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Comparative Analysis of E. coli, Salmonella, and Fungus in Portable Water of District Faisalabad

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Abstract

Increasing industrialization and urbanizationpose a significant threat of waterborne diseases to the region. The main cause of many diseases, such as typhoid, gastroenteritis, and diarrhea, is contaminated water, which is due to waterborne pathogens like coliforms and Salmonella, etc. Water spreads various infections that lead to endemic or epidemic events around the globe. Coliforms have been utilized as indicator organisms for a long time and are crucial to the regulatory benchmarks governing the standard of portable and recreational water. The purpose of this study is to identify a comparative evaluation of coliforms and Salmonella water contamination. The samples of 90 potable waters were

selected from different localities of Faisalabad. Enumeration of bacterial load was done through the viable count method. In order to detect bacteria, the membrane filtration method was employed. For isolation, membranes were placed on MacConkey agar and SS agar, respectively, for 24 hours. Colonies were characterized by Gram's staining and biochemical tests. For the detection of fungus, slide culture was done, and confirmation of fungal colonies was made by colony morphology and microscopy. All the data were recorded and analyzed by SPSS software.

Keywords: E. coli, Microbial Contamination, Salmonella, Fungus, Drinking Water

Introduction

The fresh water accessible for human use constitutes merely 0.6% of the total global water resources found in glaciers, flowing surface water, and groundwater (Novak Babič et al., 2017). All living creatures require water to thrive. It has been demonstrated that it accounts for roughly 70% of human body mass. For microbial life, water serves both a vehicle and a habitat. Biofilm formation in distribution systems, fecal contamination, and soil runoff are among the ways that naturally occurring bacteria might get into water supplies. Once present, these microbes can multiply in an ideal environment, making water unfit for human consumption(Barbosa, 2025).

Almost 1.8 billion individuals around the world are using water sources contaminated with feces, with more than a billion facing levels assessed as moderately hazardous(Bain et al., 2014).It is very important to know that prevalence of these pathogens in potable water, particularly in the Faisalabad district, which is commonly known as the industrial core of Pakistan, where increasing urbanization and industrial activities may impair water quality. Faisalabad relies on groundwater for over 90% of both drinking and industrial water supply. Recent evaluations of drinking water in

Faisalabad indicate that while certain regions comply with the WHO's physicochemical criteria, microbiological contamination continues to pose a risk in multiple areas (Anwar et al., 2024).

Coliforms are rod-shaped, gram-negative bacteria belonging to the Enterobacteriaceae family, which includes different genera, including *Escherichia, Klebsiella, Enterobacter*, and *Citrobacte*r are usually indicators of drinking water quality (Tambi et al., 2023).the presence of total coliform in treated water, which is typically coliform-free, may be a sign of inadequate treatment, disinfectant loss, breakthrough, contaminated water interfering with the potable water supply, or distribution system regrowth issues (Khan et al., 2021). Coliforms may enter portable water through soil, vegetation, or cracks in the water system. *E. coli* in food or water typically indicates recent fecal contamination or unsanitary conditions in food processing facilities (Agensi et al., 2019). Therefore, an important factor influencing the *E. coli* population is fecal contamination, inadequate sanitation practices, and unfavorable storage conditions (Kayembe et al., 2018).

On the other hand, *Salmonella* is also a gram-negative, rod-shaped bacterium. Several animals, including humans, birds, reptiles, and poultry, naturally harbor Salmonella in their digestive tracts (Luo et al., 2015). *Salmonella* infections that are waterborne usually occur when contaminated water is consumed, particularly when the water source is shared with livestock or is not properly chlorinated. Salmonella, in contrast to coliforms, is a real pathogen that can cause serious infections such as non-typhoidal salmonellosis, typhoid fever, and paratyphoid fever.

Abdominal cramps, fever, and diarrhea are among the symptoms; in susceptible people, systemic problems may develop.From all the food-related gastroenteritis cases, non-

typhoidal salmonellosis is one of the most common, compromising the health of almost a million individuals a year in the US alone and costing the country $3.7 billion in medical expenses (Whiley et al., 2017). An estimated 93.8 million gastroenteritis cases and 155,000 fatalities are attributed to Salmonella species per year worldwide (Brandl et al., 2013).

Salmonella is highly persistent in aquatic environments; it may live in sediments and biofilms, avoid disinfection procedures, and even become resistant to antibiotics. According to a 2025 study of African water systems, Salmonella was frequently recovered from surface and groundwater sources, frequently in combination with inadequate sanitary facilities (Rabiu et al., 2025). Salmonella typically results in self-limiting gastroenteritis, making it one of the most common foodborne illnesses in developing nations. Nevertheless, it can also serve as the root cause for invasive systemic diseases like paratyphoid fever and typhoid fever, which are uncommon in the US but persistent in less developing nations (Chatham-Stephens, 2019).

The presence of fungal genera in potable water not only alters its smell and flavor but also leads to various technical challenges. Fungal contamination of water can result in myotoxicity and allergic reactions when inhaled or transmitted while showering (Grabińska-Łoniewska et al., 2007). It can be absorbed by various cavities like the cornea, skin, and oral areas into the human body and may enter the bloodstream directly during surgical operations (Hussain et al., 2024).

The current research will work to find the extent of contamination with coliforms, Salmonella, and fungi in drinking water sources in Faisalabad. When creating effective water safety guidelines, public health agencies will need to have access to this

information.Due to the importance of clean water for life and the challenges posed by contamination in portable water, this study is designed.

Methodology

Samples of potable waterwere collected from different localities in Faisalabad. A total of 90 water samples were collected in sterile bottles following standard procedures, obtained from various household taps and municipal water supply points. For the best results, samples were transferred to the lab and analyzed within 24 hours. Research work were conducted in the Postgraduate Medical and Molecular Microbiology Laboratories of the Institute of Microbiology, Government College University, Faisalabad.

The viable count method was used to assess bacterial load in potable water. The membrane filtration method was used for the detection of *E. coli*and *Salmonella* in potable water. For the detection of *E coli*, the membrane filter was placed on EMB agar and incubated at 37℃ for 24-48 hours.While for the detection of *Salmonella*, the membrane will first be placed in enrichment media Selenite cystine broth for 24 hours and then this broth will be plated on MacConkey agar / Salmonella Shigella agar and incubated for 24 hours.

*E. coli*colonies were identified primarily using Gram's stain and by colony morphology. *E. coli* were appear pink on MacConkey agar and showed a green metallic shine on eosin methylene blue agar (EMB)(Najm & Hussein, 2023). *Salmonella* colonies were identified by Gram's stain, cultural characteristics, and morphology of the colony. *Salmonella* was colorless on MacConkey agar and show smooth and opaque colonies with a black center on Salmonella Shigella agar.For further confirmation, various biochemical tests such as Oxidase, Catalase, and Coagulase will be utilized to identify the organism(Ahmad et al., 2021).Based on colony counts, the results for *E.coli* were

expressed as CFUs per 100 mL of water.The number of positive samples (presence/absence) and the proportion of positive samples relative to the total were computed to detect*Salmonella*.

Similarly, for the detection of fungus, SDA agar was used, and plates were incubated for 4,5 days at 25℃. For the isolation of pure colonies, sub-culturing was also done and the slide culture technique was used for their morphological identification. A compound microscope was used to examine the slide at magnifications of 10x and 40x to identify the fungus based on conidiophores, hyphal structure, spore arrangement, and colony morphology.

Results and Discussion

There were total 90 water samples which were collected from different localities of Faisalabad. Over 90 samples, only 5 samples were positive for *E. coli*and 1 sample were positive for *Salmonella*. Table 1, and Graph 1 showing prevalence of Bacteria in Portable Water of district Faisalabad.

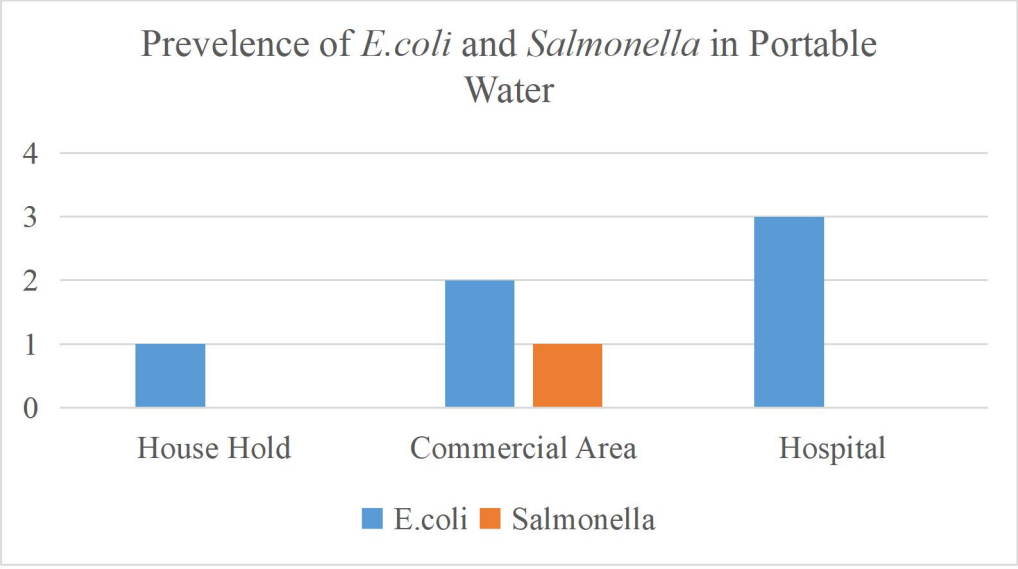

Figure 1: Graphical Representation of Prevalence of Bacteria in Portable Water

Samples Location	*E.coli*	*Salmonella*
House Hold	1	0
Commercial Area	2	1
Hospital	3	0
Total Samples		90

Table 1 Prevalence of bacterial contamination based on different Localities of Faisalabad

Similarly, fungus was also studied in this research, and Aspergillus were more dominant in these samples as shown in below table.

Sample Location	No. of Samples	Positive Samples
House Hold	30	5
Commercial Area	30	7
Hospital	30	10
Total Samples		90

Table 2: *Prevalence of Fungi in Portable Water*

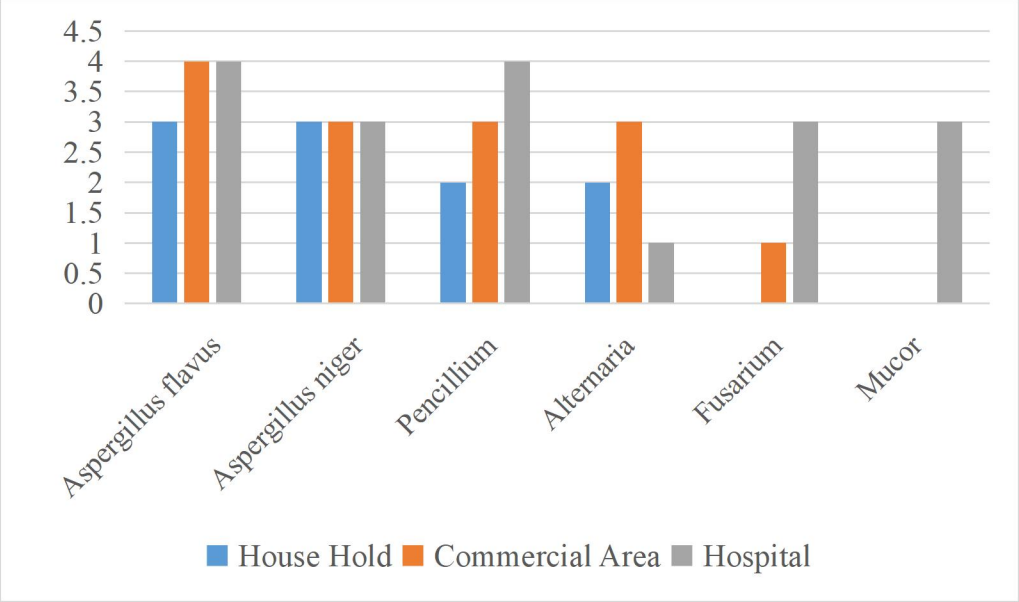

Figure 2 Graphical representation of fungus prevalence isolated from Portable Water

When comparison was done, it was showed that water were more contaminated with fungus than bacterial contamination.

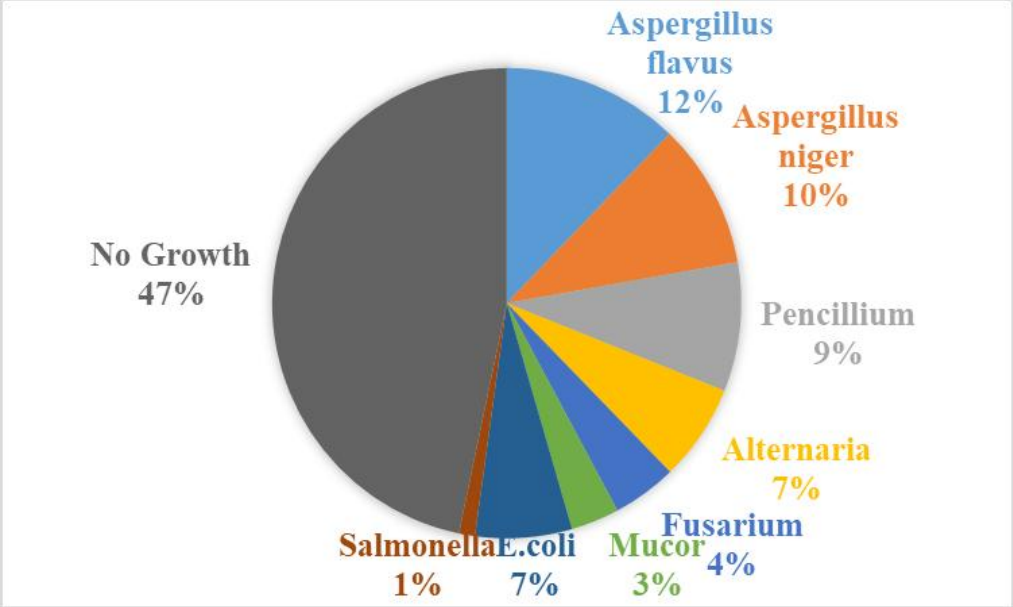

Figure 3: Types of Microbes Isolated From Portable Water

Discussion

The findings of this investigation gave a significant awareness of the prevalence and antimicrobial resistance patterns of bacteria and the prevalence of fungi isolated from the portable water of District Faisalabad, sourced from diverse locations including households, commercial areas, and Hospitals. Microbes such as (12%) *Aspergillus flavus*, (10%) *Aspergillus niger*(9%)*Pencillium*, (7%) *Alternaria*, (7%) *E. coli*, (4%) *Fusarium*, (3%) *Mucor* and (1%) *Salmonella* were isolated from the house hold, commercial area and hospitals water sample in Faisalabad. Overall, 53% contamination was observed in water samples in which *Aspergillus* was most dominant genus, and Penicillium was the second

most prevalent fungus in water. These findings show the potential risk to public health due to fecal and fungal contamination in portable water.

The WHO guideline states that coliform bacteria should be absent in any 100ml of drinking water. The detection of *E. coli* in various water samples signifies the occurrence of enteric pathogen contamination from various human or animal sources, which may lead to many gastrointestinal problems in the human population. One of the most common causes of gastrointestinal diseases in the world is drinking water, which may mainly occur because of fecal contamination of raw water, and water treatment process failure of drinking water at source or point of use.

The detection of *Salmonella* and *E. coli*confirms earlier reports that fecal impurities in urban water supply points are frequently linked to cross-connections between drinking water and sewage lines, pipelines, and inadequate chlorination procedures. The existence of Aspergillus and Penicillium, which are known to induce hypersensitive reactions and opportunistic infections, especially in immunocompromised people, suggests a neglected component of microbial water safety that needs further focus.

These results of this study show flaws of local water safety infrastructure while also being consistent with trends in developing nations. So immediate actions are required to improve disinfection procedures, fungus assessments into routine testingand establish strict water quality monitoring. These initiatives are essential to safeguarding the community's health. Some limitations in the current study as the samples were not collected across the whole year, which could influence the microbial load and detection of microbes was done by conventional methods.

In conclusion, the current study results show that Faisalabad's potable water sources are vulnerable to fecal and fungal pollution, which poses severe health risks to the community. These findings support the need for improved methods of monitoring water quality that take into account both bacterial and fungal characteristics. In order to provide safe and sanitary portable water for metropolitan residents, it is important to strengthen microbiological monitoring systems and upgrade water treatment systems.

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