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ZAM and TA designed; AF and SU performed experiments and analyzed data. MNK helped in data analysis. AF and SU wrote and revised the paper.

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*Correspondence

Shaista Urooj
Email:
shaistaurooj_map@yahoo.com

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Fecal Coliform Contamination of Drinking Water in Karachi, Pakistan

Aiman Fatima¹, Shaista Urooj^{1,2,3*}, Zulfiqar Ali Mirani³, Tanveer Abbas¹, Muhammad Naseem Khan³

¹Department of Microbiology, University of Karachi, Pakistan.

²Department of Life Sciences, Shaheed Zulfiqar Ali Bhutto Institute of Science and Technology, Karachi, Pakistan.

³Food and Marine Resources Research Centre, Pakistan Council of Scientific and Industrial Research Laboratories, Karachi, Pakistan.

Abstract:

Water is one of the most essential nutrients present in the world for the survival of mankind. But nowadays this nutrient is the source of many water-borne diseases that seriously affect human beings. Many pathogenic microorganisms contaminate the drinking water, washing water, and food processing water. Water-borne diseases like botulism, cholera, diarrhea, and typhoid, etc. can severely affect human health. In this study, different water samples were collected in 4 different groups based on seasons from June 2018 to August 2020. The water samples were processed for the detection of fecal coliforms. The results demonstrated that In group 1, among a total of 100 collected samples, 31% were fit (potable) whereas the unfit samples (non-potable) were 69%. In group 2, from 84 total samples, 38.09% were fit while 61.9% were unfit. In group 3, out of 135 water samples, the fit and unfit samples were 39.2% and 60.7% respectively. Whereas, in group 4, among 165 collected samples, the fit and unfit samples were 35.15% and 64.84% respectively. We observed that most of the water samples which were collected in the summer season were unfit for drinking purposes as compared to the winter season because the environmental temperature was high which lead to an increase in the number of microorganisms in drinking water indicating inappropriate processing and chlorination of drinking water. Therefore, an awareness campaign is necessary, and the contaminated sources should be replaced in order to provide safe drinking water to the people.



INTRODUCTION

Safe and clean drinking water is one of the basic rights and essential needs of all living beings (Verma, 2017). Bacterial contamination in drinking water results in serious health problems. Many enteric microorganisms can infect human beings. These enteric microorganisms are released from the feces of an infected person and indirectly or directly contaminate the water (Kostyla *et al.*, 2015). Water contamination with these pathogens lead to many water-borne diseases like gastroenteritis, dysentery, and diarrhea (Iqbal and Ashraf, 2020; Neupane *et al.*, 2018) and predominantly drinking water is considered as a primary source of transmission of diarrhea-causing pathogens (Chen *et al.*, 2017; Iqbal *et al.*, 2019).

In recent years, WHO strongly recommended using *Escherichia coli* as a fecal indicator in drinking water (Brooks *et al.*, 2020, Chen and Walker, 2012). Bacterial contamination in drinking water is not always due to fecal sources. There are many other causes such as damaging of water supply lines, improper storage of water, and sometimes testing methods and instruments that affect the quality of water. Poor sanitation and hygiene conditions are also responsible for bacterial contamination, temperature and climate change can also influence the transportation of microbiological agents to water (Mahato, 2019). Temperature affects the physical and chemical properties of water. Climate change has direct influence on the quality of water as well as indirect effects as the consequences of human activities. Microbiological analysis of water depends upon the detection of total coliforms, fecal coliforms, and *E.coli* from drinking water. Coliform bacteria are not always disease-causing, but their presence in drinking water indicates that water might be contaminated with disease-causing pathogens, fecal contamination in drinking water sources can lead to outbreaks of water-borne diseases (Mirzaei *et al.*, 2015).

Total coliforms refer to the group of bacteria found in the environment (soil, vegetation). Water can be contaminated by surface water,

animal or human waste. Fecal coliforms are the sub-group of total coliforms and their presence indicates that water is recently contaminated with fecal sources (Saxena *et al.*, 2015; Odonkor and Addo, 2018).

Karachi is the biggest city of Pakistan, where people consume water from various sources. However, the quality of water is not addressed as per the standards of public health. Therefore, it is imperative to assess the quality of drinking water to ensure that it is acceptable for human consumption. The objective of the research work was to detect the presence of fecal coliforms in drinking water samples with respect to different seasons.

MATERIALS AND METHODS

Collection of Samples

This study was conducted from 2018 to 2020. In this city-based cross-sectional study, a total of 484 samples were collected from drinking water sources. These water samples were processed and collected in different seasons. Summer, winter groups, and the rest two groups were collected in mid of these seasons. The purpose was to explore water quality throughout the year. Samples were aseptically collected from each sampling site in sterile plastic bottles (vol. 1000ml) and transported in icebox within 6 h of collection. For the chlorinated water samples, about 2.5 ml of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) (10% w/v, Merck-Darmstadt, Germany) was added into each sampling bottle to prevent the chlorination process during transportation.

Bacteriological Analysis of Water Samples

Qualitative analysis

Water samples were processed as described by ISO-9308, Part 1 & 2 (1998). Briefly, three tubes of double strength MacConkey's broth (OXOID-Ltd, Basingstoke-UK) with Durham tubes were inoculated with 10 ml water sample (in each tube) and two sets of three tubes of single strength with 1.0 ml and 0.1 ml respectively and incubated at 35°C for 48 h. The production of

acid and the presence of gas in any of the Durham tubes indicate the growth of coliform in water samples. The number of the positive tubes was recorded, and the most probable number (MPN) was calculated according to MPN tables. Samples were considered “fit” that contain <1 CFU/100ml of coliform where those considered “unfit” contain more than 1 CFU/100ml of coliform.

Confirmatory test for coliform and fecal coliforms

One ml from each positive tube of presumptive coliforms and fecal coliforms was inoculated in Brilliant Green Lactose Bile Broth (BGLB) (OXOID-Ltd, Basingstoke-UK) tube and EC (OXOID-Ltd, Basingstoke-UK) tubes and incubated at 35°C and 44.5°C respectively in a water bath for 24 hours. Tubes with gas and turbidity were considered positive. BGLB indicates the presence of coliforms and EC tubes indicate the presence of thermotolerant fecal coliforms (ISO-9308, 1998).

RESULTS

A total of 484 samples were collected from different sources from June 2018 to August 2020. These water samples are distributed in 4 different groups.

In group 1 (summer group), a total of 100 samples were collected from May 2018 to September 2018, the ratio of fit to unfit samples was 31% and 69% respectively. In group 2 (autumn + summer), 84 samples were collected from April 2019 to October 2019. In these samples, the ratio of fit to unfit samples was 38.09% and 61.9% respectively. In group 3 (winter), 135 samples were collected from November 2019 to February 2020 and the ratio of fit to unfit samples was 39.2% and 60.7% respectively. Whereas in group 4 (spring + summer), 165 samples were collected from April 2018 to August 2018 and the ratio of fit to unfit water was 35.15% and 64.84% respectively (Table 1).

Table 1. The distribution of fit and unfit samples in defined groups.

Group	No of Samples	% of fit samples	% of unfit samples
1	100	31.00%	69%
2	84	38.09%	61.9%
3	135	39.2%	60.7%
4	165	35.15%	64.84%

DISCUSSION

The results obtained in the present study are highly alarming that need immediate action and follows ups as they show very high bacterial load in all categories, especially the percentage of unfit samples was more as compared to fit samples in summer than winter. In the 4th group, the percentage of unfit samples was more as compared to all other groups because in this season heavy rainfall occurred and coliforms may enter into the water supply chains due to floods and improper management. Heavy rainfall if not properly managed adversely affects the quality of water. Urooj et al. (2018) observed that in the wet season the concentration of coliforms was greater as compared to the dry season. An

increase in temperature also affects the quality of water by decreasing dissolved oxygen and increasing microorganisms (Urooj *et al.*, 2018).

Heterotrophic plate count (HPC) is a widely used method for the critical assessment of the quality of drinking water (Reasoner, 2004). HPC is not an acceptable index for the presence of pathogens but its high values indicate inefficacious water treatment or faults in the distribution system or maybe the presence of biofilms (Derlon *et al.*, 2014). HPC is also indicative of a variety of micro-flora or may indicate the presence of pathogenic, facultative, or opportunistic organisms. Heterotrophs that may include bacteria, yeast, and mold play a dominating role in water discoloration, changes

in water taste and odor, corrosion, scale formation, and even pipe blockage (Allen *et al.*, 2004; Pecl *et al.*, 2017).

World Health Organization (WHO) and National Standards for Drinking Water Quality (NSDWQ), state that coliform, fecal coliform, such as *E. coli*, as thermo-tolerant organisms should be undetectable in potable water (Ahmed *et al.*, 2019). These indicator organisms are used to define the certainty of drinking water quality and to measure the degree of pollution in drinking water because testing of all pathogens is difficult and time-consuming process (Mahmud *et al.*, 2019). However, it is evident from the conducted research that coliform and fecal coliforms were detected in most of the water samples in their respective groups. As it was observed that most of the samples are unfit for drinking purposes. The condition is the same as the researchers found the contamination of drinking water in other parts of the Sindh (Khan *et al.*, 2017; Ahmed *et al.*, 2020; Abia *et al.*, 2015). The presence of fecal coliform organisms made the water unfit for drinking (Saleem *et al.*, 2020). The contaminated water is responsible for contaminated food products resulting in waterborne diseases (Ashraf *et al.*, 2019; Iqbal *et al.*, 2015,16). If this unfit water is consumed by any other means then this can lead to severe consequences for health.

There should be proper measurements taken to improve the quality of water. The most important step is the proper chlorination of the water not only in the collection centers but also at the distribution points. Natural plant extracts are used for the treatment of water to improve the physicochemical and microbiological quality of water (Ashraf and Iqbal, 2021; Ibiene *et al.*, 2021). There is a need to enforce extensive testing of water quality and provide data regarding water quality and deploy effective water treatment strategies for the removal of heavy metals from water sources. (Iqbal and Ashraf, 2018; Ashraf and Iqbal, 2020).

RECOMMENDATIONS

- Professional assessments should be employed for best cleaning, filtrations, and disinfection for the water treatment plants before the distribution of water in public networks, and rigorous inspection and follow-up are needed for water testing and future water contamination.
- Seasonal assessments should be carried out because it is proven by research that warm and humid environment results in high microbial growth.
- Rapid advance methods to analyze microbial and environmental parameters such as 16S DNA-based sequencing (root analysis of bacterial community), flow cytometry (targeting abundance of the bacterial population) should be adopted.
- The development of a highly precise, cost-effective kit for the rapid screening of water pathogens is of paramount importance.
- People should adopt good hygiene and sanitation practices i.e. WASH practices should be made common among the people.
- Water network pipelines should be changed if they are rusty and old. The network of drinking water supplies and sewage pipelines should have a proper distance so that due to breakage this water will not intermix.
- Proper wastewater treatment plants should be installed in industries before discharging into the water bodies.
- Installation of filtration plants on pumping stations of every town is of great importance to double-check the quality of municipality-provided water.
- Comprehensive public awareness programs and campaigns should be launched through the media. Educational institutions, hospitals, or even masjids can play their best role for this purpose. Awareness at the domestic level should be spread such as

consumption of properly boiled water reduces the risk of waterborne disease.

- Gutters should be positioned correctly and maintenance work of pipelines and water distributions systems should be assigned to qualified and experienced professionals.
- Cost-effective installation of household water treatment systems should be adopted.
- Waste material should be incinerated but not dispose of in water bodies.
- Hand washing practices with soap should be initiated and Hygiene training programs should be incorporated into the school curriculum.
- Government should promote and patronize research work for the development of efficient and modern water purification systems, indigenously.
- National water and wastewater policies should be implemented strictly and should be revised comprehensively every five years.
- Annual budget allocations to overcome this problem should be the priority of the Government without any political interference into this profession.

CONCLUSION

This research work indicates insightful results on the overall microbiological quality of various drinking water sources in different seasonal groups in Karachi, the provincial capital city of Sindh. The findings of this research study suggest that various samples were unsatisfactory for human consumption concerning WHO and NSDWQ guidelines. Therefore, water should be treated properly before it reaches consumers. There is a dire need for a coherent and comprehensive policy framework and proper allocation of budgets on the current problem especially on the infrastructure of the slum areas of Karachi. The role of federal and local governments, strategic

and developmental partners, private sector, policymakers, and researchers is of prime importance, so they should understand the current scenario of water and sanitation problems keeping in view different seasons and should support the increase in the share of water and sanitation total expenditure on national GDP level.

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CONFLICT OF INTEREST

There is no conflict of interest.

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