IJABR Vol. 7(2): 9 - 17 (2016)

Original Article

MULTIDRUG RESISTANT *Escherichia coli* ISOLATED FROM DRINKING WATER FROM PARTS OF KADUNA SOUTH, KADUNA STATE, NIGERIA

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Submitted: November, 2016; Accepted: November, 2016; Published: December, 2016

ABSTRACT

Drinking water from parts of Kaduna South was investigated for the presence of multidrug resistant *Escherichia coli (E. coli).* A total of one hundred and sixty-seven water sources (borehole, tap, well, stream and packaged water) were sampled from two Local Government Areas in the zone. The samples were collected over a period of twelve months (March 2014– February 2015). *E. coli* was isolated after enrichment in Tryptone soy broth and streaking on Eosin Methylene Blue agar. From these samples, 17 isolates were confirmed to be *Escherichia coli*, out of which 10 were selected for antibiotic susceptibility testing. The isolates were tested for resistance to ten commonly used antibiotics using the Kirby-Bauer method. The highest resistance observed was to Tetracycline (80%), while all the isolates showed susceptibility to Chloramphenicol, Cefotaxime and Gentamicin. Two of the isolates (20%) showed resistance to 7 antibiotics while 3(30%) were resistant to 3 antibiotics respectively. The isolates that were resistant to the highest number of antibiotics were obtained from tap water and well water samples. An isolate obtained from sachet water was also found to be resistant to four antibiotics. These results indicate that these water sources are generally unfit for consumption. The isolation of antibiotic resistant bacteria in drinking water, especially water that has been treated is of public health concern due to the possibility of transferring multiple antibiotic resistances to normal flora in the intestinal tract leading to more complicated antibiotic resistance. The isolates could also be possible reservoirs of antibiotic resistance as they would harbour and disseminate antibiotic resistant genes to other bacteria.

Key words: *Escherichia coli,* multidrug resistance, antibiotics, Kaduna south.

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INTRODUCTION

Water of poor quality has continually become a leading cause of health problems especially in developing countries where it is estimated that about 3.4 million deaths occur every year especially among children (UNICEF, 2008). Many pathogenic bacteria potentially transmitted by water infect the gastrointestinal tract and are excreted in the faeces of infected humans and other animals, from where they might end up in water sources via sewage (WHO, 2011).

Isolation of pathogens such as *Salmonella* spp, *Escherichia coli* (*E. coli*), *Campylobacter*, *Shigella* species and *Vibrio cholerae* from water sources, is indicative of possibly serious public health risk to consumers. This risk is further worsened by the widely reported cases of resistance of enteric bacterial pathogens to several antibiotics (Ash *et al*., 2002; Okeke *et al*., 2007).

Antimicrobials have helped in the reduction of mortality and morbidity resulting from communicable diseases, but the increased rate of resistance has led to a reduction in their effectiveness (Nontongana *et al.,* 2014). Increased outbreaks of enteric illnesses have invariably led to increased use of antibiotics for managing infections. A very large percentage of the antibiotics used in animal husbandry are mainly for prophylaxis and growth promotion, hence, they usually get excreted from the body of the animals in an unchanged state (Titilawo *et al.,* 2015).

E. coli from drinking water has been previously associated with the transfer of antibiotic-resistant gene pools to other bacteria including normal flora of humans and animals. This has led to a selective pressure which encourages the survival of resistant strains. Eventually, they find their way into nature through wastewater, compost and sewage (Kinge *et al.,* 2010). Monitoring of water bodies for pathogenic bacteria and antimicrobial resistance is therefore of great importance (Doughari *et al.,* 2010).

MATERIALS AND METHODS

Site description

The study area was Kaduna South Senatorial district of Kaduna State which is in the centre of Northern Nigeria located on a latitude of 11° 12` N and a longitude of 07° 37`E.

Sample collection

A total of 167 samples were collected from two Local Government Areas (Jema'a and Kagarko) between March 2014 and February 2015 using a convenience sampling method and depending on the availability of the sample types. The samples comprised of tap, wells, boreholes, packaged and stream water, depending on which was available at the time of sampling. They were collected in sterile 1litre containers, except for the packaged water which were purchased at selling points and transported to the laboratory under cold storage for analysis within 24 hours.

Sample analysis

Escherichia coli was isolated from the water samples using the method of LeJejune *et al.* (2001). Twenty (20) millilitres of each water sample were inoculated into duplicate flasks containing 20ml of sterile double strength Tryptone Soy Broth. The tubes were incubated at 44°C for 24 hours. After this time, a loopful of the broth was streaked on plates of eosin methylene blue (EMB) agar and incubated at 44°C for 24 hours. Mixed cultures were re-streaked for purity on plates of sterile EMB agar and then colonies that were observed to have greenish sheen with dark centres were transferred on to nutrient agar slants and subjected to Gram staining, and biochemical tests using the Microgen *Enterobacteriaceae* GN A ID kit for confirmatory tests.

The susceptibility test of the organisms to different antibiotics was carried out using the Kirby-Bauer disc diffusion method on Mueller Hinton agar (Oxoid, UK). The isolates were tested *in vitro* for susceptibility to the following commonly used antibiotics: Ampicillin (10µg), Gentamicin (10µg), Amoxycillin (10µg), Tetracycline (30µg), Ciprofloxacin (5µg), Augmentin (30µg), Cefotaxime (30µg), Nalidixic acid (30µg), Chloramphenicol (30µg) and Sulphamethoxazole-trimethoprim (25µg) (CLSI, 2014). Zone diameter for standards were compared with Clinical Laboratory Standards Institute published limits. The response of the isolates were classified either sensitive (S), intermediate (I) or 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).

RESULTS

Escherichia coli was obtained from water samples of both locations and from all the water sources that were sampled (Tables 1 and 2). From all the samples collected, *E. coli* accounted for 10.2% of all isolates.

Table 1 Distribution of *Escherichia coli* isolated from Jema'a and Kagarko LGAs

Location (N)	Sample distribution	*Escherichia coli* n (%)
Jema'a(87)	Tap(24), well(39), Borehole(17),packaged(5), Stream(2)	4(4.60)
Kagarko(80)	Tap(0),well(45), Borehole(22),packaged(9), Stream(4)	13(16.25)
TOTAL -167		17(10.2)

KEY: N- number of samples collected; n- number of isolates obtained; %- percentage

Table 2: Distribution of *Escherichia coli* isolated from various water sources

Sample type(N)	*Escherichia coli* n (%)
Sachet water(15)	1(6.67)
Tap water(25)	2(8)
Well water(83)	8(9.64)
Borehole water(38)	5(13.16)
Stream water(6)	1(16.67)
Total- 167	17(10.2)

KEY: N- number of samples collected; n- number of isolates obtained; %- percentage

A total of 80% *E. coli* isolates were susceptible to Amoxycillin-clavulanic acid, 100% were susceptile to Chloramphenicol, Cefotaxime and Gentamicin, and 50% to Sulphamethoxazole-trimethoprim (Table 3). The highest resistance observed was to Tetracycline (80%), followed by Ampicillin and Amoxycillin (70%).

Seventy (70) percent of the isolates tested were multidrug resistant (MDR). Two of the isolates (20%) were resistant to seven antibiotics each (Figure 1). On the other hand, one isolate showed intermediate and complete susceptibility to all the antibiotics. The organisms with the highest multidrug resistance were obtained from tap water and well water. Interestingly, one isolate obtained from sachet water was resistant to four antibiotics. Most of the resistant isolates showed the TeNaAmpAml resistance pattern. These antibiotics belong to the tetracycline, quinolone and Penicillin families. tetracycline.

The results showed that the isolates produced 4 resistance patterns (Table 4), and were resistant to 1-7 antibiotics. The most common resistance patterns was TeSxtAmpAml. Two isolates had the resistance pattern TeNaSxtAmpAmlAmcCip.

Table 3: Antimicrobial susceptibility patterns of *Escherichia coli* isolates from water samples

			E.coli(N=10)		
Antimicrobial agent	Symbol	Disc strength (µg)	R n(%)	I n(%)	S n(%)
Amoxicillin	AML	10	7(70)	1(10)	2(20)
Amoxicillin-clavulanic acid	AMC	30	2(20)	0(0)	8(80)
Ampicillin	AMP	10	7(70)	1(10)	2(20)
Chloramphenicol	CHL	30	0(0)	0(0)	10(100)
Ciprofloxacin	CIP	10	2(20)	4(40)	4(40)
Cefotaxime	CTX	30	0(0)	0(0)	10(100)
Gentamicin	GNT	30	0(0)	0(0)	10(100)
Nalidixic acid	NAL	30	2(20)	3(30)	5(50)
Sulphamethoxazole-trimethoprim	SXT	25	5(50)	0(0)	5(50)
Tetracycline	TET	30	8(80)	0(0)	2(20)

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

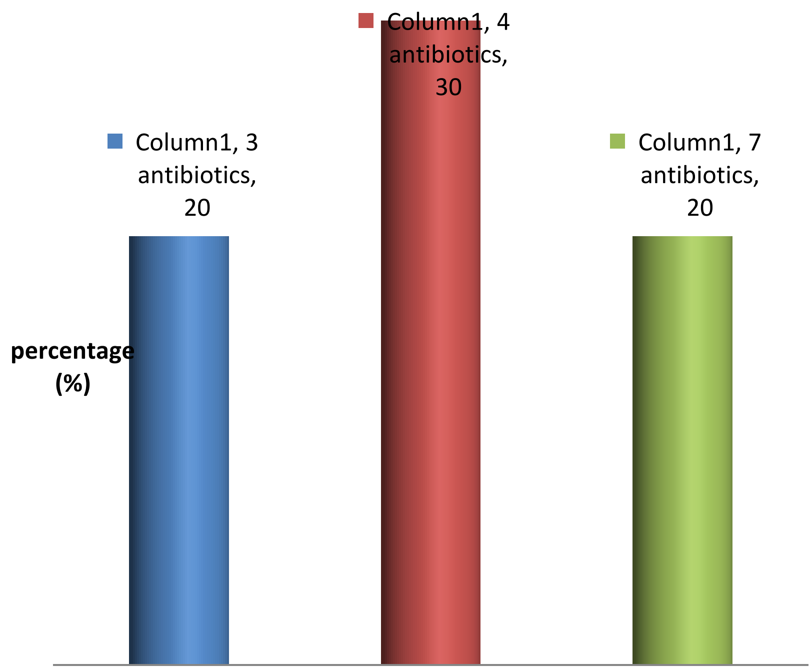

Figure 1: Percentage multidrug resistance of *E. coli* isolates (N=25)

Table 4: Antibiotic resistance patterns of *E. coli* isolates

Isolate no.	Sample location	Sample source	Resistance pattern
131E.C	Kagarko	borehole	TetAmpAml
227E.C	Kagarko	well	TetAmpAml
236E.C	Kagarko	packaged water	TetSxtAmpAml
239E.C	Kagarko	borehole	TetSxtAmpAml
234E.C	Kagarko	borehole	TetSxtAmpAml
262E.C	Jema'a	Tap	TetNalAmcSxtCipAmpAml
261E.C	Jema'a	Tap	TetNalAmcSxtCipAmpAml

KEY: E.C- *E.coli,* AML-Amoxycillin,AMC- Amoxycillin-clavulanic acid, AMP- Ampicillin, CHL-Chloramphenicol, CIP-Ciprofloxacin, CTX-Cefotaxime, GNT-Gentamicin, NAL-Nalidixic acid, SXT-Sulphamethoxazole-trimrthoprim, TET-Tetracycline

DISCUSSION

The isolation of *E. coli* from drinking water showed that hygiene is generally poor in the locations that were sampled. More worrisome, is the isolation from sachet water and tap water which are supposed to have undergone adequate treatment. The presence of these organisms is an indicator of possible faecal contamination of the water sources and the likely presence of other harmful organisms like *Vibrio cholerae,* Rotavirus, *Cryptosporidium* spp and so on. (Halablab *et al.*, 2010). The presence of *E.*

coli is an indication of the poor bacteriological quality of water (Chigor *et al.*, 2012). The organisms have also been isolated by other researchers who worked with drinking water in other parts of the country (Edema *et al.,* 2011; Ibiene *et al.,* 2012; Egbe *et al.*, 2013; Tula and Osaretin*,* 2014; Oluyege *et al.,* 2014; Aboh *et al.,* 2015).

A previous survey of Kaduna State showed that there is improper sewage disposal in most of the parts, indiscriminate defaecation and improper siting of wells and boreholes close to latrines and soak-aways (Olukosi *et al.,* 2008). Sachet water is considered by most people as being cleaner compared to ordinary tap water. Unfortunately, it was observed in this study that most of the sachet water samples were not fit for consumption. This is in agreement with the observations of other researchers (Dodoo *et al.* (2006), Oyedeji *et al.* (2011) and Akinyemi *et al.* (2011)). The faecal contamination of the sachet water may not be unconnected to the fact that the water may have been sourced from shallow and contaminated boreholes or wells and may not have be properly treated before sealing.

The high resistance rate of isolates to tetracycline could be explained due to that the antibiotic is used in animal husbandry to treat or prevent disease and also to promote growth (Lapierre *et al.,* 2006), while the low resistance to gentamicin could be due to the fact that it is administered parenterally and therefore cannot be easily taken without the aid of a skilled medical personnel.

The extremely low toxicity of the antimicrobial agents in the tetracycline, sulfonamide and β-lactam classes is one of the factors that has encouraged their excessive use leading to increased resistance (Titilawo, 2015).

This result slightly differed from that obtained by Oyedeji *et al.* (2011) where it was observed that none of the enteric bacteria isolated from water was resistant to ciprofloxacin. Also, the resistances that were observed in that study were more from stream water. However, they also reported high level of resistance to ampicillin, Similar results were also reported by other researchers (Patoli *et al.,* 2010; Nimri and Azaizeh, 2012; Tula and Osaretin, 2014).

The high level of resistance is an evidence 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).

When people use sub-therapeutic doses of antibiotics, as obtained when the dosage for the drugs are not strictly adhered to, highly resistant strains of the organisms are selected sequentially. Poor quality drugs can provide sub-inhibitory selective pressure, of which neither the patient nor the prescriber may be aware. There have been numerous reports of sub-standard drugs in Nigeria. These reports describe drug preparations containing between 0-80% of the stated label claim. Some of them contain such low concentrations that they can only be considered counterfeit, meaning that they were deliberately manufactured with low or no active drug content. Others may have complied with pharmacopoeia standards at some time but have, in the course of distribution and display, been degraded by heat and humidity (Okeke and Sosa, 2003).

The isolation of antibiotic resistant bacteria in drinking water samples is of public health concern because of the possibility of transferring multiple antibiotic resistances to normal flora in the intestinal tract leading to more complicated antibiotic resistance. They may also act as reservoirs which will contribute to the maintenance and spread of antibiotic resistance genes(Guardabassi *et al.*, 2004; Constanzo *et al.*, 2005).

Many of the *E. coli* isolates from this study were observed to have patterns of resistance that indicate that the genes responsible were acquired from other organisms, such as resistances to antibiotics of different classes, and resistance that show that they produce extended spectrum beta-lactamases.

CONCLUSION

This study showed that drinking water sources sampled in Kaduna state were generally of low quality and were reservoirs of antibiotic resistance in the study area. It is therefore advisable, that these waters should not be consumed without further adequate treatment.

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