Microbiological quality of water processed and bottled in Zimbabwe

R.N. Okagbue, N.R. Dlamini, M. Siwela* and F. Mpofu

Department of Applied Biology and Biochemistry, National University of Science and Technology (NUST), P.O. Box AC 939 Ascot, Bulawayo, Zimbabwe; Email: msiwela@nust.ac.zw; Fax: 263-9-286803

* To whom correspondence should be addressed.

SUMMARY

A total of sixty samples of bottled water processed in Zimbabwe by three companies, were analysed microbiologically, to assess the relative safety of locally processed bottled water. The samples were from different batches and from different storage conditions and the analyses were for total viable counts and coliforms. Four (6.7%) and seven (11.7%) samples were found to exceed the recommended maximum total viable and coliform counts, respectively. There was a low incidence of *Staphylococus aureus (3.3%), Pseudomonas* species (6.7%) and *Bacillus* species (5%). Overall, the work shows that locally bottled water is generally safe, microbiologically, though it is necessary to continue with precautionary measures because any lapse in hygiene may lead to microbial proliferation.

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Introduction

Bottled waters are commonly consumed as a safe form of water where municipal water supplies are of unacceptable quality or because of the therapeutic effects of the minerals present [1]. In Bulawayo, Zimbabwe, there has been an upsurge in the consumption of bottled water, prompted by a scare reported in the mass media, that one of the local municipal water sources might have been contaminated by mud containing cyanide residues from disused gold mine dumps.

There are various categories of bottled water, viz. mineral water, spring water, purified water and carbonated water. Water described or presented as mineral or spring water is, by legal definition, potable water obtained from an approved underground source, and not from public water supply. Such water should contain neither coliform bacteria nor more that 500 mg/L dissolved solids present [2]; it may be treated to remove unwanted chemical and biological elements. Other bottled waters such as purified or carbonated water are produced by distillation or deionisation or reverse osmosis from spring, well or public water supply, and should not contain more that 10 mg/L dissolved solids; also they should not contain coliforms or more than 100 aerobic bacteria per milliliter [2]. In most instances, before bottling water from an underground or other source, the water is micro-filtered with membranes of 0-10 μm pore size to remove microorganisms. This process is usually coupled with UV disinfection or is complemented by ozone gas treatment directly before bottling or prior to microfiltration [1].

Unfortunately, there are suggestions that bottled water is not always microbiologically safe. For example, a Canadian study [2], and other studies [3, 4] indicate that bottled water may be unsafe, microbiologically. It was found [5] that 3 out of 30 samples of bottled water randomly selected from retail markets in Harare contained coliforms. From the literature available to us, it appears that the microbiological quality of water which is processed and bottled in Zimbabwe has

not been studied. The aim of this project is to establish and document the microbiological quality of locally bottled water.

Materials and Methods

Samples

A total of sixty bottles of water processed by three local companies, were purchased randomly over a period of two months from shops in Bulawayo, Zimbabwe. By means of date code, bottles from different batches were collected. For each company, refrigerated and non-refrigerated bottles were considered.

Microbiological analyses

Conventional tests for bacteriological examination of water [6] were applied to the contents of each bottle which was inverted three times to ensure suspension of any sedimented organisms. Additionally, *Pseudomonas* species and *Staphylococus aureus* and *Bacillus* species were detected.

For the total viable count, duplicate one milliliter volumes of the sample were placed in sterile petri dishes and 10 ml of nutrient agar (NA) cooled to about 47^0 C poured and mixed thoroughly. A negative control, that is a plate containing NA only was prepared in duplicate. The plates were incubated at 37^0 C for 18-24 h after which colonies growing on the plates were counted.

Colonies suspected to be *Pseudomonas* species on the basis of pigmentation and Gram negative reactions were picked, purified on NA and preserved on slants of the same medium. Subsequently, they were tested for lactose fermentation, oxidase reactions and fermentative/oxidative metabolism on triple sugar iron (TSI) afar. *Bacillus* species were identified from the NA plates by subjecting the colonies to Gram and spore staining.

Mannitol Salt agar (MSA) was used to isolate *Staphylococcus aureus* by culturing one milliliter of the water sample by the pour plate method. The plates were incubated at 37^0C for 18-24 h and *Staphylococcus aureus* was identified by its ability to ferment mannitol producing a yellow colouring around the colonies. Colonies were picked, purified on NA plates and preserved on slants of the same medium NA slants for further biochemical tests which were the Grams reaction, tests for catalase, coagulase, haemolysis and DNAse. *Staphylococcus aureus* tests positive to all the mentioned tests.

Coliforms were determined by a 5-tube most probable number procedure (MPN) using brilliant green lactose bile broth (BGLB). The BGLB containing inverted Durham tubes to indicate gas production were incubated at 37^0 C for 24 h. The tubes showing gas production were considered positive for presumptive coliforms, and the tubes showing negative results were re-incubated for another 24 h. The MPN was computed from a 5-tube MPN (MacGrady Table) [6]. The presumptive coliforms were further characterised as faecal coliforms or *Escherichia coli (E. coli)* as follows: From gas-positive BGLB tubes and incubated at 45^0C for up to 48 h. Gas production confirmed the presence of faecal coliforms. To detect the presence of *E.coli*, 0.1mL aliquots from gas-positive BGLB tubes which had been incubated at 45^0C were streaked onto plates of eosin methylene blue (EMB) afar and incubated at 37^0C for 24 h. *E. coli* appear with a brilliant green metallic sheen whilst *Enterobacter aerogenes* and related species produce gummy pink colonies with dark centres. From EMB agar, distinct presumptive *E.coli* colonies were confirmed by the IMViC (indole, methly red, Voges-Proskauer, citrate) tests.

Results

Table 1 shows that four (6.7%) out of the sixty samples tested had total viable counts ranging from 103 to 151 c.f.u/ml. These values are above the bottled water standard of 100 c.f.u/ml as was previously reported [2].

Interestingly, these very high viable counts were obtained from water bottled in one Company C and kept at room temperature. Microbial loads from a few samples (at room temperature) from the other two companies were also appreciable high (range 64-76 c.f.u./mL). The results thus suggest that refrigeration of bottled water results in lower microbial loads. However, cold storage is not necessarily a foolproof way of ensuring a low microbial load since psychrophiles such as *Pseudomonas* can grow at refrigeration temperature.

Coliforms were detected in seven (11.7%) samples of bottled water and thus these are considered to be unsatisfactory. According to a previous report [7], bottled water at the time of bottling should contain less than one coliform per 100 ml and a total viable count of less than 500 c.f.u./ml. Generally, the presence of coliforms in mineral and spring water is considered an adequate indicator that there is potential contamination by intestinal pathogens, and thus this serves as an indicator of lack of good hygiene. The occurrence of faecal coliforms (8.3%), *E. coli* (5.0%) and *Staphylococcus aureus* (3.3%) (Table 2) in the bottled water was probably contaminated at some stage of its preparation and bottling.

Table 1. Total viable counts of coliforms in bottled water produced by different companies.

		COMPANY					
		A		B		C	
	Sample	TVC[a]	MPN[b]	TVC	MPN	TVC	MPN
Refrigerated	1	53[c]	0[d]	0	0	0	0
	2	40	0	0	0	46	1
	3	49	0	0	0	17	0
	4	10	0	0	0	7	0
	5	5	0	0	0	0	0
	6	7	0	0	0	2	0
	7	30	0	0	0	13	0
	8	8	0	0	0	7	0
	9	2	0	71	0	0	0
	10	7	0	0	0	0	0
Shelf-temperature	11	6	2	6	0	19	0
	12	9	0	2	0	40	0
	13	0	0	3	0	22	0
	14	0	0	0	0	10	0
	15	72	0	76	0	103	2
	16	73	0	65	0	118	1
	17	13	0	0	0	116	1
	18	16	0	0	0	151	2
	19	0	0	0	0	0	0
	20	64	0	0	0	0	0

[a] total viable count (c.f.u/ml); [b] Most probable numbers/100ml; [c,d] Means of duplicate data

Tabel 2. Frequency of occurrence of indicator and other Microorganisms in bottled water

Microorganisms	Number Positive	% Positive
Colifoms	7	11.7
Feacal coliforms	5	8.3
E. coli	3	5.0
Staphylococcus aureus	2	3.3
Pseudomonas spp.	4	6.7
Bacillus spp.	3	5.0

Discussion

Staphylococcus aureus strains which are haemolysis, coagulase and DNAse positive are undesirable because the mentioned characteristics are virulence factors. *Pseudomonas* are opportunistic pathogens which can become harmful in immuno-compromised individuals: Also, some *Bacillus* species are pathogenic. Although the above discussed microorganisms occurred in very low numbers in the water samples it would be necessary to improve the processing and bottling operations in order to virtually eliminate them.

Generally, the microbiological quality of bottled water can be improved by good manufacturing practices and improved storage conditions. It appears that the type of treatment given to the water prior to bottling has an influence on the microbial load of the water. In the samples tested, companies A and C filter the water and then use UV to disinfect it. Company B filters and chlorinates the water. From Table 1, it is clear that bottled water from Company B has the lowest microbial load. The results suggest that a combination of filtration and chlorination more effectively eliminates microorganisms in the water than filtration followed by UV treatment. While filtration alone can reduce the microbial load of the water, it has been stated [8] that there was a chance for contamination of the effluent if the filtration equipment was not maintained properly. The microbial contaminants of the bottled water, especially those that have simple nutrient requirements such as *Flavobacterium* and *Pseudomonas* species, proliferate during storage, particularly at room temperature [3].

Conclusion

Although our sample size was relatively small, this study shows that the water which is processed and bottled in Zimbabwe is microbiologically safe. However, it is necessary to be cautious during processing, bottling and subsequent storage of the product because any lapse in hygiene may lead to microbial proliferation.

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