

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Evaluation of Bacteriological and Sanitary Quality of Drinking Water Stations and Water Tankers in Makkah Al-Mokarama

Alaa A. Mihdhdhir

Department of Biology (Applied Microbiology), Faculty of Applied Science,
Umm Al-Qra University, Makka Al-Mukarramah, Saudi Arabia

Abstract: The present study was conducted to evaluate the bacteriological and sanitary quality of drinking water produced in Makkah Al-Mokaramah during the high season in the month of Ramadan. Water samples were collected both from the drinking water stations and the water tankers (in Arabic language called whitats) used to transport and distribute water in different places in the Holy city. Water samples were analyzed to determine the densities of HPC at 22 and 37°C, total coliforms, *E. coli* and *S. aureus*. The bacteriological analysis of drinking water samples at 37°C proved that 6.7-33.3, 20-46.7, 0-20 and 0-6.7% of total water samples contained HPC, total coliforms, *E. coli* and *S. aureus*, respectively which were higher than the safe limits for drinking water. The bacterial analysis of drinking water varied from one water station to another. On the other hand, drinking water transported by tankers appeared to be in the lowest category of water quality. Because out of total water samples 40-59%, 60-68.8%, 31.2-37.5%, 10-25% contained HPC, total coliforms, *E. coli* and *S. aureus*, respectively, which were higher than the established safe limits of drinking water. One possible reason for poor quality of drinking water could be attributed to the application of inadequate water disinfection treatments and also the absence of sanitary aspects as supported by the bacteriological analysis which holds true especially for water supplied by tankers. In conclusion, it is important to apply proper water disinfection measures and provide sanitary monitoring programs during the production of drinking water as a whole and for the water tanker in particular.

Key words: Water quality, sanitation, bacteriology, total coliform, *E. coli*, *S. aureus*, disinfection, water tankers

INTRODUCTION

Drinking water quality is important for the safety of consumers. The pathogenic contamination of drinking water poses significant health risks to consumers especially to human beings. On a global scale, there have been countless numbers of disease outbreaks and poisoning around the world resulting from the consumption of untreated or poorly treated drinking water (Moyer, 1987; Hurst, 1997; Moor *et al.*, 2001; Shaw *et al.*, 2005; Fong *et al.*, 2007). Levy *et al.* (1998) stated that untreated and poorly treated drinking water was responsible for 50% of infection by the water borne-diseases. Moore *et al.* (2001) found significant correlation between drinking water contaminated with campylobacter and the distribution of gastroenteritis among population in Northern Ireland. Surveillance for water borne-disease outbreaks in United States (Lee *et al.*, 2002) showed that a total of 39 outbreaks associated with drinking water caused illness among 2068 persons in 25 states of America. They further stated that more than 50% of these outbreaks were associated with microbial pathogens as etiological agents.

The quality of private water supplies within Aberdeen shire in UK was analyzed to determine total coliform and faecal coliforms. Out of 1750 water samples, 41 and 30% were contaminated with total coliform and faecal coliforms, respectively (Reid *et al.*, 2003). A study was carried out by Vollaard *et al.* (2005) in Jakarta, Indonesia to assess the water supply, water quality and human waste disposal and their association with diarrheal illness. They isolated faecal coliforms in 56% of the samples. Also, diarrheal episodes, reported in one third of householders, were significantly associated with water contamination with >100 faecal coliforms per 100 mL. Recently, microbiological quality of groundwater wells located in Ohio (USA) was examined for total bacteria, faecal indicators, enteric viruses and protozoa. All the wells were positive both for the total coliform and faecal coliform (*E. coli*), but 30% of wells were positive for enterococci and arcobacter (an emerging bacterial pathogen) and 25% of wells were positive for all microbiological tested (Fong *et al.*, 2007).

Adequate supply of good quality drinking water is an important issue in the Holy city of Makkah Al-Mokarramah which receives large numbers of pilgrims

during the Holy months of Ramadan and Haj season. Among the various problems faced for supplying good quality water, microbial contamination is considered as one of the most dangerous causes of water born diseases especially those which are characterized by quick spreading at high rates in the population. As such, the demand for good quality drinking water increases manifold as compared to the normal days and leads to enhance production of drinking water. Consequently, it increases the wastewater production and creates its disposal problem thus resulting in sanitary conditions conducive to public health and water born diseases. There is every possibility that poor sanitary conditions might develop due to high rate of drinking water production by small units, spillage from water tankers (usually called as whitats) used to supply drinking water to different areas and improper disposal of drainage water. The poor drinking water quality can be associated also to the poor storage facilities of drinking water and the conditions of the water supply tankers.

The main objective of the present study was to evaluate bacteriological and sanitary quality of drinking water produced at different water stations which is distributed through water supply network and transported by water tankers (whitatt), during the Holy month of Ramadan every year.

MATERIALS AND METHODS

Water sampling: Five drinking water stations representing different districts of Makkah Al-Mokkaramah and three water tankers (whitatt) used for transportation and distribution of drinking water to houses and establishments were selected. The bacteriological and sanitary evaluation was carried out during the month of Ramadan, 1427 H. Eight to sixteen drinking water samples (each 2 L volume) were collected in sterilized containers. All the water samples were transferred directly to the analytical laboratory for bacteriological analysis instantly or at least within a maximum period of two hours after sampling.

Bacteriological analysis: The bacteriological analysis and other water quality parameters were determined according to the standard methods of American Public Health Association (APHA, 1998) for the examination of different types of waters.

Determination of Hetero Plate Count (HPC): Hetero plate count of all drinking water samples was determined using dilution plate method technique and standard plate count agar medium. Decimal dilution was done and three replicates (1 mL each) were used from each dilution. Plates

were incubated for 48 h at 22 and 37°C. Suitable dilutions were selected and average colony counts were calculated and expressed as (cfu mL⁻¹).

Determination of total coliforms: Membrane filtration technique (which is quick, accurate and highly reproducible), was used for drinking water samples. One hundred drinking water samples were filtered through 0.45 µm, diameter 47 mm cellulose nitrate membrane filters. After filtration, the filter was removed from the filter base using sterile forceps and placed carefully on Eosin Methylene Blue (EMB) agar culture medium. After incubation for 24 h at 37°C, characterized and verified coliform colonies were enumerated and expressed as total coliform/100 mL of drinking water samples.

Detection of faecal coliforms (*Escherichia coli*): The faecal coliforms are part of the total coliform group. The major species in the faecal coliform group is *E. coli*, a specie indicative of faecal pollution and the possible presence of enteric pathogens. Typical characterized faecal coliform colonies small colonies with metallic sheen characteristic were picked from total coliforms EMB agar plates and subjected to verification procedures according to Maria *et al.* (1999). Cultures that produced gas in EC broth tubes at 44.5°C and followed by subsequent positive reaction in Indol test were considered as faecal coliforms (*E. coli*).

Detection of *Staphylococcus aureus*: The membrane filter technique was employed for enumerating total Staphylococci and *S. aureus* after verification using known confirmation tests. Membrane filter technique was applied using 100 mL drinking water sample by following the above mentioned procedure except by using Baird-Parker agar as a selective medium for enumerating members of genus *Staphylococcus*. After incubating the Baird-Parker medium plates containing filter for 25-48 h at 37°C, circular bright gray to black colonies were picked, purified and sub-cultured on nutrient agar. Growth cultures were subjected to Gram stain and catalase test. Gram-positive cocci in clusters and catalase positive were enumerated and expressed as total Staphylococci/100 mL. For the detection of *S. aureus*, verified *Staphylococcus* cultures were again subjected to two confirmed tests i.e., mannitol fermentation under aerobic and anaerobic conditions and the coagulase test (Jawetz *et al.*, 1991). Gram-positive cocci in clusters, positive reaction in mannitol fermentation, catalase and coagulase tests were considered as *S. aureus*.

RESULTS AND DISCUSSION

Bacteriological and sanitary quality of drinking water in Makkah Al-Mokarama: Evaluation of sanitary quality of drinking water in Makkah Al-Mokarama was carried out through bacteriological analysis of drinking water samples collected from five water stations and three water tankers (whitat). All the water samples were analyzed to determine the densities of Heterotrophic Plate Count (HPC) at 22 and 37°C, total coliforms at 37°C and *E. coli* as indicators of faecal pollution, while the total Staphylococci and *S. aureus* determined as an indicator of hygienic conditions.

Heterotrophic Plate Count (HPC): Heterotrophic plate count determined at 22 and 37°C is considered as a direct quantitative measurement of the viable aerobic and facultative anaerobic bacteria (Table 1). The values of HPC at 22 and 37°C revealed that out of total water samples, 53.3-63.6 and 6.7-33.3%, respectively were above the established standards (MCL of HPC allowable are 100 cfu/1 mL at 22°C and cfu/1 mL at 37°C according to EEC (1998). On the other hand, high percentage of drinking water samples from tankers (whitat) were also above the same standard since the values of HPC were 70-75% and 40-50% at 22 and 37°C, respectively of total water samples (Table 2). In fact the levels of bacteria determined by HPC is considered a key value for assessing the efficiency of water treatment processes which provide an estimate of the general quality of drinking water which seems to be poor. In spite of that, such bacteria determined by HPC have been implicated as potential pathogens in drinking water (Lye and Dufour, 1991).

Total coliform group: The density of total coliform group is a major criterion of the degree of pollution and the sanitary quality of drinking water. The coliform group includes bacteria genera of faecal and non-faecal origin. According to World Health Organization (WHO, 1996), the standard limits for total coliform numbers should be zero/100 mL in drinking water. According to this criterion, 20-46.7% of total drinking water samples from water station distributed in Makkah Al-Mokarama were above the established standards (Table 1). In comparison to this, 60-68.8% of total drinking water samples from water tankers (whitat) were also above the established standard (WHO, 1996). Primarily, these results indicate that application of proper sanitary conditions is inadequate during the production of drinking water in all water stations. These findings agree with of Maria *et al.* (1999) who reported the presence of various degrees of pollution in tankers used for transportation and distribution of drinking water to different houses and establishments.

Faecal coliform bacteria (*E. coli*): The faecal coliform bacteria are a part of the total coliform group and is the major specie of faecal contamination (Pam *et al.*, 2005), because it originates primarily in the mammalian intestine. Therefore, the detection of *E. coli* is considered as the most specific recommended test for evaluating the drinking water quality from sanitary point of view. According to standard limits of EEC (1998) and WHO (1996), drinking water should be free from *E. coli* in 100 mL sample i.e., zero/100 mL in drinking water. Based on this criterion, drinking water samples from four out of five water stations were positive for the presence of *E. coli* and ranged from 5.7-20% of the total samples expect in sample No. 4 (Table 1). In comparison, data in

Table 1: Bacteriological analysis of drinking water samples from different water stations in Makkah Al-Mokarama

| Water station No. | Total samples | Ratio of water samples above the established standards * | | | | |
|-------------------|---------------|--|------|-----------|----------------|------------------|
| | | HPC** | | Coliforms | <i>E. coli</i> | <i>S. aureus</i> |
| 22°C | 37°C | | | | | |
| 1 | 15 | 60.0 | 33.3 | 46.7 | 20.0 | 6.7 |
| 2 | 14 | 63.6 | 14.3 | 28.6 | 5.7 | 5.7 |
| 3 | 15 | 60.0 | 26.7 | 40.0 | 13.3 | 6.7 |
| 4 | 15 | 53.3 | 6.7 | 20.0 | 0.0 | 0.0 |
| 5 | 16 | 62.5 | 18.7 | 31.2 | 12.5 | 0.0 |

Table 2: Bacterial analysis of drinking water samples from different water tankers (Whitat) in Makkah Al-Mokarama

| Water station No. | Total samples | Ratio of water samples above the established standards * | | | | |
|-------------------|---------------|--|----|-----------|----------------|------------------|
| | | HPC** | | Coliforms | <i>E. coli</i> | <i>S. aureus</i> |
| 22°C | 37°C | | | | | |
| 1 | 16 | 75 | 50 | 68.8 | 31.2 | 18.7 |
| 2 | 10 | 70 | 40 | 60.0 | 30.0 | 10.0 |
| 3 | 8 | 75 | 50 | 60.0 | 37.5 | 25.0 |

*According to World Health Organization (WHO) (1996). **According to Economic Community (EEC) (1998)

Table 2 reveal that high portion of drinking water samples from all the three water tankers were positive for *E. coli* (30-37.5% of total samples). The presence of *E. coli* in drinking water samples from water stations and in particular from water tankers clearly advocates shortage of sanitary conditions and the water treatment processes. One water sample showed decreases in the total count of coliforms group during the third and 4th week of Holy month of Ramadan. This decrease may be due to high level of chlorine (0.30 mg L⁻¹) according to standard limits of EEC (1998) and WHO (1996) where the maximum limit of chlorine is 0.20 mg L⁻¹. There was no growth in low concentration in the samples due to high level of chlorine while it was in high concentration in the absence of chlorine. Actually the presence of *E. coli* in drinking water indicates faecal pollution resulting in the presence of enteric pathogens and related to other public health problems. These results agree with the findings of Lee *et al.* (2002), Reid *et al.* (2003), Volloard *et al.* (2005) and Fong *et al.* (2007).

***Staphylococcus aureus*:** Members of the genus *Staphylococcus*, mostly *Staphylococcus aureus* is considered as an indicator of hygienic status employed in the field of production or distribution of drinking water. Although, there are no known guidelines either for total *Staphylococci* or *Staphylococcus aureus* in drinking water, but in spite of that, drinking water should be free of *Staphylococcus aureus* as a bacterial pathogen. The drinking water samples from three out of five water stations were positive for the presence of *Staphylococcus aureus* as 5.7-6.7% of total water samples were above the established standards expect sample No. 4 and 5 (Table 1). The density of *S. aureus* increased from 10-25% of the total drinking water samples from all the three water tankers. Although, the percentage of *S. aureus* is limited in drinking water samples, but its presence may be attributed to infected employees who reflect improper hygienic status.

CONCLUSION AND RECOMMENDATIONS

The bacteriological and sanitary quality of drinking water produced in Makkah Al-Mokarama varied significantly from one station to another during high season during the Holy month of Ramazan. The HPC at 37°C, total coliforms, *E. coli* and *S. aureus* were 6.7-33.3%, 20-46.7%, 0-20% and 0-6.7%, respectively for the sanitary measures applied during drinking water production and inadequate water treatment processes. On the other hand, the high percentage of bacteriological parameters such as HPC at 37°C, total coliforms, *E. coli* and *S. aureus* were 40-50%, 60-68.8%, 31.2-37.5% and 10-25%, respectively

in drinking water samples from tankers which clearly indicated the absence of necessary sanitary conditions required in these water tankers.

Overall, the bacteriological and sanitary results indicate that the quality of drinking water from water stations in Makkah Al-Mokarama during high season (Holy month of Ramadan) is not acceptable. This could be attributed to the failure in the application of effective sanitary measures of drinking water. This is further polarized due to the absence of adequate water treatment processes and to the deficiency of proper sanitary conditions in water tankers. It is, therefore, recommended that the water production equipments and water tankers should be cleaned and washed with disinfectants on regular basis to maintain high quality drinking water, to prevent survival of multiply microorganisms and to avoid accumulation of any health hazardous metal in the water tankers. Most importantly, a comprehensive sanitary program should be formulated and implemented during water production and transportation along with the issue of valid health certificate to employees by regular inspection by the water authority.

REFERENCES

- APHA, 1998. Standard Methods for Examination of Water and Waste Water. 20th Edn., American Public Health Association, Washington, DC.
- European Economic Community (ECC), 1998. Council directive 98/83/EC relating to the quality of water intended for human consumption. Off. J. Eur. Communities, 330: 32-54.
- Fong, T.T., L.S. Mansfield, D.L. Wilson, D.J. Schwab, S.J. Molloy and J.B. Rose, 2007. Massive microbiological ground water contamination associated with a water borne outbreak in lake Erie, South Bass Island, Ohio. Environ. Health Perspect, 115: 856-864.
- Hurst, 1997. Water Microbiology in Public Health. Manual of Environmental Microbiology. ASM Press, Washington, DC.
- Jawetz, E., J., Melnick, E. Adelberg, G. Brooks, J. Buteland and L. Orniston, 1991. The Staphylococci, In: Medical Microbiology. 19th Edn., Appleton and Lange, Norwalk, Connecticut, California.
- Lee, S.H., D.A. Levy, G.F. Craun, M.G. Beach and R.L. Calderon, 2002. Surveillance for water borne-disease outbreak-United States, 1999-2000. MMWR Surveil Summ, 22: 1-47.
- Levy, D.A., M.S. Bebs, G.F. Craun, R.L. Calderon and B.L. Herwaldt, 1998. Surveillance for waterborne-disease outbreak-United States, 1995-1996. MMWR Surveil, 47: 1-34.

- Lye, D.E and A.P. Dufour, 1991. A membrane filter procedure for assaying cytotoxic activity in heterotrophic bacteria isolated from drinking water. *J. Applied Bact.*, 70: 89-94.
- Maria, C., C. Csba and V. Klara, 1999. *Microbiological Examination of Water and Waste Water*. Lewis Publishers, London, New York, Washington, DC.
- Moore, J.E., P.S. Cardwell, B.C Millar and P.G Murphy, 2001. Occurrence of *Campylobacter* for public health. *Ulser. Med. J.*, 70: 102-107.
- Moyer, N.P., 1987. Clinical significance of *Aeromonas* sp. isolated from patients with diarrhea. *J. Clin. Microbiol.*, 25: 2044-2048.
- Pam, T., M. Brenda, L. Cassandra and T. Kam, 2005. Microbial indicators of faecal contamination in water: A current perspective. *Water Air soil poll.*, 166: 139-166.
- Reid, D.C., A.C. Edwards, D. Cooper, E. Wilson and B.A. McGaw, 2003. The quality of drinking water from private water supplies in Aberdeen shire, U.K. *Water Res.*, 37: 245-254.
- Shaw, W.D., M. Walker and M. Benson, 2005. Treating and drinking well water in the presence of health risks from arsenic contamination: Results from a US hot spot. *Risk Anal.*, 6: 1531-1543.
- Vollaard, A.M., S. Ali, J. Smet, H. Van Asten and S. Widjaja *et al.*, 2005. A survey of the supply and bacteriologic quality of drinking water and sanitation in Jakarta, Indonesia. *South East Asian J. Trop. Med. Public. Health*, 36: 1552-1561.
- WHO, 1996. *Guidelines for Drinking-Water Quality*. 2nd Edn., World Health Organization, Geneva.