International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 3 issue 2 May, 2024 Research Article

Microbial Assessment of Bonny Drinking Water Sources As A Direct Link to the Ongoing Outbreak of Typhoid Fever and Diarrhoea

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ABSTRACT

Water void of microbial contaminants is said to be safe for drinking as the chances of diarrhoea and other gastrointestinal diseases transmitted by water is limited. The microbial assessment of Bonny drinking water sources was evaluated to determine the sources of ongoing outbreaks of typhoid and diarrhea in Bonny. Water samples were collected from taps of Kiosks located in four communities within the Local Government Area. The communities included: Bypass water kiosk, Akiama water Kiosk, Oguede water kiosk, and Shell water tap. These tap waters are known to be supplied by two sources: Bonny Utility Company and the Shell Water. The microbiological parameters were analysed using standard microbiological methods. The total heterotrophic bacterial counts ranged from $4.8\pm0.7\times10^4$ to 4.0×10^5 CFU/mL, while the fungal counts ranged from $0.81\pm0.8\times10^3$ to 1.8±0.8SFU/mL. The coliform and faecal coliform counts were 0. The total heterotrophic bacterial load of the Akiama water kiosk was significantly ($P<0.05$) higher than counts of the other kiosk water samples. *Staphylococcus* sp, *Bacillus* sp and *Pseudomonas* sp were isolated from the water samples. *Salmonella thyphi* which is the causative agent of thyphoid fever was not isolated during the study. The fungal isolates associated with the water samples included *Penicillium*, *Mucor*, *Aspergillus niger* and *Candida* sp. Despite the high THB counts, the absence of faecal coliform and total coliform implied that the water could be safe for drinking and treatment measures to eliminate fungal isolates is highly recommended.

Keywords: Bonny drinking water, microbial assessment, *Staphylococcus* sp, *Bacillus* sp, typhoid outbreak.

Introduction

Water availability and water quality are of critical concern due to their importance in human health (Ollis *et al.*, 2006). Onojake and Frank (2013) opined that the coastal areas of Nigeria, particularly the Niger Delta Basin, have suffered debilitating environmental degradation and pollution from human activities such as oil industry operations, manufacturing, and municipal discharges. More so, urbanisation and municipal activities have also contributed to the number of wastes, including solid, liquid, gaseous emissions and heavy metals deposited on the environment, which may contaminate our environment (Onojake and Frank, 2013).

Obire *et al.*, (2008) reported that the indiscriminate dumping of untreated waste into nearby rivers and streams including the low standards of health in the Niger Delta region are caused by a general lack of awareness of good hygiene practices, direct contamination of beach waters through bathing and washing and uncontrolled waste disposal around the shoreline is prevalent in most developing countries like Nigeria.

The contamination of water resources by faecal pollutants poses significant risks to human and animal health since numerous pathogens are often associated with faeces (Reischer *et al*., 2008).

Thus, the safety of water, in public health terms, is determined by its microbial, physical, chemical and radiological quality (Aleruchi *et al.*, 2023). Interest in water quality assay is to ascertain the level of pollution in the water body (Kgabi, 2015).

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Water quality assessment is the complete process of evaluation of the physical, chemical and biological nature of water based on human effects and intended uses (Mwangi, 2014). Obire and Osigwe (2016), opined that the microbiological analysis of water quality is vital and a significant technique to assess the level of biological or microbial contaminants which may not be detected during chemical assessment. Thus, assessing the water for the presence of heterotrophic bacteria and coliforms gives a proper understanding of the quality of water and whether there are pathogenic microorganisms which could cause diseases or not. Globally, numerous deaths have been reported due to water resources not meeting the health criteria in terms of their constituents' concentration (Afiti *et al.,* 2015). Improved water resource management ensures that water resources have less risk of contamination and the water is suitable for both human lives and the environment at large (WHO/UNICEF/WSSCC, 2004). Bacteria such as *E. coli, Salmonella, Proteus, Shigella,* and *Enterococcus* has been reported in previous studies to be associated with polluted water and most of these pathogens are known to cause diarrhoea and other water-borne diseases (Ding *et al.,* 2015, Hald *et al.,* 2013, WHO, 2016). The present study therefore investigated the microorganisms present in tap water located in different communities in Bonny Local Government, Rivers State, Nigeria.

Materials and Methods

Sample Collection

Water samples were collected from outlets (taps) of Bypass water kiosk, Akiama water Kiosk, Oguede water kiosk and the Shell water tap. The Bypass water kiosk, Akiama water Kiosk, and Oguede water kiosk taps are supplied by the Bonny Utility Company while the Shell water is supplied by Shell Company. These are the major sources of drinking water supply to various communities in Bonny, Rivers State, Nigeria.

Enumeration and Isolation of Bacteria

The water samples were analysed using the method described by Aleruchi *et al.,* (2023). Aliquot (0.1 mL) of 10^{-2} and 10^{-3} dilutions of samples were inoculated on nutrient agar, McConkey and eosin methlyne blue (EMB) agar plates for the isolation of heterotrophic bacteria, coliforms and faecal coliforms respectively. The plates were evenly spread with the aid of sterile bent glass rod and incubated at 37°C and 44°C for total heterotrophic bacteria, coliform and faecal coliforms, respectively. After incubation, the counts in the respective plates were counted and recorded as colony forming unit of bacterial per millilitre (CFU/ml) of water sample. The colonies were also isolated for identification.

Enumeration and Isolation of Fungi

The fungi in the water samples were enumerated using the method described by Aleruchi *et al.,* (2023). Aliquot (0.1 mL) of 10^{-1} and 10^{-2} dilutions of the samples were inoculated on Saburoud dextrose agar plates in duplicates. The plates were evenly spread with the aid of sterile bent glass rod and incubated at 25°C for 3-5days (Robinson *et al.*, 2020).

After incubation, the colonies which developed in the respective plates were counted and recorded as colony forming unit of fungi per millilitre (CFU/ml) of water sample. The colonies were also isolated for identification while the spores of the fungal isolates were subcultured on freshly prepared SDA plates and incubated.

Pure fungal isolates were subjected to macroscopic and macroscopic identification (Douglas and Robinson, 2019) and the features of the isolates were compared to those in the book of fungi (Sarah *et al*., 2016).

Characterization and Identification of Bacterial Isolates

The bacterial isolates after ensuring their purity by continuous subculturing were subjected to morphological identification (Gram reaction and motility) and biochemical tests: Methyl red, citrate utilisation, oxidase, catalase, sugar fermentation tests (glucose, lactose, mannitol, fructose, sucrose, and arabinose), voges proscauer and indole tests. The procedures were as described in Prescott *et al.* (2011).

Statistical Analysis

The obtained counts were subjected to statiscal analysis. The descriptive statistics was used to calculate the mean and standard deviations while the One-way Analysis of variance was used to check for significant differences. The Turkey's b was used in separating the mean at P = 0.05.

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Results

The bacterial count of the water samples presented in Table 1 showed that the total heterotrophic bacterial counts ranged from $4.8\pm0.7\times10^4$ to 4.0×10^5 CFU/mL. There were no detectable counts for the total coliform and faecal coliform in the water samples, while the fungal counts ranged from $0.81\pm0.8\times10^3$ to 1.8±0.8SFU/mL. Results also showed that the total heterotrophic bacterial load of the Akiama kiosk water was significantly ($P<0.05$) higher than the total heterotrophic bacterial counts of the other water samples.

The result of the distribution of these isolates across the water samples is presented in Table 2. It shows that, all the isolates *Staphylococcus* sp, *Bacillus* sp and *Pseudomonas* sp were isolated from By-pass kiosk water, Akiama kiosk water, and Shell water. *Bacillus* sp and *Staphylococcus* sp were isolated from all four water sources while *Pseudomonas* sp was from three water sources (By-pass, Akiama, and Shell water). *Pseudomonas* sp was not isolated from the Oguede Kiosk water.

The fungal isolates associated with the water samples included *Penicillium, Mucor, Aspergillus niger* and *Candida* sp. The fungal isolates were not evenly distributed across the water samples as some of the isolates found in a particular water sample were not isolated in other samples. The distribution of the fungal isolates across the water sources showed uneven distribution (Table 3).

Table 1: Microbial Count of Drinking Water Samples from Kiosks in Bonny

Water Samples	Total Heterotrophic Bacteria ($\times10^4$)	Total Coliform (CFU/100mL)	Faecal Coliform (CFU/100mL)	Fungal Count ($\times10^3$)
Akiama kiosk	40.2±2.5[a]	0.0±0.0	0.0±0.0	0.81±0.8[a]
Bypass kiosk	3.6±0.4[b]	0.0±0.0	0.0±0.0	1.2±0.5[a]
Oguede kiosk	8.7±1.5[b]	0.0±0.0	0.0±0.0	1.8±0.8[a]
Shell water	4.8±0.7[b]	0.0±0.0	0.0±0.0	1.0±1.0[a]
P-value	0.005	-		0.269

*Means with similar superscript down the group showed no significant difference ($P>0.05$)

Table 2: Distribution of Bacterial Isolates Across the Drinking Water Samples from Kiosks in Bonny

Bacterial Isolate	Oguede Kiosk	Akiama Kiosk	Bypass Kiosk	Shell Water
Staphylococcus sp	+	+	+	+
Bacillus sp	+	+	+	+
Pseudomonas sp	-	+	+	+

Key: + = isolated; - = not isolated

Table 3: Distribution of Fungal Isolates across the Drinking Water Samples from Kiosks In Bonny

Fungal Isolate	Oguede Kiosk	Akiama Kiosk	Bypass Kiosk	Shell Water
Penicillium sp	+	+	-	-
Aspergillus niger	-		+	-
Mucor sp	-	+	-	+
Candida sp	-	+	-	+

Key: + = isolated; - = not isolated

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Discussion

The total heterotrophic bacterial and fungal load in the water samples were generally high and above the recommended limit for drinking water. The coliform and faecal coliform counts are within the recommended limit for drinking water. The recommended limits for total heterotrophic bacteria, coliform and faecal coliforms were 1.0×10^2 CFU/mL, < 3 and 0 CFU/100mL, respectively (WHO, 2016). Based on this recommendation, the bacteriological quality of the water being void of coliforms and faecal coliforms might not pose serious health issues. The presence of faecal coliform in drinking water sources has been attributed to be an indicator of pathogenic bacteria (Obire *et al.*, 2008). Wilcox *et al*., (2023) in their study reported a high total heterotrophic bacterial load and 0 faecal coliforms which agreed with the present study. More so, the presence of total coliforms in their study contradicts the present study which showed 0 coliform counts. The total heterotrophic bacterial and coliform counts in the present study are lower than those reported in a previous study of mono-pumps and borehole water (tap water sources) in Rivers State (Kpormon *et al*., 2023).

Bacillus sp, *Staphylococcus* sp and *Pseudomonas* sp in the present study have been reported in previous studies. Wilcox *et al*., (2023) isolated *Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp, *Shigella* sp, *Enterococcus* sp, *Serratia* sp, and *Erythrobacter* sp from tap waters in Bonny. Unlike the presents study, these isolates except *Bacillus* sp, *Staphylococcus* sp and *Pseudomonas* sp were not isolated. This could imply that the water has undergone treatment. Kpormon *et al*., 2023) reported the presence of *E. coli, Staphylococcus aureus*, *Proteus* sp and *Salmonella* sp., in tap water sources in Rivers State. The presence of *Bacillus* sp in the water sources could be due to their adaptation in extreme environments especially with the presence of endospores (Prescott *et al.*, 2011).

Most of the fungal isolates in the present study are filamentous fungi and could contain pathogenic strains especially with the presence of *Penicillium* and *Aspergillus niger* which have been associated with secretion of mycotoxins and aspergillosis, respectively (Prescott *et al.*, 2011). Secondary metabolites produced by fungi, particularly those growing in localised pockets near the consumer end may be responsible for altering the taste and odour of drinking water.

It is thought that the threshold level for several fungi that can cause such issues may be around 102-103 CFU/mL. While problems with taste and odour do not necessarily imply a health risk but are often perceived as such by the consumer (Sonigo *et al*., 2011).

In conclusion, the study showed that the various tap water from the two sources of water supply in Bonny, Rivers State are not contaminated with pathogenic bacteria isolates. Although the total heterotrophic bacterial and fungal counts were high, the absence of both total coliform and faecal coliform in all the water samples implied that diarrhoea and other gastrointestinal disease-causing bacteria are not present in the drinking water. Thus these drinking water sources in Bonny are not associated with neither do they have a direct link to the present ongoing outbreak of typhoid fever and diarrhea in Bonny. However, the presence of filamentous fungi and *Candida* sp in the water sources is of public health significance. Thus, attention should not only be given to treatment of bacterial contaminants but also of fungal contaminants.

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