

ORIGINAL ARTICLE

ISOLATION AND IDENTIFICATION OF VARIOUS BACTERIA IN DRINKING WATER IN BAHAWALPUR CITY, PAKISTAN.

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ABSTRACT

Introduction: Waterborne diseases remain a major global public health concern, especially in low- and middle-income countries like Pakistan. Contamination of drinking water due to poor sanitation and unhygienic storage practices contributes significantly to the disease burden. Bacterial pathogens such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* have been frequently isolated from drinking water in affected regions.

Objective: To isolate and identify bacterial contaminants in drinking water samples from various residential colonies of Bahawalpur City, Pakistan.

Methods: This cross-sectional study was conducted from January 2024 to August 2024 after ethical approval from the Bahawalpur Medical and Dental College, Bahawalpur. A total of 250 water samples were collected from different colonies using sterilized 250 ml glass bottles. Samples were transported and stored under strict aseptic and cold chain conditions. Water was cultured on MacConkey, CLED, Blood, and Chocolate agar media. Bacterial isolates were identified using Gram staining and standard biochemical tests.

Results: Public filtration plant water samples showed no microbial growth. In contrast, water with added market ice revealed mixed growth of *E. coli* and *Salmonella*. Water stored in plastic tanks was found to contain *Salmonella*, *Pseudomonas*, and *Staph. aureus*. Filtered dispenser water was free from contamination. Poor households storing water in earthen pots showed bacterial contamination. Overall, *E. coli* (60%) and *Salmonella typhi* (54%) were the most frequently isolated organisms.

Conclusion: Unsafe storage and handling practices, along with poverty and illiteracy, contribute significantly to microbial contamination of drinking water. Community education and improved sanitation are essential to reduce disease transmission.

Keywords: *Drinking water, bacterial contamination, sanitation, public health, Bahawalpur.*

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INTRODUCTION:

In Pakistan, waterborne diseases are a major disease burden on the health care system.¹ It is necessary to determine the bacteria that can cause disease via

human water consumption, and for all different domestic purposes. This measure is necessary to prevent outbreaks of diseases in a community and thus improve the healthcare system.² Diseases that spread via water are a major global issue.^{2,3} It has been found that *E. Coli* bacterial contamination of water usually occurs through fecal contamination of water.⁴ This can be easily prevented by taking

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proper preventive measures. However, Salmonella, S. aureus, and P. aeruginosa have also been isolated from water in different outbreaks.^{4,5} If these environmental factors are addressed seriously, the burden of cost to cure the ailing community can be reduced quite a lot and there can be an easy way of preventing people to get disease and suffering.⁶ By improving health care and educating the illiterate poor people, improving water supply sanitation and hygiene the annual budget of government being utilized on these preventable diseases can be saved. The World Health Organization's 2024 study shows, almost 10 % of the diseases of the world can be prevented by improving the quality, sanitation, and management of water resources.⁷ From these, more than 50 % are microbial intestinal infections, with cholera standing out in the first place. Water-related diseases continue to be one of the major health problems globally.⁸ Water can be defined as safe if it does not cause any significant hazard to health over a lifetime of consumption. Safe drinking water is thus suitable for all purposes, including personal hygiene.⁹ The microbiological quality of drinking water is a concern to consumers, water suppliers, regulators, and public health authorities. The potential of drinking water to transmit microbial pathogens to a great number of people, causing subsequent illness, is well documented in many countries at all levels of economic development.^{10,11} The objective of the study is to determine the bacterial contamination in drinking water in Bahawalpur City, Pakistan.

METHODS:

Ethical Approval and Study Duration: This cross-sectional study was approved by the Ethical Review Committee of Bahawalpur Medical and Dental College, Bahawalpur. The study was conducted over

eight months, from January 2024 to August 2024. All study expenses were borne by the researchers.

Sample Collection: A total of 250 water samples were collected from various residential colonies in Bahawalpur City, including Medical Colony, Model Town (A, B, C), Satellite Town, Kauser Colony, Bindra Basti, Behari Colony, Muslim Colony, Mohammadia Colony, and Fauji Basti. Sterilized, capped glass bottles with a 250 ml capacity were used for sample collection. Each sample (200 ml) was collected directly from household taps using strict aseptic techniques, with consent from residents.

Sample Transport and Storage: Immediately after collection, the bottles were sealed and placed in ice packs to preserve microbial integrity. Samples were transported vertically and stored at 1–4°C. All samples were processed within one hour of collection.

Culture Media and Incubation: Each water sample was inoculated onto four types of culture media: MacConkey agar, CLED agar, Blood agar, and Chocolate agar. The inoculated plates were incubated at 37°C for 24 hours.

Bacterial Identification: Plates showing microbial growth were examined under a light microscope for Gram staining. Biochemical tests were conducted to identify bacterial species.

- **E. coli** was identified on MacConkey agar as pink, dry, lactose-fermenting colonies. It appeared as Gram-negative motile rods and tested catalase-negative, coagulase-negative, oxidase-negative, and citrate-negative. Gas was produced on TSI agar, but H₂S was not.
- **Staphylococcus aureus** appeared as large golden-yellow beta-hemolytic colonies on Blood agar. Gram staining showed Gram-

positive, non-motile cocci in clusters. It tested catalase-positive, coagulase-positive, oxidase-negative, and TSI-negative for gas or H₂S.

- **Salmonella typhi** appeared as transparent pink colonies on MacConkey and lactose non-fermenting colonies on CLED. It was Gram-negative, catalase-positive, citrate-positive, and produced black H₂S on TSI. It was indole- and oxidase-negative but positive for methyl red and nitrate reduction.
- **Pseudomonas aeruginosa** appeared as greenish fluorescent colonies. It was Gram-negative, motile, catalase-positive, oxidase-positive, and citrate-positive. It produced gas from nitrates but not H₂S.

Control Sample: A tap water sample boiled for 20 minutes was used as a control and showed no microbial growth.

RESULTS:

The study revealed that households in different residential colonies of Bahawalpur used a variety of sources for drinking water. A majority (55%) of residents collected water from public filtration plants and stored it in household water coolers. Microbiological analysis of these samples showed no bacterial growth, indicating effective filtration. However, contamination was observed in samples where residents had added commercially purchased ice to the stored filtered water. These samples exhibited mixed growth of *Escherichia coli* and *Salmonella typhi*, likely due to unhygienic handling and transportation of ice from the market, followed by its storage in water coolers.

Approximately 40% of households used donkey pumps or direct bore systems to draw groundwater. This water was either directly added to coolers or stored in rooftop plastic tanks before distribution through internal pipelines. Water samples from rooftop tanks showed mixed bacterial contamination

with *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Residents using plastic tanks were advised about the importance of routine cleaning and maintenance of their storage systems.

A small proportion (5%) of families used electric water dispensers or domestic filtration units. Samples from these households were consistently free from microbial contamination, reflecting good hygiene practices.

In contrast, low-income households typically stored water in uncovered earthenware pots. It was observed that water was often retrieved by hand from these containers, contributing to contamination. Samples from such sources frequently revealed *Salmonella typhi*, *E. coli*, and *Staph. aureus*. Representative culture plates of the isolated bacteria are presented in Figures 1–5.

The findings also highlighted a correlation between socioeconomic status, awareness of hygiene, and water safety. Poor and uneducated households demonstrated limited knowledge of safe water handling, while middle-class and educated families were more aware of proper sanitation practices.

Residents consuming contaminated water frequently reported episodes of diarrhea and abdominal cramps, especially among children under five and elderly individuals. The distribution of bacterial isolates is presented in Table 1.

Table: 1. Percentage of various bacteria isolated in water samples (n=250)

Bacteria	Percentage (%)
E Coli	60
Salmonella Typhi	54
Staphylococcus Aueus	42
Pseudomonas Aeruginosa	02
Shigella	0



Fig 1: E. coli Citrate negative: TSI agar showing yellow butt and yellow slant with gas

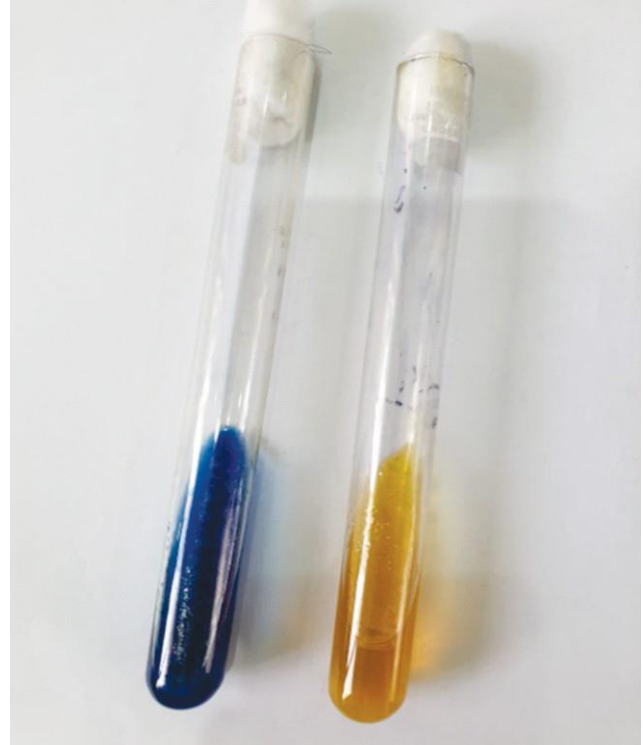


Fig 3: Salmonella Typhi-Citrate positive: TSI agar showing yellow butt and yellow slant with blackening due to H₂S.



Fig 2: Pseudomonas Aeruginosa-Citrate positive: TSI agar red butt and red slant and no gas



Fig 4: Staphylococcus growth on blood agar



Fig 5: Growth of E Coli on MacConkey agar

DISCUSSION:

This study found that 66% of drinking water samples collected from various residential colonies in Bahawalpur were contaminated with bacterial pathogens. *Escherichia coli* and *Salmonella typhi* were the most frequently isolated organisms, particularly in samples stored in rooftop plastic tanks or mixed with market-sourced ice. In contrast, water from public filtration plants and domestic dispensers showed no microbial growth, indicating the effectiveness of proper treatment and storage. According to recent studies in the United States of America, waterborne disease was found to be moderate to severe in approximately 560,000 people, and approximately 7.1 million suffer from a mild-to-moderate waterborne infection every year. Similarly, approximately 37.7 million Indians suffer from waterborne diseases each year. Around 1.5 million kids are estimated to die because of loose motions leading to dehydration alone.⁷ Clean drinking water should be made available to improve health. It is

important to consider the health and development issues at national and regional centers. In some areas, the investment in clean water availability and sanitation outweighs the burden of treating the ill patients and providing a cure. Spending on clean water supply in homes by people themselves should be encouraged, and proper guidance and education should be given to the general population to prevent them.¹² It has been observed by different studies that measures which are taken to improve water purification basically affect poor and illiterate people rather than middle-class, rich, and educated people.¹³ Our study's results corroborate this conclusion. This observation is common in both urban and rural areas of Pakistan. Many studies have proved that availability of clean and fresh water is a major step in improving many health issues and this supply of clean water should be considered an essential element when various health issues are dealt with.⁷ Acute diarrhea is one of the commonest diseases which is a major public health problem leading to dehydration and ultimately death in Pakistan.¹⁰ Our research findings are consistent with this observation. It has also been shown in other studies that diarrhea is more common in people with poor hygiene.¹³⁻¹⁵ Our research project also yielded similar results to those found in these studies, except for the fact that our study had a higher percentage of *Salmonella* and *Staphylococcus aureus*. This may be because the studies are carried out in diverse settings, differing from those of other research studies. In our study, it was found that children under 5 years had the highest frequency of developing waterborne diseases via different microbial agents. A similar incidence has also been found in other Asian and African countries.¹³ So that shows that our drinking water has microbial agents in it and has to be boiled for at least 20 minutes before drinking, according to the World Health Organisation.⁷ But again, poverty becomes the main hurdle. As a matter of fact, poor

people cannot afford enough fuel to boil water for 20 minutes. This is a very important financial matter. Even educating these poor people cannot save them from the dreadful outcomes of the water that contains harmful infectious microbiological agents. This biological contamination needs to be considered seriously by the government. The clean and safe water will then further lower the burden on the Health Department. Water and sanitation are a global problem. The microbiological quality of drinking water is a concern to consumers, water suppliers, regulators, and public health authorities. The potential of drinking water to transmit microbial pathogens to a great number of people, causing subsequent illness, is well documented in many countries at all levels of economic development.

CONCLUSION: This study highlights a high rate of bacterial contamination in drinking water, mainly due to poor storage practices and a lack of hygiene awareness. Safe sources like public filtration plants and domestic dispensers were free of contamination. Promoting hygienic storage and community education is essential to prevent waterborne illnesses and protect public health.

DECLARATION OF INTEREST: The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS:

A.I.: Data collection, data analysis, literature review, write-up and references

A.G.: Conceptualization, proofreading, and final editing of manuscript.

REFERENCES:

1. Pakistan Council of Research in Water Resources (PCWR). Launching of National Water Quality Monitoring Program Report 2020–21 [Internet]. 2021 [cited 2024 September 10]. Available from: <https://pcwr.gov.pk/launching-of-national-water-quality-monitoring-program-report-2020-21/>

2. UNICEF Pakistan. Water, Sanitation and Hygiene [Internet]. 2022 [cited 2024 September 10]. Available from: <https://www.unicef.org/pakistan/wash-water-sanitation-and-hygiene-0>
3. Eckstein D, Kunzel V, Schafer L. Global Climate Risk Index 2021. Berlin: German Watch; 2021. Available from: https://www.germanwatch.org/sites/default/files/Global%20Climate%20Risk%20Index%202021_2.pdf
4. Acciarri G, Gizzi FO, Torres Manno MA, Stülke J, Espariz M, Blancato VS, Magni C. Redundant potassium transporter systems guarantee the survival of *Enterococcus faecalis* under stress conditions. *Front Microbiol.* 2023;14:Article 123456.
5. Ahmed L, Wong LP, Chua YP, Channa N, Mahar RB, Yasmin A, et al. Quantitative microbial risk assessment of drinking water quality to predict the risk of waterborne diseases in primary-school children. *Int J Environ Res Public Health.* 2020;17:2774. doi:10.3390/ijerph17082774
6. Akram N. Consumption of safe drinking water in Pakistan: its dimensions and determinants. *Drink Water Eng Sci.* 2020;13:43–50. doi:10.5194/dwes-13-43-2020
7. World Health Organization. Guidelines for drinking-water quality: small water supplies. Geneva: WHO; 2024 Feb 15.
8. Alsalmeh A, Al-Zaqri N, Ullah R, Yaqub S. Approximation of ground water quality for microbial and chemical contamination. *Saudi J Biol Sci.* 2021;28:1757–62. doi:10.1016/j.sjbs.2020.12.017
9. Ali SA, Ahmad A. Analysing water-borne diseases susceptibility in Kolkata municipal corporation using WQI and GIS based kriging interpolation. *GeoJournal.* 2020;85:1151–74. doi:10.1007/s10708-019-10015-3
10. Amin RM, Zaidi MB, Bashir S, Khanani R, Nawaz R, Ali S, et al. Microbial contamination levels in the drinking water and associated health risks in

Karachi, Pakistan. *J Water Sanit Hyg Dev.* 2019. doi:10.2166/WASHDEV.2019.147

11. Aram SA, Saalidong BM, Lartey PO. Comparative assessment of the relationship between coliform bacteria and water geochemistry in surface and ground water systems. *PLoS One.* 2021;16:e0257715. doi:10.1371/journal.pone.0257715

12. Guterres A. In flood-hit Pakistan, Guterres appeals for ‘massive’ global support, tougher action on climate change. *UN News* [Internet]. 2022 [cited 2024 September 25]. Available from: <https://news.un.org/en/story/2022/09/1126331>

13. Asomaku SO. Quality assessment of groundwater sourced from nearby abandoned landfills from Industrial City in Nigeria: water pollution indices approach. *HydroResearch.* 2023;6:130–7. doi:10.1016/j.hydres.2023.03.002

14. Gunter H, Bradley C, Hannah DM, Manaseki-Holland S, Stevens R, Khamis K. Advances in quantifying microbial contamination in potable water: potential of fluorescence-based sensor technology. *WIREs Water.* 2023;10:e1622. doi:10.1002/wat2.1622

15. Janjua S, Hassan I, Muhammad S, Ahmed S, Ahmed A. Water management in Pakistan’s Indus Basin: challenges and opportunities. *Water Policy.* 2021;23(6):1329–43. doi:10.2166/wp.2021.068

16. Yasin HQ, Breadsell J, Tahir MN. Climate-water governance: a systematic analysis of the water sector resilience and adaptation to combat climate change in Pakistan. *Water Policy.* 2021;23(1):1–35. doi:10.2166/wp.2020.113

17. Ishaque W, Mukhtar M, Tanvir R. Pakistan’s water resource management: ensuring water security for sustainable development. *Front Environ Sci.* 2023;11:1096747. doi:10.3389/fenvs.2023.1096747

18. Ishaque W, Tanvir R, Mukhtar M. Climate change and water crises in Pakistan: implications on water quality and health risks. *J Environ Public Health.* 2022;2022:e5484561. doi:10.1155/2022/5484561

19. Sohail MT, Ehsan M, Riaz S, Elkaeed EB, Awwad NS, Ibrahim HA, et al. Investigating the drinking water quality and associated health risks in metropolis area of Pakistan. *Front Mater.* 2022;9:864254. doi:10.3389/fmats.2022.864254