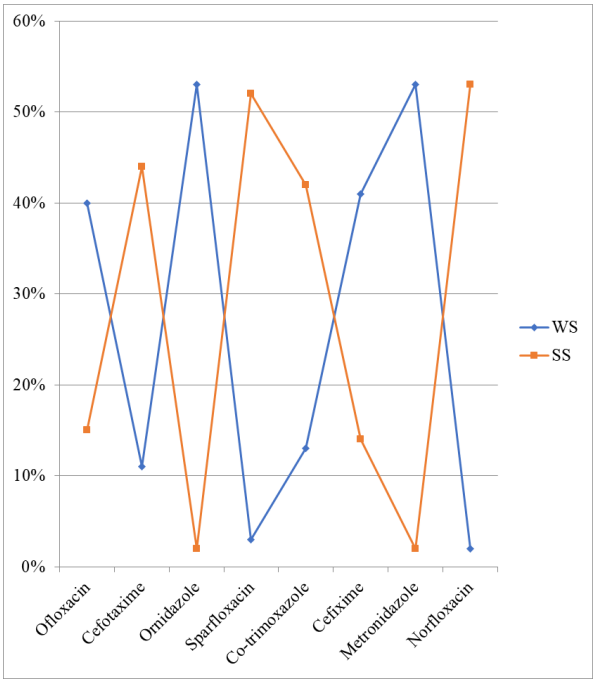

Figure 2. Comparison of the percentage of resistant isolates by season with different antibiotics

3.3 Results of Pathogenicity Analysis

Regarding Table 2, out of 55 isolated bacteria, 38 bacterial samples were found to be resistant during winter. The bile esculin test confirmed that 17 samples were positive and 21 were negative, while the D-mannitol test showed 10 positive and 28 negative results. Pathogenic bacteria were identified as standard from each area sample, with 5 samples showing resistance and pathogenicity in both the bile esculin and D-mannitol tests (Fig. 3). In the summer season, 36 bacteria exhibited resistance in the selected isolated samples. Among them, 13 bacteria tested positive in the bile esculin test, and 23 tested negative**.** In contrast, the D-mannitol test analysis showed that 8 samples were positive, while the remaining 28 were negative**.** Of these, 3 samples displayed resistance and pathogenicity in both the bile esculin and D-mannitol tests (Fig. 4).

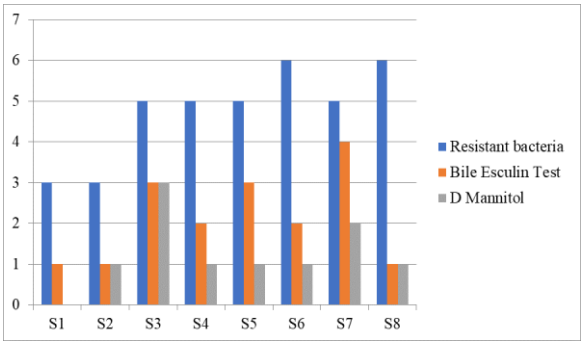

Figure 3. Resistance and pathogenicity of bacteria in the winter season

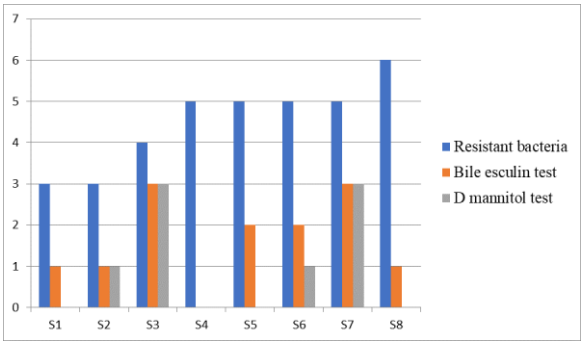

Figure 4. Resistance and pathogenicity of bacteria in the summer season

3.4 Results of Biochemical Analysis

Biochemical analysis was conducted for the winter and summer season for the selected colonies of isolated bacteria using a set of reactions, including a test for dextrose production, lactose fermentation, glucose production, glycerol production, sucrose production, maltose production, D-mannitol, gelatine production, indole production, methylred, V.P test, citrate utilization, oxidase

activity, urease activity, pigment production, nitrate reduction, starch hydrolysis, casein hydrolysis, H_2S production, motility test, and bile esculin test and for gram-positive *Bacillus* (rod) and *Streptococcus* (cocci) growth at 45^0C, growth at 65^0C, growth at 7% NaCl test were also conducted. It was observed that during the winter and summer seasons, gram-negative *E.coli, E. harmanni, Pseudescherichia vulneris, Klebsiella pneumoniae. subsp pneumonia, Bacillus licheniformis, Citrobacter gillenii, Brenneria rubrifaciens, Londaleaquercina, Kluyvera ascorbata***,** were dominant bacteria. However, *Staphylococcus nepalensis Enterococcus lemanii,* and *Bacillus licheniformis are* gram-positive bacteria, which were also identified in the collected samples. Identification was carried out using Advanced Bacterial Identification Software [38] (Tables 3 and 4). The total number of identified bacteria in the winter and summer seasons is 22 and 18, respectively (Fig. 5).

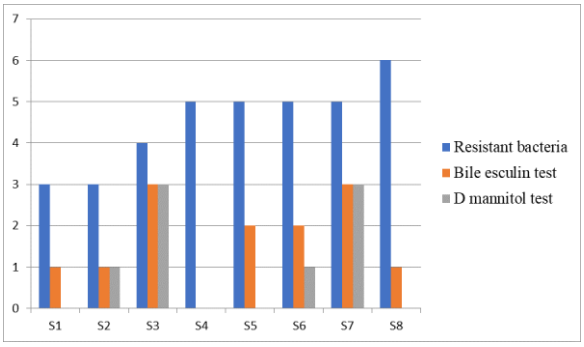

Figure 5. Total number of identified bacteria in winter and summer seasons

4. DISCUSSION

The emergence and presence of antibiotic-resistant bacteria in tap water resources pose a significant threat to human health [48,49]**.** Monitoring the appearance of antibiotic-resistant bacteria in tap water is essential, as there is insufficient awareness of their toxicity in water and the surrounding environments. Although drinking water is treated before distribution, it must still be safe for consumption[50]. This study aimed to investigate the presence of antibiotic-resistant bacteria in tap water. 48 water samples were collected in triplicate during the winter and summer to determine the presence of antibiotic-resistant bacteria at the selected sites. Ninety-nine bacterial isolates (55 from winter and 44 from summer) were identified based on their similar morphological features. Twelve bacterial genera were identified, showing an unexpectedly high level of bacterial diversity, likely due to multiple sources of environmental pollution. Seven and ten categories of bacteria were found in the winter and summer seasons.*E. coli, E. harmanni, Pseudescherichia vulneris, Klebsiella pneumoniae subsp pneumoniae, Kluyvera Ascorbata, Bacillus licheniformis,* and *Staphylococcus nepalensis,* found in both winter and summer seasons**.** However**,** *Trabulsiella gaumensis* was found only during the winter season, while *Citrobacter gillenii*, *Brenneria rubrifaciens*, *Londalea quercina*, and *Enterococcus lemanii* were found only during the summer season. Overall, the most commonly identified bacterial genera were *E. coli*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Salmonella*, *Bacillus*, and *Streptococcus* [51]. Solar radiation was found to negatively affect bacterial growth during the summer season [52], while continuous rainfall during the summer eliminated a substantial number of microbes and reduced their activity [39]. The current findings indicate fecal contamination of drinking water in the sampling sites with the majority of drinking water sources showing rapid bacterial growth and the presence of coliforms. The presence of *E. coli* in drinking water is primarily attributed to human activities, which must be addressed by implementing targeted policies, particularly in rural areas [53]. *Pseudescherichia vulneris,* which is naturally resistant to various antibiotics or exhibits multidrug resistance, was widely detected [54].

The presence of *Streptococcus*, *Citrobacter*, and *Klebsiella* further indicates fecal contamination in the drinking water systems [55]. Additionally, the presence of *Bacillus licheniformis, Kluyvera ascorbata,* and *Enterococcus faecalis poses* a serious public health threat due to their presence in drinking water [56,57]. Consumption of water containing fecal pathogenic microorganisms seriously endangers human health. Further studies are necessary to identify the microorganisms responsible for antibiotic resistance in drinking water and the environment and to monitor these in all states and regions worldwide. The implementation of preventive measures and a global surveillance program is essential to protect water bodies from antibiotic-resistant bacteria.

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Ethical approval: Not applicable.

Informed Consent: All authors have consented to participate.

Author contribution: Rich Jain (R J) performed the analysis and contributed to the write-up and final draft of the manuscript. **Vinita Vishwakarma** (V V) coined the title and idea, designed the study, wrote a section, and finalized the write-up.

Data Availability Statement: Not applicable.

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Table 3 Biochemical analysis of bacteria in the winter season

	S1	S2	S3	S3	S3	S3	S3	S4	S4	S5	S5	S5	S6	S6	S6	S7	S7	S7	S7	S7	S8	S8
	C1	C4	C1	C2	C3	C4	C7	C3	C5	C6	C7	C9	C1	C4	C7	C1	C2	C3	C4	C5	C3	C4
G. Staining	-ve road entero	-ve road entero	-ve road entero	-ve road entero	-ve road entero	-ve road entero	+ve cocci	-ve road	-ve road entero	-ve road entero	-ve road entero	-ve road entero	+ve road	-ve road entero	-ve road	-ve road entero	-ve road entero	-ve road entero	-ve road entero	-ve road	+ve cocci	-ve road entero
Dextrose	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Lactose	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Glycerol	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Sucrose	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
Maltose	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve
Mannitol	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
Gelatine	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
Indole	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
Methyl Red	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
V.P.	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Citrate	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Urease	-ve	-ve	-ve	-ve	-ve	-ve	+ve		-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Pigment Production	-ve	-ve	-ve	+ve	ve	-ve	+ve	-ve	-ve	-ve	-ve	ve	-ve	-ve	-ve	ve	-ve	ve	-ve	-ve	+ve	ve
Nitrate Reduction	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Starch Hydrolysis	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Casein Hydrolysis	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve
Blood Haemolysis	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Growth at 7% NaCl	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve
Growth with Lysozym	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

e																						
H_2S Production	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Motility Test	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Bile esculin	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve
bacteria	*E.coli*	*E.coli*	*E.coli*	*Pseudescherichia.vulneris*	*E. harmanni*	*Pseudescherichia.vulneris*	*Staphylococcus.nepalensis*	*Klebsiella .pneumoniae .subsp pneumoniae*	*E.coli*	*E. coli*	*Pseudescherichia.vulneris*	*E. harmanni*	*Bcillus. licheniformis*	*Kluyvera. ascorbata*	*trabulsiella.gaumensis*	*E. harmanni*	*Pseudescherichia.vulneris*	*E. harmanni*	*Kluyvera. ascorbata*	*trabulsiella.gaumensis*	*Staphylococcus.nepalensis*	*E. harmanni*

Table 4 Biochemical analysis of bacteria in the summer season

	S1	S2	S2	S3	S3	S3	S3	S5	S5	S6	S6	S6	S7	S7	S7	S7	S7	S8
	C2	C3	C4	C1	C2	C3	C4	C6	C7	C1	C4	C6	C1	C2	C3	C4	C5	C4
G. Staining	-ve road entero	+ve road	-ve road entero	-ve road entero	-ve road entero	-ve road entero	-ve road	+ve cocci	-ve road entero	+ve cocci	-ve road entero	-ve road entero	-ve road entero	+ve road	-ve road entero	+ve cocci	-ve road entero	-ve road entero
Dextrose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Glycerol	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Sucrose	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
Maltose	-ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
Mannitol	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
Gelatine	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Indole	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve
Methyl Red	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
V.P.	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Citrate	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Urease	-ve	+ve	-ve	-ve	-ve	-ve		+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Pigment	-ve	-ve	+ve	-ve	ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	ve	-ve

Production																		
Nitrate Reduction	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Starch Hydrolysis	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve
Casein Hydrolysis	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve
Growth at 45	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Blood Haemolysis	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Growth at 65	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
Growth at 7% NaCl	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve
Growth at pH 5.7	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Growth with Lysozyme	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
H2S Production	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve
Motility Test	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Bile esculin	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
bacteria	*E.coli*	*Bcillus. licheniformis*	*Brenneria.rubrifaciens*	*Londalea.querc ina*	*E. harmanni*	*Pseudescheric hia.vulneris*	*Klebsiella.pneu moniae.subsp pneumoniae*	*Staphylococcus .nepalensis*	*E.coli*	*Staphylococcus .nepalensis*	*E.coli*	*Citrobactor.gille nii*	*Citrobactor.gille nii*	*Bcillus. licheniformis*	*Kluyvera. ascorbata*	*Enterococcus.l emanii*	*E. harmanni*	*Pseudescheric hia.vulneris*

IZVOD

SEZONSKA PROCENA BAKTERIJA REZISTENTNIH NA ANTIBIOTIKE I PATOGENOSTI U RURALNOJ VODI

Obični ljudi nemaju stalnu vodu za piće i oslanjaju se na korišćenje vode iz slavine za svoje svakodnevne potrebe. Među njima, približno 20% porodica koristilo je prečišćivač vode, a 80% direktno vodu iz slavine. U ruralnim područjima, ova voda se dovodi do kuća kroz cevovode, koji prolaze kroz drenažu i kontaminiraju se zbog curenja iz cevi. Uglavnom, ove vode sadrže bakterije otporne na antibiotike, a direktna upotreba vode izaziva visok rizik od bolesti koje se prenose vodom kod ljudi. U ovoj studiji, procena bakterija otpornih na antibiotike urađena je tokom zimske i letnje sezone vode iz slavine. Izvršena je analiza patogenosti žučnim eskulinom, D-manitol testom i njihova identifikacija biohemijskim reakcijama uz pomoć softvera za naprednu identifikaciju bakterija (ABIS). Bakterijske kolonije su izolovane sa agar ploča pomoću ukupnog broja održivih ćelija (TVC) kako bi se utvrdila specifičnost mikroba prisutnih u uzorku vode. Izolati bakterija su identifikovani na osnovu njihovog morfološkog, fiziološkog i biohemijskog izgleda. Obrasci osetljivosti izolata bili su rezistentni na ofloksacin, cefotaksim, ornidazol, sparfloksacin, ko-trimoksazol, cefiksim, metronidazol i norfloksacin putem analize zone inhibicije. Ovo je utvrdilo obrazac otpornosti kod izolovanih bakterijskih sojeva prema Institutu za kliničke i laboratorijske standarde, 2020, u Petripločama nakon 24 sata. Ova studija je osmišljena da utvrdi prisustvo bakterija otpornih na antibiotike i njihovu patogenost u sakupljenoj vodi za piće, što je alarmantno za ljude koji insistiraju na zahtevanju dodatnog tretmana iz različitih izvora vode.

Ključne reči: Bakterije otporne na antibiotike, Voda iz slavine, Osetljivost na antibiotike

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