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Antibiotic Resistance Phenotypes among *Escherichia coli* Isolated from Sachet and Pipe-Borne Water from Selected Local Government Areas of Kaduna State, Nigeria

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Abstract

The presence of coliform bacteria such as Escherichia coli (E. coli) in water used for drinking is of great public health concern due to the risk it poses to consumers. This risk is exacerbated when the bacteria are resistant to commonly used antibiotics. This study aimed at determining the antibiotic resistant phenotypes among E. coli isolated from sachet water (Pure water) and municipal pipe-borne water in selected local government areas of Kaduna state, Nigeria. A total of 105 water samples (69 sachet water and 36 municipal pipe-borne water) were subjected to bacterial isolation following standard methods and the isolated E. coli were subjected to antibiotic susceptibility testing to ten (10) commonly used antibiotics. The isolation rates of the organism were 14.4% and 22.9% in sachet and pipe-borne water respectively. The isolates were all (100%) susceptible to gentamicin, and also showed high susceptibility to amoxicillin-clavulanic acid (83.3%), chloramphenicol (94.5%), cefotaxime (94.5%) and sulphamethoxazole - trimethoprim (100%). On the other hand, 9 isolates (50%) showed various resistance patterns with 3 isolates being resistant to up to seven (7) antibiotics, with resistance patterns TE, NA, C, CIP, CTX, AMP, AML and TE, NA, AMC, SXT, CIP, AMP, AML. Eight out of the 9 resistant isolates showed resistance to Nalidixic acid and 7 showed resistance to Tetracycline. These results indicate that drinking water in the study area is a source of antibiotic resistance. It is obvious that more attention needs to be focused on ensuring the safety and potability of drinking water in the study area.

Key words: *Escherichia coli, Antibiotic resistance, sachet water, pipe-borne water*

INTRODUCTION

The detection of enteric bacteria such as *Escherichia coli* in water which is used for drinking is of public health concern. Their presence, apart from being a possible source of water-borne diseases, could also signify the possible presence of other enteric organisms indicating faecal pollution (WHO, 2011). Recently, there has been increasing interest in antibiotic resistant bacteria in food and environmental samples such as water (Chen *et al.*, 2017). The presence of such organisms in water that is considered safe enough for drinking and use in domestic settings, is of grave concern. The use of water packaged in polythene sachets commonly referred to as "Pure water" is considered as being better than most other sources of water because it is assumed that they have undergone proper treatment and checks before they are packaged and sold to consumers (Daniel and Daodu, 2016). The implications of antibiotic resistant bacteria being isolated from such water samples are enormous. Firstly, there is the likelihood of transfer of antibiotic resistance determinants to other bacteria in the environment, and in the consumers (human or animal), leading to selective pressure and an increased proliferation of antibiotic resistance in the environment (Waila *et al.*, 2004). Furthermore, because water from these sources are usually not treated further before they are consumed, if these organisms cause illness in consumers, it could lead to fewer treatment options, the use of more expensive antibiotics, longer hospital stays, increased risk of complications and even death (Tula and Osaretin, 2014). This study aimed at determining the antibiotic resistant phenotypes of *E. coli* isolated from sachet water, commonly referred to as "Pure water" and municipal pipe-borne water in parts of Kaduna state, North-Western Nigeria.

MATERIALS AND METHODS

Study Area

The study area comprised of some Local Government Areas (LGA) of Kaduna State, which is in the center of Northern Nigeria and is about 960km from the Atlantic Ocean. It is located on a latitude of 11° 12` N and a longitude of 07° 37`E. (Ukegbu, 2005).

Sample Collection

One hundred and five (105) samples were collected from four locations in Kaduna state, namely: Ikara, Sabon-gari, Kaduna north and Kajuru Local Government Areas (LGA), comprising 69 Sachet water and 36 pipe-borne water samples. The pipe-borne water samples were collected in clean 1litre containers after flaming the mouths of the tap and allowing the water to run for some minutes. The sachet water samples were purchased from vendors and all samples were transported to the laboratory under cold storage for analysis within 24 hours of collection.

Sample Analysis

E. coli was isolated from the water samples by inoculating 20 millilitres (ml) of each water sample into duplicate flasks containing 20ml of sterile double strength Tryptone Soy Broth. The tubes were afterward incubated at 44°C for 24 hours. After this time, a loopful of the enrichment culture was streaked on plates of Eosin Methylene Blue (EMB) agar (Oxoid,UK) and incubated at 44°C for 24 hours (LeJejune *et al* 2001). The suspected colonies were subjected to Gram staining and conventional biochemical tests including indole, methy red voges-proskaeur, citrate, motility, triple sugar iron and urease tests. Furthermore, the Microgen Gram Negative-A (GN-A) ID kit for *Enterobacteriaceae* was used for the confirmation of the organism following the manufacturer's instructions. This test kit consisted of a plastic strip with twelve (12) microtubes containing dehydrated substances that detect the indicated biochemical characteristics. The test substrates were inoculated with a pure culture of three colonies of the organism evenly suspended in sterile physiological saline. After incubation for 24hours, the 12 test results were converted to a 4-digit octal code which was used to confirm the identity using Microgen ID computer software, version 1.2.5.26.

Antibiotic Susceptibility Test

This was carried out using Mueller Hinton agar (Oxoid, UK). The organisms were tested *in vitro* for susceptibility to ten commonly used antibiotics namely: Ampicillin (10µg), Gentamicin (10µg), Amoxycillin(10µg), Tetracycline(30µg), Ciprofloxacin (5µg), Augmentin (30µg), Cefotaxime (30µg), Nalidixic acid (30µg), Chloramphenicol (30µg) and Sulphamethoxazole-trimethoprim (25µg) (Eziekel *et al.*,2011).

Using a sterile wire loop, three well isolated colonies that had been freshly grown on nutrient agar, were picked and emulsified in sterile normal saline. The prepared bacterial suspension was matched with a turbidity standard of 0.5 McFarland, which was prepared by mixing 0.05ml of 1% Barium chloride solution and 9.95ml of 1% sulphuric acid. This produced a solution with a turbidity that matched a bacterial suspension of approximately 1.5×10^8 cfu/ml of bacterial cells. A sterile swab was used to inoculate the suspension by streaking on the prepared and dried sterile Mueller-Hinton agar plate evenly after which it was allowed to stand for 3 minutes. Sterile forceps were then used to place the antimicrobial discs on the inoculated plates. Within 30 minutes of applying the disc, the plates were incubated at 37°C for 24 hours. By using a meter rule on the underside of plate, the diameter of each zone of inhibition was measured in millimetres (CLSI, 2014).

Zone diameter for standards were compared with Clinical Laboratory Standards Institute published limits. Each isolate was classified as; sensitive (S), intermediate (I) and resistant(R). Organisms that were observed to be resistant to at least three different antibiotics were classified as being multidrug resistant (Eziekel *et al.*, 2011).The MAR Index was obtained by calculating the ratio of the number of antibiotics an organism was exposed to with regards to the total number of antibiotics that were used in the testing (Furtula *et al.*, 2013).

RESULTS

The sachet water samples from Sabon-gari had the highest isolation rate of *E. coli* (5.8%). The lowest rate was observed in samples from Kaduna North as shown in Table 1. The overall isolation rate in sachet water samples was 14.4%.

Table 1: Isolation frequency of *Escherichia coli* in Sachet water samples

Location (N)	n (%)
Sabon-gari LGA (28)	4(5.8)
Ikara LGA (19)	3(4.3)
Kaduna North LGA (19)	2(2.9)
Kajuru LGA (3)	1(1.4)
Total (69)	**10(14.4)**

Key: N- Number of samples collected, n- Number of samples containing *E. coli*

The isolation rate of *E. coli* in the pipe-borne water samples are presented in Table 2. At the time of sampling, pipe-borne water supply was not available in Kajuru LGA. The highest isolation rate was observed in Kaduna North LGA with16.7% isolation rate of *E. coli*.

Table 2: Isolation frequency of *Escherichia coli* in pipe-borne water samples

Location(N)	n (%)
Sabon-gari LGA(11)	1(2.8)
Ikara LGA (5)	1(2.8)
Kaduna North LGA (20)	6(16.7)
Kajuru LGA (0)	0
Total (36)	**8(22.9)**

Key: N- Number of samples collected, n- Number of samples containing *E. coli*

The antibiotic susceptibility patterns of the isolates to the 10 commonly used antibiotics are shown in Table 3. The highest resistance was observed to Nalidixic acid (44.4%) followed by Tetracycline (38.9%), while all the isolates were susceptible to Gentamicin (100%).

Table 3: Antibiotic susceptibility patterns of *Escherichia coli* isolates from water samples (N=18)

Antimicrobial agent	Symbol	Concentration (µg)	R n (%)	I n(%)	S n(%)
Amoxicillin	AML	10	5(27.8)	3(16.7)	9(50)
Amoxicillin-clavulanic acid	AMC	30	3(16.7)	0(0)	15(83.3)
Ampicillin	AMP	10	4(22.2)	2(11.1)	12(66.7)
Chloramphenicol	C	30	1(5.6)	0(0)	17(94.5)
Ciprofloxacin	CIP	10	4(22.2)	9(50)	5(27.8)
Cefotaxime	CTX	30	1(5.6)	0(0)	17(94.5)
Gentamicin	GN	30	0(0)	0(0)	18(100)
Nalidixic acid	NA	30	8(44.4)	2(11.1)	8(44.4)
Sulphamethoxazole-trimethoprim	SXT	25	3(16.7)	0(0)	15(83.3)
Tetracycline	TE	30	7(38.9)	3(16.7)	8(44.4)

KEY
N-Number of isolates tested
n-Number of isolates susceptible/intermediate/resistant
R- Resistant
I-Intermediate
S-Susceptible

Table 4 shows the resistance patterns and multiple antibiotic resistance (MAR) index of the isolates. Three of the isolates were resistant to up to 7 antibiotics.

Table 4: Antibiotic resistance patterns and multiple antibiotic resistance (MAR) index of *E. coli* isolated from water

Isolate no.	Sample location	Sample source	Resistance pattern	MAR Index
1E.C	Kaduna north	PBW	NA, TE	0.2
2E.C	Kaduna north	PGW	NA, CIP	0.2
3E.C	Sabon-gari	PGW	NA, TE	0.2
4E.C	Kaduna north	PBW	NA, AML, AMC	0.3
5E.C	Sabon-gari	PGW	TE, AMP, AML	0.3
6E.C	Kaduna north	PGW	TE, NA, SXT	0.3
7E.C	Sabon-gari	PGW	TE, NA, C, CIP, CTX, AMP, AML	0.7
8E.C	Kaduna north	PBW	TE, NA, AMC, SXT, CIP, AMP, AML	0.7
9E.C	Kaduna north	PBW	TE, NA, AMC, SXT, CIP, AMP, AML	0.7

Key: E.C- *E. coli*, PBW- Pipe-borne water, PGW- Packaged water, NA- Nalidixic acid, AMC- Amoxycillin Clavulanic acid, SXT- Sulphamethoxazole, CIP- Ciprofloxacin, AMP- Ampicillin, AML- Amoxycillin, TE- Tetracycline, C-Ciprofloxacin, CTX- Cefotaxime,

DISCUSSION

The isolation of faecal *E. coli* in packaged and pipe-borne water sold in the various study areas is of public health concern. The implication of isolating *E. coli* from the packaged water and pipe-borne water samples is that it indicates the possible presence of other pathogenic organisms which are of human or animal intestinal origin. This gives a strong indication that the contaminated water samples have been in contact with faecal material (Patoli *et al.,* 2010).Furthermore, there are a few strains which have been shown to be pathogenic, causing diarrheoa. In some persons, particularly children under 5 years of age and the elderly infection with some strains can also cause a complication called haemolytic uraemic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail (Kaper *et al*., 2004).

Our findings also indicate that the water was not properly treated before being packaged for sale. This is unfortunate because the packaged water popularly referred to as 'pure water' in the local parlance are believed to have undergone various treatments and are also perceived to be of better quality than other untreated water sources. This finding is similar to the results obtained by other researchers where the isolation frequency of *E. coli* in sachet water in Ghana and Ile-Ife, Nigeria ranged from 13.3 to 40% (Dodoo *et al.,* 2006; Tagoe *et al.,* 2011 and Oyedeji *et al.,* 2011).

According to the WHO, *E. coli* should not be detected at all in treated water. It is therefore safe to say that these contaminated packaged water brands sampled are not fit for drinking (WHO, 2011).Interestingly, for a packaged water brand to be sold, it is ideally supposed to have undergone checks by the Food and Drug regulatory agency in Nigeria. There is a possibility that many unscrupulous entities just package any source of water that may look clean to the naked eye, and sell to unsuspecting citizens (Tagoe *et al.,* 2011). There is also a possibility that packaged water could get contaminated post-production due to improper storage, poor handling of sachet water by the vendors and back-seepage into bags that are not well sealed, though the evidence showing this is scarce. In a study carried out by Dada (2009),it was reported that the quality of sachet water, despite having the mandatory National Agency for Food and Drug Administration and Control (NAFDAC) certification number, can be compromised significantly due to handling as it moves from the manufacturer to the consumer, and must not be assumed to be generally safe for drinking.

The municipal water supply in Nigeria is quite poor (Aboh *et al.,* 2015), and it was not available in Kajuru LGA at the time of sampling. Even where it is available, many of the pipes are most likely very old, and could have points of leakage through which there could be the influx of sewage into the pipes. The water is supposedly chlorinated from the water treatment plants, so it could be that it is at the point of distribution that contamination occurs. Furthermore, some of the pipes may also be sited very close to toilets and septic tanks, which could also be a source of contamination.

The 100% susceptibility rate to gentamicin observed in this study agrees with the findings of other researchers (Oyetayo *et al.,* 2007; Tagoe *et al.* 2011 and Odonkor and Addo 2018), where the bacteria isolated from drinking water in Akure, Nigeria and Ghana also showed 100% susceptibility to gentamicin. The excellent susceptibility to gentamicin could be due to the fact that it is administered parenterally and therefore cannot be easily taken without the aid of skilled medical personnel.

The findings of high susceptibility to cefotaxime, chloramphenicol, sulphamethoxazole- trimethoprim and amoxycillin- clavulanic acid are in consonance with those of Walia *et al.* (2004) where 81% of their isolates from drinking water were susceptible to chloramphenicol. Larson *et al.* (2019) also observed 78% susceptibility of their isolates to sulphamethoxazole-trimethoprim. Tetracycline and other antimicrobials are used in food animals to treat or prevent disease and also to promote growth (Lapierre *et al.*, 2006 and Olatoye, 2010). This implies that the unused drug could be discarded into the environment thereby exposing environmental isolates, which could be a contributing factor to resistance.

The extremely low toxicity of the antimicrobial agents in the tetracycline, sulfonamide and β-lactam classes has resulted in their overuse in the medical world, hence the observed increased resistance. The relatively high level of resistance to antimicrobial agents in the environment depicts a true reflection of their indiscriminate and excessive usage (Titilawo, 2015). There was no resistance observed in the isolates from samples collected from Kajuru and Ikara LGAs. The isolation of antibiotic resistant bacteria from the water samples shows that these bacteria could be a source of transmission of antibiotic resistance to other bacteria in the environment (Waila *et al.*, 2004).

The level of resistance as an evidenced by the Multiple Antibiotic Resistance (MAR) Index of 0.2-0.7 is an indication of abuse and overuse of antibiotics in the environment and in the hospitals (Okeke *et al.*, 2007). This could be worsened by the over the counter sale of antibiotics, inappropriate prescription by clinicians and poor regulations on the retail and purchase of antibiotics (Tula and Osaretin, 2014).

CONCLUSION

From the findings in this study, it can be seen that the quality of the sachet and pipe-borne water in the study area is of low quality generally and are not fit for drinking. The isolates obtained were highly susceptible to gentamicin, cefotaxime and chloramphenicol, sulphamethoxazole- trimethoprim and Amoxycillin- Clavulanic acid. The resistance pattern observed was majorly in response to Nalidixic acid, Tetracycline, Ampicillin and Amoxycillin. Three of the isolates were resistant to 7 antibiotics. This shows that there is a problem of abuse of antibiotics as evidenced by the isolation of multidrug resistant isolates from the drinking water samples. The populace living in these areas must therefore be advised to treat their drinking water properly before consumption. The National regulatory agency must also step up their activities to ensure strict monitoring of all commercially available sachet water brands and proper treatment of the municipal pipe-borne water.

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