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SHIGA, TOXIN-PRODUCING *ESCHERICHIA COLI* O157:H7 IN PACKAGED DRINKING-WATER IN ABEOKUTA, NIGERIA

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Abstract

There is a need to carry out regular microbiological analysis of packaged sachet water (PSW) sold in Nigeria to determine whether they comply with drinking-water quality standards. One hundred and fifty three PSW samples from fifty-one packaged sachet water facilities (PWMFs) in Abeokuta were collected from retail outlets between April – July, 2014. Samples were analyzed for *Escherichia coli* using standard culture media. Latex agglutination serological test; sequencing of 16S rRNA gene and sequence similarity analysis yielded *E. coli* O157:H7 from two PSW (1.32%) from one PWMF (1.96%). Both *E. coli* O157:H7 isolates were Shiga toxin (stx1) positive but stx2 negative. The two *E. coli* O157:H7 strains exhibited different resistance patterns to ten (10) antibiotics belonging to seven (7) different classes. Each *E. coli* O157:H7 strain showed resistance to more than two classes of antibiotics with MIC ≥ 8 μg/ml. This paper showed that the not all packaged sachet water analyzed in this study are fit for drinking, and can even be source of multidrug resistant and Shiga, toxin-producing *E. coli*.

Introduction

Packaged water has become another source of drinking water in most countries of the world (Oyedeji et al. 2010). In Nigeria, many people are currently involved in the production of PSW using different complex tech-

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niques such as ozonization and reverse osmosis, however, many producers fail to adhere to standards for the production of packaged sachet water (PSW). Findings from quality assessment using *Escherichia coli* as faecal indicator revealed that there are some PSW with microbiological limits exceeding WHO drinking-water guidelines of zero coliform count per 100 ml of water (WHO 2002, 2017). Although most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans and are occasionally responsible for product recalls (TARR et al. 2005).

Shiga-like toxin-producing *Escherichia coli* (STEC) O157:H7 are known zoonotic, food and water-borne pathogens and toxin-producing strains associated with human illness (GYLES 2007) and STEC also harbor the phage-encoded genes *stx1* and *stx2* coding for potent cytotoxins that cause severe tissue damage with fatal consequences (WALKER et al. 2012).

In Nigeria, especially the southwest, a lot of work has been done on the microbiological quality of vended sachet water (OLUYEGE et al. 2014, ONILUDE et al. 2013, OYEDEJI et al. 2010) with *E. coli* as an indicator of water quality. There is no index study on the serotype of *E. coli* isolated from such vended sachet water. Also in Nigeria, *E. coli* 0157:H7 have been reported from clinical and food samples (OKEKE et al. 2003, OLATOYE 2010), tap water and surface water (CHIGOR et al. 2010, LUGA et al. 2007, SMITH et al. 2009) but none from packaged sachet water. There is a need to subject suspected *Escherichia coli* from sachet water to further analysis (phenotypic, serotyping and molecular). As such, this study investigates the presence of Shiga, toxin-producing *Escherichia coli* O157:H7 in packaged sachet water samples in Abeokuta, Nigeria, with the goal to assess the possible risk of human exposure to *Escherichia coli* O157:H7 through consumption of sachet water, which are packaged essentially as drinking-water in the study area.

Methodology

The study area, Abeokuta, southwest Nigeria, has three local government areas (LGA). Samples (PSW) were collected over a period of 4 months from April to July 2014 from 39 different locations within the study area. The main selection criterion is population density. As such, the selected locations are highly populated areas (in terms of residential and business activities) within each of the three LGA.

In Nigeria, The National Agency for Food and Drug Administration and Control (NAFDAC) is the agency saddled with regulation of food and

water that is packaged for commercial purposes. As at April 2014, the number of registered PSW companies in Abeokuta was 72. There were only sixty registered PSW companies that were in operation as at the time of collection of samples. Using Yamane's formula (YAMANE 1967) the sample size was determined as follows:

$$n = N/1 + Ne2,$$

where:
n – required sample size
N – population size which is 60
e = 0.05 at 95% confidence interval.

The calculation gives a total of 51 which was used as the sample size. Samples were purchased thrice. Therefore, in total there was one hundred and fifty-three (153) packaged sachet water samples from 51 different packaged water manufacturing facilities (PWMFs) purchased randomly from retailers across the city of Abeokuta at the thirty nine different locations. All samples were transported in ice packs to the laboratory for microbiological analysis within 6 hours (h).

One ml from each sample was pre-enriched in Brain Heart Infusion (BHI) broth at ratio 1:9 for 3 h at 35°C. An aliquot (1 ml) of the pre-enrichment was transferred to 9 ml Tryptone Soya broth (TSB) and incubated at 44°C for 20 h followed by subsequent culture on Eosin-methylene blue (EMB) agar, incubated at 37°C for 24 h (Pathogenic *Escherichia coli*... 2006). Both gram and biochemical reactions were carried out. Typical green metallic sheen (presumptive *E. coli*) colonies which were gram negative and indole positive were subjected to further characterization such as test for sorbitol fermentation using Sorbitol MacConkey Agar (SMAC) incubated at 37°C for 18–24 h. Non-sorbitol fermenting *E. coli* colonies were serologically confirmed as O157:H7 using *E. coli* latex agglutination serotyping kit (Dryspot *E. coli* O157 latex test) for *E. coli* O157 (Oxoid, Basingstoke, UK). Colonies that agglutinated to the antisera were considered to be *E. coli* O157:H7.

Each of the isolates (*Escherichia coli*) was subjected to antibiotic sensitivity testing by the disk diffusion method (KIRBY-BAUER et al. 1966). The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted using Clinical and Laboratory Standards Institute guidelines (CLSI 2012). The antibiotics used are: penicillin, cefixime, streptomycin, erythromycin, amoxicillin, ampicillin, tetraxycline, co-trimoxazole, gentamycin, ciprofloxacin. The antibiotic disks were produced by Randox laboratories, UK.

Extraction of genomic DNA was conducted at Biotechnology Centre, Federal University of Agriculture, Abeokuta, Nigeria. Extraction was per-

formed using DNA extraction kit (Norgen, Canada) following manufacturer's instruction. The extracted DNA was viewed by agarose gel electrophoresis followed by PCR amplification obtained with universal primer for detection of bacteria (5pMol forward (5′-CGAGCAGCCGCGGAATACG -3′) and backward primer (5′ ATCGGTACCTTGTACGACTTC -3′)) (RAHMANI et al. 2006).

Bi-directional sequences were edited and aligned to generate a consensus sequence using BioEdit Sequence Alignment Editor (version 7.1.9). Consensus sequences were then subjected to similarity searches on National Centre for Biotechnological Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) to establish identities of the bacteria strains (both sequences have been submitted with accession numbers (OQ311321 and OQ311322). Also, PCR amplification for *stx*1 and *stx*2 gene was carried out (WILLNER et al. 2012) USING universal selective primers. Table 1 shows the PCR primers used for Shiga toxin detection in this study.

Table 1

Genomic sequence of primers for detection of Siga toxin genes in *Escherichia coli* O157:H7 strains from sachet water packaged as drinking-water in Abeokuta, Nigeria

Primer	Primer sequence (5'- 3')	Target gene	Size of amplicon (base pair, bp)	Reference
*Stx*1-F	ACACTGGATGATCTCAGTGG	Stx-1	614	(FERENS and HOVDE 2011)
*Stx*1-R	CTGAATCCCCCTCCATTATG	–	–	–
*Stx*2-F	CCATGACAACGGACAGCAGTT	Stx-2	779	(FERENS and HOVDE 2011)
*Stx*2-R	CCTGTCAATGAGCAGCACTTTG	–	–	–

Abbreviations: F – Forward; R – Reverse

Results

Occurrence of *Escherichia coli* in packaged sachet water samples, phenotypic reaction of isolated *E. coli* and serotyping

Out of 153 packaged sachet water samples analyzed, *E. coli* was isolated from only two sachet water samples (1.32%). The organism (*E. coli*) was isolated from the same PSW collected at different months (two months apart, with different batch number) from the same PWMF. The two Isolates were confirmed to be *Escherichia coli* O157:H7 with sorbitol fermentation and latex agglutination test as shown by the clumps in plate (Figure 1).

Table 2 shows that there was a positive reaction for the 2 isolates in terms of fermentation and gas production at 37° and 44°C respectively, which shows that the *E. coli* isolates are from feacal origin. The isolates were negative for tube haemagglutination. The hemolytic action of the isolates on blood agar shows that the isolates were not able to cause any hemolytic action on the blood agar (γ-hemolysis) while the isolates were pale coloured (negative reaction) on sorbitol MacConkey agar which shows that they were non-sorbitol fermenters.

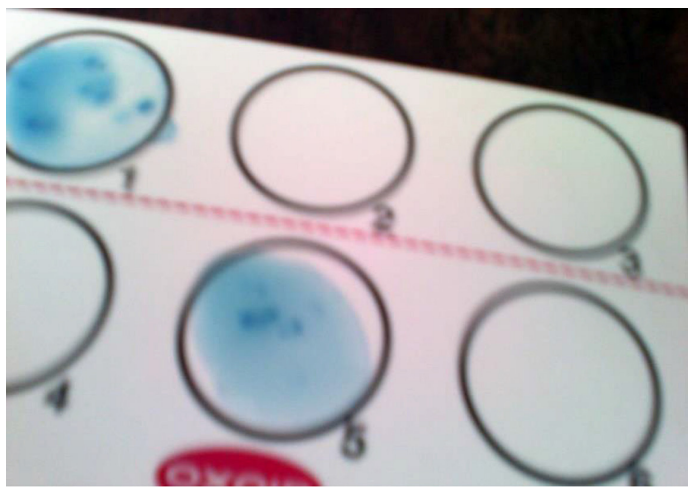

Fig. 1. Agglutination reaction of *E. coli* from packaged water sold in Abeokuta, Ogun State, Nigeria to oxoid O157:H7 antiserum

Table 2

Phenotypic characterization of *E. coli* isolates in packaged water sold in Abeokuta, Ogun State, Nigeria

Phenotypic conditions	Isolates	
	Escherichia coli[a]	*Escherichia coli*[b]
Growth at 37°C	+	+
Growth at 44°C	+	+
Haemmaglutination assay	–	–
Hemolytic reaction	–	–
Sorbitol fermentation	–	–

Abbreviations: + positive; – negative

Antibiotics Sensitivity Test (AST)

Table 3 shows the pattern of each bacteria strain to different classes of antibiotics used. Strain A showed resistance to five antibiotics of four classes while *E. coli* strain B showed resistance to seven antibiotics belonging to six classes which indicate that the antibiotic resistance pattern differed in the two strains.

Table 3

Minimum inhibitory concentration and minimum bactericidal concentration of *E. coli* isolates in packaged sachet water sold in Abeokuta, Ogun State, Nigeria

Antibiotics	*Escherichia coli*[a]	*Escherichia coli*[b]
PEN	R	S
CEFX	S	R
STREP	R	S
ERY	R	R
AMX	S	R
AMP	S	R
TET	S	R
COT	S	R
GEN	R	R
CIPX	R	S

Abbreviations: [a] – first *E. coli* isolate; [b] – second *E. coli* isolate; S – sensitive; R – resistance; Antibiotics (CLSI, 2012): PEN – Penicillin; CEFX – Cefixime; STREP – Streptomycin; ERY – Erythromycin; AMX – Amoxicillin; AMP – Ampicillin; TET – Tetracycline; COT – Co-trimoxazole; GEN – Gentamycin; CIPX – Ciprofloxacin

Molecular characterization of *E. coli* O157:H7 isolates

Figure 2 described a gel picture showing the successful extraction of deoxyribonucleic acid (DNA) material of isolates.

STX genes in *Escherichia coli* O157:H7 strains

Gel electrophoresis of PCR amplified Shiga toxin genes shows that both *E. coli* strains produced *stx*1 with 503 bp (Figure 3) but none had *stx*2 (Figure 4).

Fig. 2. Gel picture of extracted genomic DNA of *E. coli* O157:H7 from packaged water sold in Abeokuta, Ogun State, Nigeria. Abbreviations: *M* – DNA marker; *A* – *E. coli* isolate *A*; *B* – *E. coli* isolate B; - – control

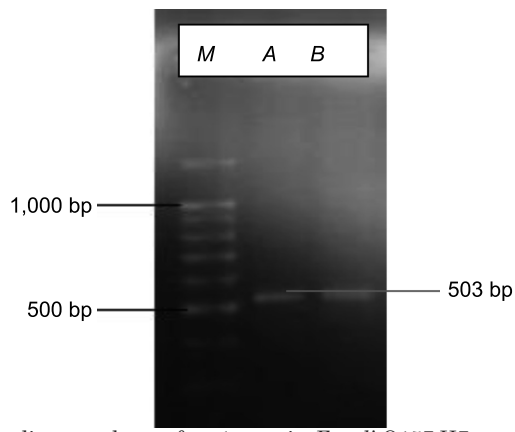

Fig. 3. PCR amplicon products of *stx1* gene in *E. coli* O157:H7 strains from sachet waterpackaged as drinking-water in Abeokuta, Nigeria. Abbreviations: *M* – molecular marker; *A* – *Escherichia coli* strain A; *B* – *Escherichia coli* strain B

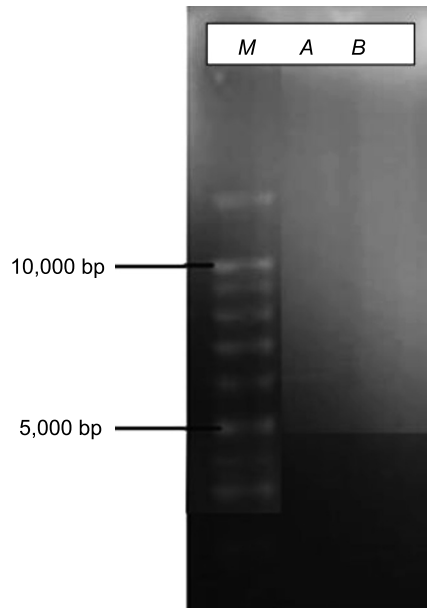

Fig. 4. PCR amplicon products of *stx2* gene in *Escherichia coli* O157:H7 strains isolated from sachet water packaged as drinking-water in Abeokuta, Nigeria. Abbreviations: *M* – molecular marker; *A* – *Escherichia coli* strain A; *B* – *Escherichia coli* strain B

Discussion

Shiga, toxin-producing Escherichia coli O157:H7 in packaged drinking-water: implications for public health

This study isolated *Escherichia coli* O157:H7 from sachet water that are essentially packaged as drinking-water in Abeokuta, southwest Nigeria, at a prevalence of 1.3% from one brand of PWMF. Arguably, the percentage prevalence of 1.3 is low, however, it should be noted that the object of study (Packaged sachet water) is treated water and should, according to WHO and NAFDAC, have zero coliforms cfu/100ml of water. Also, though the percentage prevalence is low (1.32%), the strain of *E. coli* (0157:H7) that was isolated from the samples is of public health interest considering the bacterial pathogenicity, low infectious dose and ability to survive in extra-intestinal environments (AIBINU et al. 2007). Similarly, the population at risk (more than 60% of over 170 Million Nigerian population take sachet water) when factored in makes the presence of coliforms a public health concern to a wide human population regardless of the low prevalence rate.

Serological and molecular techniques confirmed the strain of *E. coli* as O157:H7. Although, this strain has been isolated in tap water and surface water in Nigeria (CHIGOR et al. 2010, LUGA et al. 2007, SMITH et al. 2009), but it not been isolated from PSW, due to the assumption that PSW, being treated water should be free from bacteria not to talk of the highly pathogenic *E. coli* O157:H7. Therefore, most research on PSW in Nigeria are benchmarked at biochemical characterization.

The two strains from this study were positive for PCR detection of Shiga toxin (stx1) confirming their virulence though stx2 was not detected. Shiga toxin is the critical virulence factor in STEC diseases. The isolated *E. coli* O157:H7 strains were also multidrug resistant, which is not a new occurrence in Nigeria (AIBINU et al. 2007). Multi-drug resistant Shiga toxin-producing *E. coli* O157:H7 constitutes a significant public health problem in Nigeria (SOUZA et al. 2011). In addition, the occurrence of *E. coli* O157:H7 from packaged sachet water in this study is of public health significance, particularly, considering the percentage of households that depend on sachet water for drinking in Nigeria. The people that are most at risk of infection are the very young, the elderly and people with an already weakened immune system (KALGI and MARTINS 2015), pregnant women and HIV/AIDS patients (ABONGO et al. 2008, REUBEN and GYAR 2015). Therefore, packaged sachet water in Nigeria should be given serious attention by carrying out more studies on the pathotypes of *E. coli* isolated in water beyond the sole focus given to the occurrence of *E. coli* as an indicator for water quality.

Furthermore, urgent attention should be geared towards the enforcement of the adoption and development of water safety interventions by drinking-water suppliers to prevent outbreaks of waterborne diseases, particularly, the application of Water Safety Plans (WSP). Water Safety Plans are preventive, comprehensive and systematic assessment of drinking-water supply systems from source to tap, using risks assessments and management tools (WHO 2017). Enforcing the application of water safety planning is to ultimately ensure water and public health protection.

Conclusion

Shiga toxin producing multi-drug resistant *Escherichia coli* O157:H7 is identified as a microbiological hazard transmitted to a wide population through sachet water that is packaged essentially for drinking in Abeokuta, Nigeria.

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