Water quality assessment and plasmid analysis of multiple antibiotic-resistant *Escherichia coli* O157:H7 from well-water sources in Ado-Ekiti metropolis, Nigeria

Jacob Olaoluwa Oluyege, Monisade Omolade Adeoye and Busayo Mutiat Olowe

**ABSTRACT**

This research aimed to assess the physicochemical and bacteriological quality of underground water, and determine the antibiotic susceptibility and presence of plasmids in multiple antibiotic-resistant (MAR) *Escherichia coli* O157:H7 in underground water sources in Ado-Ekiti. Physicochemical and bacteriological analysis of water samples were carried out using standard methods, an antibiotic susceptibility test was investigated using the standard disc diffusion method and plasmid analysis of MAR isolates was carried out using the polymerase chain reaction (PCR) technique. The physicochemical parameters analyzed were within WHO recommendations except for pH and potassium while the water samples did not conform to the WHO bacteriological recommendations for drinking water. A total of 272 *E. coli* were isolated and identified, among which 150 isolates were non-sorbitol fermenters (NSF) and taken as presumptive *E. coli* O157. MAR to three and more classes of antibiotics used were observed among these NSF with high MAR-Index, >0.2. Plasmid analysis of selected 15 isolates among the MAR NSF showed that their resistance to antibiotics was likely plasmid-mediated as they carry one to two plasmids on them. The study revealed that the water samples from Ado-Ekiti metropolis are unsafe for consumption.

**Key words |** *E. coli* O157, MAR-Index, multiple antibiotic-resistance, plasmid analysis, water quality

**INTRODUCTION**

Safe drinking water remains an indispensable, essential item for human and other life-forms. It also plays an important role in the world’s economy, as it functions as a solvent for a wide variety of chemical substances and facilitates industrial cooling and transportation (Baroni et al. 2007). Although access to safe drinking water has improved over recent decades in almost every part of the world, approximately one billion people still lack access to safe water and over 2.5 billion do not have adequate sanitation (Ludwig et al. 1995).

Ado-Ekiti is the capital of Ekiti State, Nigeria. The city has had rapid population growth from 274,000 in 1995 to over 1.1 million in 2013, of which 82% live in the urban centre (Oriye 2013). Most homes in this city rely on self-constructed wells as their source of water. The issue with these wells is inadequate sanitation in the environment, which contributes to their contamination. Seepage from septic tanks, leachate streams from agricultural runoff, and faecal loading of water sources by birds and animals has also contributed immensely to water contamination (Olowe et al. 2016a). This faecal contamination of water sources has led to the emergence of pathogenic organisms such as pathogenic *Escherichia coli*, *Salmonella typhi*, *Shigella* sp., and *Vibrio cholera*, among others, which pose a threat to the health of the country (Abdelrahman & Eltahir 2011).

*Escherichia coli*, a family member of *Enterobacteriaceae*, found in the intestines of warm-blooded animals including humans, is a Gram-negative, facultative anaerobic,
non-sporulating and rod-shaped bacterium (Odonkor & Ampofo 2013). Commensal E. coli strains are harmless, but some pathogenic strains such as E. coli O157 can cause serious waterborne infection if consumed (Aboaba et al. 2006). E. coli O157 has been recognized as an emerging pathogen in drinking water sources (Selim et al. 2015). Many of the E. coli O157 which are resident in water bodies exhibit virulence traits such as resistance to antibiotics and heavy metals and possession of plasmids which encode their resistance to multiple antibiotics found in the environment. An antibiotic resistance trait in bacteria is of significant concern for human health because it brings about severe illness, increases mortality rates, risk of complications and admission to hospital (Livermore 2012). It also leads to increased costs of healthcare. Water bodies such as wells are liable to contamination from sources which are laden with heavy metals or antibiotics. Therefore, the study examines water quality, isolates and identifies E. coli O157 and investigates the presence of plasmids in multiple antibiotic-resistant (MAR) E. coli O157 from water samples randomly collected from Ado-Ekiti metropolis.

**MATERIALS AND METHODS**

**Collection of well-water samples**

A total of 25 well-water samples were randomly collected from five separate communities in Ado-Ekiti metropolis, which are far apart in Ado-Ekiti metropolis, as described by the Standing Committee of Analysts (2010) between April and June, 2015. Sterilized bottles were used for sample collection. At the well sites, a long rope was attached to the neck of each sterile bottle, which was gently let down into the well. Five samples each were collected from Adebayo, Ajilosun, Aba-Ebira, Government Residential Area (GRA) and Iworoko Communities. These were immediately transported in ice-pack to the laboratory and analyzed within 6 hours of collection.

**Physicochemical analysis of water samples**

The physicochemical analysis was carried out in the central science laboratory of Obafemi Awolowo University OAU, Ile-Ife, Osun State, Nigeria. The water samples were analyzed for pH, total dissolved solids (TDS), conductivity, alkalinity, turbidity, magnesium, calcium, potassium and sodium as described by Standard Methods (21st edition) of APHA et al. (2005).

**Bacteriological analyses of water samples**

The heterotrophic bacterial count of the water samples was carried out as described by Olowe et al. (2016a). Coliform counts of the water samples were determined by a standard membrane filtration technique; 100 ml of tenfold dilution (10^-6) of each water sample was filtered through a membrane filter, after which the membrane filter was inoculated with its face retaining bacteria upward on eosin methylene blue (EMB) agar.

Also, the presence and isolation of E. coli on EMB agar was carried out using the standard pour plate method; 1 ml of dilution 10^-3 was plated on EMB agar, and the plate was incubated at 37 °C for 24 hours and examined for a greenish metallic sheen which is a distinguishing feature of E. coli. A discrete colony was picked and subcultured on EMB agar until a pure culture of E. coli was obtained, after which it was kept on nutrient agar slant and incubated at 37 °C for 24 hours, and slants were stored at 4 °C in a refrigerator for further studies. Gram staining and biochemical tests which included indole, oxidase test, methyl red, the Voges–Proskauer test, and citrate utilization were carried out to further confirm the identity of E. coli.

Presumptive identification of E. coli O157 was carried out on Sorbitol MacConkey (SMAC) agar using standard procedures described by Public Health England (2013). Biochemically identified E. coli colony was streaked onto freshly prepared SMAC agar and incubated for 24 hours at 37 °C. After 24 hours of incubation, the plates were examined for growth of colourless, non-sorbitol fermenting colonies.

**Determination of antibiotic susceptibility**

Antibiotic susceptibility testing was performed using the standard disc diffusion method according to Clinical and Laboratory Standards Institute (2013). A total of eight different antibiotics of different classes were used. These included: cephems: ceftazidime (30 μg disk^-1), cefuroxime...
(30 μg disk⁻¹), cefixime (5 μg disk⁻¹); fluoroquinolones: ofloxacin (5 μg disk⁻¹), ciprofloxacin (5 μg disk⁻¹); nitrofurantoin: nitrofurantoin (300 μg disk⁻¹); beta-lactam: augmentin (30 μg disk⁻¹) and aminoglycoside: Gentamicin (10 μg disk⁻¹).

### Plasmid analysis

The isolates that showed multiple resistance to the antibiotics used were subjected to plasmid analysis. This was investigated using the TENS method as described by Smith et al. (2003). The DNA of the isolates was electrophoresed on 0.8% agarose gel, stained with ethidium bromide, visualized by UV trans-illumination. Molecular weights were calculated based on molecular weight standards.

### RESULTS AND DISCUSSION

The results shown in Table 1 depict the mean physicochemical values and standard deviation for each parameter determined. This table reveals that the mean pH values of the water samples vary in the range 5.88–6.68, TDS values in the range 73.5–184 ppm, conductivity values in the range 2.5–6.2 ppm and turbidity values in the range 0.57–10.35 NTU. The presence of cations Ca²⁺, K⁺, Na⁺ and Mg²⁺ in the water samples was also determined and included in Table 1. For Ca²⁺ and Mg²⁺, the mean values were both highest in GRA water samples and least in Ajilosun and Adebayo water samples respectively. Mean values for K⁺ and Na⁺ were highest in Ajilosun and Aba-Ebira respectively and both least in Iworoko water samples. Among all the parameters examined, only turbidity in the Aba-Ebira water samples and potassium in all the water samples were above the WHO recommended maximum allowable limits. However, the mean values of the parameters examined were below the maximum allowable limits of the Nigerian Standard for Drinking Water Quality (NSDQW) except the pH of water samples from Aba-Ebira and Ajilosun, which were above the allowable limit.

Table 2 presents the mean microbiological quality of the water samples. The results show that all the water samples from the communities had a high mean heterotrophic bacterial count, which ranged from 8.1 log cfu/ml in Iworoko water samples to 8.6 log cfu/ml in GRA water samples. Mean coliform count was extremely high, ranging from 7.3 log cfu/ml in Iworoko water samples and 8.3 log cfu/ml in Adebayo water samples. The presence of E. coli was confirmed in all the water samples with mean E. coli count varying between 5.18 log cfu/ml in Iworoko and 6.5 log cfu/ml in GRA water samples.

A total number of 272 E. coli isolates were recovered from all the water samples. These were then screened on

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aba-Ebira</th>
<th>Ajilosun</th>
<th>*GRA</th>
<th>Iworoko</th>
<th>Adebayo</th>
<th>Nigerian standard for drinking water quality (maximum allowable limit)</th>
<th>WHO recommended limits (highest desirable – maximum allowable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH at 20 °C</td>
<td>6.7 ± 0.2</td>
<td>6.7 ± 0.0</td>
<td>6.2 ± 0.4</td>
<td>6.1 ± 0.1</td>
<td>5.9 ± 0.2</td>
<td>6.5–8.5</td>
<td>6.5–8.5</td>
</tr>
<tr>
<td>TDS (ppm)</td>
<td>93.5 ± 12.0</td>
<td>184 ± 1.4</td>
<td>77 ± 12.9</td>
<td>73.5 ± 3.5</td>
<td>108 ± 7.1</td>
<td>500</td>
<td>600–1,000</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>155.8 ± 20.0</td>
<td>306.6 ± 2.3</td>
<td>128.3 ± 21.2</td>
<td>122.4 ± 5.9</td>
<td>180 ± 11.8</td>
<td>1,000</td>
<td>90–1,200</td>
</tr>
<tr>
<td>Alkalinity (ppm)</td>
<td>4.3 ± 0.1</td>
<td>6.2 ± 0.3</td>
<td>2.5 ± 0.0</td>
<td>3.0 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>NA</td>
<td>30–500</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>10.4 ± 0.6</td>
<td>19 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>2.2 ± 0.1</td>
<td>1.5 ± 1.3</td>
<td>5</td>
<td>1–5</td>
</tr>
<tr>
<td>Calcium (ppm)</td>
<td>1.2 ± 0.1</td>
<td>7.4 ± 0.0</td>
<td>0.8 ± 0.0</td>
<td>2.1 ± 0.0</td>
<td>2.0 ± 0.0</td>
<td>NA</td>
<td>75–200</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>1.4 ± 0.1</td>
<td>5.5 ± 0.5</td>
<td>3.3 ± 2.1</td>
<td>0.9 ± 0.0</td>
<td>1.2 ± 0.0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Sodium (ppm)</td>
<td>2.4 ± 2.6</td>
<td>0.4 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>200</td>
<td>0–200</td>
</tr>
<tr>
<td>Magnesium (ppm)</td>
<td>1.0 ± 0.0</td>
<td>1.2 ± 0.0</td>
<td>0.6 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>0.2</td>
<td>30–150</td>
</tr>
</tbody>
</table>

NA: Not available.

*GRA: Government Residential Area.
SMAC and it was observed that 122 (44.8%) isolates were sorbitol fermenters and 150 (55.14%) isolates were non-sorbitol fermenters (Table 3). The 150 isolates were taken as presumptive E. coli O157 and then antibiotic susceptibility testing was carried out on them.

Overall resistance of the isolates to the antibiotics showed that 147 (98%) out of 150 isolates were resistant to at least one of the antibiotics used in this study. Figure 1 shows the resistance profile of presumptive E. coli O157 results revealed that resistance to augmentin was highest with 126 (84%) isolates showing resistance to the antibiotic while resistance to ciprofloxacin was least with seven (4.6%) isolates resistant to the antibiotic. Also, isolates showed resistance to nitrofuratoin, cefixime, gentamicin, cefuroxime and ceftazidime while all the isolates were susceptible to ofloxacain (Figure 1). From the 147 isolates, 70 (47.6%) showed multiple antibiotic resistance with different combinations of antibiotics (Table 4). The table shows that 64 isolates were multiply resistant to three different classes of antibiotics. Also simultaneously, five isolates were resistant to four antibiotics and one isolates to all five classes of antibiotics. In addition, the table shows the MAR-Index for each multiple antibiotic-resistant isolate. The MAR-Index estimated for each isolate that demonstrated resistance to ≥3 classes of antibiotics ranged from 0.6 to 1.0 (Table 4).

A total of 15 multiple antibiotic-resistant isolates were screened for plasmids and they were found to harbour one or more plasmids of different sizes (Figure 2). Plasmid profiles of these isolates are detailed in Figure 2. The plasmid profiling demonstrated that all the isolates except isolates in lanes 5 and 13 had one plasmid of size 23,100 bp, while isolates in lanes 5 and 13 contained two plasmids of different sizes. The isolate in lane 5 contained plasmids of sizes 1,100 bp and 3,100 bp while the isolate in lane 13 had plasmids of sizes 1,100 bp and 2,100 bp (Figure 2).

One of the primary goals of assessing drinking water quality is to protect public health. Therefore, regular assessment of drinking water in any community becomes necessary to ascertain the quality of such water and if it is

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### Table 2
Mean heterotrophic plate count (HPC), total coliform count and E. coli count of well-water samples from Ado-Ekiti metropolis

<table>
<thead>
<tr>
<th>s/n</th>
<th>Sampling locations</th>
<th>HPC ($\log_{10}$ cfu/ml) Mean</th>
<th>SD</th>
<th>Coliform count ($\log_{10}$ cfu/ml) Mean</th>
<th>SD</th>
<th>E. coli ($\log_{10}$ cfu/ml) Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Adebayo</td>
<td>8.3</td>
<td>0.1</td>
<td>8.3</td>
<td>0.1</td>
<td>5.5</td>
<td>0.3</td>
</tr>
<tr>
<td>2.</td>
<td>GRA</td>
<td>8.6</td>
<td>0.1</td>
<td>7.6</td>
<td>1.8</td>
<td>6.5</td>
<td>0.4</td>
</tr>
<tr>
<td>3.</td>
<td>Iworoko</td>
<td>8.1</td>
<td>0.5</td>
<td>7.3</td>
<td>1.6</td>
<td>5.2</td>
<td>1.2</td>
</tr>
<tr>
<td>4.</td>
<td>Aba-Ebira</td>
<td>8.1</td>
<td>0.3</td>
<td>8.1</td>
<td>0.3</td>
<td>5.9</td>
<td>0.4</td>
</tr>
<tr>
<td>5.</td>
<td>Ajilosun</td>
<td>8.2</td>
<td>0.1</td>
<td>7.9</td>
<td>0.6</td>
<td>6.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*GRA: Government Residential Area.

### Table 3
Percentage distribution of sorbitol and non-sorbitol fermenter E. coli isolated from well-water samples from Ado-Ekiti metropolis

<table>
<thead>
<tr>
<th></th>
<th>Number of isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorbitol fermenters</td>
<td>122</td>
<td>44.9</td>
</tr>
<tr>
<td>Non-sorbitol fermenters</td>
<td>150</td>
<td>55.1</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 4
Antibiotic resistance pattern and MAR-Index of presumptive E. coli O157 isolated from well-water samples from Ado-Ekiti metropolis

<table>
<thead>
<tr>
<th>s/n</th>
<th>Resistance pattern</th>
<th>No. of isolates</th>
<th>No. of classes to which isolate demonstrated resistance</th>
<th>MAR-Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AUG/CPX/GEN/CXM; AUG/CAZ/CPX/GEN/CXM</td>
<td>64</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>2.</td>
<td>AUG/NIT/CAZ/GEN/CRX/CXM</td>
<td>5</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>3.</td>
<td>AUG/NIT/CPR/CAZ/CPX/GEN/CXM</td>
<td>1</td>
<td>5</td>
<td>1.0</td>
</tr>
</tbody>
</table>
actually fit for human consumption. In this study, the physicochemical analysis of the water samples was carried out. This remains pivotal for the safety of drinking water because physicochemical attributes are used to determine the acceptability and use of water. WHO (2011) provides maximum allowable limits, which refer to the limits that should not be exceeded and which will cause rejection of the water. However, the highest desirable level is one which is likely to be objectionable to an appreciable number of people. From the study, it was observed that all the parameters examined except pH, TDS, conductivity and sodium were below the maximum allowable limit (Table 1) in all the water samples. The mean pH of water samples collected from Adebayo communities was slightly acidic. This may be as a result of the mineralogical composition of the study area or discharge composition (Talabi & Ogundana 2014) and runoff from various sources (Olowe et al. 2016b). Also, it was found that potassium is not expected in the water but considerable quantities above the recommended limits by WHO (2011) were detected in each of the tested water samples. This could arise from inorganic fertilizers (e.g. NPK fertilizer) being used on crops and consequently found in the environment and water bodies as a result of runoff and percolation during the rainy season (Lathamani et al. 2014).

Microbial analysis of the quality of the water sampled in this study failed to meet the NSDQW and WHO standard set for heterotrophic plate count (100 cfu/ml), total coliform count (10 cfu/100 ml) and E. coli (0 cfu/ml) (NSDQW 2007; WHO 2011). This result implied that the water samples from these communities are not suitable for drinking purposes. The result revealed the poor construction of the wells and unhygienic practices around the well water. The consumption of such water if not properly treated may constitute a health risk to the populace of these communities. This result is similar to a study by Olowe et al. (2016a). They also recorded poor bacteriological quality of water samples from their study areas.

The study also recorded an incidence of antibiotic-resistant presumptive E. coli O157 in the water samples with high MAR-Index > 0.2. This suggests that in the communities where the well water was sampled, the water sources had been exposed to contamination by sources which are laden with antibiotics such as veterinary medicine, feed additives, biocides in crop and food production or as growth-promoting agents in animals (Akhter et al. 2014), which end up in the environment. The GRA and Ajilosun areas are home to the city’s elite or wealthier inhabitants. Most of the residents in these communities rear security dogs. These dogs may be administered drugs when there are symptoms of illnesses and the regimen of the drugs may not be properly followed. Iworoko, Aba-Ebira and Adebayo areas constitute an environment for students who stay off-campus. In these
community students normally treat themselves with several kinds of antibiotics to treat their illnesses without actually consulting physicians. To worsen the situation, they use antibiotics haphazardly without following strictly the normal regimen of taking the drugs. The Iworoko area not only constitutes a student environment but also farmers combined. These farmers have farmlands close to residential areas where pesticides and fertilizers are applied. All these may contribute to the incidence of antibiotic-resistant E. coli O157 in drinking water samples from these communities and this is of significant concern. This is due to the danger in promoting multiple antibiotic-resistant organisms in humans through possible colonization of the gastrointestinal tract and conjugal transfer of plasmids, which code for antibiotic resistance, to the normal flora leading to more multiple antibiotic-resistant organisms (McKeon et al. 1995). Moreover, it also poses a great challenge to clinicians as the consumption of such water containing these antibiotic-resistant organisms may have negative impacts on veterinary and human medicine. Those results strengthen reports of other studies on the incidence of antibiotic-resistant E. coli in aquatic environments including potable water (Oliveira et al. 2012; Akhter et al. 2014; Oluyege et al. 2014; Olowe et al. 2015). The MAR-Index is a good tool for risk assessment (Mishra et al. 2015) and also gives an idea of the number of bacteria showing antibiotic resistance in the risk zone in the study’s routine susceptibility testing. Moreover, the MAR-Index also shows that antibiotic-resistant isolates, somehow, originated from the environment where antibiotics were over-used (Moon 2013).

Plasmid profiling of isolates is generally a useful tool for obtaining knowledge about resistance of bacteria to antimicrobial substances and transfer of plasmids among closely related bacteria from different sources (Losada et al. 2016). Many researchers have shown that antibiotic-resistant bacteria from the environment harbour a conjugative R-plasmid (Abdelatey et al. 2011; McArthur et al. 2011; Martínez 2012; Losada et al. 2016). The proliferation of plasmid-borne antibiotic-resistant bacteria presents a potential health hazard because it represents therapeutic-failure sources. The result of the plasmid profile in this study implies that not all the multiple antibiotic-resistance pattern observed in these organisms is plasmid-mediated as some of the organisms did not contain plasmids. This suggests that not all antibiotic resistance genes are located in plasmids. Some of the genes conferring resistance may be located on bacterial chromosomes. The finding is similar to the study of Molina-Aja et al. (2002). In their study of Vibrio strains isolated from cultured shrimps, they reported that some strains which were multiple antibiotic-resistant all contained one plasmid of 21.2 kb pairs and they suggested that resistance of some of the strains could be encoded in plasmids and others in the chromosomes.

CONCLUSION

The findings from this study showed that the well-water samples from these communities did not conform to recommendations by WHO and some also contained multiple antibiotic-resistant E. coli. These well-water samples are unsafe for consumption. Therefore, efforts should be made by individuals and government agencies to ensure that safe drinking water is available to each community. Moreover, unhealthy disposal and irrational use of antibiotics or substances laden with antibiotics should be checked among the individuals in the communities so as to forestall the problem of antibiotic resistance exhibited by microorganisms in the environment.

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