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Assessment of the microbial contaminant of water bottles used by primary school pupils in Amai, Ukwuani L.G.A, Delta State

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Abstract

The study was aimed at identifying and isolating bacterial and fungal from bottle water of school children in Amai community. Samples for microbiological investigation were collected randomly with a swab stick from bottle water of some school children. The isolated bacterial and fungal was confirmed and enumerated using biochemical test and colony counter expressed in colony forming unit (CFU/ml). The result showed the presence of *Staphylococcus aureus, Citrobacter sp, Pseudomonas sp, Klebsiella sp,E.coli, Salmonella sp* and *Proteus* sp as bacterial isolates while *Aspergilus flavus*, *Aspergilus niger* and *Penicillum* sp were the fungal isolates respectively. The frequency of bacterial isolates ranged from $1.0x10^2$ to $2.0x10^2$cfu/ml with *Staphylococcus aureus* and *Citrobacter* as the most predominant while the frequency of fungal isolate ranged from $1.0x10^2$ to 2.10^2cfu/ml with *Aspergilus flavus* as the most predominant. The study has shown that bacteria and fungi can be isolated from water bottles of school children in Amai community schools. This is a great public health concern because it poses health threat to the children if their water bottle is not properly washed.

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Introduction

Bottled water is a potable water that is bottled and distributed for sale and specifically intended for human consumption (Osei, 2005). It is assumed that ‘’environmental realities will soon force us to change our resource-costly behaviour”. These changes must include modifications in both quantity and ways in which we use or drink water on a daily basis.

The privatization of water resources and selling of water bottle has raised serious concern in western cultures about the utilization of, and linked to the use of water bottle containers. It has been reported that bacteria will grow in bottle water. A population of approximately 10^2 to 10^5 colony forming unit per ml (cfu/ml) was formed in the mineral water after bottling (WHO, 2016) . Despite worldwide efforts and the modern technology employed for production of safe drinking water, transmission of water borne diseases is still a matter of major concern. Some common food borne organisms associated with water include *Campylobacter*, *E. coli* 0157:H7, *Salmonella*, and’ *Vibrio cholera*, to name a few (WHO, 2016). These can lead to severe illness and death. Clearly there are health implications associated with unclean water consumption. Safe drinking water has always been one of the primary requirements for healthy and sustainable human life. Water is used every day by every single person.

However, World Health Organization (WHO, 2006) reported that 884 million people do not have access to safe drinking water, and 2.2 million deaths (mainly children) are attributed to diarrhoea, which is transmitted through contaminated water, inadequate sanitation or hygiene. One of the biggest threats to public health is caused by bacteria pathogen in drinking water, which lead to the outbreaks of diseases such as giardiasis, gastroenteritis, cholera, cryptosporidiosis etc., having access to safe drinking water is essential to health. This study was aimed at assessing the microbial contaminant of water bottles used by primary school pupils in Amai, Ukwuani L.G.A, Delta State

Study Area: This study was carried out in Amai, is located in Ukwani local Government Area of Delta state. It is divided into five quarters namely; Umuosele, Ishikaguma, Umuekum, Umubu and Amai Nge. The major occupation of the indigenes is mainly

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farming, their main source of water is hand dug well and boreholes.

Sample Collection: Sterile swabs were used to collect samples randomly directly from the cork and inner walls of the water bottles of some students at primary schools in Amai, Delta state. The samples were then transported to the laboratory at the department of biological sciences (Microbiological laboratory), Novena University Ogume, Delta state for microbiological examination.

Culture Media for Isolation of Microorganisms: Commercially prepared sobouraud agar was used for isolation of fungi. Similarly, Mannitol salt agar, MacConkey agar (MA), Blood agar (BA), *Salmonella-Shigella* agar was used for isolation of bacteria. Nutrient agar was used for total heterophic bacteria counts. All the media were prepared according to the manufacturer's instruction.

Preparation Of Culture Media For Isolation Of Bacteria And Fungi: The recommended quality of all culture media was weighed into conical flask and the appropriate quantity of distilled water based on manufacturer's instructions was added to it. The substance was boiled to completely dissolve the agar. All the media were sterilized by autoclaving at 121°C at a pressure of 15 pound for 15minutes. After sterilization, 15ml of each medium were poured into sterile petri dishes and allowed to solidify. The petri dishes were labelled accordingly.

Identification of Fungi: Fungi isolates were identified based on characteristics. The macroscopic characteristics such as shape, pigment, and textures of colonies on sabouraud agar plates were examined. On the reversed, microscopic characteristics such as the vegetative and reproductive structures were studied, little portion of the colony was picked using sterilized inoculating loop, placed on the lacto phenol cotton blue, gently covered with cover slip and viewed under the microscope objective as described by (Samson *et al.*, 2004).

Isolation, Enumeration and Dentification of Bacteria

The sample (swab) was inoculated directly onto Nutrient agar (NA) and Blood agar (BA). The plates were incubated at 37°C for 24 hours. The number of colonies were counted using a colony counter and recorded as colony forming unit per gram (cfu/g) for swabs.

Serial Dilution

The culture was taken in a test tube and six test tubes, each with 9ml of sterile diluents. A sterile pipette is taken 1ml of culture were properly mixed and drawn into the pipette. The sample were then added into the first tube to make the total volume of 10ml and provide the initial dilution of 10'1. The dilution was thoroughly mixed by emptying and filling the pipette several times. The pipette tip was discarded, and a new pipette tip were attached to the pipette. 1ml of mixture was taken from the 10^{-1} dilution and was emptied into the second tube. The second tube has a total dilution factor of 10^{-2}. the same process were then repeated for the remaining tubes, 1ml was taking from the previous tube and adding it to the next 9ml diluents. As six tubes are used, the final dilution for the bacteria cell will be 10^{-6}

Result

Table 1: Cultural and Morphological Characteristics of Fungal Isolates

	1	2	3
Cultural Characteristic	yellow colony with reverse Side yellow	colonies were green on the upper surface White border and brown on the reverse side	Black colony with reverse side yellow
Microscopic Characteristic			
Nature of hyphae	Septate	Septate	Round Shape
Type of Spore	Conidiophores	Conidiophores	Conidiophores
Appearance of special Structure	Foot cells	Foot cells	Foot cells
Color of Spore	Brown	Yellow	Green
Possible Isolates	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium* sp

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Table 1 shows the cultural and morphological characterisitics of fungal isolated from water bottles of school children from Amai community schools. The possible isolates were *Aspergillus niger* , *Aspergillus flavus* and *Penicillium* species

Table 2. Characterization and identification of Bacteria Isolates

Test	**Isolate A**	**Isolate B**	**Isolate C**	**Isolate D**	**Isolate E**	**Isolate F**
Colonial morphology	Milk white round colonies with yellow pigment	Large cream white round colonies with smooth edge	Slightly raised cream round colonies		Raised	pink
Gram reaction	+	+	+		-	-
Shape	Cocci in clusters	Cocci in chains	Rod		Rod	Rod
Citrate test	-	-	+		-	+
Motility test	-	-	+		-	-
Catalase test	+	-	+			
Coagulase test	+	-	-			
Oxidase test	+	-	+			+
Spore	-	-	+			
Lactose	-	+	+		+	+
Indole test	-	-	+			
Mannitol test	+	-	+			
Organisms isolated	*Staphylococcus aureus*	*Klebsiella sp.*	*Pseudomonas* sp. *Citrobacter* sp *E.coli Samonella* sp *Proteus* sp			

Table 2 shows the cultural and morphological characteristics of bacterial isolated from water bottles samples from school children from Amai community school. The possible isolates were *Staphylococcus aureus, Klebsiella sp, Pseudomonas* sp, *Citrobacter* sp,, *E.coli, Samonella* sp, and *Proteus* sp,

Table 3: Frequency of Bacteria Isolates from the water Bottle

Bacteria Isolates	Water Bottle Inside wall	Cork	NO (cfu/ml)
Staphylococcus aureus	+	+	2
Escherichia coli	-	+	1
Citrobacter sp	+	+	2
Klebsiella sp	-	+	1
Proteus sp	+	-	1
Salmonella sp	-	+	1
Acinetobacter sp	+	-	1
Pseudomonas sp	-	+	1
TOTAL	4	6	10

Table 3 shows the frequency of ten (10) different bacteria isolated from water bottles of school children from community schools in Amai. *Staphylococcus aureus* and *Citrobacter* sp had the highest number of occurrences followed by other isolates with just one occurrence each.

Key: + =Present, – = Absent, NO_B =Number of occurrences of bacterial isolate

CFU/Ml=colony forming unit per mililiter

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Table:4 **Frequency of Fungal Isolated from water bottle**

Fungi Species	Water Bottle		NO(CFU/ml)
	Inside wall	Cork	
Aspergilus niger	-	+	1
Aspergilus flavus	+	+	2
Penicillium sp	+	+	2
TOTAL	2	3	5

Key: + = Present , - = absent, NO_F = number of occurrence of fungal isolate
CFU/ml=colony forming unit per mililiter

Table 4 shows the total frequency of occurrence of fungal isolates from water bottle of school children in Amai was five (5). *Aspergilus flavus* and *Penicillium* sp had two each proving to be predominant isolates.

Discussion

Provision of safe drinking water is one of the most essential amenities to be made available for citizens in the modern world. Particularly, for long-distance travelers who need to be extra careful of their health but do not have other options, depend on packaged water sources. The cultural and morphological characteristics of fungal isolated from water bottles of school children in Amai has been studied. The findings in table 1 and 2 suggest that significant fungal and bacterial contamination can occur in individual water bottle . The results in this study is in consonance with the previous results of (Osei-Tutu and Anto, 2016) who isolated and identified some fungal and bacteria using standard mycological and bacteriological methods. In another research done by (WHO, 2016) also concluded that fungal and bacterial can be isolated from water bottle due to the presence of water as a source of fungal and enteric bacteria growth. Inadequate and proper hand washing after students have used the bathroom facilities could result in fecal coliforms and therefore cause fecal transmission from the environment to the student water bottles. Provision of safe drinking water is one of the most essential amenities to be made available for citizens in the modern world. Particularly, for long-distance travelers who need to be extra careful of their health but do not have other options, depend on these packaged water sources. Therefore, it is a matter of concern that only about two-thirds of the bottled water tested was suitable for drinking in the present study. This was similar to the findings of another study done in Mangalore in 2002 which reported 66.7% of the sampled bottled water safe for consumption [WHO, 2014]. This meant that the situation of hygienic status of bottled water available in this city has not shown any improvement with time. It could be because of the reason that, this issue was not given priority as much as other public health issues concerning this city. In other studies done in India, the acceptability of bottled water ranged from [WHO, 2014a]. In studies done in other parts of Asia, the acceptability of bottled miner. In a study done in different parts of North India, around 2% of the samples tested had bacterial counts of more than 1000 CFU/ml [WHO, 2015b. The contamination level of water samples reported in these above-mentioned studies was therefore much more than our observations. However, another study done in Chennai, India, reported that bacterial counts ranged from 0 to 41 CFU/ml among all the water samples tested, which was much lesser than that observed in the present study [WHO, 2016]. The presence of heterotrophic bacteria in the bottled water causes significant health risk particularly for children, elderly, and immunocompromised individuals [WHO, 2015]. Its presence in bottled water is also an indicator of poor practices involved in the manufacturing processes.

The kind of bacteria found in the bottled water has previously been reported to have multiple drug resistance in samples collected from different parts of India. Safety of bottled drinking water can be ensured with sealed caps on bottles, hygienic filling systems, the minimal time between production and sale, and use of nonreturnable plastic containers. It was observed in a Nigerian study that contamination of packaged water aggravates as the product moves down the distribution chain [Osei-Tutu and Anto, 2016]. Assessment of water quality is therefore required not only at various stages of production but also in postproduction stage . This will ensure improvement in transportation and storage practices in the supply chain. Government and other stakeholders need to intensify surveillance activities of water treatment processes at packaged water industries. This will ensure that strict hygienic measures are followed, resulting in safe and quality bottled water being available at various retail outlets for public use.

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Study done in Telangana, India, all the bottled water samples were colorless and had no objectionable odor and taste [Parashar *et al.*, 1999].

The batch number, period of manufacture, and period of expiry were not mentioned on three bottles, all of which were manufactured by local companies. A Nigerian study also reported that none of the bottled water brands had mentioned the batch number (Parashar *et al.*, 1999). Batch number is very essential for any manufactured product. In the event of discovery of any abnormality, with the help of the batch number, the entire lot the product can be identified and recalled from the market by the company (Parashar *et al.*, 1999)

However, all the bottle water samples in the latter study had mentioned manufacturing and expiry dates, unlike our observations (WHO, 2015). All the samples without batch number, manufacture date, and expiry date were found unacceptable for drinking in the present study. Therefore, public enlightenment on particulars which they need to look out for on the package label before purchasing bottled water is essential. The local companies that manufacture products without complete label need to be questioned on these issues.

Moreover, samples of locally manufactured bottled water were found unfit for consumption in this study. Springing up of several small-scale entrepreneurs engaging in the production of mineral water, without due regard to hygienic practices, has resulted in Mangalore. This might be due to the high demand of water as a consequence of the hot and humid weather seen mostly at this place. Packaged water manufactured by these regional companies may lack the guarantee to meet the set standards for drinking water quality. Therefore, identification of all local companies involved in its production, licensing, and renewal of licensing of these companies, by concerned authorities, is required in order to safeguard the health of the consumers (WHO 2012). The occurrence of positive cases of diarrhea-related diseases from

Table 1,2 and 3 indicates that diarrhea-related diseases is still a public health challenge in Amai. The highest number of positive cases came from community primary while the lowest number of positive cases were recorded for residents coming from nearby villages with clean and potable water supply. The socio-economic status of a locality may influence the occurrence of the disease, with affluent areas expected to have less cases Understandably, poorer areas may have little sanitary infrastructure and less hygienic conditions for drinking water or food preparation (Motarjemi *et al.*,1993). Personal hygiene of individuals is also likely to improve with better standard of living. This may explain the lower number of positive cases coming from Amai schools and its environ as opposed to other areas. Hospital records showed that males (463 patients) had significantly more positive cases (6.9%) than females (5.6%) although Legon and Adenta showed a contrary trend (Table 3). Studies by Tawiah (2004) at the Princess Marie Louis Children's Hospital in Ghana observed more female children having cases of diarrhea-related illness than male children. This they attributed to the presence of more female children in the community. Hospital visits among male children is known to be higher than among female children (Parashar,1999; Boccolini, 2012) and this observation is consistent with the findings of this study (Table3). This may explain the higher overall percentage of positive cases of diarrhea-related illness among male children observed in this study. Bacteria counts of swab samples (Total Coliforms, Pseudomonas aeruginosa and Total Heterotrophic bacteria Table 3, 4, 5 and 6) were

also high and well above the recommended GSA (2013) levels. This was unexpected as

swabbed samples tend to be lower in count due to low recovery of bacteria by this technique(Eissa & Mahmoud, 2012).The Ghana drinking water standards requires that potable water must have zero detectable counts of TC, EC and PA per 100 ml of water analyzed while THB counts must remain below 500 cfu/100 ml. Thus, samples contaminated by only one bacteriological parameter would stillnot be adjudged as potable. Fifty (50) water samples were analyzed (for both morning and afternoon samples), and out of this, only six (6) satisfied the criteria for potability (Table 2). The presence of coliforms and THB above recommended levels is indicative of inadequate treatment of the water or contamination.

The presence of PA can result in illness in persons with compromised immune system while presence of EC is indicative of faecal contamination of drinking water. Drinking water or food contaminated with faecal matter can result in the spread of diarrhea-related diseases. School 5 (Table 4) showed contamination with faecal matter for both swab and water samples, both in the morning and afternoon based on ECcount observed. Contamination of drinking water bottles of the pre-school children is obviously due to poor handling of drinking water package as well as poor personal hygiene. School 2 (Table 4) showed contamination of drinking water with faecal matter in the afternoon but not in the morning suggesting that contamination occurred at school as a result of inadequate hygienic practices. Sixty-eight percent (68%) of parents who use water bottle or used PET

bottles to package their wards drinking water claimed they sterilize the water. However, drinking water and swabbed samples were contaminated (Table 2), suggesting that their method of sterilization is not effective. Methods of sterilization used by parents included washing with soap and brush (48% of parents), washing in warm water (23% of parents) and others (5%). It was observed during the sampling period that some of the water bottles had sophisticated bottle heads with several levels of crevices that can make cleaning difficult. This may contribute to the ineffectiveness of the cleaning methods. WHO (2011) and (2015a) recommends the use of water heated to rolling boil point for rinsing after washing with brush and detergent. Stainless steel water bottles should also be preferred to plastic reusable ones as it is easier to clean.

A previous study conducted in a primary school in England indicated that hygienic training significant decrease the levels of fecal *streptococci* isolated from the hand of primary school students (Dutta *et al*., 2013). The study also indicates that the level of bacterial contamination in the cork of student water bottle were on the high side, the study suggests that use of hot water and soap to wash water bottles will reduce or eliminate microbes.

Table 3 and 4 also showed the frequency of fungal and bacterial isolated from the cork and inside wall of the water bottles. The highest were *Staphylococcus aureus* and *Citrobacter* sp with 2.0 $x10^2$ cfu/ml followed by *Pseudomonas* sp, *E.coli*, *Klebsiella* sp and *Proteus* sp with a range of $1.0x10^2$ cfu/ml while Aspergilus flavus were the most predominant with 2.0 $x10^2$cfu/ml followed by Aspergilus niger and Penicillum with $1.0x10^2$ cfu/ml .The findings from this study is in line with the previous results of (Boccolini *et al*., 2012) who carried out similar research in some kindagerteen schools in England and concluded that enteric bacteria , *Staphylococcus aureus*, *Aspergilus flavus* were the common organisms associated with water bottle. In another study carried out by (APHA, 2012) investigated the likely cause of enteric fever and boil amongst school children in southern Nigeria. He concluded that *Staphylococcus* sp and *Salmonella* sp were the likely organisms. He went further to state that children play during recreation and even during school hours around the rest room in the school premises without adhering to proper handwashing hygiene could be attributed to the presence of the isolates.

Conclusion

Children are susceptible to any manner of infection because of their immunity and immature nature .Parents, guardian should make sure children water bottles are properly washed daily and clean water is given to these children. There should be thorough survey and monitoring of school children by those in authority not only in Amai locality but the world at large so as to maintain a proper hygiene before and after school hours.

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