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Assessment of Antibiotic-Resistant Bacterial Contamination in Diverse Drinking Water Sources: Implications for Water Safety and AMR Control Strategies

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ORIGINAL ARTICLE

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ABSTRACT

Background: Antimicrobial resistance (AMR) poses a significant global public health threat, particularly through environmental routes like drinking water. In low- and middle-income countries, contamination of water sources with antibiotic-resistant bacteria (ARB) is exacerbated by inadequate sanitation and unregulated antibiotic use.
Objective: To assess the prevalence and antibiotic resistance profiles of bacteria in various drinking water sources in Lahore, Pakistan.
Methods: A cross-sectional study was conducted using 30 drinking water samples from tap, filtered, and bottled water. Microbiological analysis included membrane filtration and pour plate methods. Bacterial identification involved Gram staining, biochemical tests, and Analytical Profile Index (API) kits. Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method with interpretation per Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: Bacterial contamination was found in 70% of samples, highest in tap water (90%). Common isolates included *Escherichia coli* (35.4%), *Pseudomonas aeruginosa* (27.1%), and *Staphylococcus aureus* (20.8%). High resistance was observed to ampicillin (80.2%) and tetracycline (70.1%). Multidrug resistance (MDR) was identified in 62.5% of isolates, with tap water showing the highest MDR frequency (72.7%).
Conclusion: The widespread presence of ARB, especially MDR strains, in drinking water underscores the urgent need for improved water treatment, routine monitoring, and stringent regulation of antibiotic use. These interventions are critical to mitigating AMR risks and ensuring safe water access.

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Introduction

One of the biggest issues in public health of the 21st century is antimicrobial resistance, which makes it difficult to check and treat infections by bacteria, viruses, fungi, and parasites. According to the World Health Organization, AMR currently leads to about 1.27 million deaths each year, and this number may increase to 10 million annually unless steps are taken right now [1]. The frequent and improper use of antibiotics in people, animals, and agricultural activities has made many drugs less effective against certain bacteria, rendering many life-saving drugs ineffective [2]. All people have a right to drink clean and safe water to protect them from diseases spread through water. Lack of proper sanitation and treatment of wastewater is the reason water in many LMICs is easily contaminated with medicinal and hospital waste. So, the contamination leads to antibiotic-resistant bacteria and antibiotic resistance genes ending up in drinking water, which seriously affects public health. [3]. This contamination facilitates the spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) into drinking water systems, posing a severe public health risk.

Many infections come from bacteria in contaminated water, and Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae are known to cause serious gastrointestinal and body infections. When different kinds of antibiotics fail to eliminate these bacteria, they become dangerous since they make treatment more complicated and increase death rates. Besides, genetic materials in bodies of water allow HGT among different bacteria, which leads to an even bigger problem with AMR globally [4]. Furthermore, the aquatic environment serves as a reservoir for resistance genes, enabling horizontal gene transfer (HGT) among bacterial populations, which exacerbates the global AMR crisis [5].

Recent studies have detected MDR bacteria in various drinking water sources, including tap water, bottled water, and filtered water systems, in both urban and rural settings [6][7]. These findings underscore the urgent need for continuous water quality monitoring, improved sanitation infrastructure, and stricter regulations on antibiotic use to curb the environmental spread of AMR. This study aims to assess the prevalence of antibiotic-resistant bacteria in drinking water from different sources, providing critical insights into the role of water as a transmission pathway for AMR. The findings will contribute to global efforts in combating antimicrobial resistance and ensuring safe drinking water for all.

Methods

Study Design and Sample Collection

This cross-sectional study was conducted in Lahore, Pakistan, where a total of 30 drinking water samples were collected from three different sources: household tap water (n=10), filtered water from domestic filtration units (n=10), and commercially available bottled water (n=10). Each sample was collected aseptically in sterile 500 mL glass bottles, labeled with the source, date, and lahore, and transported to the microbiology laboratory in an icebox at 4°C to prevent microbial proliferation. All samples were processed within two hours of collection to ensure accurate microbial analysis.

Microbiological Analysis

For bacterial isolation, two techniques were employed: membrane filtration and the pour plate method. In the membrane filtration method, 100 mL of each water sample was passed through a sterile 0.45 µm pore-size membrane filter (Millipore, USA). The filter was then placed on selective and differential media, including MacConkey agar (Oxoid, UK) for Gram-negative enteric bacteria such as Escherichia coli, Cetrimide agar (HiMedia, India) for Pseudomonas aeruginosa, and Mannitol Salt Agar (MSA, Merck, Germany) for Staphylococcus aureus. In parallel, the pour plate method was conducted by adding 1 mL of each water sample to sterile Petri dishes, followed by the addition of molten nutrient agar (NA, Difco, USA). All plates were incubated aerobically at 37°C for 24 to 48 hours.

Bacterial Identification

Bacterial colonies exhibiting distinct morphological characteristics were selected for further identification. Preliminary identification was performed using Gram staining followed by standard biochemical tests, including catalase, oxidase, indole, citrate utilization, urease, and coagulase tests. For ambiguous isolates, additional confirmation was carried out using Analytical Profile Index (API) kits (bioMérieux, France).

Antibiotic Susceptibility Testing

Antibiotic resistance profiles were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA, Oxoid, UK). A bacterial suspension equivalent to the 0.5 McFarland standard was prepared for each isolate and uniformly spread over the agar surface. Antibiotic discs (Oxoid, UK) tested included ampicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), and ceftriaxone (30 µg). The plates were incubated at 37°C for 18 to 24 hours, and the inhibition zones were measured in millimeters. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines.

Data Analysis

All data were recorded in Microsoft Excel (Version 16.0), and descriptive statistical analysis was performed to determine the prevalence of bacterial contamination and antibiotic resistance patterns. The frequency of multidrug-resistant (MDR) strains defined as resistance to three or more antibiotic classes was calculated and compared across different water sources.

Results

Prevalence of Bacterial Contamination

Out of the 30 drinking water samples analyzed, bacterial contamination was detected in 21 samples (70%). The highest contamination rate was observed in tap water samples (90%), followed by filtered water (70%), while bottled water exhibited the lowest contamination rate (50), as shown in Table 1.

A total of 48 bacterial isolates were recovered from the contaminated samples. The most frequently identified species were Escherichia coli (35.4%), followed by Pseudomonas aeruginosa (27.1%) and Staphylococcus aureus (20.8%). Less prevalent isolates included Klebsiella spp. (10.4%) and Enterococcus faecalis (6.3%) as shown in Table 2.

Antibiotic susceptibility testing revealed significant resistance among the isolates. Escherichia coli exhibited the highest resistance to ampicillin (85%) and tetracycline (70%), while remaining largely susceptible to ciprofloxacin (75%). Pseudomonas aeruginosa demonstrated complete resistance to ampicillin (100%) and moderate resistance to ceftriaxone (65%). Staphylococcus aureus isolates showed resistance to ampicillin (80%) and chloramphenicol (50%) but retained sensitivity to gentamicin (85%) as shown in Table 3.

Overall, 62.5% of the bacterial isolates were classified as MDR, exhibiting resistance to three or more antibiotic classes. The highest proportion of MDR strains was found in tap water (70%), followed by filtered water (60%) and bottled water (40%).

These results indicate a concerning prevalence of antibiotic-resistant bacteria in drinking water, particularly in tap and filtered sources. The high frequency of MDR strains underscores the potential public health risks associated with contaminated water and highlights the need for enhanced water quality monitoring and antimicrobial stewardship programs.

Table 1: Bacterial contamination in drinking water samples

Water Source	Total Samples	Contaminated Samples	Contamination Rate (%)
Tap water	10	9	90%
Filtered water	10	7	70%
Bottled water	10	5	50%
Total	30	21	70%

Table 2: Distribution of bacterial isolates (n=48)

Bacterial Species	Tap Water (n=22)	Filtered Water (n=16)	Bottled Water (n=10)	Total (%)
Escherichia coli	8 (36.4%)	6 (37.5%)	3 (30.0%)	17 (35.4%)
Pseudomonas aeruginosa	7 (31.8%)	4 (25.0%)	2 (20.0%)	13 (27.1%)
Staphylococcus aureus	4 (18.2%)	3 (18.8%)	3 (30.0%)	10 (20.8%)
***Klebsiella* spp.**	2 (9.1%)	2 (12.5%)	1 (10.0%)	5 (10.4%)
Enterococcus faecalis	1 (4.5%)	1 (6.2%)	1 (10.0%)	3 (6.3%)

Table 3: Antibiotic resistance patterns of bacterial isolates (n=48)

Antibiotic	Tap Water (n=22)	Filtered Water (n=16)	Bottled Water (n=10)	Overall Resistance (%)
Ampicillin	18 (81.8%)	13 (81.3%)	7 (70.0%)	80.2%
Tetracycline	16 (72.7%)	11 (68.8%)	6 (60.0%)	70.1%
Ciprofloxacin	5 (22.7%)	3 (18.8%)	2 (20.0%)	22.3%
Ceftriaxone	13 (59.1%)	10 (62.5%)	5 (50.0%)	58.5%
Chloramphenicol	10 (45.5%)	8 (50.0%)	4 (40.0%)	45.8%
Gentamicin	4 (18.2%)	4 (25.0%)	1 (10.0%)	19.6%
MDR isolates	16 (72.7%)	10 (62.5%)	4 (40.0%)	62.5%

Discussion

The research found that antibiotic resistance among bacteria is common in drinking water drawn from various places in lahore. In our study, it was found that 90% of the bacterial samples from tap water were contaminated, 70% of those from filtered water, and 50% from bottled water. According to Ahmed et al. [8] and Khan et al. [9], the same challenges lead to the presence of pathogens in water sources in emerging countries.

It was observed in this study that the main offenders were Escherichia coli (35.4%), Pseudomonas aeruginosa (27.1%), and Staphylococcus aureus (20.8%), each of which are famous for leading to gastrointestinal and body-wide diseases. Since E. coli is found in large numbers, there could be leaks from sewage or not enough treatment given to water supply [10]. We should pay special attention to this, given that the pathogens are developing resistance to many of our most common antibiotics, which makes infections harder to manage [4].

Ampicillin had the highest level of resistance at 80.2%, while resistance to tetracycline was at 70.1% of the incidences. They agree with what the World Health Organization (WHO) reported in 2021 [1], showing that these are antibiotics with the greatest resistance rates all over the world. Even though antibiotics such as gentamicin and ciprofloxacin face the high rate of MDR infections, they may still be effective for treating waterborne infections in our area because their resistance rates are relatively low (19.6% and 22.3%, respectively).

It is important to notice how much more contaminated water sources can be. Most tap water is highly polluted (90%) mainly due to problems with the water system and not enough disinfection carried out by the residual chlorine [11]. Despite the fact that filtered water revealed less contamination (70%), this means that not all germy bacteria might be completely removed by domestic filters. There are fewer problems with resistant bacteria in bottled water, so some form of quality control is in place, nevertheless, Wright et al. point out that resistant bacteria were still found in nearly half of these samples [12].

Bacteria that are resistant to medicines in our drinking water may seriously harm public health. Water helps to store stubborn bacteria and enables genes with resistance to pass between various bacterial types (Bengtsson-Palme et al., 2018). Much alike, when such strains spread through the community, they might not be helped by antibiotics given without testing first, leading to more people dying [2].

Some factors may have led to the high resistance to antibiotics we saw in our research. Using antibiotics in humans and in animal farming at a wide scale probably both play big parts [13]. As well, poor water treatment systems can let through methicillin-resistant bacteria and antibiotic wastes [6]. The tropical environment in Lahore may also create conditions that are helpful for the growth of harmful bacteria in water supply pipes [14].

There are some things that limit our study. The number of people in our sample was not very large and represented only one region, so we cannot say if the results apply to the whole population. Because we did not do genomic research on resistance genes, we could not learn much about the ways the fungi resist pesticides [5]. It would be helpful for future studies to include many samples from various regions and study different resistance genes.

These discoveries point out how critical it is to develop complete plans to deal with antibiotic resistance in water supplies. Recommendations include:

1. Enhancing water treatment methods to strictly target bacteria that are hard to kill
2. Conducting continuous tests on the water to find drug-resistant microbes
3. Making better sanitation systems to keep water free from contamination
4. Strengthening the policies that control how antibiotics are given to people and animals
5. Advocating for proper care with water and how to take antibiotics

All things considered, drinking water in Lahore is found to hold a large amount of antibiotic-resistant bacteria, which can risk the well-being of the public. The high number of drug-resistant bacteria makes it urgent to increase water safety and fight back against antimicrobial resistance. To deal with this, it will be important for water groups, medical staff, the agricultural sector, and policymakers to join efforts.

Authors' contributions

ICMJE criteria	Details	Author(s)
1. Substantial contributions	Conception, OR	1
	Design of the work, OR	1
	Data acquisition, analysis, or interpretation	1
2. Drafting or reviewing	Draft the work, OR	1
	Review critically for important intellectual content	1
3. Final approval	Approve the version to be published	1
4. Accountable	Agree to be accountable for all aspects of the work	1

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The Ethics Review Committee of University of the Punjab, Lahore, Pakistan approved the study. Informed consent was taken from all volunteer participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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