Occurrence and Antibiotic Profile of some Enteric Bacteria in Retailed Sachet Water sold in Oluponna, Osun State, Nigeria

ABSTRACT

The research reports the occurrence and antibiogram pattern of the pathogenic organisms *Shigella* spp., *Salmonella* spp. and *Escherichia coli* in retailed sachet water ~~in order~~ to assess their microbial quality and potential health impact on consumers. A total of 50 sachet water, consisting of three different brands, were bought from sale outlets in Oluponna, Osun State, Nigeria and screened on Salmonella-Shigella agar for *Shigella* spp.and *Salmonella* spp. and on Eosin-methylene agar for *E. coli* using the pour plating technique. Antibiotic sensitivity profile using 0.5 McFarland of each of the obtained isolate was carried out on Mueller Hinton agar using the disc diffusion method. Results showed that the percentage occurrence ~~were~~ was *E. coli* (98%), *Salmonella* spp. (26%) and *Shigella* spp. (98%). *E. coli* isolates were 100% resistant to cefixime while *Salmonella* spp. and *Shigella* spp. were 100% resistant to cefuroxime. Furthermore, the different strains of isolates phenotypically exhibited multidrug resistance, with *E. coli* having the highest multidrug resistance of 73.33% to the combinations of cefuroxime, cefixime, ceftazidime, augmentin, and nitrofurantion. It is suggested that if adequate process treatment is given to packaged water, during production and the microbial quality kept within the World Health Organization (WHO) and Standards Organization of Nigeria (SON) standards, the presence of these bacterial pathogens, as well as their antibiotic resistant and multi-drug resistant forms would be eliminated in the water, hence, would make the drinking water safe for public consumption.

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Keywords: Sachet water, microbial quality, antibiotic resistance, multi-antibiotic resistance

1. INTRODUCTION

Portable water is water that has been treated, cleaned or filtered to meet established drinking standards and which could have been sourced from surface waters such as rivers, streams or the ground water~~s~~ such as spring, wells, and boreholes (1, 2). However, the inadequacy of humans to access safe drinking water, as well as government's inability to provide enough of the same, have collectively, triggered ~~a number of~~many small-~~-~~scale water producing industries towards packaging and marketing factory filled sachet drinking water (3, 4). Sachet drinking water ~~are~~ is small nylon sachets containing 0.5 L of water which ~~are~~ is electrically heated and sealed at both ends (5). The sale and consumption of packaged water continue to grow astronomically and rapidly in most countries of the world (6, 7, 8).

Sachet water is easily affordable and accessible in the rural and semi-~~-~~urban environments of many developing countries (9), particularly by the general populace consisting mostly of low-~~-~~income individuals. Generally, investigating the microbial quality of this widely consumed and highly in-demand product is an effort towards assessing its potential health impact on consumers, as well as providing information for improving the drinking water standard within the study area. This research was aimed at determining the prevalence of *Shigella* spp., *Salmonella* spp. and *Escherichia coli* in retailed sachet water, in Oluponna, Osun State, Nigeria, establish the antibiogram profile of the same organisms and possibly predict the potential public health risks associated with the consumption of this product.

2. MATERIAL AND METHODS

Sample Collection

Sachet water samples, of three different brands (MX, GZ, and QL), were bought from various sale's points in Oluponna, Osun State, Nigeria. Samples were collected in February/March 2018, for a total of three weeks.

Isolation of target organisms

For each sachet water brand sample, 1mL of its water content was aseptically inoculated into a test tube containing *non-selective pre-enrichment broth of* 9 mL sterile maximum recovery diluent (MRD) and mixed thoroughly. The test tube was plugged with the cotton wool and incubated at 37˚C for 24 hours. Afterwards, 1 mL of the incubated diluent was serially diluted, and 0.5 mL of the final diluent pour plated into sterile petri-dishes containing Eosin Methylene Blue (EMB) agar and Salmonella-Shigella agar (SSA) for the isolation of *E. scherichia coli*, *Salmonella* spp., and *Shigella* spp, respectively. The dishes were allowed to solidify and incubated for 24 hours at 37˚C. Presumptive colonies for *Salmonella* spp (colourless and black centered), *Shigella* spp (colourless) and *E. coli* (green metallic sheen) were counted and determined after confirming their identity through biochemical tests (10).

Characterization and Identification of Bacterial Isolates

The obtained bacterial isolates were characterized on the basis ofby their Gram staining, and biochemical tests such as catalase, indole, citrate utilization, starch hydrolysis, methyl-red, Voges-Proskauer, motility, and sugar fermentation tests as described by (11, 12, 13).

Antibiotic sensitivity test

The disc diffusion method was used to examine the susceptibility pattern of each affirmed bacterial isolate to antimicrobial agents. A 0.5 McFarland turbidity standard of each identified bacterial isolate was inoculated uniformly on sterile Mueller Hinton agar plates using cotton swabs and multi-disc antibiotics placed on the plates, using sterile forceps. The antibiotic discs used were ceftzidime (30 µg), cefuroxime (30 µg), gentamycin (10 µg), cefixime (5 µg), ofloxacine (5 µg), augmentin (30 µg), nitrofurantion (300 µg) and ciprofloxacin (5 µg). The plates were incubated for 24 hours at 37˚C. The zones of inhibition produced were measured in millimeters, and the values obtained interpreted according to the Clinical and Laboratory Standards Institute (13).

Comment [S2]: Ceftazidime please confirm which one is correct???

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Statistical analysis

The analysis of variance (ANOVA), using the IBM SPSS Statistics 20 software, was used to compare the significance of mean differences (*P≤0.05*) between the *E. coli, Salmonella* spp. *and Shigella* spp counts in each of the sachet water sample brands as well as for each particular bacterial pathogen counts among the different brands used in the study.

3. RESULTS AND DISCUSSION

Identification and prevalence of obtained isolates

A total of 50 sachet water samples were collected and 111 bacterial isolates obtained. Based on morphological appearances and biochemical characteristics, a sum of 49, 49 and 13 of these isolates were identified as *Shigella* spp., *E. scherichia coli*, and *Salmonella* spp., respectively. The *E. coli*, *Shigella* spp., and *Salmonella* spp. counts in sachet water brands MX, GZ and QL are shown in Tables 1, 2 and 3, respectively.

Table 1: *Escherichia coli*, *Shigella* and *Salmonella* spp. counts (CFU/mL) in brand MX sachet water

	Count (CFU/mL)		
Sample	***E. coli***	***Shigella* spp.**	***Salmonella* spp.**
MX 1	4×10^3	6×10^3	No growth
MX 2	2×10^2	4×10^3	No growth
MX 3	4×10^3	5×10^3	No growth
MX 4	5×10^3	4×10^3	2×10^2
MX 5	4×10^3	5×10^3	No growth
MX 6	4×10^3	2×10^3	No growth
MX 7	5×10^3	5×10^3	6×10^2
MX 8	8×10^3	4×10^3	No growth
MX 9	5×10^3	4×10^3	No growth
MX 10	5×10^3	2×10^3	No growth
MX 11	6×10^3	2×10^3	1×10^2
MX 12	3×10^3	1×10^3	7×10^2
MX 13	3×10^3	1×10^3	1×10^3
MX 14	5×10^3	5×10^3	3×10^3

MX 15	3×10^3	4×10^3	No growth
MX 16	3×10^3	2×10^3	8×10^1
MX 17	5×10^3	6×10^3	No growth
MX 18	4×10^3	1×10^3	5×10^3
MX 19	No growth	5×10^3	3×10^2
MX 20	4×10^3	2×10^3	4×10^3
Mean	$3.61 \times 10^3{}_{ac}$	$3.50 \times 10^3{}_{c}$	$7.49 \times 10^2{}_{b}$
Std. Dev.	$\pm 1.73 \times 10^3$	$\pm 1.70 \times 10^3$	$\pm 1.47 \times 10^3$
SON	0/1mL	-	-
WHO	0/ 1mL	-	-

Std. Dev = Standard deviation; SON= Standard Organization of Nigeria; WHO=World Health Organization; Means with different subscripts across a row are significantly different at *P≤0.05*

Table 2: *Escherichia coli*, *Shigella* and S*almonella* spp. counts (CFU/mL) in brand GZ sachet water

	Count (CFU/mL)		
Sample	***E. coli***	***Shigella* spp.**	***Salmonella* spp.**
GZ 1	4×10^3	2×10^3	No growth
GZ 2	3×10^2	3×10^3	No growth
GZ 3	2×10^3	4×10^3	No growth
GZ 4	5×10^2	5×10^2	No growth
GZ 5	4×10^3	4×10^3	No growth
GZ 6	6×10^3	5×10^3	No growth
GZ 7	5×10^3	3×10^3	No growth
GZ 8	6×10^3	4×10^3	No growth
GZ 9	5×10^3	5×10^3	No growth
GZ 10	4×10^3	4×10^3	No growth
GZ 11	4×10^3	4×10^3	No growth
GZ 12	3×10^3	6×10^2	No growth
GZ 13	4×10^3	1×10^3	No growth
GZ 14	5×10^3	6×10^2	No growth
GZ 15	4×10^3	3×10^3	No growth
GZ 16	3×10^3	4×10^3	No growth
GZ 17	5×10^3	4×10^3	No growth
GZ 18	4×10^3	4×10^3	No growth
GZ 19	5×10^3	5×10^3	No growth
GZ 20	5×10^3	5×10^2	No growth
Mean	$4.08 \times 10^3{}_{a}$	$3.06 \times 10^3{}_{b}$	0.00_{c}
Std. Dev.	$\pm 1.32 \times 10^3$	$\pm 1.61 \times 10^3$	±0.00
SON	0/1ml	-	-
WHO	0/ 1ml	-	-

Std. Dev = Standard deviation; SON= Standard Organization of Nigeria; WHO=World Health Organization; Means with different subscripts across a row are significantly different at *P≤0.05*

Table 3: *Escherichia coli*, *Shigella* and S*almonella* spp. counts (CFU/mL) in brand QL sachet water

	Count (CFU/mL)		
Sample	***E. coli***	***Shigella* spp.**	***Salmonella* spp.**
QL 1	5×10^3	4×10^3	5×10^3
QL 2	$3x\ 10^3$	3×10^3	5×10^2
QL 3	4×10^3	5×10^3	5×10^2
QL 4	9×10^2	4×10^3	No growth
QL 5	4×10^3	4×10^3	No growth
QL 6	5×10^3	6×10^3	No growth
QL 7	5×10^3	4×10^3	No growth
QL 8	4×10^3	3×10^3	No growth
QL 9	1×10^3	5×10^3	No growth

QL 10	3×10^3	No growth	No growth
Mean	3.49×10^{3} $_{ac}$	3.80×10^{3} $_{c}$	6.00×10^{2} $_{b}$
Std. Dev.	$\pm 1.53 \times 10^3$	$\pm 1.62 \times 10^3$	$\pm 1.56 \times 10^3$
SON	0/1mL	-	-
WHO	0/ 1mL	-	-

Std. Dev = Standard deviation; SON= Standard Organization of Nigeria; WHO=World Health Organization; Means with different subscripts across a row are significantly different at *P≤0.05*

The mean *E. coli*, *Shigella* spp. and *Salmonella* spp. counts in each of the different sachet water brands were significantly different at *P≤0.05* (Table 1, 2 and 3).Nevertheless, there was no significant difference (*P≤0.05*) in the mean counts of each specific pathogen among the different sachet water brands (Table 4).

Table 4: ANOVA of each pathogen's mean count in the different sachet water brands

	Mean counts (CFU/mL)			
Organism	**MX**	**GZ**	**QL**	**Remark**
E. coli	$3.61 \times 10^{3}{}_{a}$	$4.08 \times 10^{3}{}_{a}$	$3.49 \times 10^{3}{}_{a}$	NS
Shigella spp.	$3.50 \times 10^{3}{}_{a}$	$3.06 \times 10^{3}{}_{a}$	$3.80 \times 10^{3}{}_{a}$	NS
Salmonella spp.	$7.49 \times 10^{2}{}_{a}$	0.00_{a}	$6.00 \times 10^{2}{}_{a}$	NS

NS = Not significant; Means with same subscripts across a row are not significantly different at *P≤0.05*; ~~*E. coli = Escherichia coli*~~

Shigella spp. had 100% occurrence in all the brands sampled while *E. coli* had 100% occurrence in sachet water brands GZ and QL (Table 5).The overall incidence of *E. coli*, *Shigella* spp. and *Salmonella* spp. in the study were 98%, 98%, and 26%, respectively

Table 5: Occurrence of *Escherichia coli*, *Shigella* and *Salmonella* spp. in sachet water

Organism	Water Sachet Brand	N	Occurrence (%)	Overall Occurrence (%)
E. coli	MX	20	95	98
	GZ	20	100	
	QL	10	100	
Shigella spp.	MX	20	100	98
	GZ	20	100	
	QL	10	100	
Salmonella spp.	MX	20	90	26
	GZ	20	0	
	QL	10	30	

N= Sample size; ~~*E. coli = Escherichia coli*~~

Generally, drinking water should contain no pathogens (15,16). The high prevalence of 98% for both *E. coli* and *Shigella* spp. is worrisome. The detection of these organisms implied that the water samples have been contaminated with faecal matter and are therefore not safe for human consumption. Furthermore, the occurrence of pathogens or indicator organisms in water sources depends on the intrinsic physical and chemical characteristics of the catchment area, the magnitude and range of the human activities/animal sources that release pathogens to the environment. as well as the level of treatment given to the water (17). The microbes may break through inadequate treatment process. Presence of these bacteria in water may be unnoticed even in transparent packaged water and may eventually pose a potential risk to consumers, when ingested. Even the consumption of such contaminated water may facilitate widespread infections which could ultimately lead to an epidemic outbreak (18).

Microbial pollution of packaged water particularly in developing countries has grave implications on public health (19). It threatens the population's existence is causing diseases such as gastroenteritis (20), typhoid fever and shigellosis (21, 22) and it may be possible that prevalence of these diseases within the study area may be connected with the consumption of these products. Diarrhea caused by enteric infections is a ~~major~~ significant factor in morbidity and mortality worldwide, with mortalities due to water--associated diseases and symptoms being asserted to exceed 5 million people per year (23).

Antibiotic resistance pattern of the isolates

Table 6 shows the antibiogram pattern of *E. ~~scherichia~~ coli*, *Shigella* spp., and *Salmonella* spp. *E. ~~scherichia~~ coli* were 100% resistant to cefixime and 93.3% resistant to both gentamycin and cefuroxime.

Table 6: Antibiotic resistance profile of the isolated pathogens

Antibiotic	*Escherichia coli* N = 49 (%)	*Salmonella* spp N = 49 (%)	*Shigella* spp N = 13 (%)
Ceftazidime	86.67	0	21.74
Cefuroxime	93.30	100	100
Gentamycin	93.30	0	13.40
Cefixime	100	91.67	95.65
Ofloxacin	0	0	0
Augmentin	80	66.67	95.65
Nitrofurantion	73.30	0	73.91
Ciprofloxacin	0	0	0

N= Number of isolates

Salmonella species and *Shigella* species were though together 100% resistant to cefuroxime but nonetheless, separately 91.67% and 95.65% resistant to cefixime, respectively. *E.coli* isolated from surface waters in South Eastern, Nigeria have been reported to be 100% resistant to cefixime(24).

Furthermore, this study has observed that all the isolated pathogens were susceptible to ofloxacin. and ciprofloxacin. These antibiotics are fluoroquinolones and could be considered as the antibiotic drug for treatment of bacterial infections derived from consuming the contaminated water in the locality. Quinolones are ~~considered~~ regarded as drugs of choice for treatment of Salmonella infections (25). Nevertheless, it is worth noting that, *E.coli* isolated from sachet water in Abakiliki, Ebonyi State, Nigeria, have been asserted to be 83% resistant to ofloxacin(26).

This study has also shown the presence of multidrug--resistant strains (Tables 7, 8 and 9) with 73.33% of the *E. ~~scherichia~~ coli* isolates having the highest multi--drug resistance incidence to the antibiotic combinations of cefuroxime, cefixime, ceftazidime, augmentin and nitrofurantoin (Table 7).

Table 7: Multidrug resistance pattern of *E. ~~scherichia~~ coli* isolated from sachet water

Resistance	Number of occurrence	Percentage of occurrence
CXM, CRX, CAZ, AUG, NIT	11	73.33
CXM, CRX, CAZ, AUG	1	6.67
CXM, CRX	4	26.67

Keys: CAZ=Ceftazidime; CRX= Cefuroxime; CXM= Cefixime; AUG=Augmentin; NIT= Nitrofurantion

Table 8: Multidrug resistance pattern of *Salmonella* species isolated from sachet water

Resistance	Number of occurrence	Percentage of occurrence
CXM, CRX, NIT, AUG	8	66.67
CXM, CRX, AUG	1	8.33
CXM, CRX, NIT	1	8.33
CXM, CRX	2	16.67

Keys: CAZ=Ceftazidime; CRX= Cefuroxime; CXM= Cefixime; AUG=Augmentin; NIT= Nitrofurantion

Table 9: Multidrug resistance pattern of *Shigella* species isolated from sachet water

Resistance	Number of occurrence	Percentage of occurrence
CXM, CRX, NIT, AUG, CAZ	11	47.83
CXM, CRX, NIT, AUG, CAZ, GEN	3	13.04
CXM, CRX, NIT, AUG	3	13.04
CXM, CRX, AUG	4	17.39
CRX, AUG	1	4.35
CXM, AUG, CRX, CAZ	1	4.35

Keys: CAZ=Ceftazidime; CRX= Cefuroxime; CXM= Cefixime; AUG= Augmentin; NIT= Nitrofurantion; GEN= Gentamycin

Furthermore, *Shigella* spp. had the highest multidrug resistance of 47.83% to cefixime, cefuroxime, ceftazidime, augmentin and nitrofurantion, and 13.04% of the same isolates resistant to cefuroxime, cefixime, ceftazidime, augmentin, gentamycin and nitrofurantoin (Table 8). *Salmonella* spp. had the highest multidrug resistance of 66.67% to cefixime, cefuroxime, nitrofurantion and augmentin (Table 9). The relatively high level of resistance to antimicrobial agent as well as the development of multidrug- resistant strains could be a reflection of misuse or abuse of these antibiotics in the environment (27). Bacteria become resistant to the antimicrobial agents by ~~a number of~~many mechanisms which are; production of enzymes which inactivate or modify antibiotics, changes in the bacterial cell membrane, preventing the uptake of antibiotics and development of metabolic pathways by resistant strains, hence, enabling the site of an antimicrobial action to be by-passed (27).

Several mechanisms have evolved in bacteria which confer them with antibiotic resistance. These mechanisms can chemically modify the antibiotic, or ~~modify~~ change the target site so that it is not recognized by the antibiotics (28). The emergence of resistance to fluoroquinolones in virtually all species of bacteria was recognized soon after the introduction of these compounds for clinical use more than 10 years ago. Various resistance mechanisms, often interdependent, may explain different levels of resistance. Epidemiological factors, local antibiotics policies, the origin of the strains, and geographic location (29) are among the factors contributing to highly variable resistance rates. The presence of these antibiotic- resistant bacteria in sachet water is of health significance because of the potential danger of promoting multiple antibiotic resistance through possible colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistance to the normal floral leading to more multiple antibiotic resistant organisms (30).

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4. CONCLUSION

In summary, this study has revealed that three retailed brands of sachet water samples, MX, GZ and QL, from Oluponna, Osun State, Nigeria, contained varied microbial counts of *E. ~~scherichia~~ coli*, *Shigella* spp. and *Salmonella* spp. and with an overall prevalence of 98%, 98%, and 26%, respectively. These implicated pathogens also showed phenotypic antibiotic and multidrug resistance. The presence of these pathogenic bacteria in sachet water may be a~~s~~ result of inadequate treatment of the water during production. Proper assessment of the microbial quality of water at some important stages of production; pre-production, production and post-production stages at the factories is, therefore, suggested ~~in order~~ to ensure their quality and safety, particularly to ensure that they meet the required WHO and SON standard for portable water. Adequate treatment of water would also eliminate the presence and spread of these antibiotic- resistant strains in water, hence, making the sachet water safe for drinking.

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