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Microbiological Quality of Drinking Water and Beverages in Karachi, Pakistan

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Abstract. Microbiological assay of 780 water samples and 1220 beverage samples (412 branded and 808 unbranded), collected from 490 different schools, both government (98 schools) and private (392 schools), situated in different areas of the city of Karachi, was conducted for bacterial heterotrophic plate count, total coliforms, faecal coliforms, *E. coli*, faecal streptococci, *Pseudomonas* and *Salmonella* species. The counts ranged from 0 to 2.5×10^5 cfu/mL and from 0 to 10^6 cfu/mL in water and beverage samples, respectively. About 36% of water samples and 48% of unbranded beverage samples were contaminated with the indicator and the pathogenic bacteria; all the branded beverage samples were found fit for human consumption from microbiological viewpoint.

Keywords: drinking water, beverages, microbiological quality

Introduction

Raw water itself does not contain large number of micro-organisms. Drinking water contains assimilable organic compounds that allow a certain degree of bacterial growth (Exner *et al*., 2005). Improperly installed hand pumps permit infiltration of contaminated surface water, whereas unclean storage devices and other factors contribute to disease cycle, malnutrition and high mortality. Infectious diseases caused by pathogenic bacteria are the most common and wide spread health risk associated with drinking water. Some of the pathogens, which are transmitted through contaminated drinking water, lead to severe and sometimes life threatening diseases particularly in children. The potential of drinking water to transport microbial pathogens to large number of population, causing subsequent illness, is well documented in different countries.

The most common and widespread risk associated with drinking water is its contamination by human or animal excreta. The potential consequences of microbial contamination necessitates that its control be of paramount importance. Pathogens in drinking water, presenting serious risk of diseases, include *Salmonella* sp., pathogenic *Escherichia coli*, *Pseudomonas*, *Vibrio cholerae* etc. Faecal specific bacteria such as coliforms, faecal coliforms and *E. coli* are the parameters of importance in monitoring faecal pollution.

The survival and growth of microorganisms in processing environments of foods, such as beverages, sherbets, ice creams, ice-lollies, etc. may lead to contamination of the finished products, resulting in reduction of microbiological safety and quality (Kohnen *et al*., 2005). However, several studies have demonstrated that the total counts and number of pathogens may get reduced due to the acidity and the effect of CO_2 during storage. (Mugochi *et al*., 1999; Simango and Rukure, 1992; Sheth *et al*., 1988; Zschaler, 1979; King and Nagel, 1975; 1967).

In Pakistan people lack access to adequate supply of safe water for daily use. The basic sanitary facilities are very poor and are not available for half of the population. Sources of microbial contamination of water and beverages include raw materials, processing equipment or utensils, human activities, sanitation practices, workers or handlers, waste materials, animal and insect pests and microbial growth niches. Chemical composition of foods and beverages and the environmental factors, such as water activity, pH, temperature, etc., determine the types of organisms that can grow there.

The present study was undertaken keeping in view the hazards of polluted or contaminated drinking water and the effect of its use in beverages and ice lollies etc. The survey provides base line data for authorities to set the guidelines for microbiological quality of water and beverages within the country.

Materials and Methods

Sample collection. A total of 780 water and 1220 beverage samples were collected from 490 different schools/educational institutes of Karachi, located in various areas including those inhabited by low income, middle class and upper middle class population, as well as rich and wealthy people. Both the government and the private institutions were included in the

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study, out of these 98 were government schools whereas 392 were private institutions. Insulated ice chest with ice packs was used for collection and transportation of samples. The collected samples were labelled with date and laboratory code. Other necessary information about the samples, like area or location of school, collection point etc. was recorded on prescribed forms. Samples were collected in sterilized screw-capped glass bottles.

Beverage samples were categorized as branded and unbranded. The unbranded beverages consisted of gola-gandas (local name for an item made by crushed ice and additives), sherbets (drinks) and carbonated and other drinks sold on vending carts (thailas). All the water and beverage samples were tested microbiologically for their heterotrophic plate counts, total coliforms, fecal coliforms, *E. coli* 0157:H7, faecal streptococci, *Pseudomonas* and *Salmonella* species.

Heterotrophic plate count (HPC), coliforms and faecal coliforms were tested according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998; ISO 9308-1: 2000; 9308-2: 1990; 6222: 1988a; 8199: 1988b). *E. coli* 0157:H7 was tested by serological kit method (Pro-Lab Diagnostics) according to Thompson *et al*. (1990) and faecal streptococci were determined using ISO method 7899-1 (1984). Other parameters were tested according to on-line Bacteriological Analytical Manual of US FDA (2006; 2002; 2001). *Salmonella* was confirmed using antisera (Pro-Lab Diagnostics).

Results and Discussion

Figures 1 and 2 present total heterotrophic plate count (HPC) in water and beverage samples, respectively. The heterotrophic plate count in water and beverage samples ranged from 0 to 2.5×10^5 cfu/ml and from 0 to $<10^6$ cfu/ml, respectively. HPC may be used to assess the general bacterial content as well as the efficiency of water treatment. The HPC standards for drinking water vary a lot from country to country. According

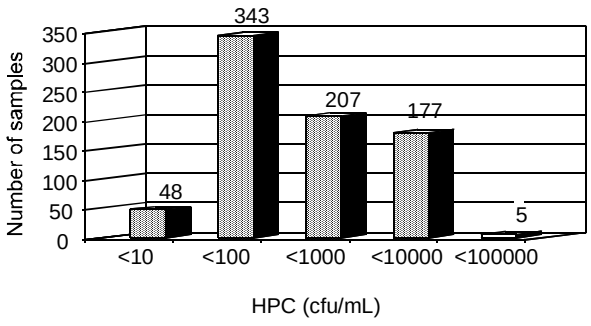

Fig. 1. Heterotrophic plate counts of water samples.

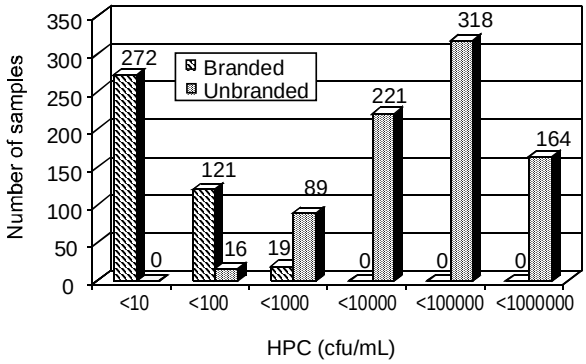

Fig. 2. Heterotrophic plate counts of beverage samples.

to WHO (1999) guidelines for drinking water quality, the limit for HPC is 100 cfu/mL which has also been adopted by Pakistan Standards and Quality Control Authority (PSQCA; 2004).

However, WHO guidelines for drinking water quality are intended to be used as a basis for the development of national standards in the context of local or national environmental, socio-economical and cultural conditions (WHO, 1999). The HPC standard for drinking water in Sri Lanka is 10,000 cfu/mL (SLS, 2001; 1995), which is a pretty relaxed standard, compared to the stringent standard of WHO. According to Canadian standard, HPC is not considered as a parameter of drinking water quality. A review study conducted by Allen *et al*. (2004) reveals that there is no evidence to support health-based regulations of HPC concentrations.

The national standards should be influenced by national priorities and economic factors. Stringent standards could limit the availability of water supplies, which is a significant consideration in regions of water shortage. However, public health must never be endangered. For this very reason, it is suggested that the WHO standard for HPC must not be adopted as the criterion for rejection of line water samples for drinking purpose and the acceptance limit must be adopted according to Sri Lankan standard or the Canadian guidelines. The presence of indicator organisms and/or pathogens only must be considered as the criterion for declaring a sample unfit for human consumption. However, we do support adoption of WHO guidelines for bottled water according to which the samples must not contain any count at all as the manufacturers/bottlers are claiming it to be pure and charging extra money for that.

Figure 2 presents total bacterial counts in beverage samples. According to the PSQCA (2002) standard PS: 1654-2002 R, the freshly prepared carbonated drinks may contain <100 bacteria per mL of the sample whereas the count must

decrease to <30 within three days of storage. The counts in the branded samples were mostly within the limit of PS standard with only 19 of the 412 tested samples having counts exceeding 100 mL.

Coliform organisms are recognized as suitable microbial indicators of drinking water quality. Coliforms include heterogeneous lactose fermenting bacteria found in faeces and environment. Detection of coliforms suggests inadequate treatment, post-treatment contamination or excessive nutrients. Although coliforms may not always be related to the presence of faecal contamination or pathogens, they are useful for monitoring the microbial quality of public water supplies. Presence of coliforms in the absence of faecal coliforms suggests the use of secondary indicators like faecal streptococci for confirmation of faecal contamination.

Figures 3 and 4 present the occurrence of organisms of public health significance in water and beverage samples, respectively. The microbiological analyses revealed that a total of 36% water samples and 48% of unbranded beverages were unfit for human consumption due to the presence of organisms of public health significance i.e. indicator organisms and/or pathogenic bacteria. The branded samples, on the other hand were all free from organisms of public health significance.

In the present study, a total of 269 out of 780 water samples (34.5%; Fig. 3) and 549 out of 808 unbranded beverages samples (45%, Fig. 4) were found contaminated with coliform bacteria (Noble *et al*., 2004, 2003; Reasoner and Galdreich, 1985).

Thermotolerant (faecal) coliform group comprises of *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*, which are able to ferment lactose at 44-45 °C. Out of these organisms, only *E. coli* is specifically of faecal origin; other thermotolerant coliforms may originate from organically enriched waters such as industrial effluents or from decaying plant materials and soils.

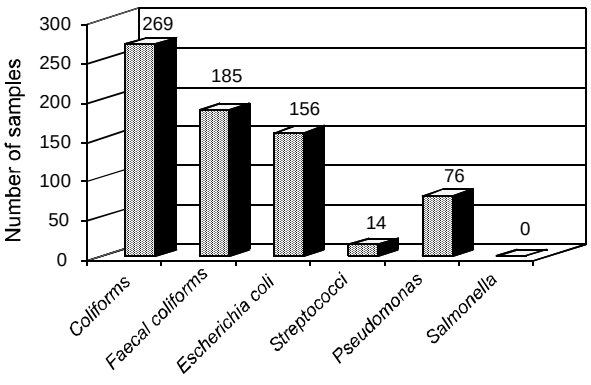

Fig. 3. Incidence of organisms of public health significance in water samples.

Out of 780 water samples, 185 (23.5%) and 156 (20%) (Fig. 3) and out of 808 unbranded beverages, 549 (45%) and 463 (38%) (Fig. 4) samples were found contaminated with faecal coliforms and *E. coli*, respectively.

The presence of *Pseudomonas aeruginosa* in potable water also indicates serious deterioration in bacteriological quality and is often associated with complaints about taste, odour and turbidity linked to low rates of flow in distribution system and a rise in water temperature.

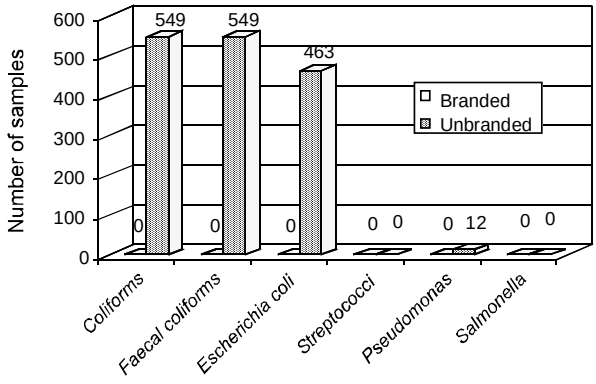

Fig. 4. Incidence of organisms of public health significance in beverage samples.

Conclusion

The present study has shown that the microbiological quality of water and locally made beverages vended in schools is not satisfactory and approximately 36% water samples and 48% unbranded beverage samples were found to be unfit for human consumption due to the presence of organisms of public health significance i.e. indicator organisms and/or pathogenic bacteria.

Water sources must be protected from contamination by the human and animal excreta and other wastes to protect the community from the risks of outbreaks of intestinal and other infectious diseases. Moreover, effective treatment and regular monitoring must be carried out in order to protect the water reservoirs. Furthermore, strict sanitary and hygienic regulations must be imposed on the local manufacturers of beverages; the sanitary conditions of the vending carts and the beverages being sold through them must also be effectively monitored.

References

Allen, M.J., Edberg, S.C., Reasoner, D.J. 2004. Heterotrophic plate count bacteria - what is their significance in drinking water. *Intternational Journal of Food and Microbiology*, **92:** 265-274.

APHA, 1998. Microbiological examination. In: *Standard Methods for the Examination of Water and Wastewater*, 20th edition, American Public Health Association, Washington DC., USA.

Exner, M., Vacata, V., Gebel, J. 2005. Public health aspects of the role of HPC - An introduction. In: *Heterotrophic Plate Counts and Drinking Water Safety*, J. Bartram Cotruvo, M. Exner and A. Glasmacher (eds.), AITBS Publishers, New Delhi, India.

ISO 9308-1:2000(E). Detection and enumeration of *Escherichia coli* and coliform bacteria. Part 1. Membrane Filtration Method.

ISO 9308-2:1990(E). Water quality - detection and enumeration of coliform organisms, thermotolerant coliform organisms, and presumptive *Escherichia coli*. Part 2: Multiple Tube (Most Probable Number) Method.

ISO 6222:1988a. Enumeration of viable microorganisms. Colony count by inoculation in or on nutrient culture medium.

ISO 8199:1988b. General guide to enumeration of microorganisms by inoculation in or on culture medium.

ISO 7899-1:1984. Detection and enumeration of faecal streptococci-Part 1: Method by enrichment in a liquid medium.

King, Jr., A.D., Nagel, Ch. W. 1975. lnfluence of carbon dioxide upon the metabolism of *Pseudomonas aeruginosa*. *Journal of Food Science*, **40:** 362-366.

King, Jr., A.D., Nagel, Ch. W. 1967. Growth inhibition of a *Pseudomonas* by carbon dioxide. *Journal of Food Science*, **32:** 575-579.

Kohnen, W., Teske-Keiser, S., Meyer, H.G., Loos, A.H., Pietsch, M., Jansen, B. 2005. Microbiological quality of carbonated drinking water produced with in-home carbonation systems. *International Journal of Hygiene and Environmental Health*, **208:** 415-423.

Mugochi, T., Parawira, W., Mpofu, A., Simango, C., Zvauya, R. 1999. Survival of some species of *Salmonella* and *Shigella* in mukumbi, a traditional Zimbabwean wine. *International Journal of Food Science and Nutrition*, **50:** 451-455.

Noble, R.T., Leecaster, M.K., McGee, C.D., Weisberg, S.B., Ritter, K. 2004. Comparison of bacterial indicator analysis methods in stormwater-affected coastal waters. *Water Research*, **38:** 1183-1188.

Noble, R.T., Moore, D.F., Leecaster, M.K., McGee, C.D., Weisberg, S.B. 2003. Comparison of total coliform, fecal coliform and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research*, **37:** 1637-1643.

PSQCA, 2004. Pakistan Standard Specification for Drinking Water. PS: 4639-2004 (R), Pakistan Standards & Quality Control Authority, Standards Development Centre.

PSQCA, 2002. Pakistan Standard Specification for Carbonated Beverages. PS: 1654-2002 (R), Pakistan Standards and Quality Control Authority, Standards Development Centre.

Reasoner, D.J., Geldreich, E.E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Applied Environmental Microbiology*, **49:** 1-7.

SLS, 2001. Sri Lankan Standards 894, specification for packaged bottled drinking water.

SLS, 1995. Sri Lankan Standards 1038. Specification for bottled natural mineral water.

Sheth, N.K., Wisniewski, T.R., Franson, T.R. 1988. Survival of enteric pathogens in common beverages: an *in vitro* study. *American Journal of Gastroenterology*, **83:** 658-660.

Simango, C., Rukure, G. 1992. Survival of bacterial enteric pathogens in traditional fermented foods. *Journal of Applied Bacteriology*, **73:** 37-40.

Thompson, J.S., Hodge, D.S., Borczyk, A.A. 1990. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. *Journal of Clinical Microbiology*, **28:** 2165-2168.

US FDA, 2006. *Bacteriological Analytical Manual*, An Online publication of Center for Food Safety & Applied Nutrition of USFDA. Chapter 3 (2001), Chapter 4 (2002), Chapter 5 (2006).

WHO, 1999. *Guidelines for Drinking-Water Quality*. *Health Criteria and Other Supporting Information*, vol. **2**, 2nd edition, AITBS Publishers, World Health Organization, Geneva, Switzerland.

Zschaler, R. 1979. Influence of carbon dioxide on the microbiology of beverages. *Antonie van Leeuwenhoek* **45:** 158-160.