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RESEARCH PAPER

TITLE:

MICROBIAL CONTAMINATION IN DRINKING WATER: A STUDY OF GOVERNMENT GIRLS DEGREE COLLEGE DARGAI MALAKAND AND SURROUNDING AREAS

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MICROBIAL CONTAMINATION IN DRINKING WATER: A STUDY OF GOVERNMENT GIRLS DEGREE COLLEGE DARGAI MALAKAND AND SURROUNDING AREAS

ABSTRACT

About 80% population of Khyber Pakhtunkhwa (KP) are using surface and ground water as prime source of drinking water. World health Organization (WHO) has been reported transmission of diseases such as cholera, diarrhea, dysentery and polio are linked with contamination of drinking water. Therefore, assessment of the prime source of drinking water in least developed areas gained significant importance. The present investigation aimed to assess the quality of drinking water and evaluate the contamination of microorganisms (bacteria and fungi) in drinking water sources at Dargai, Malakand and nearby places in Khyber Pakhtunkhwa, Pakistan, between October 2021 and March 2022. The contamination level of the collected samples from different sources were determined through gram staining and other microbiological techniques. The results revealed the presence of microorganism, which consisted predominantly of cocci (81.81%) and Bacilli (9.09%). Among these, gram-positive accounted for 36.36%, while gram-negative microorganisms comprised 90.90%. Additionally, 72.72% of the microorganisms were identified as Fungi. The results revealed substantial microbial presence, including various bacterial and fungal species, indicating potential health risks. These findings highlight the critical need for enhanced water management planning and risk assessment of community water sources in Dargai Malakand and surrounding areas, and recommend further research by using advanced microbial identification techniques and evaluate the broader public health implications.

KEYWORDS: Microbial contamination, Drinking water quality, fungal contamination, bacterial assessment, Dargai Malakand, gram staining, water safety

1. INTRODUCTION

The world health organization defines safe drinking water as water that will not cause substantial harm to human health (Oliveira et al., 2013). Access to safe drinking water is a basic requirement in development countries. However, ensuring the supply of clean drinking water requires vigilance and responsiveness to potential microbial contamination events. Further, climatic changes such as floods, and drought are also a major factor in affecting drinking resources of water (Taylor et al., 2024). Water is a basic nutrient of the human body and is critical for sustaining life. It aids in the digestion, adsorption, transportation and use of nutrients and as well as the elimination of toxins and wastes from the body (Kleiner, 1999). The quality of the water we consumed is well-recognized as an important transmission route for infectious diarrheal

and other diseases (Baltazar et al., 1993). Therefore, access to safe drinking water has become a widely debated issue within the global community, with concerns about chronic health issues caused microbial contamination. The importance of water quality continues to be emphasized by its role in epidemics and contribution to endemic disease from Pathogens (Ford, 1999). Pakistan is among the top ten countries in the world with the lowest access to clean and safe water near home (The State of the World's Water, 2018). Studies shows that more than 50% of well water samples have elevated levels of microbial contamination. Additionally, approximately 30% of all wells and 90% of those located in towns or villages are contaminated with nitrates (Staradumskyte and Paulauskas, 2014)

Bacteria are probably the most frequently studied group of microorganisms with respect to the water-borne diseases in most developing countries where drinking water sources are communally shared since 1911 (Amann, 1911). The number of bacteria reaches its lowest levels in water tanks after treatment procedures (e.g., chlorination). However, in tap water, bacteria levels increase again due to the presence of biofilms in plumbing systems (Kormas et al., 2010). Gram stain is a rapid and important method for bacterial differentiation (Beveridge, 2001). The SCOPE project (Surveillance and Control of Pathogens of Epidemiologic Importance) found that in individuals with an underlying malignancy, Gram positive organisms in those accounted for 62% of all bloodstream infections in 1995 and 76% in 2000, while Gram-negative organisms accounted for 22% and 14%, respectively (Wisplinghoff et al., 2003). Gram-negative bacteria that grow after 4-7 days were isolated and examined for their ability to grow on media with and without cysteine (Matuszewska and Krogulska, 2000). Non-fermentative Gram-negative bacilli (non-fermenters) are ubiquitous in the environment and can cause a vast variety of infections (Dijkshoorn et al., 2007; LiPuma et al., 2011; Su et al., 2009).

Fungi are eukaryotic, heterotrophic organisms that include both single-celled yeasts and multicellular filamentous fungi (Paterson and Lima, 2005). Fungi have been implicated in a number of diseases, including allergies, respiratory illness, cutaneous infection and life-threatening meningitis (Pfaller & Diekema, 2004; Sulaiman et al., 2014). Bacteria and fungi coexist and interact in many environments, as they often share a common substrate. Fungi produce most enzymes due their higher biomass, while bacteria benefit from the enzymatic capacity of fungi, particularly in degradation of plant polymers (Mille-lindblom, 2005). Peptidoglycan, which forms bacterial cell walls, has been shown to stimulate hyphal growth in *C. albicans* (Xu et al., 2008, Shank and Kolter, 2009).

Dargai Malakand which is located in the northern part of Pakistan, faces significant challenges in accessing good quality drinking water. A recent study reported that heavy metals from the marble plants in Malakand significantly increase the heavy metal contamination in the soil, which ultimately affect the drinking water (Khan et al., 2024). Similarly, another study reported that untreated effluents from steel mills in Dragai Malakand further enhanced the soil and water

contamination (Khan et al., 2022). Moreover, a previous review highlighted that distribution networks and the consumer tap, from which drinking water is collected, are significantly populated with microbes (Nabeela at al., 2014). Thus, the aim of this study was to assess the microbial contamination of drinking water in GGDC Dargai Malakand and nearby areas, with a focus on identifying bacteria and fungi using gram staining. We investigated the presence of the microbes in the drinking water of GGDC Dargai Malakand and nearby places via gram staining method. These findings highlight the critical need for comprehensive management programs to improve the quality and safety of drinking water in the studied locations.

2. MATERIAL AND METHODS

The Figure 1 describes the steps of this study starting with sample collection and culminating in the microscopic analysis of the samples.

2.1 Study design and setting

The study was conducted at GGDC Dargai from October 2021 to March 2022 in the Microbiology Laboratory. GGDC Dargai is a government college located in District Malakand, Khyber Pakhtunkhwa, Pakistan. The laboratory provided a controlled environment for microbiological analysis, including sample collection, media preparation, microbial growth, and staining.

2.2 Sample collection

Water samples were collected from various locations in Tehsil Dargai to study the microbial contamination of water sources. The specific locations included:

GGDC Dargai Malakand Tank-1
GGDC Dargai Malakand Tank-2
GGDC Dargai Malakand Tank-3
Filter-1 of GGDC Dargai Malakand
Filter-2 of GGDC Dargai Malakand
Well Water of GGDC Dargai Malakand
Government Higher Secondary School Dargai No. 2
Dargai Bazar, Malakand
Sorana Dargai, Malakand
Government Girls Primary School No. 2 Sakhakot, Malakand
Sakhakot Model School, Malakand

Water samples were collected in sterile containers and transported to the laboratory for analysis to ensure no contamination during the sampling process.

2.3 Preparation of PDA media

Potato Dextrose Agar (PDA) was prepared by dissolving 3.9 grams of PDA powder in 100 mL of distilled water in a clean flask. The flask was covered with aluminum foil to prevent contamination and then autoclaved at 121°C for 30 minutes to sterilize the medium. Sterilization ensures that only the target microorganisms from the water samples can grow on the medium, avoiding unwanted contamination.

2.4 Inoculation

After sterilization, PDA solution was poured into sterile Petri plates to create a solid medium for culturing microbial growth. Once the agar solidified, inoculation was performed by streaking samples from the collected water onto the surface of the plates using a sterile inoculating loop. This process was done under a sterile environment to minimize external contamination. The inoculated Petri plates were then incubated at 37°C for one week to allow microbial

growth. The temperature was maintained at 37°C to simulate the optimal conditions for the growth of many microorganisms.

2.5 Staining

To identify the type of microorganism's present, Gram staining was performed on the grown cultures. The procedure was as follows:

Heating the inoculating loop until it glowed red to ensure sterility.

A drop of autoclaved water was placed on a clean glass slide.

A small amount of the microbial culture was mixed with the water drop and spread evenly over the slide, then allowed to air dry.

Crystal violet stain was applied for 30 seconds, followed by washing off the excess stain with distilled water.

Gram iodine was applied for one minute to form a complex with the crystal violet, then rinsed with water.

The smear was washed with 95% alcohol for 20-30 seconds to decolorize the sample.

The slide was rinsed again with water and counterstained with safranin for 30 seconds, followed by rinsing with water.

The slide was then blotted to remove excess water and allowed to dry.

The Gram staining technique differentiates microorganisms into Gram-positive (purple) and Gram-negative (pink) based on their cell wall structure, which is essential for classifying and identifying bacterial species.

2.6 Microscopic analysis

After staining, the slides were examined under an electric light microscope at various magnification powers starting from 10x. The examination aimed to observe the morphological characteristics of the microorganisms, such as shape (cocci, bacilli, etc.), size, and arrangement, which are key in identifying bacterial species. The oil immersion lens (100x) was used for detailed examination to observe cellular structures more clearly.

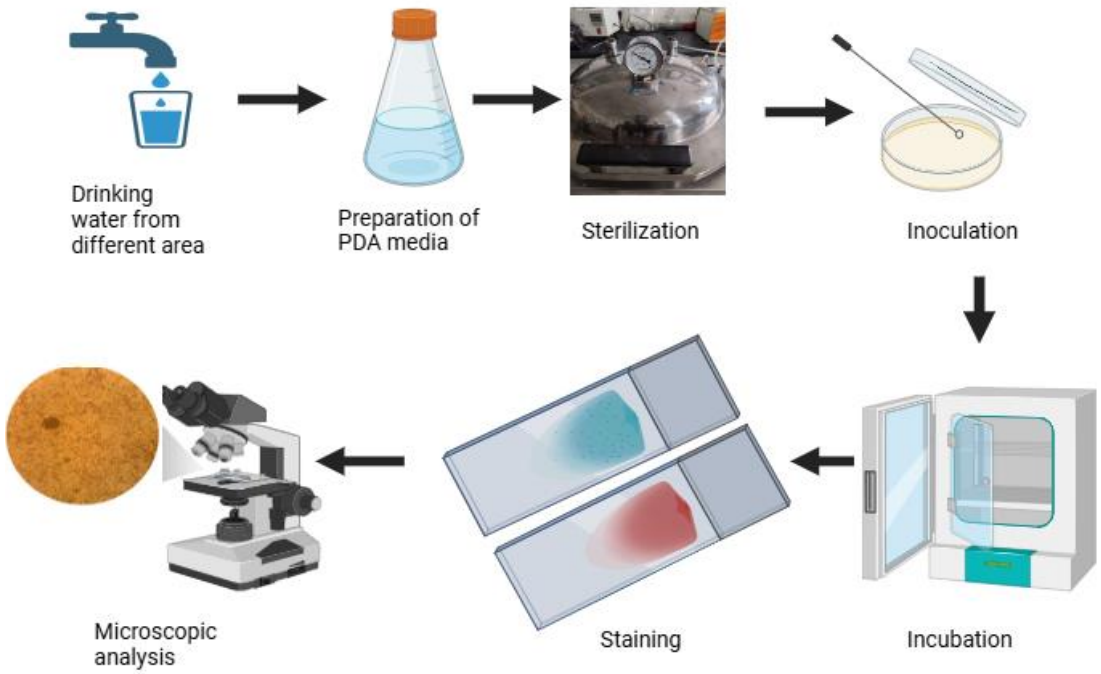

Figure 1: Flowchart of the current study. The flowchart describes the steps of the study starting with sample collection and culminating in the microscopic analysis of the samples. Each step is represented to show the progression from initial data acquisition to the final analytical procedure, highlighting key stages involved in the study.

3. RESULTS

A total of eleven water samples from different areas of Dargai, Malakand Division, were examined. Out of these, eight samples contained fungi, while ten samples contained bacteria.

3.1 Evolution of Fungi in the studied water samples

The fungal isolates from the samples were examined under a microscope at a magnification power of 10X. We observed that only one plate, named Sorana Dargai, was exclusively occupied by fungi, with black-colored fungal spores (Figure 2A). Seven plates, including Tank-1, Tank-2, Tank-3, Filter-1, Filter-2, GHS Dargai No-2, and Government Girls Primary School Sakhakot No.2, contained both fungi and bacteria (Figures 2B–H). Tank-1 and Filter-2 slides contained green spore-producing fungi, whereas Tank-2 slides contained black spores (Figures 2B, F, C).

Tank-3 slides exhibited clear hyphae, while Filter-1 slides contained green spores, and a distinct reproductive structure was also observed (Figures 2D, E). The GHS Dargai No-2 sample contained black spores and exhibited a clear reproductive structure similar to that of Filter-1 (Figure 2G). Additionally, the GGPS Sakhakot No-2 slides contained black spores (Figure 2F).

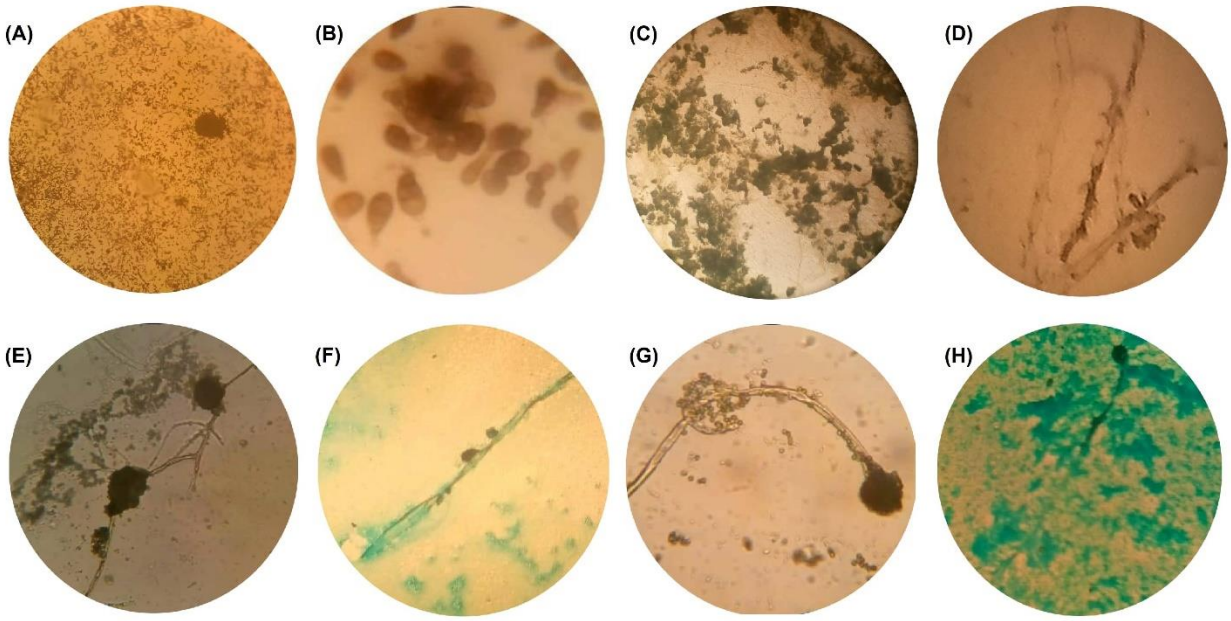

Figure 2: Identification of fungi in drinking water collected from the Government Girls Degree College Dargai Malakand and nearby locations. Fungal growth was examined under a microscope at 10X magnification. **(A)** Dargai Sorana, **(B)** Tank-1 of Government Girls Degree College Dargai Malakand **(C)** Tank-2 of Government

Girls Degree College Dargai Malakand **(D)** Tank-3 of Government Girls Degree College Dargai Malakand **(E)** Filter-1 of Government Girls Degree College Dargai Malakand **(F)** Filter-2 of Government Girls Degree College Dargai Malakand **(G)** Government High School (GHS) No-2 Dargai **(H)** Government Girls Primary School (GGPS) Sakhakot No-2.

3.2 Bacterial assessment in the collected samples

All bacterial isolates were examined under a microscope at a magnification power of 100X. Among the ten positive bacterial samples, all were gram-negative, while four samples also contained gram-positive bacteria. The gram-positive bacteria were cocci in shape and were found in Tank-2, Tank-3, GHS Dargai No-2, and Dargai Bazar samples (Figures 3A–D). All gram-negative bacteria were cocci in shape (Figures 3A–I), except for the Sakhakot Model School sample, which contained bacilli (Figure 3J).

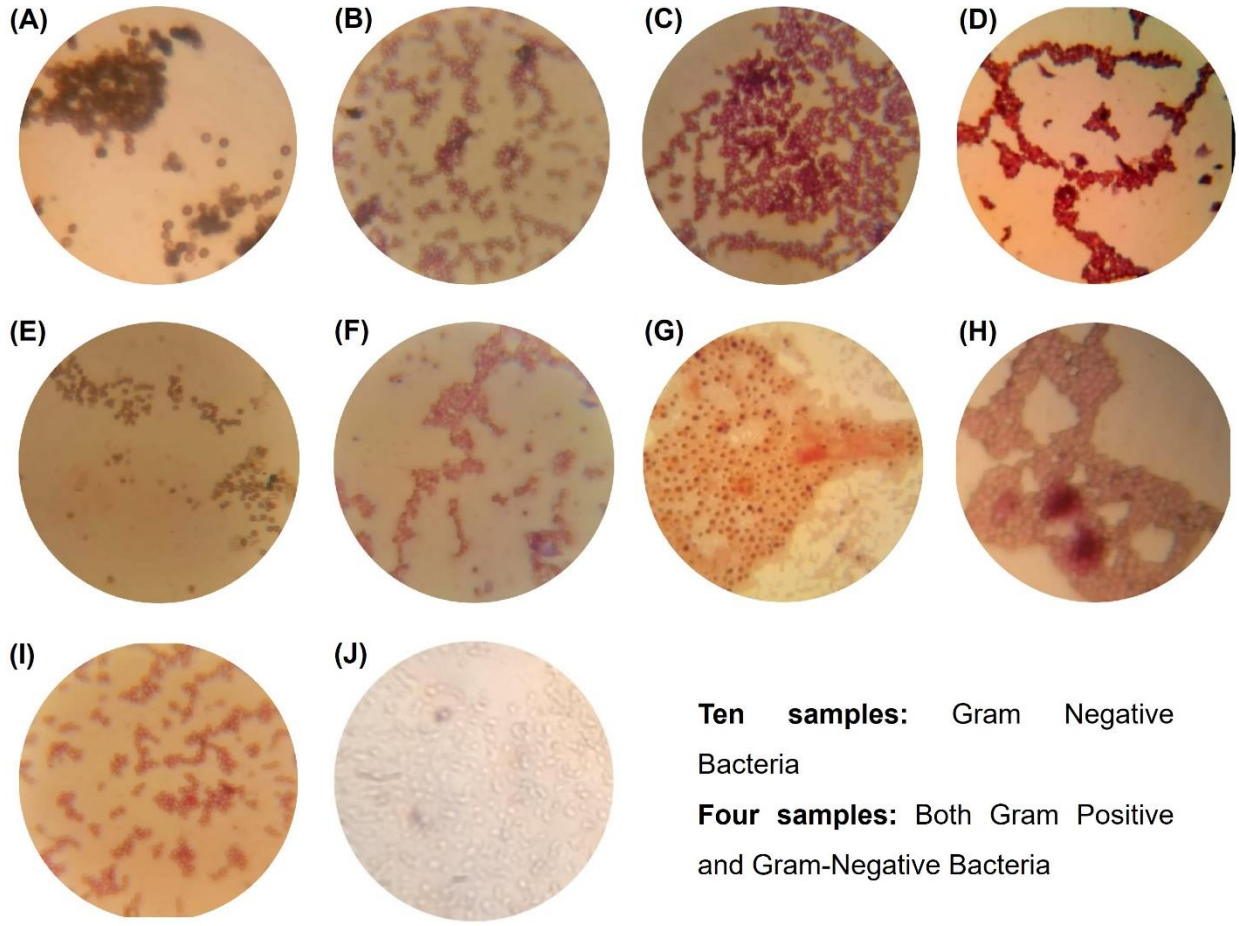

Figure 3: Identification and classification of bacteria in drinking water collected from the Government Girls Degree College Dargai Malakand and nearby places.

Bacterial growth was examined under a microscope at 100X magnification. **(A)** Tank-2 of GGD College Dargai (Gram positive +Gram negative Cocci in shape) **(B)** Tank-3 GGD College Dargai (Gram positive +Gram negative Cocci in shape) **(C)** GHS No-2 Dargai (Gram positive +Gram negative Cocci in shape) **(D)** Dargai bazar (Gram positive +Gram negative Cocci in shape) **(E)** Tank-1 GGD College Dargai (Gram negative and cocci in shape) **(F)** Filter-1 of GGD College Dargai (Gram negative and cocci in shape) **(G)** Filter-2 of GGD College Dargai (Gram negative) **(H)** Well water of Dargai bazar (Gram negative) **(I)** GGPS Sakhakot No-2 (Gram negative and cocci in shape) **(J)** Sakhakot Model School 2 (Gram negative, bacilli in shape).

3.3 Microbial frequency in the collected samples

The frequency of fungi and bacteria in the studied samples was also evaluated. A total of eleven water samples were collected from different areas of Dargai Malakand to assess fungal contamination in drinking water (Table 1). The results revealed that eight out of eleven samples (72.72%) were contaminated with fungi. Similarly, bacterial contamination was assessed using gram staining (Table 1). The gram-staining analysis showed that ten samples (90.90%) contained gram-negative bacteria, while four samples (36.36%) contained a mixed population of gram-positive and gram-negative bacteria.

Our study further categorized bacterial isolates based on their shape. Most water samples (81.81%) contained cocci-shaped bacteria, whereas only one sample (9.09%) contained bacilli (Table 2).

Table 1: Distribution of bacteria and fungi in drinking water collected from the Government Girls Degree College Dargai Malakand and nearby places. (+) reparents the presence of the microbe, whereas (–) represent the absence of the microbe.

Region	Tank1	Tank2	Tank3	Filter1	Filter2	Well water	GHS Dargai No-2	Dargai bazar	Dargai sorana	GGPS Sakhakot No-2	Sakhakot model school
Fungi	+	+	+	+	+	-	+	-	+	+	-
Gram positive	-	+	+	-	-	-	+	+	-	-	-
Gram negative	+	+	+	+	+	+	+	+	-	+	+

Table 2: Classification of identified bacteria from drinking water collected at the Government Girls Degree College Dargai Malakand and nearby places. (+) represents the presence of the microbe, while (-) represents its absence.

Bacteria	Tank1	Tank2	Tank3	Filter1	Filter2	Well water	GHS Dargai No-2	Dargai bazar	Dargai sorana	GGPS Sakhakot No-2	Sakhakot model school
Cocci	+	+	+	+	+	+	+	+	-	+	-
Bacilli	-	-	-	-	-	-	-	-	-	-	+
Spirila	-	-	-	-	-	-	-	-	-	-	-

4. DISCUSSION

In the current study, drinking water samples were analyzed for contamination from different areas of district Malakand tehsil Dargai, Khyber Pakhtunkhwa province, Pakistan. We found that the number of Gram-negative bacteria was high. Gram-negative bacteria are highly pathogenic and can cause many diseases, such as diarrhea, allergies, asthma and superficial infections of hair, skin and nails. The result revealed the presence of Fungi and Bacteria in drinking water, with the frequency of fungi at 72.72% and bacteria at 90.90%. Among the bacteria, 36.36% were gram-positive, and 90.90% were gram-negative. These results indicated the very high contamination of drinking water of Tehsil Dargai and high risk for the drinking water diseases. Drinking water contamination is also reported in other parts of Pakistan. Researchers have already been concluded studies on the Gram negative and Gram-positive bacteria in drinking water of different areas. For instance, Yousaf et al. (2014) conducted research in Karachi, Pakistan, where Gram negative bacteria were found to be higher (76%) than Gram positive (14%), with mixed bacteria present at 10% (Yousaf et al., 2014).

Ahmad et al. (2014) conducted a study on the drinking water quality of Peshawar, Khyber Pakhtunkhwa province, Pakistan. According to their findings, 16.67% of the samples were contaminated with Salmonella typhi, 26.67% with Pseudomonas, and 26.67% with *Shigella spp* (Ahmad et al., 2014). These differences may be due to better sanitary conditions in Peshawar, where people are more aware of the importance of personal hygiene as compared to Dargai, which is less developed. Qureshi and Aiman (2019) conducted research in district Swabi, Khyber Pakhtunkhwa province, Pakistan. The total number of positive samples of *salmonella spp* were 68.9% s. aureus were 88.34% and *p. aeruginosa* were 24.27% (Qureshi and Aiman, 2019). This indicates a very high level of contamination in the drinking water of Swabi, posing a high risk for disease. Additionally, there are notable differences in temperature between Swabi and Dargai.

Ahmed et al. (2020) studied the microbial quality of drinking water and waterborne diseases in primary school children in Sindh. Their study showed that across all schools, about 49% of the drinking water were contaminated with *E. coli*, 54% with *Salmonella spp*., 49% with *V.cholerae*, and 63% were contaminated with *shigella* spp (Ahmed et al., 2020). The water was highly contaminated with *E. coli*, a Gram-negative bacterium, indicating a as high risk of infections. Hussain et al. (2011) studied drinking water fungi and their pathogenic effects on human in District Bhimber Azad Kashmir, Pakistan. Their study identified 11

fungal species in well-water, 6 species in spring water, 6 species in hand pump water, and 7 species in tap water sample (Hussain et al., 2011). This study provides critical evidence of severe microbial contamination in drinking water in Tehsil Dargai, highlighting major health risks and the urgent need for improved water sanitation practices. The findings should serve as a call to action for policymakers, public health officials, and researchers to develop effective intervention strategies for ensuring safe drinking water in the region.

5. CONCLUSION

One of the serious effects of diarrheal disease in school children is illness, leading to a loss of school time. One study estimates that an average of 670,000 Pakistani children are unable to attend school daily due to water-related illness. Our study demonstrated that drinking water is highly contaminated with bacteria and fungi, posing a significant risk of infections and other health problems. This study found that 36.36% of bacterial isolates consisted of a mixed population of gram-positive and gram-negative bacteria, while gram-negative bacteria were predominant in 90.90% of the samples. Microscopic evaluation confirmed variations in fungal spores and bacterial morphology across different water sources. Notably, some samples contained green or black fungal spores, while others exhibited distinct bacterial shapes, with cocci being the most prevalent (81.81%) and bacilli present in only one sample (9.09%). The high level of microbial contamination in drinking water presents a serious public health concern. These results must be communicated to the concerned local government units and policymakers to serve as a basis for policy making and implementation.

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