Microbiological quality of packaged bottled water product sold in Ovia North East and Ovia South-West Local Government Areas of Edo State, South-South Region of Nigeria

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Abstract

Non-portable drinking water can predispose human consumers to preventable life-threatening diseases. Hence, this research evaluated the extent of contamination of commercially packaged bottled water product sold in Ovia North East and Ovia South-West Local Government Areas (LGAs) of Edo State, South-South region of Nigeria. Eight popular brands of bottled water product that were sold in these LGAs were selected for sampling. Heterotrophic plate count (HPC) was carried out on the water samples with the pour-plate technique. The most probable number technique (MPN) was used to estimate the total coliform count (TCC). Bacterial isolates were subsequently identified with phenotypic tests and 16S rRNA gene sequencing techniques. In the brands of bottled water product where bacterial colonies were isolated, mean HPC of the samples from these brands ranged from 0.25 ± 0.25 CFU/ml to 4.75 ± 2.93 CFU/ml. However, these HPC values were within recommended limits of ≤ 100 CFU/ml stipulated by the World Health Organization (WHO) and the National Agency for Food and Drug Administration and Control (NAFDAC). Values of TCC were less than 1.00 ± 0.00 MPN/100 ml in all the brands of bottled water product examined, and were, therefore, within recommended limits of 10 MPN/100 ml stipulated by WHO and NAFDAC. *Bacillus subtilis*, such as *B. subtilis* strain PDA 231 and *B. subtilis* strain NVS 11, were the main bacterial species that were isolated. Hence based on the recommended microbiological limits of WHO and NAFDAC, all the different brands of bottled water product examined were confirmed to be fit for human consumption and would not pose any adverse health effects.

Key words: *Bacillus subtilis, Portable, Diseases*

1. Introduction

Water which is regarded as one of the vital ingredients of life is also one of the commonest routes of a plethora of infectious diseases. Eighty per cent of the diseases in the tropical countries have been linked to unsafe drinking water which ultimately leads to more than 30% of preventable deaths [1, 2]. Thus, it is recommended that the quality and safety of drinking water must be assured [3]. The coliform group, particularly *Escherichia coli*, has been widely used as a microbial indicator of water quality and safety since the presence of *E. coli* in water is an indication of faecal contamination and the potential presence of intestinal pathogens [4] such as *Salmonella typhi* and *Shigella dysenteriae* that can cause typhoid fever and dysentery respectively. Hence it is necessary to purify and disinfect water before it can be certified safe as potable drinking water. Little efforts have been made by governments, especially in developing countries, in the provision of potable drinking water. Hence this has prompted the proliferation of water packaging companies to ameliorate the inadequacies of the government in fulfilling one of its social contract to the populace [5]. In Nigeria, the operations of the drinking water packaging companies are mainly regulated by the National Agency for Food and Drug Administration and Control (NAFDAC) with the Standards Organization of Nigeria also playing a complementary role [6, 7, 8].

Bottled drinking water is a term referring to water that is presumed to be processed, packaged and sold in bottles [9]. Some studies [10, 11] have negated the public perception that packaged bottled

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water may not be of high quality. Hence this study was carried on some popular brands of packaged bottled water product sold in Ovia North East and Ovia South-West Local Government Areas of Edo State South Southern Nigeria to justify public perception as regards the microbiological quality of the drinking water product.

2. Materials and Methods

2.1 *Experimental design*

Eight popular brands of bottled water product that were sold in Ovia North East and South- West Local Government Areas (LGA) were selected for sampling. They were collected in two batches, with the first batch of samples collected in September 2019 and the second batch in October 2019. One packet (12 bottled water product per packet) of each brand of the bottled water product was collected from five different localities (Iguobazuwa, Iguomo, Okada, Usen and Igbogor) in the selected LGAs. The samples collected for each batch were analyzed in an attempt to predict the degree of compliance of the manufacturers to the Standard Operational Procedure (SOP) stipulated by food regulatory agencies. The treatments which were performed include total heterotrophic plate count (HPC) and total coliform count (TCC). Levene test of homogeneity was used to deduce homoscedasticity (equality of variance) of the HPC and TCC datasets. Student's t-test was used to measure the statistical significance in the mean of the HPC and TCC datasets obtained from the bottled water samples.

2.2 *Enumeration of total coliform count (TCC)*

TCC was estimated with the most probable number (MPN) technique as previously described [12, 13]. The MPN technique includes the presumptive, confirmed, and completed tests.

2.3 *Determination of total heterotrophic plate count (HPC)*

HPC was performed with the pour-plate technique [14]. One ml portion of each of the samples was used to prepare 10-fold serial dilutions to 10^{-3} in 1.5 % W/V sterile peptone water diluents. One ml of each of the undiluted and diluted samples was transferred into a sterile Petri dish and 15 ml of sterile molten nutrient agar medium was poured into the Petri dish. The sample and agar were mixed thoroughly by rotating the plate several times and were then allowed to solidify. The Petri plates were subsequently incubated at 37°C for 48 hours. After incubation, suitable Petri plates from different dilutions were selected and the distinct colonies were counted with a colony counter. The HPC was expressed as colony-forming unit per ml (CFU/ml) of the sample.

2.4 *Genus- and species- level identification of bacterial isolates*

The phenotypic techniques employed for the genus-level identification of bacterial isolates were performed with standard methods [15]. The phenotypic tests that were performed include Gram staining, citrate utilization, urea utilization, indole production, methyl red test, Voges Proskauer test, catalase test and haemolysis test.

Species-level identification employed a technique which involved partial 16S rRNA gene analysis that was performed with polymerase chain reaction (PCR) and gene sequencing methods [16, 17]. Ultrapure DNA templates were extracted with the Zymo-Spin column as prescribed by the manufacturer (Zymo Research Corporation, USA) and used for polymerase chain reaction (PCR) and sequencing. Universal 16S rRNA bacterial primers (27F [forward primer]: AGA GTT TGA TCM TGG CTC AG; 1492R [reverse primer]: GGT TAC CTT GTT ACG ACT T) often used for bacterial taxonomy were employed.

2.5 *Statistical analysis*

Descriptive statistics of the bacterial concentration datasets was performed with NCSS ver. 12 data analysis software. Also performed with NCSS ver. 12 data analysis software was the Levene test of homogeneity and Student's t-test.

3. Results

3.1 Extent of Microbial Contamination

The HPC and TCC obtained from the two batches of samples are presented in Table 1. No bacterial colony was isolated from nutrient agar plates containing samples from brands 1, 2, 3 and 7 but was isolated in samples from brand 4, 5, 6 and 8. Mean HPC of the 10 samples from brand 4, 5, 6 and 8 ranged from 0.25 ± 0.25 CFU/ml to 4.75 ± 2.93 CFU/ml. There was an unequal variance ($P < 0.05$) between the HPC values obtained from the first batch of samples and those obtained from the second batch of samples examined. Results of the Student's t-test indicated that there was no statistically significant difference ($P > 0.05$) between the HPC values of the first batch and second batch of samples. As indicated by the TCC values, infinitesimal concentrations of total coliform bacteria (< 1.00 ± 0.00 MPN/100 ml) were probably present in all the samples examined.

Table 1: Microbial concentration in the different brands of bottled water product sold in Ovia North East and Ovia South-West LGAs of Edo State, South-South region of Nigeria

Microbial analysis									
Total Heterotrophic Plate Count (HPC) analysis					Total Coliform Count (TCC) analysis				
First batch of samples (N = 5)		Second batch of samples (N = 5)		Mean HPC (N = 10) (CFU/ml)	First batch of samples (N = 5)		Second batch of samples (N = 5)		Mean TCC (N = 10) (MPN/100 ml)
Brands of bottled water product	Bacterial counts (CFU/ml)	Brands of bottled water product	Bacterial counts (CFU/ml)		Brands of bottled water product	Coliform counts (MPN/100 ml)	Brands of bottled water product	Coliform counts (MPN/100 ml)	
1	0.00 ± 0.00	1	0.00 ± 0.00	0.00 ± 0.00	1	< 1.00 ± 0.00	1	< 1.00 ± 0.00	< 1.00 ± 0.00
2	0.00 ± 0.00	2	0.00 ± 0.00	0.00 ± 0.00	2	< 1.00 ± 0.00	2	< 1.00 ± 0.00	< 1.00 ± 0.00
3	0.00 ± 0.00	3	0.00 ± 0.00	0.00 ± 0.00	3	< 1.00 ± 0.00	3	< 1.00 ± 0.00	< 1.00 ± 0.00
4	9.50 ± 2.00	4	0.00 ± 0.00	4.75 ± 2.93	4	< 1.00 ± 0.00	4	< 1.00 ± 0.00	< 1.00 ± 0.00
5	4.00 ± 1.00	5	0.00 ± 0.00	2.00 ± 1.23	5	< 1.00 ± 0.00	5	< 1.00 ± 0.00	< 1.00 ± 0.00
6	3.00 ± 0.00	6	1.50 ± 0.50	2.25 ± 0.48	6	< 1.00 ± 0.00	6	< 1.00 ± 0.00	< 1.00 ± 0.00
7	0.00 ± 0.00	7	0.00 ± 0.00	0.00 ± 0.00	7	< 1.00 ± 0.00	7	< 1.00 ± 0.00	< 1.00 ± 0.00
8	0.50 ± 0.50	8	0.00 ± 0.00	0.25 ± 0.25	8	< 1.00 ± 0.00	8	< 1.00 ± 0.00	< 1.00 ± 0.00

N is the total number of samples examined.

Table 2: Characterization of bacteria isolated from some of the bottled water samples

Isolates	Cultural/morphological characteristics			Biochemical characteristics						16S rRNA sequence homology		Identified organisms
	Nutrient agar plates	Gram staining	Haemolysis test	Ca	Ur	Ci	In	Mr	VP	16S similarity	16S identity	
1	Mucoid colony	Positive rods	γ	+	-	+	-	-	-	100.00%	95.27%	*Bacillus subtilis*
2	Mucoid colony	Positive rods	γ	+	-	+	-	-	-	100.00%	96.13%	*Bacillus subtilis*
3	Dry colony	Positive rods	γ	+	-	+	-	-	-	100.00%	95.92%	*Bacillus subtilis*
4	Mucoid colony	Positive rods	γ	+	-	+	-	-	-	100.00%	95.64%	*Bacillus subtilis*

Ca, Ur, Ci, In, Mr and Vp indicate catalase, urease, citrate, indole, methyl red and Voges Proskauer test respectively; γ indicates gamma none haemolytic bacterial isolates.

Figure 1: Some partial 16S rRNA gene sequences obtained from the bacterial strains isolated from some brands of the bottled water product

3.2 Characterization of Microbial Isolates

The identity of the bacterial isolates obtained from some brands of the bottled water product is presented in Table 2. All the bacterial isolates obtained from some brands of bottled water product were identified as Bacillus species via the phenotypic analysis. Haemolysis test performed on all the Bacillus species, which is often used to detect the probable production of haemolysin toxin, showed that the isolated Bacillus species were gamma (none) haemolytic. Sequence analysis identified all the bacterial isolates as Bacillus subtilis. As shown in Figure 1, GenBank accession numbers for representative B. subtilis strain PDA 231 and B. subtilis strain NVS 11 isolated from the bottled water product were MN198140 and MN197861.

4. Discussion

Findings from studies conducted by the World Health Organization [18] indicated that 88 % of global diarrhoeal disease burden was attributed to unsafe water supply, sanitation, and hygiene. In the present study, all the samples from the different brands of bottled water product had TCC values (Table 1) that were within the limits (≤ 10 MPN/100 ml) stipulated by the World Health Organization, European Federation of Bottled Waters and the National Agency for Food and Drug Administration and Control (NAFDAC). The present findings agreed with the previous work carried out by Adesiji [10], but were at variance with the work of Ogundipe et al. [11] who reported a total coliform count ranging from 3 to > 1100 MPN/100 ml in the bottled water product that were examined. The presence of coliforms within the recommended limits (TCC ≤ 10 MPN/100 ml) in the examined brands of bottled water sold in Ovia North East and Ovia South-West LGAs (Table 1) suggests that these brands of bottled water product are of better microbiological quality and suitable for human consumption.

The presence of indicator coliform organisms indicates that water is contaminated by potentially dangerous faecal matter and hence their absence de-

notes that the water is generally safe for consumption [19]. 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 the production of quality and safely packaged drinking waters [20].

The presence of coliform bacteria within recommended limits in the brands of bottled water product examined in this study could be attributed to more stringent compliance to Standard Operational Procedures (SOP) stipulated by the regulatory agency during the production of the bottled water product. These include use of protective sealed caps on bottles, improved hygienic filling system and the use of non-returnable plastic containers [21].

In contrast to the findings in the present study (Table 1), Ogundipe et al. [11] reported HPC values of 250 CFU/ml which exceeds recommended limits (≤ 100 CFU/ml) in some of the bottled water product that they examined.

According to the WHO 2002 report [12], 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 [22]. However based on the recommended standard limits of ≤ 100 CFU per ml of drinking water, all the examined brands of bottled water product sold in Ovia North East and Ovia South-West LGAs were considered fit for human consumption.

Bacillus subtilis was the main bacterial species that were isolated from the brands of bottled water product that were examined (Table 2). Previous studies have also reported the presence of Bacillus species in packaged drinking water [10, 12, 22]. The presence of the Bacillus species in some of the brands of bottled water product that were examined could be largely attributed to imperfect purification procedures during the processing of the source water.

5. Conclusion

The different brands of bottled water product examined in this study had HPC and TCC values that were within the limits set by international and national regulatory authorities. Hence from the microbiological point of view, it appeared that stricter adherence to Good Manufacturing Practice (GMP) was implemented during the production of these different brands of bottled water product sold in Ovia North East and Ovia South-West LGAs. Thus, they were confirmed to be fit for human consumption and would pose no adverse health effects.

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