IJASRM
International Journal of Advanced Scientific Research and Management, Vol. 2 Issue 9, Sept 2017.
www.ijasrm.com
ISSN 2455-6378

Microbiological Quality Analysis of Commercially Prepared Bottled Drinking Water in Kandy, Sri Lanka

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Abstract

During the past few years bottled drinking water industry has risen dramatically. In Sri Lanka also there are many brands of commercially prepared bottled drinking water available. Water sources may be contaminated with fecal matter and disease causing pathogenic organisms may be present. If the bottled drinking water is not subjected to sufficient water treatments these pathogenic microorganisms may be present in these products and may cause water borne illnesses in consumers. This study is done in order to determine the microbiological quality of commercially manufactured bottled drinking water available in the local market of Kandy, Sri Lanka. For this study 10 sample brands of commercially manufactured bottled drinking water samples were selected. These samples were subjected to microbiological analyzing to determine their microbiological quality. These samples were subjected to total coliform testing, fecal coliform testing and *E.coli* testing. Only three out of ten samples showed positive results for total coliform test. Out of these samples only one sample showed positive results for presumptive, confirmed and completed test of total coliform test. Out of all samples only one sample gave positive result for fecal coliform test and showed growth of *E.coli* in EMB medium. The presence of coliforms is may be due to either contamination of water source, unsatisfactory water treatment techniques or unhygienic practices during packaging process. The study reveals that seven out of ten brands of bottled drinking water samples were within the acceptable microbiological parameters set by WHO.

Keywords: *coliforms, fecal contamination, E.coli, multiple tube fermentation, MPN*

1. Introduction

Water is essential to maintain the life of all living beings. Living organisms cannot survive without water. Just as other organisms humans also greatly depend on water. People obtain water from many sources. But the quality of these water sources may vary greatly.

During the past few years bottled drinking water industry has risen dramatically. This increased demand for bottled drinking water is may be due to the fact that they pose less harmful effects on health comparatively to other beverages. In Sri Lanka also there are many brands of commercially prepared bottled drinking water are available.

According to weekly Epidemiological Reports of Ministry of Health of Sri Lanka, no water borne illness has been reported in Sri Lanka due to consumption of bottled drinking water. Nevertheless, in some countries outbreaks of water borne illnesses have been reported. It has been estimated that there are over 250 million cases reported throughout the world (Esrey et al., 1985) .So there is a great need in ensuring in microbiological quality of bottled drinking water.

Disease causing pathogens may be present in the bottled water due to contamination of water sources. The greatest risk is caused by water sources contaminated by human and animal feces (WHO. Guidelines for drinking water quality, 2008) Consumption of water contaminated with pathogenic microorganisms may cause diseases such as, cholera, gastroeneteritis, typhoid fever, salmonellosis, bacillary dysentery and shigellosis (Water Microbiology. Bacterial Pathogens and Water, 2010).

Microbiological analysis of these disease causing microbial pathogens directly is difficult. This is due

to the fact that they are, present in small numbers, sporadic and isolating and culturing is difficult (George *et al*., 2002). Hence an indicator organism is used to determine the microbiological quality of drinking water. There are many characters that should be present in microorganism to select them as an indicator organism of fecal contamination. These characters include can detect easily, present in high numbers in human intestine and feces, not pathogenic, present in greater numbers than the pathogenic organism, does not multiply outside the enteric environment and have similar die-off behavior as the pathogenic organism. Coliform bacteria are selected as a good candidate for indicator organism in testing of microbiological quality of drinking water, as they meet with the above mentioned criteria (WHO. Guidelines for drinking water quality, 2008) (Grabow, 1996) (Medema *et al*.,2003) (George *et al*., 2002) (Gauthier *et al*., 2001). Total coliforms refers to a large group of gram negative, non-spore forming, rod shaped bacteria that ferment lactose and produce gas after incubating for 48 hours at 35–37 °C, in a medium that contains detergents and bile salts (WHO. Guidelines for drinking water quality, 2008) (Grabow, 1996) (George *et al*., 2002) (Payment *et al*., 2003). Fecal coliforms ferment lactose at 44.5C in a medium that contains bile salts (WHO. Guidelines for drinking water quality, 2008) (Grabow, 1996) (George *et al*., 2002) (Payment *et al*., 2003). Analyzing the microbiological quality of drinking water was done by detecting the presence of coliforms by using multiple-tube fermentation method (UNICEF, 2002).

The objective of this study is to determine the microbiological quality of commercially manufactured bottled drinking water available in the local market of Kandy, Sri Lanka.

2.Materials & Methods

2.1 Sample Collection

10 samples of different brands of bottled drinking water were selected from the local market of Kandy, Sri Lanka. The samples were selected in such a way that they were manufactured within the same month.

2.2 Microbiological Analysis

2.2.1Total coliforms

The selected samples were tested for the microbiological quality. Microbiological quality was tested by testing the water samples for presence of total coliforms. Presumptive test, Confirmed test and completed test were done to detect the presence of total coliforms.

2.2.1.1Presumptive Test

Lactose broth was inoculated using the collected water samples. The tubes were incubated at 37ºC for 4 hours and observed for production of gas. The Most Probable Number (MPN) of coliforms was determined using the number of positive tubes.

2.2.1.2Confirmed Test

Eosin Methylene Blue (EMB) agar plates were inoculated using positive test tubes of presumptive test. The plates were incubated at 37ºC for 24 hours.

2.2.1.3 Completed Test

Lactose broth and Nutrient agar slants were inoculated using colonies that grew on EMB plates. Lactose broth and NA slants were incubated at 37ºC for 24 hours. Lactose broth was observed for production of gas and Gram staining is done for organisms that grow in NA slants.

2.2.2 Fecal coliforms

Samples that gave positive results for total coliform test were used to inoculate the EC broth. The test tubes were incubated at 44.5ºC for 24 hours.

2.2.3 Test for *E.coli*

Samples that gave positive results in EC broth were used to inoculate EMB agar medium. Streak plates were prepared and the plates were incubated at 37ºC for 24-48 hours.

3. Results & Discussion

As indicated in Table 1 out of all the 10 sample brands of bottled drinking water only 3 samples gave positive results to total coliform test. These samples are sample C, E and J. Samples C and J only gave positive results for the presumptive test. The test tubes of lactose broth inoculated with these samples showed accumulation of gas and turbidity in broth. Sample E gave positive results for all presumptive, confirmed and completed tests. Lactose broth inoculated with sample E showed gas production and turbidity in broth, colonies were grown in EMB medium and gas production in lactose broth and growth in NA slants. When the colonies were subjected to gram staining small, gram negative, rod shaped bacteria were observed. This indicates that sample E definitely contains coliforms.

Table 1 – Results of microbiological analysis

Sample	Parameter		
	Total coliforms (MPN/100ml)	Fecal Coliforms	*E.coli*
A	<2	-ve	-ve
B	<2	-ve	-ve
C	4	-ve	-ve
D	<2	-ve	-ve
E	40	+ve	+ve
F	<2	-ve	-ve
G	<2	-ve	-ve
H	<2	-ve	-ve
I	<2	-ve	-ve
J	2	-ve	-ve

When tested for fecal coliforms only sample E showed positive results. When sample E is inoculated in EC broth it showed gas production and turbidity in the medium.

When sample E is tested for *E.coli* it showed growth of colonies in EMB agar plates. The colonies had a green metallic sheen indicating the presence of *E.coli*.

According to the results of microbiological analysis by detecting coliforms, one sample out of ten samples used in this study is unsuitable for consumption. It is contaminated with fecal matter and may contain pathogenic microorganisms. According to WHO permissible level of coliforms in drinking water should be 0MPN/100ml. In this study out 10 samples 3 samples showed higher coliform levels than the permissible levels (2MPN/100ml, 4MPN/100ml and 40MPN/100ml). Hence these samples were not suitable for consumption.

The presence of coliforms in the samples is may be due to unsatisfactory treatment of water during the water purification plant of the bottled drinking water factory. Coliforms would have entered the bottled drinking water samples either at the source, which may be contaminated with fecal matter or during the manufacturing process due to inadequate water treatment techniques or during packaging due to unsatisfactory sanitary practices of the workers.

4. Conclusion

The present study reveals that the microbiological quality of most of the commercially prepared drinking bottled water samples collected from Kandy, Sri Lanka were within the acceptable standards, i.e. seven out of ten samples were of satisfactory microbiological quality. Two samples showed some degree of contamination while only one sample showed definite fecal contamination due to presence of *E.coli* and unsuitable for consumption. In order to prevent outbreak of water borne diseases bottled drinking water manufactures should take relevant steps to maintain the quality of the product according to WHO standards.

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