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Bacterial Contamination of Potable Processed, Packaged, And Commercialized Water in Parts of Kaduna Metropolis, Nigeria

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Abstract

A study was conducted to isolate and characterize *Salmonella typhi* and *Vibrio cholerae* from three (3) types of packaged commercial water (sachet, bottle and dispenser jar) in Kaduna metropolis. Membrane filtration technique was employed in screening for viable pathogenic bacteria (*Salmonella typhi* and *Vibrio cholerae*); coliform indicator (*Escherichia coli*) and the opportunistic pathogenic bacteria (*Pseudomonas* sp.). A total of 270 samples from 90 brands were collected randomly comprising of 171, 69 and 30 of sachet, bottle and dispenser jar using a patronage ratio of 6:2:1 of the three types of packaged water by consumers. *Salmonella typhi* and *Vibrio cholerae* appeared more with the prevalence of 11(15.9%) and 9(13.1%) of the 69 bottles; followed by 3(10.0%) and 2(6.7%) in dispenser jars; then 15(8.8%) and 5(2.9%) in sachet samples respectively. The probability of a consumer likely picking a bacteria contaminated water from sachet, bottle and dispenser jar was found to be 0.117, 0.290 and 0.166 respectively. There was no significant difference in level of contamination at $p \geq 0.01$ (99%) for all the three types of packaged water. Molecular confirmation of cholera toxigenic gene (CtxA) in *Vibrio cholerae* isolates is suggestive that virulent strains were present and clinical infection is possible for consumers of the contaminated water. In conclusion, there is therefore an equal possibility of contracting food-borne bacterial infection and intoxication without any relative safety advantage between the three different packaged brands. Manufacturers of the water might need to reinvigorate and improve their method of processing to ensure almost complete sterility in the end product as the presence of even one bacterial pathogen may adversely affect the health of consumers of the water. This development could spell negative consequences for the manufacturers of the product, as consumers might be scared of consuming their products.

Keywords: Salmonella tyhi, Vibrio cholerae, Pseudomonas sp. Escherichia coli

INTRODUCTION

Next to air, water is no doubt the most essential element to human life as the body cannot survive longer than several days without it. Not only that water is essential to every single cell and organ in the body, it makes up two third of the human body weight. Given its importance, the need to ensure that every individual gets access to safe water cannot be over emphasized (Ameto, 2011).

The insalubrious situation and scarcity of water in many Nigerian cosmopolitan cities has made the quest for potable water indispensable. Potable water is thought to have been treated to eliminate most contaminants (infectious and toxigenic substances) in it and is regarded as safe for human consumption.

Over the years, consumption of packaged water commonly known as “pure water” has increased substantially in Nigeria. A common perception is that packaged water is safe for consumption. However, the microbiological quality of some packaged water has been questioned with reported cases of presence of fecal indicator and heterotrophic bacteria with levels exceeding drinking water guidelines (Ahmed *el al*., 2013).

Kaduna as one of such cosmopolitan cities in Nigeria has witnessed a boost in growth in the number of water treatment industries today. Many of whom process and supply commercialized packaged water to interested consumers under different brand names. The purified water is commonly sold out in three major packaged forms in sachets, bottles and dispenser jars. All three packaged types are subject to certification after meeting all the minimum safety standards for potable water by the regulatory authorities, National Agency for Food and Drug Administration and Control (NAFDAC) before sales to the public.

In bacteriological testing of the potability of processed, packaged water, NAFDAC employs the detection of total coliforms especially *Escherichia coli* that are present in relatively larger numbers in the faeces of humans which are easier and quicker to detect/or count than the pathogenic bacteria as an indicator of contamination (Madigan *et al*., 2009; Brown *et al.,* 2012).

Waterborne illnesses are caused by various bacteria, viruses, protozoa and other pathogenic microorganisms that usually occur due to ingestion of untreated and poorly treated drinking water and or disposal of waste water or natural disaster like flooding and environmental pollution that contaminate sources of drinking water. Some of the common water borne diseases in Nigeria include; typhoid, cholera, hepatitis and dracunculiasis (Yusuf *et al.,* 2014). Typhoid fever and cholera are illnesses caused by the bacteria *Salmonella typhi* and *Vibrio cholerae,* respectively.

Salmonella bacteria are facultative intracellular pathogens which are contacted by ingestion of contaminated water and food. They are divided into two groups: Typhoidal and non – typhoidal *Salmonella* serovars. Non–typhoidal serovars are more common and usually cause self – limiting gastro intestinal disease. They can infect a range of animals and are zoonotic while the typhoidal salmonellosis is caused by *Salmonella enterica* serovar Typhi and Paratyphi A, B and C which are adapted to humans and do not occur in other animals (Jantsch *et al*., 2011).

Epidemiological data in Kaduna between 1990 and 1992 reported by Abbas (1994) and cited by Denwe (2005), suggested an upsurge of typhoid incidences (hyperendemicity) in the Kaduna metropolis.

Cholera is an infectious disease of the gastrointestinal tract, caused by drinking contaminated water and or food with *Vibrio cholerae* (CDC, 2013). It is potentially epidemic and life threatening, characterized by numerous secretory diarrhea,

voluminous "rice-water" stool, often accompanied by vomiting and culminating in hypovolemic shock and acidosis (Finkelstein, 1996; Pelczar *et al*., 2005).

Epidemiological data from The United Nations Children's Fund (UNICEF, 2004) factsheet between 2004 and 2014 reported cholera outbreaks in Kaduna with 9,520 cases, 170 deaths and case fatality rate (CFR) of 1.8%.

Cholera is a major public health concern because of its high transmissibility, death – to – case ratio and ability to occur in epidemic and pandemic magnitudes (Riyan, 2004). Several incidences of outbreaks of cholera have been documented in parts of Nigeria (Adagbada *et al.*, 2012). The largest outbreaks were reported in the northern states of Nigeria (UNICEF, 2014).

Moreso, enteric fever has also continued to pose serious threat to public health especially in economically poor countries where level of hygiene is low (Okome and Ayo, 2000).

The present study intended to compare the degree of potability of the three kinds of packaged water by specifically investigating the presence of some bacteria pathogens using Thiosulphate bile salt sucrose (TCBS) agar, Salmonella-Shigella agar (SSA), Eosin methylene blue (EMB) agar and Pseudomonas agar (PA) for isolation of *Vibrio* sp. *Salmonella* sp. *Escherichia coli, and Pseudomonas* sp. respectively. The investigation was to note the relative abundance of the four genera of bacteria as an index of relative safety (potability) between the three types of packaged water.

The present study adopted the use of membrane filtration technique to capture any available viable food-borne pathogen or indicator bacteria in the water sample. The finding could give a better overview of the safety standard of the packaged water as no bacteria could escape through the membrane with 0.45µm pore size unlike the routine method used by NAFDAC.

MATERIALS AND METHODS

Sample collection

Samples were collected randomly at various retail points of Kaduna metropolis. Sampled sachet and bottled water were stored and transported in ice boxes (Anwar, 2013). The water samples were collected at the ratio of 6: 2: 1 for sachets, plastic bottle and dispenser jars respectively based on their patronage by the public.

Isolation of *S. typhi* and *V. cholerae*

Membrane filtration method was employed where the membrane filtration unit was assembled aseptically by inserting the filter holder base into the neck of a one litre side armed flask. Then, 0.45µm pore sized nitrocellulose membrane filter was inserted into the filter holder base with a flamed sterilized forceps. A filter funnel was placed on top of the filter disk and clamped carefully to the base. Using a calibrated graduated measuring cylinder, a volume of 100ml of the sample water was filtered through the funnel (Brown, 2012). The filter membrane was transferred with a sterile forceps into already prepared culture media, (Salmonella-Shigella Agar (SSA) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar) for isolation of *Salmonella* sp. and *Vibrio* sp. respectively. Eosin methylene Blue (EMB) and Pseudomonas agar (PA) for selective isolation of *Escherichia coli* and *Pseudomonas* sp. Plates were incubated at 35ºC for 48 hours. Discrete suspected colonies that appeared colorless on SSA and yellow on TCBS were picked for further characterization of salmonellae and vibros respectively. *Pseudomonas spp.* was greenish on PA, while *E. coli* exhibited greenish metallic sheen on EMB agar (Momta *et al*., 2013).

Gram staining

Gram staining of bacteria isolates from each positive plate was conducted according to the method of Cheesbrough (2006). Bacteria cells that appeared blue-purple were read Gram positive, while those that appeared reddish were the Gram negatives. All the Gram negative rod-shaped bacilli were biochemically screened for all four genera of bacteria (salmonellae, Vibrios, *Escherichia* and *Pseudomonas)*

Biochemical characterization of suspected gram negative bacteria isolates.

Suspect bacteria isolates that were gram negative and phenotypically consistent with any of the two pathogenic genera of bacteria in the selective media were further characterized biochemically using Triple Sugar Iron (TSI) agar, Indole, Oxidase and Methyl red tests.

Molecular confirmation of presumptive *S. typhi* and *V. cholerae* isolates

DNA Extraction and Amplification

Extraction and purification of DNA directly from bacteria was carried out using standard protocol (Qiagen USA, 2016).

PCR (Polymerase Chain Reaction) for *Salmonella typhi*

PCR was carried out in 20µL containing 16µL premix (Taq polymerase, dNTP, $MgCl_2$, PCR buffer and distilled H_2O), 2µL prepared sample (template), 1µL of primer 1 and 1µL of primer 2. The reaction procedure consisted of 35cycles of predenaturation at 94˚C for 5minutes, denaturation at 94˚C for 30seconds, primer annealing at 54˚C for 30seconds, extension at 72˚C for 1minute and final extension at 72˚C for 5minutes. Negative control reaction mixture contained sterile distilled water (DW) in place of template DNA and was subjected to the protocols consistent with the DNA extraction described above.

Primers utilized for *S. typhi* was:

Salmo (InVA) F 5' CGA GCA GCC GCT TAG TAT TGA G 3'

R 5' CCA TCA AAT TAG CGG AGG CTT C 3' (Kumar *et al.,* 2006).

PCR for *Vibrio cholerae*

PCR was carried out in 20µL reaction containing 16µL premix, 2µL prepared sample (template), 1µL primer 1 and 1µL primer 2. An initial denaturation was set at 94˚C for five minutes, followed by 30 amplification cycle denaturation for 1 minute at 94˚C, annealing for 1minute at 51˚C, extension for 30 seconds at 72˚C and the final extension for seven minutes at 72˚C. Negative control reaction mixture contained sterile distilled water (DW) in place of template DNA and was subjected to the protocols of DNA extraction above.

Primer utilized for *V. cholerae* was:

CtxA F 5' CAA ATG ATG ATA AGT TAT ATC GG 3'

R 5' GAC CAG ACG ACA ATA TAG TTT GAC C 3' (Behnaz *et al.,* 2014).

Visualization of Amplicon (Agarose gel electrophoresis)

Amplified samples were visualized using agarose gel of 1.5% with 5µL ethidium bromide, covered with Tri-acetate EDTA (TAE) buffer, operated at 100V and molecular weight DNA marker with 100bp increment (100bp ladder, Qiagen, USA, 2016) was used as size standard for both organisms. DNA fragment bands after electrophoresis were visualized under Ultra-Violet (UV) light on a transilluminator and photographed by image documentation system (laboratory Germany).

RESULTS

Bacterial contamination of packaged commercial water samples in Kaduna metropolis.

From Table 1: All three brands of packaged commercial water recorded the two pathogenic bacteria, *Salmonella* sp. and *Vibrio* sp. *Escherichia coli* and *Psuedomonas* sp. were also isolated from them. Plastic bottled samples recorded the highest number of suspected *Vibrio* sp. with 33.3% whereas dispenser jar recorded the highest number of suspected *Salmonella* sp. bacteria with 46.7%. From the cumulative totals, *Salmonella* sp. showed the highest number with 26.3% from the 3 packaging types followed by *Vibrio* sp. with 19.6%. Except for *Vibrio* sp. all the other three bacteria *Salmonella* sp. *Escherichia coli and Pseudomonas* sp. appeared more in dispenser with 46.7%, 10.0% and 40.0% respectively, as against their lowest in sachet water with 14.6%, 22.2% and 5.3% respectively.

Table 1: Bacteria isolates from each of the three different Packaged commercial water Sampled from 4 L.G.As in Kaduna metropolis.

Package type	No. of brands sampled	Quantity sampled	← Media and no. of bacteria isolated →			
			TCBS	SSA	EMBA	PA
Sachet	57	171	25(14.6%)	38(22.2%)	5(2.9%)	9(5.3%)
Bottle	23	69	23(33.3%)	19(27.5%)	5(7.2%)	4(5.8%)
Dispenser jar	10	30	5(16.7%)	14(46.9%)	3(10%)	12(40%)
Total	90	270	53(19.6%)	71(26.3%)	13(4.8%)	25(9.3%)

Key: TCBS - Thiosulphate Citrate bile salt sucrose agar, SSA – Salmonella-Shigella
EMBA – Eosin methylene Blue Agar, PA – Pseudomonas Agar

Table 3: Phenotypic And Biochemical Characteristics Of Bacteria Isolated from The 3 Commercially Package Water Samples

Cultural and Biochemical Characteristics	Appearance and probable bacteria isolates	
	A	B
Colony Color	colorless on SSA	ye
Gram's Stain	-ve	
Cell Shape	rod	
Oxidase	-ve	
Methyl Red	+ve	
Indole	-ve	
Sugar Fermentation		
Glucose	+ve	
Lactose	-ve	
Sucrose	-ve	
H_2S Production	+ve	
Gas	-ve	
Probable bacteria	*Salmonella typhi*	*Vi*

Key: + positive, - Negative, SSA - Salmonella-Shigella Agar TCBS - Thiosulphate Citrate Bile Salt Sucrose Agar

Prevalence of Salmonella typhi and Vibrio cholerae between the three different packaged commercial water in Kaduna metropolis

From Table 4: *Salmonella typhi* and *Vibrio cholerae* appeared more with a prevalence of 11(15.9%) and 9(13.1%) in bottle water; followed by 3(10.0%) and 2(6.7%) in dispenser jars, then 15(8.8%) and 5(2.9%) in sachet samples respectively. Out of 90 brands sampled from different manufacturers, 31(34.5%) recorded either *S. typhi* or *Vibrio cholerae.* Four brands recorded both *S. typhi* and *V. cholerae;* three bottled (B) samples (052, 053 and 054) and one dispenser jar (D) sample (083) as against 18(20.0%) with *S. typhi* (thirteen of the sachet, four bottled and one dispenser jar), then 9(10.0%) with *V. cholerae* (four of the sachet, four bottled and one dispenser jar) only.

TABLE 4: Percentages and proportion of molecularly confirmed bacteria isolates from sampled packaged commercial water.

Package Type	No. of Samples	Bacteria isolated *S. typhi*	*V. cholarae*	Total Bacteria Present	Proportion (ratio) of Contaminated Water
Sachet	171	15(8.8%)	5(2.9%)	20(11.7%)	0.117
Bottle	69	11(15.9%)	9(13.1%)	20(29.0%)	0.29
Dispenser	30	3(10.0%)	2(6.7%)	5(16.7%)	0.166
Total	270	29(10.7%)	16(5.9%)	45(16.7%)	0.166

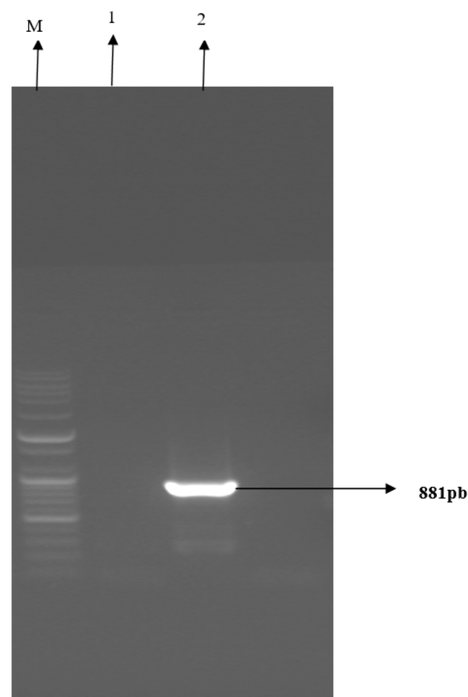

Figure 1: Electrophoresis of PCR product of *Salmonella typhi* at 881bp

Key: M - DNA marker of 100bp increment, 1 - Negative control, 2 - Isolated with 881 bp gene band for *Salmonella typhi*

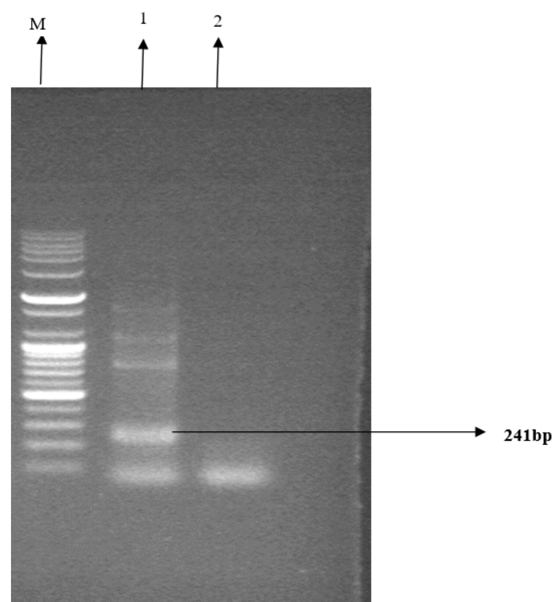

Figure 2: Electrophoresis of PCR product of *Vibro cholerae* at 241bp

Key: M - DNA marker of 100bp increment, 1 - Isolate with 241 bp gene bands for *Vibro cholerae,* 2 - Negative control.

DISCUSSION AND CONCLUSION

Since all the three brands of packaged water were contaminated with both *S. typhi* and *V. cholerae,* the bacterial purity of all the three processed commercial packaged water is questionable. Hence, package type (sachet, bottle and dispenser jar) is inconsequential to bacterial potability of the water content. The cumulative prevalence of 53(19.6%) for *Vibrio cholerae* and 71(26.3%) for *Salmonella typhi* are both higher than those of the fecal coliform *Escherichia coli* with 13(4.8%) and the opportunistic pathogenic bacteria *Pseudomonas* sp. with

25(9.3%) (Table 1) that are routinely used to test the quality of drinking water (Madigan *et al.,* 2009). The probable high occurance incidences of *Vibrio* sp. and *Salmonella* sp. might not be unconnected with the use of more than one selective differential medium, Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar and Salmonella-Shigella agar (SSA), respectively. The collaborative use of membrane filter of 0.45µm pore size that would not have allowed the escape of any viable bacteria in the water sample, might have added to the higher chances of isolating the bacteria.

The relatively large quantity of 100ml of water per sample filtered through the membrane filter also increased the chances of capturing viable available bacteria than with the direct plating method that uses 1ml only. The finding concurs with that of Momtaz *et al. (*2013) that used the same filtration method, but differs from that of Ugochukwu *et al.* (2015) who used direct method of inoculation practiced by NAFDAC. The occurrence of viable coliform and specific pathogens is indicative that some of the water samples have some level of contamination before ingestion by consumers.

In the findings, the sachet water harbored less bacteria with 15(8.8%) of *Salmonella typhi* and 5(2.9%) of *Vibrio cholerae* than the plastic bottles and dispenser jars that had 11(15.9%), 3(10.0%) and 9(13.1%), 2(6.7%) of *Salmonella typhi* and *Vibrio cholerae* respectively (Table 4). The impression that packaged water in sachet as compared to bottle and dispenser jars is less purified and hence inferior to the two others has been called to question from the present work. Despite, the fact that more samples were collected from sachet water; the former was found to be at par with the latter bacteriologically (Tables 1 and 4). There was no significant difference in occurance 0f *S. typhi* and *V. cholerae* at P> 0.01(99%) for all the three packaging types connoting that there is no relative advantage of one brand of packaged water (sachet, bottled and dispenser jar) over another (Table 2). The probability of drinking sachet, bottled and dispenser jar water contaminated with any two of the pathogenic bacteria is almost the same (Table 4). Signifying that there is an equal possibility of contracting foodborne bacterial infection and intoxication without relative safety advantage between the three different packaged types. The prevalence of *Salmonella typhi* 29 (10.7%) in all the three different commercial packaged water were higher than *Vibrio cholerae* with 16(5.9%) implying higher chances of contracting enteric fever than cholera (Table 4).

More occurrence of *V. cholerae* and *S. typhi* in the water samples could be indicative of some health risk of infection. However, the size of the inoculum ingested, the source of the bacteria and the prevailing condition in the gastrointestinal tract could influence the success of an infection. One with a normal gastric acidity might be infected when exposed to a higher infection dose of about 10^{10} *V. cholerae* when water is the source of the bacteria. But with other foods that might neutralize the acidity in the gut as the bacterial source, lower inoculums of between 10^{2}-10^{9} *V. cholerae* might suffice to initiate an infection. Circumstances that decrease stomach acidity predispose consumers of contaminated water to *V. cholerae* that attach to the microvilli, multiply and elaborate toxigenic substances that cause cholera disease (Brooks et *al*., 2007).

The presence of viable *S. typhi* and *V. cholerae* has called to question the safety standards of all three brands of packaged water sold in Kaduna Metropolis. The preponderance of the pathogenic *Salmonella typhi* and *Vibrio cholerae* in all three (3) types of commercial packaged water (sachet, bottle and dispenser jar) is suggestive that the likelihood of food-borne infection by bacteria is implicative. Especially in immune compromised consumers such as the infants, geriatrics and HIV patients. The secretion of immunoglobin A (IgA) antibodies in the mucosal fluid of the digestive tract could aid to abort colonization of pathogenic bacteria among consumers of the contaminated water.

The routine microbial test of water quality that is dependent on the absence of coliform indicator bacteria might not be exhaustive enough as a means of guarantying bacteria water purity. There is also the risk of cholera disease outbreak due to the isolated bacterial pathogens especially with the *Vibrio* bacteria that harbor toxigenic genes that could make them virulent. The danger is even more worrisome considering the fact that most of these commercial packaged water have NAFDAC certification of safety; the only regulatory body mandated to certify the safety of drugs and foods processed and sold in Nigeria. Packaging type does not therefore appear to have any significant relative advantage in terms of bacterial contamination and safety level of the processed, commercialized water in Kaduna metropolis.

Recommendations

i. The regulatory agency NAFDAC should adopt appropriate standard procedures- membrane filtration and varietal selective media to diversify and meet the minimum safety standards for certification of drinking water before sales to the public.
ii. The inspectorate unit of the regulatory agency should routinely embark on impromptu checks by procuring samples from the open market without the consent of the producers at regular intervals to ensure consistency in potability of finished products.

There is the need to legislate and enforce laws that could compel commercial packaged water factories with recurring cases of contamination by harboring pathogenic bacteria to suspend production

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