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Occurrences and antibiogram of bacteria isolated from some sachet drinking water brands sold in Gombe Metropolis, Gombe State, Nigeria

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ABSTRACT

Background: Diseases contracted through consuming contaminated water present health challenges globally, hence this study aimed to assess occurrence and antibiogram of bacteria isolated from various brands of sachet drinking water sold in Gombe metropolis. **Methods:** Twenty brands of samples were collected randomly, serially diluted, and cultured on nutrient agar (NA). Isolates were identified morphologically and biochemically, with antibiogram determined using Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** All the 20 samples produced positive bacterial growths with counts ranging from 1.0×10^3 to 9.8×10^3 CFU/ml with identified colonies of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Klebsiella pneumoniae* (*K. pneumoniae*). Antibiogram revealed the isolates were all resistant to augmentin, cefixime, cefuroxime and ceftazidime, but *E. coli* and *S. aureus* were also resistant to gentamicin. **Conclusion:** The samples were contaminated with potentially pathogenic bacteria that were resistant to some antibiotics. Hence there is need for enforcement of drinking water standards to avoid consequences of unsafe drinking water, thus improving the health of the population.

Introduction

The drinking of medically decent water is mandatory for sustaining upright individual healthiness and a healthy country in general [1]. Individuals that reside permanently in communities especially in rural areas of developing nations that have insufficient supplies of medically safe water have no other option than to seek home-grown sources of drinkable water. These are habitually defenseless against point and non-point sources of contamination which institute some foremost public

health hazards like enteric ailments which caused about 700,000 child mortalities in 2011 [2].

In line with the parameters established by the World Health Organization, healthy drinkable water should never hold *Escherichia coli* (*E. coli*) or thermotolerant coliform bacteria, giardia worms, viruses, *Cryptosporidium* spp, *Legionella pneumophila*, *Entamoeba histolytica* and other opportunistic disease-causing bacteria like *Clostridium* spp, *Klebsiella* spp, and *Pseudomonas* spp [3].

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Nonetheless, inadequate access to healthy drinkable water has continuously sustained the transmission level of water-borne ailments that have been reported to be responsible for up to 6.3% of human mortalities documented globally [4]. On a typical basis, 1.6 million kids perish each year owing to assimilation of polluted water, and these water-borne and water-quality-related illnesses enact unparalleled communal and financial burden to the affected persons and societies [5] and are the second greatest common reason of demise in kids below the age of five years in sub-Saharan Africa and Southern Asia [6].

In Nigeria, the Federal Ministry of Water Resources detailed that Nigeria is gifted with enormous water reserve possibilities which are projected at 267 billion cubic meters of groundwater [4]. Nevertheless, there has been a low-slung degree of clean water amount disseminated in the nation, where only around 58% of the populace have contact to harmless water sources [2]. Thus, sachet water will probably contribute a significant part in sub-Saharan Africa's attainment of sustainable development goals (SDG) 6 for "universal and equitable access to safe and affordable drinking water for all" [7].

In Gombe metropolis of Gombe State, Nigeria, there are numerous brands of commercially available sachet drinking water, yet there is a dearth of documented scholarly information regarding the occurrence of bacteria in such products, hence this study was aimed to bridge this knowledge gap and to ascertain the occurrence and antibiogram of bacteria in these brands of sachet drinking water commonly consumed daily in the study area.

Methods

Study area

The study area is Gombe metropolis, which is the capital of Gombe state, located in the north eastern part of Nigeria, and located between latitude 10°17' 13.97" N and longitude 11° 9' 58.45" E, within the Sahel savannah belt [8].

Sample collection

A total of 20 different-brand samples of sachet water were collected in March 2019 from various retail points using simple random sampling and transported to the Microbiology laboratory of Gombe State University inside an insulated carrier, for further processing and analysis [9].

Serial dilution

A 10-fold serial dilution was carried out using 1ml of sample into 9mls of sterile distilled water, the dilution factors were determined, then 0.1ml from

tube with 10^{-3} dilution was used for further analysis [10].

Isolation: inoculation and incubation

Spread plate method was used, whereby freshly prepared nutrient agar (TM MEDIA TM341) plates were distinctly inoculated each with 100µl of serially diluted sample from the tube with 10^{-3} dilution factor, these were then incubated for 24 hours at 36°C [11].

Enumeration

The viable plate count method was used, whereby the visible bacteria colonies formed after 24 hours of incubation were counted using colony counter to ascertain the total number of colonies on each of the plates which were then multiplied by the reciprocal of the dilution factor to obtain the CFU/ml for the different samples [12].

Identification

This was done through macroscopic examination of physical colony morphology which included color and texture, microscopic examination through Gram's staining, and then biochemical tests that included coagulase, oxidase, indole, citrate, urease and catalase tests, which were chosen based on observations from the physical colonial morphology examination and Gram's reaction [13].

Antibiogram assay

First, standardizations of test inocula of the various identified isolates were executed by employing direct colony suspension technique that involved formulating a suspension of different organisms equivalent to that of the 0.5 McFarland turbidity standard [14].

The antibiotic susceptibility investigations were carried out via the disc diffusion antibiotic susceptibility test which involved spread plating the standardized inocula on discrete Mueller Hinton Agar (HI MEDIA M173) (MHA)-containing petri dishes, then standard antibiotic discs (ABTEK) of augmentin, ciprofloxacin, nitrofurantoin, ofloxacin, cefixime, ceftazidime, gentamycin and cefuroxime were aseptically positioned on these inoculated petri dishes then incubated at 36°C for 18 hours after which zones of inhibition were observed and measured to the nearest whole millimeter with a meter rule, and interpreted as either resistant, intermediate, or sensitive, based on guidelines for antimicrobial susceptibility testing [14].

Results

Isolation, morphological identification, and enumeration

The results of isolation on nutrient agar revealed microbial colonies of the following organisms: *E.*

coli, *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Klebsiella* spp. The results of enumeration (Table 1) revealed that the samples contained bacteria ranging from 1.0×10^3 to 9.8×10^3 CFU/ml, while some were too many to count (TMTC).

Biochemical identification

The results of biochemical identification (Table 2) showed that the organisms suspected to be *S. aureus* tested positive for catalase and coagulase tests, those suspected to be *E. coli* tested positive to indole and catalase tests, suspected *P. aeruginosa* tested

positive to catalase, oxidase and citrate tests, while suspected *K. pneumoniae* tested positive to urease and citrate tests.

Antibiogram assay

The results of sensitivity tests for the isolates (Table 3) revealed that representative isolates were sensitive to nitrofurantoin and ofloxacin, but resistant to gentamicin, augmentin, cefixime, cefuroxime, ceftazidime, and ciprofloxacin. Nonetheless, *P. aeruginosa* and *K. pneumoniae* were sensitive to gentamicin.

Table 1. Results of enumeration.

Sample	Number of colonies	CFU/ml
1	71	7.1×10^3
2	90	9.0×10^3
3	88	8.8×10^3
4	50	5.0×10^3
5	47	4.7×10^3
6	39	3.9×10^3
7	TMTC	TMTC
8	68	6.8×10^3
9	77	7.7×10^3
10	60	6.0×10^3
11	48	4.8×10^3
12	35	3.5×10^3
13	10	1.0×10^3
14	TMTC	TMTC
15	79	7.9×10^3
16	20	2.0×10^3
17	33	3.3×10^3
18	70	7.0×10^3
19	65	6.5×10^3
20	98	9.8×10^3

Table 2. Results of biochemical identification

Suspected organism	Catalase test	Coagulase test	Indole test	Oxidase test	Citrate test	Urease test
<i>S. aureus</i>	+	+	-	-	-	-
<i>E. coli</i>	+	-	+	-	-	-
<i>P. aeruginosa</i>	+	-	-	+	+	-
<i>K. pneumoniae</i>	-	-	-	-	+	+

KEY: + = positive, - = negative

Table 3. Results of antibiogram assay.

Organisms/ZI	NIT	AUG	OFL	CXM	GEN	CRX	CAZ	CPR
<i>E. coli</i>	20 (S)	10 (R)	20 (S)	14 (R)	08 (R)	09 (R)	10 (R)	27 (S)
<i>S. aureus</i>	21 (S)	07 (R)	18 (S)	09 (R)	10 (R)	09 (R)	08 (R)	21 (S)
<i>P. aeruginosa</i>	18 (S)	08 (R)	17 (S)	07 (R)	16 (S)	07 (R)	07 (R)	20 (S)
<i>K. pneumoniae</i>	23 (S)	09 (R)	30 (S)	13 (R)	20 (S)	14 (R)	08 (R)	31 (S)

KEY; Zone of inhibition (ZI), Augmentin (AUG), Ciprofloxacin (CPR), Cefixime (CXM), Cefuroxime (CRX), Ceftazidime (CAZ), Gentamicin (GEN), Ofloxacin (OFL), Nitrofurantoin (NIT), S=Sensitive, R=Resistant.

Discussion

The bacteria isolated from sachet water brands in this study which included *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* are believed to have occurred due to improper processing and packaging during the water production process, failure to adhere to global standards of drinking water production, negligence by supervising authorities, or corruption among the staff involved in enforcing water quality in the study area [15]. These findings are in line with reports of **Olaoye & Onilude** [16] who also isolated varying levels of these bacteria from sachet water samples in western Nigeria. The biochemical identifications of these isolates such as *S. aureus* been positive to catalase and coagulase tests due to it producing catalase and coagulase enzymes agree with the work of **Talaiekhosani et al.** [17] who described identification of microorganisms based on their biochemical characteristics, and with the reports of **Omonigho** [4] who biochemically identified some of these bacteria they also isolated from sachet water.

The interpretations of the sensitivity zones of inhibition revealed that *E. coli* was sensitive to nitrofurantoin, ofloxacin, and ciprofloxacin but resistant to augmentin, cefixime, gentamicin, cefuroxime and ceftazidime. Also, *S. aureus* was sensitive to nitrofurantoin, ofloxacin, and ciprofloxacin but resistant to Augmentin, cefixime, gentamicin, cefuroxime and ceftazidime. *P. aeruginosa* was sensitive to nitrofurantoin, ofloxacin, ciprofloxacin and gentamicin but resistant to Augmentin, cefixime, cefuroxime and ceftazidime. And lastly, *K. pneumoniae* was sensitive to nitrofurantoin, ofloxacin, ciprofloxacin and gentamicin but resistant to Augmentin, cefixime, cefuroxime and ceftazidime [14]. These patterns of antibiotic resistance in the isolates are believed to be the result of antibiotic misuse [18] which is common in Gombe metropolis [19]. These findings agree with the works of **Tagoe et al.** [20]

who isolated antibiotic resistant bacteria such as *S. aureus* from sachet water in Ghana.

The strength of this study is in the distinctiveness of the brands of sachet water sampled, while the weakness of the study is that the packaging date/time of the samples could not be verified.

This study has shown that various brands of sachet water commonly sold in Gombe metropolis are contaminated with bacteria some of which are resistant to some antibiotics and can cause an array of infections which can result in reduction of quality of life or serious diseases.

It is recommended that regular and unannounced investigations into microbiological safety of sachet water should be carried out constantly in the study area and defaulters should be brought to justice.

Conflicts of interests: None exist.

Author contributions

- Umar Abdullahi Tawfiq: Research design, disc diffusion assay.
- Patas Mary: Sample collection, isolation.
- Adamu Muhammad Tukur: Biochemical identification.
- Usman Musa: Sub-culture, Pure culture.
- Jauro Hadiza Abubakar: Preparation of turbidity standard, Standardization of inocula.
- Maigana Zainab Sale: Morphological identification, enumeration.
- Puma Hamidu Umar: Literature review, article drafting, interpretation of antibiogram assay.

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