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Impact of Seasonal Variations on Bacterial, Yeast and Mold's Count in Drinking Water Collected from Karachi Pakistan

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Abstract
The present study was conducted to assess the microbiological quality of drinking water collected from different areas of Karachi, Pakistan, during 2014 to 2015. A total of 320 samples were analyzed; of those 120 were bottled water and 200 samples were collected from the municipal water supply (Tap water). Most probable number (MPN) technique in MacConkey's broth was used for the analysis of coliforms and fecal-coliforms and membrane filtration technique on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) was used for yeast and mold analysis. All of the bottle samples were free of coliforms and fecal-coliforms but tap drinking water samples were highly contaminated with total bacterial load, coliforms and fecal coliforms. It has been observed that bacterial as well as yeast and mold's count was high in all of the water samples during summer. To overcome high risk of water-borne diseases, provision of improving water quality and adaptation of wash practices considered to be a part of monitoring framework system and a strict code of conduct should be implemented.
Keywords: Yeast & Mold, Coliform, Fecal-coliform, Tap water, Bottled water.

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INTRODUCTION

Water is essential for humans and other life-forms (Popkin *et al.,* 2010). Each person on the earth requires at least 20 to 50 liters of clean water per day for routine activities e.g. cooking and washing (Gleick, 2009). Unfortunately, the majority of world population does not have access to clean and safe water (Agrawal *et al.,* 2010). Water bodies can be contaminated through landfills and septic systems, careless disposal of hazardous medical waste, household products, leakage or mixing with sewage line water or underground storage tanks, agricultural chemicals, disposed or excreted antibiotics, dyes, heavy metals (like iron, lead, arsenic, mercury, cadmium, chromium, nickel, zinc, cobalt, vanadium and copper (WHO, 2003; Yasin *et al*., 2015).

According to WHO and UNICEF in 2015, 663 million people lack safe potable water sources and 2.5 billion lack prerequisite sanitation facilities. 7% of total disease burden due to lack of WASH (Water, Sanitation and Hygiene) facilities leads to 19% children mortality across the globe (Prüss-Üstün *et al .,* 2008; Cairncross *et al.,* 2010). Consequently, about 1.8 million people die every year of diarrheal diseases. Out of these, more that 50% are microbial intestinal infections, with Cholera standing out in the first place. Categorically, waterborne infections are caused by bacteria, viruses, and parasites (protozoans and helminths) (Girones *et al*., 2010). For the assessment of quality of potable water, culture-dependent enumeration or detection, fecal indicator bacteria e.g. total coliforms, *Escherichia coli,* or *Enterococci* have been used as a gold standard. The presence of these bacteria in the water indicates the risk of pathogens or toxins that may cause waterborne illnesses (Tan *et al.,* 2015). According to Europe and Canadian guidelines maximum limits of coliforms and fecal-coliforms in surface water must be <200 and <100 cfu/100ml (Rice *et al.,* 2012). According to World Health Organization (WHO) standards all water intended for drinking and Treated water entering the distribution system must be free of coliform and fecal-coliform bacteria and the standard limits are <1cfu/100ml. Moreover, it is very important to be aware of the complete spectrum of pathogens that are potential contaminant of our water system. Fungi e.g. yeast and mold, is one of such pathogens that may also grow and survive in water system and pose serious health risk particularly in immuno-compromised or elderly persons (Saati and Faidah, 2013). Swedish regulatory authority suggested the limit for the occurrence of fungi in water is 100 cfu/ 100 ml water (Hageskal *et al.*, 2009). This study was conducted to determine the impact of yeast, mold and bacteria on drinking water quality of Karachi in summer and winter seasons.

MATERIALS AND METHODS

Water Samples

A total of 320 samples were collected from different areas of Karachi–Pakistan during 2014 – 2015. These drinking water samples were collected in sterilized plastic bottles with sodium thiosulfate (10% w/v, Merck) and transported to the laboratory in icebox with icepack. Analyses were carried out within 6h of sampling.

Enumeration of Coliforms and Fecal-coliforms:

Samples were analyzed for total coliforms (TC) using a multiple-tube technique based on lactose fermentation with production of acid and gas within 48 h in MacConkey's Broth (Oxoid-Basingstoke Hampshire-England). The water sample showing lactose fermentation with gas production was considered presumptively positive for coliforms and fecal-coliforms. For confirmation of coliforms, the samples were sub-cultured into brilliant green lactose bile (BGLB) broth (Oxoid) tubes and incubated at 35°C for 48 h; EC broth (Oxoid- Basingstoke Hampshire-England) was used for the confirmation of fecal coliforms (FC) with incubation at 44°C for 48 h. Bacterial growth in BGLB broth and EC broth (Oxoid- Basingstoke Hampshire-England) indicates the presence of coliforms and fecal coliforms. The growth in BGLB with negative EC broth indicates the presence of coliforms only (Cabral, 2010).

Yeast and Mold Count:

Membrane filter technique was employed for yeast and mold count. A volume of 100 ml of the samples was filtered through membranes of 0.45 µm pores (Millipore, Massachusettes, USA). The membranes were placed on Dichloran Rose Bengal Chloramphenicol (DRBC) Agar (Oxoid Basingstoke Hampshire-England). The plates were incubated at 25°C for 5 to 7 days (Samah *et al.,* 2014).

Total Bacterial Count:

Pour-plate method was used to estimate the number of heterotrophic bacteria in plate count agar (Oxoid, Hampshire, England), at 35°C for 48 h, after incubation colonies were counted as CFU (colonies forming per unit) per ml of the water sample (APHA, 1988).

RESULTS

The present study demonstrated the microbiological quality of drinking water collected from different areas of Karachi City of Sindh-Pakistan, during 2014 to 2015. During this period, a total of 320 samples were analyzed; of those 120 were bottled water and 200 samples were collected from municipal water supply (Tap water). All of the bottled water samples were free of coliforms and fecal-coliforms. Contrary to this, 48% of tap water showed the presence of coliforms and 20.5% samples carried fecal-coliforms (Figure 1). Total bacterial count results showed

that 24% of bottled water samples carry >100 cfu/ml and only 3.3% bottle samples have bacterial load >1000 cfu/ml. In case of tap water samples, 95.5% showed >100 cuf/ml total bacterial count, of those 66.2% samples showed >1000 cfu/ml total bacterial count. The yeast and mold count results indicated that 12.5% of bottled water carry >100 cfu/ml yeast and 22.5% samples showed >100 cfu/ml of mold count. On the other hand, 71% of tap water samples were loaded with >100cfu/ml of yeasts; of those 42.5% samples contained yeast count of >1000 cfu/ml. Similarly, mold count of tap water was also high 86.5% showed >100 cfu/ml; of those 43.7% samples had mold counts of >1000 cfu/ml. Moreover, the water samples with high bacterial load were also loaded with high number of yeasts and molds. Only, 3 (1.5%) of tap water samples showed <100cfu/ml of bacterial count while yeast count was >1000cfu/ml (Figure 1).

The present study was divided into two parts. The part one of the study was conducted during the period of November through March i.e. winter where the prevailing temperature was low. During this period, the bacterial as well as fungal count was low in all the water samples. The second part of the study was conducted during the period of April through October, when warmer temperatures prevail i.e. summer. In this period, most of the samples showed higher loads of bacteria as well as yeasts and mold. In winter, all of the bottled water samples were yeast free while in summer, 21.1% samples showed >100 cfu/ml of yeast count. Molds also showed variations in their total count (12% and 21% of the samples) in winter and summer, respectively. Variation in total count continues in case of bacteria as shown in Table 1. In summer, it seems that all samples of tap water were microbes-loaded as 53.3% of the samples were found to be positive for coliform bacteria and 24.1% for fecal-coliforms, as well. However, the occurrence of coliform and fecal-coliform was 40% and 13.7%, respectively, in tap water samples, during winter. The total bacterial count was also high during summer and 42.5% of tap water samples showed >1000 cfu/ml of total bacterial count. This rate was reduced to 23.7% in winter season in comparison to hot season (Table 2). Similarly, yeast and mold count was also high in hot season (Figure 2).

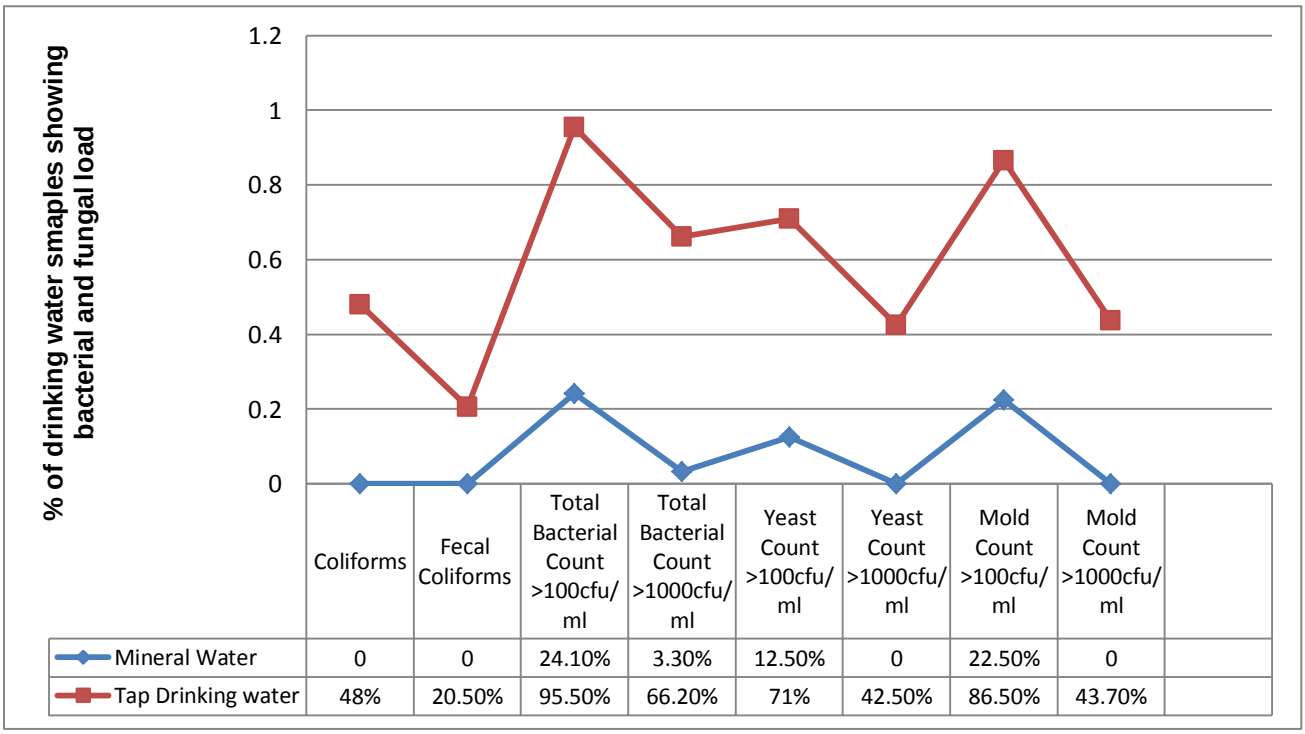

Fig. 1. Comparison of the Total bacterial load, Coliforms, Fecal – Coliforms, Yeast and mold count of tap drinking water and mineral bottled drinking water samples

Table 1. Bacterial and fungal count in bottled mineral water (n=120)

Total count of Microorganisms	November to March (n=50) (Winter)		April to October (n=70) (Summer)	
	Positive Samples	%age of Positive Samples	Positive Samples	%age of Positive Samples
Coliforms	0	0	0	0
Fecal-coliforms	0	0	0	0
Yeast >100cfu/ml	0	0	15	21.1
Mold >100cfu/ml	6	12	21	30
Total Bacterial Count >100cfu/ml	11	22	19	27
Yeast >1000cfu/ml	0	0	0	0
Mold >1000cfu/ml	0	0	0	0
Total Bacterial Count >1000cfu/ml	2	4	4	5.7

Table 2. Bacterial and fungal total count in tap drinking water (n=200).

Total count of Microorganisms	November to March (n=80) (Winter)		April to October (n=120) (Summer)	
	Positive Samples	%age of Positive Samples	Positive Samples	%age of Positive Samples
Coliforms	32	40	64	53.3
Fecal-coliforms	11	13.7	29	24.1
Yeast >100cfu/ml	51	63.7	91	75.8
Mold >100cfu/ml	66	82.5	107	89.1
Total Bacterial Count >100cfu/ml	73	91.2	118	98.3
Yeast >1000cfu/ml	12	15	33	27.5
Mold >1000cfu/ml	09	11.2	39	32.5
Total Bacterial Count >1000cfu/ml	19	23.7	51	42.5

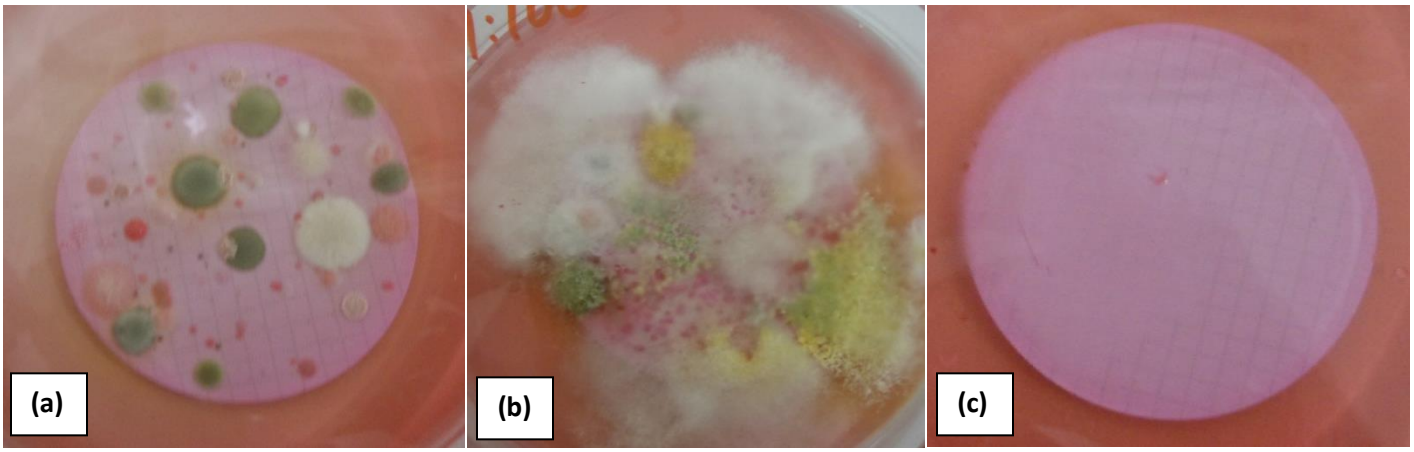

Fig. 2. High load of yeast and mold in drinking water sample, (a): Yeast count >1000 cfu/ml in tap drinking water (b): Yeast count >100 cfu/ml in mineral bottled water and (c): Control

DISCUSSION

In Pakistan, contaminated water is one of the biggest sources in the spread of infectious diseases, which costs 1.3 billion dollars every year. Worldwide out-breaks of water-borne disease are common, but incidence of serious threats is more common in developing countries. According to a recent report, waterborne infections are responsible for about 250,000 deaths of children under age of 5 years (Gillani *et al.,* 2005). The majority of waterborne illnesses are due to failure to control, treat or prevent the entry of infectious agents in water storage or distribution system (Tan *et al*., 2015). The use of contaminated water may contaminate the fruit juices (Iqbal *et al.,* 2012; 2015; 2016).

Waterborne infections are caused by a wide range of microorganisms e.g. viruses, bacteria, protozoa, parasites and fungi (Heinrich *et al.,* 2017; Girones *et al.* 2010). In case of drinking water, total coliforms, fecal-coliforms and *E. coli* are used as indicators to measure the degree of pollution and sanitary quality of drinking water, because testing for all known pathogens is a complicated and expensive process. According to WHO and others e.g. European Union, United States, Canada, United Kingdom and Australian guidelines coliforms and fecal-coliforms including *E. coli* must be absent or <1cfu/100ml in water used for drinking purposes (Tallon *et al.*, 2005). It has been reported that fungi i.e. yeast and mold may cause severe infections in patients with immune deficiency, due to e.g. HIV/AIDS, chemotherapy, immunosuppressive therapy following transplants, or other underlying health conditions, such as cystic fibrosis or diabetes mellitus. Yeast and mold may also be responsible for hypersensitivity reactions e.g. asthma in healthy individuals (Richardson & Lass-Flörl, 2008).

According to WHO criteria the counts of bacteria (coliform, fecal-coliform and total bacterial count) as well as yeast and mold in the present study were high in drinking water during summer season. As majority of tap water samples (53.3%) collected from Karachi during summer were declared not fit for drinking purposes because of the presence of high load of coliforms. In summer season these samples were loaded with heterotrophic bacteria >100cfu/ml .This might be due to high prevailing temperatures or high water demand and consumption during this period as compared to winter season. However, sometimes absence or presence of bacterial indicators may not depict the proper picture of waterborne infections. On the other hand, 40% tap drinking water samples analyzed in winter season were found to be unfit in winter season. In the case of bottled mineral drinking water coliforms and fecal coliforms were zero, which is a good indicator and very less samples were unacceptable in terms of total bacterial count, in both summer and winter season.

Assessment of seasonal variations of the drinking water plays a crucial role to maintain the quality as well as important aspect of water pollution to check the natural and anthropogenic changes (Ojok *et al*., 2017). The fungi including yeast and mold require 5 to 7 days to produce visible growth on solid media, it's important to propose standardized and authentic guidelines for the count of fungal entities in drinking water because no criteria is present for the analysis of fungal pathogens in drinking water worldwide. During the present study, it has been noticed that yeast and mold has major impact on the quality of drinking water. Yeast and mold were found not only in the tap water samples but also in bottled water. Moreover, the counts of bacteria as well as yeast and mold in the present study were high in drinking water during summer season. In Pakistan, majority of hospitalized as well as out-patients prefer to use bottled water. Therefore, presence of Yeast and Mold in bottle water should be considered as a serious threat to consumers. Moreover, yeast and mold may support waterborne bacteria in establishment of biofilm in water distribution system (Siqueira *et al.,* 2011). Therefore, necessary measures should be taken to avoid fungal growth as well as bacterial growth in water to make it safer for public health.

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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