RESEARCH ARTICLE

MICROBIOLOGICAL ANALYSIS OF PACKAGED DRINKING WATER SOLD IN CHENNAI

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ABSTRACT

Background: Adequate supply of fresh and clean drinking water is a basic need for all human beings. In terms of public and environmental health, it is essential that water sources be free of pathogenic bacteria and safe to drink. Continuous increase in the sale and indiscriminate consumption of packaged drinking water is of public health significance. In order to safeguard public health it is essential that the available packaged water is of the highest quality.

Aims & Objective: The current study was designed to assess the microbial quality of sachet and bottled drinking water sold in retail outlets in Chennai.

Materials and Methods: In the present study, fifty one samples including 36 sachet and 15 bottled drinking water samples were analyzed for the presence of bacterial indicators of water quality. Total and faecal coliform count, total viable plate count and culture were performed to determine the identities of the isolates.

Results: Of the 36 sachet water samples analysed, 33.3% failed to meet the WHO drinking water standard of zero coliform per 100 ml making them unsuitable for human consumption whereas all the 15 bottled water samples are of better quality. Faecal coliforms were not isolated from any of the sachet or bottled drinking water. The bacteria that were isolated from water samples included *Klebsiella pneumonia, Enterobacter aerogenes, Citrobacter freundii, Pseudomonas spp., Acinetobacter spp., Staphylococcus aureus, Coagulase negative Staphylococci, Micrococcus spp.* and *Bacillus spp*.

Conclusion: The bottled water samples analyzed are of better microbiological quality when compared to that of the local brands of sachet water samples. The findings therefore suggests that these sachet water are not fit for human consumption and are hazardous to health. Hence there is a need for strict and routine monitoring of the packaged drinking water with the view of raising their standards.

Key Words: Bacteriological Quality; Sachet Water; Bottled Water; Coliform Count; Heterotrophic Bacteria

Introduction

Water is an essential requirement of all life forms. Satisfactory supply of clean, safe, and hygienic drinking water is imperative for health.[1] Access to safe drinking water is a vital agent for human living.[2] Unavailability of good quality drinking water is widespread and this has serious health implications.[3] Water related diseases continue to be one of the major health problems globally. The high prevalence of diarrhea among children and infants can be traced to the use of unsafe water and unhygienic practices.[4] In developing countries, 80% of all diseases and over 30% of deaths are related to drinking water.[3]

As a preventive measure, consumption of packaged drinking water has increased in recent years in developing countries.[2] Sachet or packaged drinking water is any water that is in a sealed plastic and distributed or offered for sale and is intended for human consumption.[5] The sale and consumption of packaged drinking water continues to grow rapidly in most of the developing countries of the world.[6] In order to safeguard public health it is essential that the available packaged water is of the highest quality.[7] The continuous proliferation of these packaged drinking water and their indiscriminate consumption are of concern to public health. An understanding of their microbiological quality and safety are therefore pertinent.[8]

Several studies has documented the detection of coliforms and heterotrophic bacteria in bottled/ sachet drinking water counts which far exceeded the national and international standards set for potable water for human consumption.[2,9] Heterotrophic bacteria are non-coliform species of bacteria that utilizes organic substance for its development. The presence of heterotrophic bacteria in drinking water is not an indication that the water presents the health risk but poses significant health risk in immunocompromised individuals. Similarly total coliform bacteria may cause health risk for infants, young children, elderly people and especially to the immunocompromised individual.[10] The present study was carried out to determine the microbiological quality of sachet and bottled drinking water available in Chennai.

Materials and Methods

Bottled and sachet drinking water samples were collected from various retail outlets of Chennai during the period of

April 2013 – July 2013. Overall 51 samples belonging to 17 different brands (12 sachet and 5 bottle) were analyzed. Triplicate batches of each of the brands were purchased randomly. They were transported in cool boxes to the Microbiology laboratory for immediate analysis. The microbial quality of the drinking water samples were assessed by using total coliform count and total viable count as indices.

Enumeration and isolation of total and faecal coliform

Total coliforms were estimated using the most probable number (MPN) method. MacConkey's lactose bile salt broth with bromocresol purple as indicator was used for the presumptive tests. With a sterile pipette, 50 ml of each of the water sample was aseptically dispensed into 50ml double strength broth, another 10 ml of the sample into each of the five tubes containing 10 ml double strength broth and another one ml of the sample was then inoculated into each of the second five culture tubes containing 5ml single strength MacConkey broth with Durham's tubes. Inoculated tubes of MacConkey broth were incubated at 37° C for 24 to 48 hours. After 24-48 hours of incubation, the cultures were observed for the presence of acid production and gas formation. Reference to Mc Crady's table showed the most probable number (MPN) of presumptive coliform counts in 100ml of the sample water analyzed.

A sterile pipette was used to transfer 1ml of the culture from the positive presumptive fermentation tubes into tubes containing 5ml brilliant green lactose bile broth aseptically and incubated for 24-48 hours at 37°C. Following incubation, culture positive tubes were inoculated into MacConkey agar for total Coliform and Eosin Methylene Blue agar for faecal coliform and incubated at 37 ° C and 44 ° C respectively.

Determination of heterotrophic plate count/ total viable count

Heterotrophic plate count of all water samples were determined using dilution plate method technique and standard plate count agar medium. Serial dilutions were prepared (using peptone water) and 1 ml of the sample or dilution was transferred to a sterile, empty petridish. Plate count agar was melted by heating in boiling water and then allowed to cool in a water bath to 44 - 46° C.

Approximately 15 ml of the agar medium was poured into the petridish containing the sample. The sample and agar was mixed thoroughly by rotating the plate several times. When the media has solidified, the plates were inverted and incubated at 35 °C for 48 to 72 hours. Following the appropriate length of incubation, suitable plates from different dilutions were selected and the visible colonies were counted using a colony counter. Then the average colonies were counted and expressed as colony forming unit per ml of water.

Identification of isolates

Representative isolates from total coliforms and total viable counts were identified. Standard isolation techniques were employed. MacConkey agar was used to isolate lactose fermenting gram negative bacilli, blood agar was used to isolate fastidious organisms and Mannitol salt agar for the isolation of salt resistant bacteria .Pure isolated colonies were Gram differentiated and then biochemically identified using Indole, Catalase, Citrate, Oxidase, Coagulase and Urease tests.

Results

In the current study, overall 51 samples (36 sachet and 12 bottle), belonging to 17 different brands were analyzed. The result of coliform count using the most probable number is shown in table 1 which defines the degree of contamination and the microbiological quality of the sachet and bottled drinking water brands.

Sachet drinking water

Mean total coliform count in sachet water ranged from 1.6 to 12.6 cfu (Colony Forming Unit) /100 ml. Faecal coliform and *E. coli* was not isolated from any of the sachet drinking water. Table- 2 shows the classification of water samples based on the WHO standard for drinking water. From the 36 sachet water screened, it is noteworthy that 28 (77.7%) met the WHO standard of zero coliform, 1 (2.7%) of sachet water had 1-3 coliform, 3 (8.3%) had 4-10 coliform and 4 (11.11%).

Heterotrophic bacteria plate counts in various brands of sachet and bottled drinking water is shown in Table-4. The total heterotrophic plate count concentration ranged between 2.1 X 101 cfu/ml to 4.9 X 106 cfu/ml. The mean microbial counts of the sachet water ranged from 1.1 X 102 cfu/ml to 1.6 X 106 cfu/ml. Among the 36 sachet water samples analysed only 6 samples had a total HPC of less than 100 cfu/ml.

Bottled drinking water

Total coliform and faecal coliforms were not isolated in any of the five different brands (15 samples) of bottled

drinking water. However HPC ranged between 0 and 41 cfu/ml. Mean HPC varied from 2.3 cfu/ml to 3.4 X 101 cfu/ml.

Table-1: Most probable number of total coliforms in sachet and bottled water brands

Type of Packaged Water	Brand	1	2	3	Mean (MPN/100ml)	Range
Sachet Water	S1	0	0	0	0	-
	S2	0	0	0	0	-
	S3	0	0	0	0	-
	S4	6	9	6	7	6 - 9
	S5	0	3	2	1.6	2 - 3
	S6	0	0	0	0	-
	S7	0	0	0	0	-
	S8	14	10	14	12.6	10 – 14
	S9	0	0	0	0	-
	S10	0	0	0	0	-
	S11	7	0	10	5.6	7 – 10
	S12	0	0	0	0	-
Bottled Water	A	0	0	0	0	-
	B	0	0	0	0	-
	C	0	0	0	0	-
	D	0	0	0	0	-
	E	0	0	0	0	-

Table-2: Classification of samples according to who criteria for drinking water

Class	Grade	Presumptive Count	Sachet Water		Bottled Water	
			n=36	%	n=15	%
I	Excellent	0	28	77.8	15	100
II	Satisfactory	1-3	1	2.8	-	-
III	Suspicious	4-9	3	8.3	-	-
IV	Unsatisfactory	>10	4	11.1	-	-

Table-3: Total heterotrophic bacterial plate counts (HPC) in bottled and sachet water brands

Type of Packaged Water	Brand	Mean HPC (cfu/ml)	Range (cfu/ml)
Sachet Water	S1	2.6×10^4	$6.9 \times 10^3 – 5.4 \times 10^4$
	S2	2.6×10^3	$2.2 \times 10^3 – 3.3 \times 10^4$
	S3	5.7×10^2	$2.1 \times 10^1 – 9.3 \times 10^2$
	S4	4.3×10^4	$5 \times 10^3 – 9.8 \times 10^4$
	S5	1.6×10^6	$3.2 \times 10^2 – 4.9 \times 10^6$
	S6	5.4×10^1	$3.4 \times 10^1 - 7 \times 10^1$
	S7	3.2×10^2	$1.6 \times 10^2 - 4.1 \times 10^2$
	S8	1.3×10^5	$8.8 \times 10^3 - 3.9 \times 10^5$
	S9	1.8×10^3	$8.9 \times 10^2 - 2.1 \times 10^3$
	S10	8.9×10^1	$4.3 \times 10^1 - 1.4 \times 10^2$
	S11	5.1×10^4	$1.5 \times 10^4 – 7.3 \times 10^4$
	S12	4.7×10^3	$8.4 \times 10^2 – 1.0 \times 10^4$
Bottled Water	A	6	2 - 10
	B	9.6	6 - 12
	C	4	0 - 8
	D	3.4×10^1	$2.7 \times 10 - 4.1 \times 10^1$
	E	2.3	0 - 4

Table-4: Frequency of bacterial isolates

Bacterial Isolates	Frequency	%
Klebsiella pneumonia	05	11.9
Enterobacter aerogenes	02	4.8
Citrobacter freundii	01	2.4
Pseudomonas spp.	12	28.6
Acinetobacter spp.	02	4.8
S.aureus	04	9.5
Coagulase negative Staphylococcus	08	19.0
Micrococcus	04	9.5
Bacillus spp.	04	9.5
Total	42	100

Speciation of bacteria isolated during total coliform and HPC enumerations

A total of 42 isolates including 9 different bacteria were isolated from the samples during the sampling period. Frequency of microorganisms isolated from the water sample is shown in Table-4. Out of the 8 isolates obtained from total coliform analysis, 5 (11.9%) isolates were *Klebsiella pneumoniae*, 2 (4.8%) were *Enterobacter spp.* and 1 (2.4%) was *Citrobacter freundii*. Of the remaining 34 randomly obtained colonies from Heterotrophic Plate Count analysis, 12 (28.6%) were *Pseudomonas spp.*, 4 (9.5%) were *S. aureus*, 8 (19%) were *Coagulase negative Staphylococci*, 4 (9.5%) were *Micrococcus* and the remaining 4 (9.5%) were *Bacillus spp.*

Discussion

According to the World Health Organisation, diarrheal diseases accounts for an estimated 4.1% of the total daily global burden and is responsible for the deaths of 1.8million people every year. It was estimated that 88% of that burden is attributable to unsafe water supply, sanitation and hygiene.[11] In the current study, out of the 36 sachet water samples analysed, 11.1% (4/36) had more than 10 total coliform per 100ml of water. Ideally there should be no coliform per 100ml of treated water as per WHO guidelines for drinking water and only 77.7% of the sachet water samples fell within this criteria. Therefore 33.33% of the sachet water samples (4 of the 12 brands) failed to meet the WHO drinking water standard of zero coliform per 100ml making them unsuitable for human consumption. The bottled water brands are of better microbiological quality with all the 15 samples meeting the WHO standard of zero coliforms. This correlated well with reports of several other authors, were coliforms were not isolated from bottled water.[12,13]

The presence of indicator organisms indicates that water is contaminated by potentially dangerous faecal matter and hence their absence denotes in general the water safety. Although coliform organisms may not always be directly related to the presence of fecal contamination or pathogen in drinking water, the coliform test is still useful for monitoring the microbial quality of drinking water.[14] Ineffectiveness or malfunctioning of the treatment process employed could also result in the presence of coliform bacteria in the water samples. Appropriate treatment processes should therefore be utilized for production of quality and safe packaged drinking waters.[15]

In this study, the coliform positive samples were also tested for faecal coliform. Faecal coliform is considered

more as an indicator of faecal contamination because whereas coliform can exist in the environment, faecal coliform are non-disease causing organism which are found in the intestinal tract of warm blooded animals .Hence its presence is indicative of contamination with animal or human water. The presence of *E. coli* in water is nearly always associated with recent faecal pollution and it is the preferred indicator organism for this purpose.[2]

In the present study , although total coliforms were isolated from 4 brands of sachet waters, faecal coliforms and *E. coli* were not isolated from any of the brands of bottled and sachet water samples ensuring the absence of fecal contamination in these samples. In a study in Ghana, none of the microbial indicators were present in bottled water whereas 4.5% of sachets contained total coliforms and 2.3% had faecal coliforms.[16] In another study on packaged drinking waters in Ibadan, Nigeria, the reports showed that large proportions of sachet water were found to show positive counts compared to bottled water.[17]

The absence of coliform bacteria in all brands of bottled drinking water could be attributed to the better hygienic practices observed in the industry compared to the sachet water producing industry. These include use of protective sealed caps on bottles, improved hygienic filling system and use of non-returnable plastic containers.[18]

Further the current study revealed that the total HPCs were very high in the various brands of sachet water with 10 out of the 12 brands of sachet water having mean HPCs above 100/ml. However all the bottled water brands analysed had a mean HPCs less than 100 ml -1. Although none of the samples analysed revealed the presence of faecal coliforms, the high level of the heterotrophs in the sachet water questions the quality of sachet water when compared to that of the bottled water. According to WHO 2002 report, a high HPC concentration does not itself present a risk to human health. Nevertheless HPCs are used as good indicators of the overall quality of production.[19,20] However based on the recommended standard limits of 100 HPCs per ml of drinking water by WHO, 83.3% of locally available sachet brands were considered unfit for human consumption.

A study conducted in Lagos, Nigeria showed that, bacteriological characteristics of sachet water deteriorates considerably as products moved farther down the distribution chain. Less than 7% of sachet water contamination took place after production while between 40 and 45% of the products observed between distribution sheds and the street hawkers.[21]

The result of this study showed the presence of some microorganisms in the water samples. Previous studies in other parts of the country reported similar bacterial load indicative of poor water quality.[19,22]

Bacteria isolated from sachet water in this study includes *Pseudomonas spp.* being the most common isolate constituting for 28.6 % followed by *Coagulase negative Staphylococci* (19%), *Klebsiella pneumonia* (11.9%), *Staphylococcus aureus* (9.5%), *Bacillus spp.* (9.5%), *Micrococcus* (9.5%), *Enterobacter spp.* (4.8%) and *Citrobacter spp.* (2.4%). Presence of these bacteria in water may be unnoticed even in transparent packaged water and the presence of these microorganisms may pose a potential risk to consumers. Even the consumption of such contaminated water facilitate the widespread infections and can ultimately lead to outbreak of epidemic.[23] The presence of these bacteria in the presumed treated sachet water used in this study may be as a result of improper handling, processing and purification procedures, unhygienic handling after production. The presence of *Pseudomonas spp.*, a pathogenic organism renowned for its high resistance to antibiotic is a point of concern.[6]

Conclusion

In general, while all the tested bottled water brands are of better microbiological characteristics, the bacteriological quality of 10 brands out of 12 brands of local sachet water samples analysed ,fell below WHO drinking water standards making them unfit for human consumption. There is therefore a need for the government to intensify efforts in the routine monitoring of activities in the packaged drinking water industry with a view to supply safe and wholesome water to the public. Assessment of water quality at different stages of production and post-production is therefore suggested in order to ensure the quality and safety.

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