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Water quality and antimicrobial resistant profile of *Escherichia coli* isolated from dug wells used for domestic purposes

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ABSTRACT

The rapid spread of antimicrobial resistance (AMR) poses a significant threat to public safety. The use of water containing resistant bacteria could increase the risk of spreading AMR. This study assessed the quality of 143 dug wells used for domestic purposes in some communities in Nigeria and determined the resistance profile of isolated *Escherichia coli (E. coli)*. The Microbact™ identification kit was used to identify the isolates, and the susceptibility profile was evaluated using the Kirby-Bauer disc diffusion method. The combination disc technique was used to test all isolates for extended spectrum beta lactamase (ESBL) production. Polymerase chain reaction was used to identify ESBL genes, Integrons, and plasmid-mediated quinolone resistance genes. A total of 110 (76.9%) wells were contaminated by coliform bacteria. Of these, 94 (84.45%) wells yielded 202 *E. coli* strains. The isolates were commonly resistant to ampicillin (60.9%) but were all susceptible to meropenem. Seventy-seven (38.1%) isolates were multi-drug resistant. Two isolates harbored blaCTX-M and blaTEM separately while four (19%) ciprofloxacin-resistant isolates carried the oqxAB/aac-lb-cr gene. All isolates with resistance genes harbored class 1 and/2 Integrons. Most wells had coliform counts far above the World Health Organization's recommended limit, indicating that they are unsafe to drink. The presence of multidrug-resistant isolates in well water poses a serious risk to consumers since it might lead to outbreaks of untreatable water-borne diseases.

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1. INTRODUCTION

Poor access to safe drinking water is a major issue in many developing countries, resulting in waterborne illnesses and in some cases, death, particularly among infants [1]. In Nigeria, less than 30% of the population has access to safe drinking water due to population increase and infrastructure breakdown. The majority of the population, especially those living in rural and suburban areas, rely on dug wells for drinking water [2]. Such wells are subject to microbial and physicochemical contamination due to their shallowness and proximity to anthropogenic activities [3]. Bacteria often account for water contamination and associated infections. Of all the different microbial contaminants of water, *Escherichia coli (E. coli)*, a member of faecal coliforms has been the main indicator of faecal contamination in water quality monitoring. As a pathogen, it causes a wide spectrum of diseases ranging from diarrhoea, urinary tract infections and bacteremia to

sepsis. When rain falls, *E. coli* and other coliforms may be washed into lakes, streams, rivers, or underground water thereby contaminating drinking water [4]. Antibiotics are used in the treatment of bacterial infections but are also commonly superfluously used for non-bacterial infections particularly viral infections [5].

Antibiotic resistance in *E. coli* strains has rapidly evolved into a significant global issue, which has been related to unregulated antibiotic usage in both clinical and environmental contexts [6]. In recent years, the emergence of resistance in bacteria to beta-lactam antibiotics has become a problem, as bacteria strains that produce extended-spectrum beta-lactamases (ESBLs) have become prevalent. ESBLs render penicillins and cephalosporins useless for therapeutic use. ESBLs producing *E. coli* are commonly resistant to many antibiotics and infections by these strains are difficult to treat [7]. Also, at the start of the 20th century, strains of *E. coli* that are resistant to fluoroquinolones have become commonplace. The use of fluoroquinolones is a risk factor for isolating fluoroquinolone-resistant *E. coli* from patients in hospital settings, and resistance has been linked to treatment failure [8]. This problem of antibiotic resistance is being compounded by the ability of resistant *E. coli* to exchange its genes with other species of pathogenic bacteria. Hence, *E. coli* and other bacteria are important antibiotic resistance reservoirs [9].

Antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) have recently become a source of concern in water environments which provide an ideal setting for the acquisition and spread of antibiotic resistance because they are the main repositories of pollution from industry, agriculture, or domestic life [10]. Antibiotic-resistant infections and/or ARGs can be acquired through contact with ARB-contaminated water, and the ARGs carried by bacteria may be transferred to other microorganisms in humans by horizontal gene transfer. Such occurrence would undermine infectious disease prevention and control, posing a significant risk to public health [6].

Antibiotic resistance genes are typically found on transposons, plasmids, or integrons, which are mobile elements. Integrons have a site-specific recombination system that is used to capture, express and exchange gene cassettes. Although integrons are not mobile, their association with mobile genetic elements facilitates their movement within a genome or other bacterial species [11]. Integrons play a vital role in the spread of resistance genes in water-borne pathogens. Consequently, an increase in water-borne infections calls for quick and effective detection and treatment, but the presence of integrons and ARGS in pathogens limits the choices of antibiotics for disease containment. The detection and containment of integrons in water-borne pathogens is sine qua non to fighting illnesses, the failure of which could lead to significant morbidity and mortality [12].

Since water is inextricably linked to human activities and public health, it is critical to understand the prevalence of antibiotic resistance in drinking water sources. Therefore, the purpose of this study was to assess the quality of well water used in Ile-Ife, South-western Nigeria, and the the susceptibility profile of isolated *E. coli*, in order to provide baseline information for developing workable intervention for antimicrobial resistance control.

2. RESEARCH METHOD

This study was conducted at the six wards (Okerewe ward 1, Okerewe ward 3, Ilode ward 2, Ilode ward 1, Okerewe ward 2, and Moore ward) in Ife East local government area (LGA), Ile-Ife, Osun State, Nigeria. Ile-Ife, a city of 509,035 people in southwest Nigeria is located between the latitudes of 7°28′N and 7°45′N and longitudes of 4°30′E and 4°34′E, with an annual rainfall of 1,000 to 1,250 mm in the months of March to October and with an average humidity between 75% and 100%.

Between March and December 2019, a total of 143 open wells in the LGA were investigated for quality [13] as shown in Figure 1. The residents rely on the wells for drinking, cooking and bathing purposes. The Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria, approved the research (IPHOAU/12/863). The study included wells that had gone two months without disinfection. Wells belonging to owners who refused to consent and disinfected wells were excluded. To obtain 200 ml of water, a clean and sterile bottle was lowered into each well using a rope tied around its neck. Every sample was labelled and put in an ice-packed box before being sent to the lab, where it was immediately processed. Water quality was assessed by the multiple tube fermentation technique [14]. To detect faecal contamination in well water, the most probable number (MPN) approach was employed with MacConkey broth as the culture medium. Water samples of 10 ml, 50 ml, and 1 ml were placed in matched dilution tubes that contained inverted Durham's tubes and incubated at 37°C for 24 hours. The tubes were checked for growth and gas production, and the MPN of coliforms in 100 ml of water was calculated using McCrady's table [14], which was interpreted as "excellent", "acceptable", "unacceptable", and "grossly contaminated".

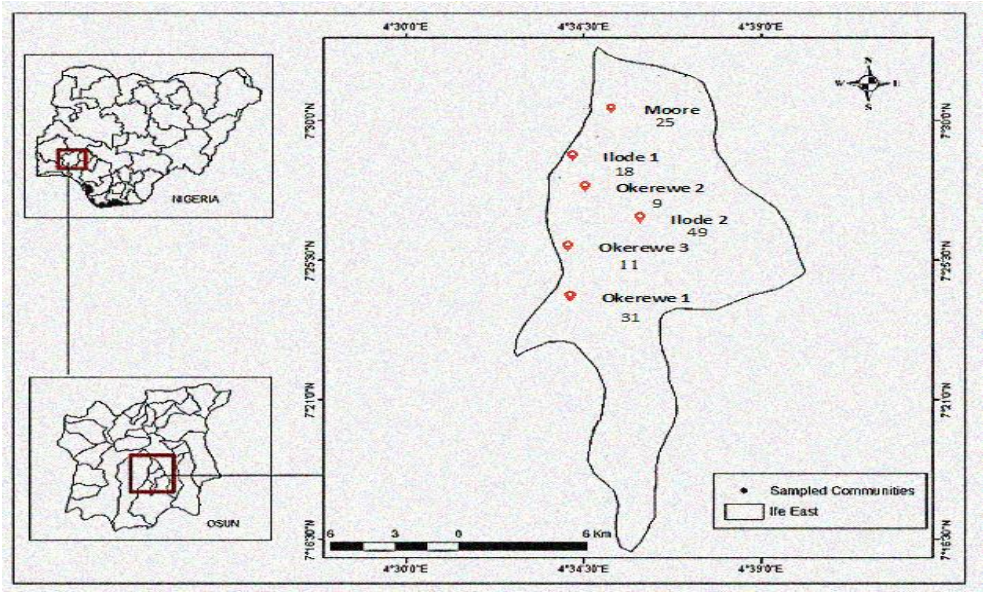

Figure 1. Map showing sampling locations

The Eijkman method was used to test the samples for *E. coli*. All positive MacConkey bottles from the previous test were subcultured into new single strength and double strength MacConkey broth and peptone water and incubated at 37^{o}C for 24 hours. Following incubation, the MacConkey bottles were examined for lactose fermentation and gas production. For the detection of indole, 3 drops of Kovac's reagent were added to each of the peptone water bottles. Upon confirmation of positive samples, they were cultured on Eosin Methylene Blue Agar plates and incubated for 24 hours at 37°C under aerobic conditions. After selecting up to three distinct colonies that had a green metallic sheen, they were transferred to Nutrient agar, which were then incubated aerobically at 37°C for 24 hours. Every presumptive *Escherichia coli* isolate was put in glycerol broth and stored at -20^{o}C for further examination. The isolates were further identified using the MicrobactTM 24E Identification kit (Oxoid Ltd., Basingstoke Hampshire, England), a standardized system for identifying Gram-negative bacilli.

Antibiotic susceptibility testing was performed on Mueller-Hinton agar (MHA) using the Kirby-Bauer disc diffusion method in line with the guidelines of the Clinical and Laboratory Standard Institute [15]. The antimicrobial discs used were ampicillin (10 µg), augmentin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), gentamicin (10 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), streptomycin (10 µg), meropenem (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), and colistin (10 µg) (Oxoid Ltd., Basingstoke Hampshire, England). The inhibition zones were measured using a graduated meter rule and the diameters of the zone of inhibition interpreted as "resistant and susceptible" according to the CLSI interpretative chart control strain *E. coli* ATCC 25922 was used as a guide. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more classes of antimicrobial agents [16].

The combined disc technique was used to screen cefotaxime or ceftazidime resistant isolates for ESBL production. This involved the use of cephalosporin discs (ceftazidime 30 µg and cefotaxime 30 µg) with and without 10 µg clavulanic acid placed on MHA earlier inoculated with the test organisms [15]. A 5-fold increase in the width of the inhibition zone when cephalosporin disk coupled with clavulanic acid was compared with cephalosporin alone indicates ESBL production. Negative and positive controls were *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 respectively.

DNA was extracted from the isolates using the boiling technique described by Odetoyin *et al.* [17]. A 1 ml aliquot of the culture was centrifuged for 2 min. at 10,000 rpm. The pellet was then suspended in 50 l of sterile water and heated for 10 min. The deoxyribonucleic acid (DNA) suspension obtained was utilised as a template for polymerase chain reaction (PCR). ESBL specific primers (TEM (TEM-F: TTTCGTGTCGCCCTTATTCC and TEM-R: ATCGTTGTCAGAAGTAAGTTGG), SHV (SHV-F: CGCCTGTGTATTATCTCCCT and SHV-R:CGAGTAGTCCACCAGATCCT), and CTX-M (CTX-M-F:CGCTGTTGTTAGGAAGTGTG and CTX-M-R: GGCTGGGTGAAGTAAGTGAC) were used to screened all ESBL producing isolates [18]. The following conditions were employed for the amplification reactions: initial denaturation at 94°C for 5 min., followed by 35 denaturation cycles at 94°C for 1 min., annealing at 52°C for TEM, SHV, and CTX-M, extension at 72°C for 1 min., and final extension at 72°C for 3 min. The negative and positive controls were *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae*

ATCC 700603 respectively. All ciprofloxacin-resistant isolates were screened for the plasmid-mediated quinolone resistance genes (PMQR) genes in two sets using specific primers for qnrA (Forward: CAGCAAGAGGATTTCTCACG and Reverse: AATCCGGCAGCACTATTACTC), qnrB (Forward: GGCTGTCAGTTCTATGATCG and Reverse: GAGCAACGATGCCTGGTAG), qnrC (Forward: GCAGAATTCAGGGGTGTGAT and Reverse: AACTGCTCCAAAAGCTGCTC), aac(6')-lb-cr (Forward: TTGCGATGCTCTATGAGTGGCTA and Reverse: CTCGAATGCCTGGCGTGTTT) and oqxAB (Forward: CCGCACCGATAAATTAGTCC and Reverse: GGCGAGGTTTTGATAGTGGA). Amplification settings for qnrA, qnrC, and qnrB were as follows: an initial denaturation at 94°C for 4 min., 30 cycles of 94°C for 30 s, optimum annealing temperature 55°C for 30 s and 72°C for 1 min., followed by a terminal extension at 72°C for 5 min. aac(6')-lb-cr and oqxAB were amplified using the following conditions: initial denaturation at 94°C for 4 min., 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 1 min. and a terminal extension at 72°C for 3 min. [19].

Also, the isolates that were positive for any of the screened genes were examined for classes 1 and 2 integrons by PCR using Lévesque5CS (GGCATCCAAGCAGCAAG) and Lévesque3CS (AAGCAGAC TTGACCTGA) primers, and Whitehep74 (CGGGATCCCGGACGGCATGCACGATTTGTA) and Whitehep51 (GATGCCATCGCAAGTACGAG) to amplify their variable regions [17]. The reaction mix for each strain was constituted as follows: 12.5 µl one TaqQuick-Load 2X master mix with standard buffer, 0.5 µl of 10 µM each of the forward primer and reverse primer, 3 µl template DNA and 8.5 µl of nuclease-free water. Amplification reactions involved initial denaturation at 94°C for 5 min., 40 cycles of denaturation at 94°C for 1 min., annealing at 50°C for 30 s, and extension at 72°C for 1 min., and a final extension at 72°C for 10 min. in GenAmp® PCR system 9700 (Applied Biosystems). *E. coli* strain 042 and 17-2 and DH5α *E. coli* strain were used as positive controls and negative control respectively. Each amplicon (10µl) was electrophoresed on a 1.5% agarose gel and examined with a UVitec transilluminator after being pre-stained with 0.5 g/ml Ethidium bromide in 1X Tris-Borate-EDTA buffer. The location of the molecular weight markers was used to determine the position of amplified products (Biolab, England). Analysis was done using the R statistical software package (version 3.4.2) [20]. Descriptive statistics (frequencies and percentages) of data was presented. Data were also presented with a bar chart and tables.

3. RESULTS AND DISCUSSION

3.1. Results

The water quality and antibiotic resistance profile of *E. coli* from 143 private wells in six wards of the LGA were examined in this cross-sectional research. Moore ard yielded 25 samples, Ilode ward 2 yielded 49 samples, Ilode ward 1 yielded 18 samples, Okerewe ward 1 yielded 31 samples, Okerewe ward 2 yielded 9 samples, and Okerewe ward 3 yielded 11 samples (Figure 1). The bulk of the wells were covered (n=108; 75.5%), while a few were partly covered (n=20; 13.99%) and a few were not covered (n=15; 10.5%). Out of 143 wells that were investigated, 33 (23.1%) were fit for consumption, 72 (50.4%) were unacceptable and 38 (26.6%) were grossly polluted, both of which indicated contamination by coliform bacteria and were unfit for consumption. The 110 (79.9%) contaminated wells were distributed across the six wards. Ilode ward 2 (n=36; 32.7%) ward 3 (n=6; 5.5 %) had the most polluted wells, whereas Okerewe ward 3 (n=6; 5.5 %) had the least as presented in Table 1.

Table 1. Well water quality and isolated *Escherichia coli* from different locations

Location	Number of wells	Water quality				Isolates of *Escherichia coli*		
		Fit for consumption	Unfit for consumption					
		Acceptable n(%)	Unacceptable n(%)	Grossly polluted n(%)	Total n(%)	No of wells with *E. coli* n(%)	No of wells without *E. coli* n(%)	No of *E. coli* strains isolated
Moore ward	25	5 (15.15)	16 (22.22)	4 (10.53)	20 (18.18)	21 (84.00)	4 (16.00)	44 (21.8)
Ilode ward 1	18	1 (3.03)	13 (18.05)	4 (10.53)	17 (15.46)	10 (55.56)	8 (44.44)	22 (10.9)
Ilode ward 2	49	13 (39.40)	22 (30.56)	14 (36.84)	36 (32.72)	30 (61.23)	19 (38.78)	66 (32.7)
Okerewe ward 1	31	7 (21.21)	14 (19.44)	10 (26.32)	24 (21.81)	20 (64.52)	11 (35.48)	42 (20.8)
Okerewe ward 2	9	2 (6.06)	5 (6.94)	2 (5.26)	7 (6.36)	7 (77.78)	2 (22.22)	15 (7.4)
Okerewe ward 3	11	5 (15.15)	2 (2.78)	4 (10.53)	6 (5.45)	6 (54.55)	5 (45.45)	13 (6.24)
Total	143	33	72	38	110	94	49	202

Escherichia coli was isolated from 94 (85.5%) of the 110 coliform bacteria-contaminated wells. Two hundred and two *E. coli* strains were recovered from the 94 contaminated wells (Table 1). Some wells yielded more than one isolate of *Escherichia coli*. The majority of the isolates (n=170; 84.1%) were resistant to at least one of the 12 antibiotics tested. The isolates were commonly resistant to ampicillin (n=127; 62.9%) followed by tetracycline (n=96; 47.5%) and streptomycin (n=80; 40%). None of the isolates was resistant to meropenem and ceftazidime. The isolates from the wells in Moore ward showed the highest rate of resistance to ampicillin (n=31; 70.5%) followed by isolates from Okerewe ward 3 (n=9; 69.2%). Besides, isolates from Okerewe ward 3 and 1 were commonly resistant to tetracycline (n=7; 53.8%) and (n=26; 61.9%) respectively as shown in Table 2.

Seventy-seven (38.1%) isolates were resistant to at least one antibiotic in at least three classes of antibiotics. Ilode ward 2 had the highest number of multidrug-resistant isolates (n=26; 33.8%) while the least number (n=6; 7.8%) was found in Okerewe ward 2. Others were Ilode ward 1 (n=8; 10.4%), Moore ward (n=14; 18.2%), Okerewe ward 1 (n=16; 20.8%) and Okerewe ward 3 (n=7; 9.1%). Of the 26 isolates that were resistant to cefotaxime, five isolates (19.2%) were confirmed phenotypically as ESBL producers. The five isolates were also screened for the presence of ESBL genes (bla_{CTX-M}, bla_{TEM}, bla_{SHV}). As shown in Figure 2(a) (see Appendix) one isolate from Okerewe ward 1 and one isolate from Ilode ward 2 harbored *blaCTX-M* (875bp) and *blaTEM* (403bp) genes respectively. The two isolates were commonly resistant to cefotaxime and ampicillin. Four isolates out of the 21 isolates that were resistant to ciprofloxacin were positive for *oqxAB* (313bp)/*aac-lb-cr* (482bp) genes Figure 2(b). All the isolates that harbored resistance genes carried classes 1 and/2 integrons with different cassettes Figures 2 (c) and (d) (Table 3).

Table 2. Antimicrobial resistance profile of *Escherichia coli* isolates

Antibiotics	Locations						Total n=202 (%)
	Moore Ward n=44	Ilode Ward 1 n=22	Ilode Ward 2 n=66	Okerewe Ward 1 n=42	Okerewe Ward 2 n=15	Okerewe Ward 3 n=13	
Ceftazidime	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chloramphenicol	0 (0.0)	4 (18.2)	3 (4.5)	0 (0.0)	1 (6.7)	2 (15.4)	10 (5.0)
Cefotaxime	5 (11.4)	7 (31.8)	12 (18.2)	2 (4.8)	0 (0.00)	0 (0.00)	26 (12.9)
Ampicillin	31(70.5)	14 (63.6)	39 (59.1)	24 (57.1)	10 (66.7)	9 (69.2)	127 (62.9)
Meropenem	0 (0.00)	0 (0.00)	0 (0.0)	0 (0.0)	0 (0.00)	0 (0.00)	0 (0.00)
Amoxicillin-clavulanate	20 (45.5)	8 (36.4)	12 (18.2)	8 (19.0)	7 (46.7)	9 (69.2)	64 (31.7)
Cotrimoxazole	11 (25)	8 (36.4)	20 (30.3)	16 (38.0)	7 (46.7)	7 (53.8)	69 (34.1)
Tetracycline	16 (36.4)	9 (40.9)	31 (47.0)	26 (61.9)	7 (46.7)	7 (53.8)	96 (47.5)
Ciprofloxacin	1 (2.3)	1 (4.5)	7 (10.6)	4 (9.5)	4 (26.7)	4 (30.8)	21 (10.4)
Colistin	1 (2.3)	0 (0.00)	3 (4.5)	2 (4.8)	0 (0.00)	0 (0.00)	6 (3.0)
Gentamicin	0 (0.00)	1 (4.5)	1 (1.5)	1 (2.4)	1 (6.7)	1 (7.7)	5 (2.5)
Streptomycin	17 (38.6)	5 (22.7)	36 (54.5)	10 (23.8)	7 (46.7)	5 (38.5)	80 (40.0)

Table 3. Resistance genes and integrons in *Escherichia coli* isolates from dug wells in different locations

Isolates	Location	Water quality	Genes	Integrons	Resistance patterns	MDR
145b	Okerewe ward 1	Contaminated	*oqxAB, aac-lb-cr*	Class 2	STR, AMP, COT, TET, CIP*	5
108a	Okerewe ward 2	Contaminated	*aac-lb-cr*	Classes 1 and 2	STR, CHL, AMP, AUG*, COT, TET*, CIP	7
129b	Okerewe ward 2	Contaminated	*aac-lb-cr*	Classes 1 and 2	STR*, AMP, COT, TET, CIP	5
130b	Okerewe ward 1	Contaminated	*aac-lb-cr*	Class 1	STR, AMP*, COT, TET, CIP	5
116a	Moore	Contaminated	bla_{CTX-M}	Class 1	CEF*, AMP,	2
79b	Ilode ward 2	Contaminated	bla_{TEM}	Class 1	CEF, AMP, AUG, COT*	4

*STR=Streptomycin; AMP=Ampicillin; COT=Cotrimoxazole; TET=Tetracycline; CIP=Ciprofloxacin; AUG=Augmentin; CEF=Cefotaxime

3.2. Discussion

In this study, the majority of the wells were contaminated by coliform bacteria, with coliform counts exceededing the limit of the World Health Organization [21]. This implies that the wells were contaminated by potentially harmful microorganisms and are therefore unfit for drinking, cooking and washing of food items since they might expose users to water-borne illnesses. Similar studies conducted across the world including Nigeria have revealed the presence of coliforms at amounts that exceeds the WHO guidelines for drinking water quality [22], [23]. The contamination of dug wells observed in our study could be caused by the shallowness of the wells, which allows for easy entry of nearby particles from runoffs, as well as poor

sanitation conditions and practices such as washing clothes near the wells or using contaminated containers to draw water from the wells.

For many decades, *E. coli* has been the primary indicator of faecal contamination in water quality monitoring which is consistent with our findings. The majority of the wells (85.5%) were contaminated by *E. coli*. Our isolation rate (85.5%) is higher than 67.74%, 23.1%, and 14.71% previously reported by Aromolaran *et al*. [24] in Ondo State, Southwest Nigeria, Odumosu and Akintimehin [22] in Ogun State, Southwest Nigeria and Onuoha [25] in Ebonyi State, Southeast, Nigeria respectively. The high rate of *E. coli* isolation could be attributed to the fact that some of the wells were not properly protected, exposing them to runoffs from nearby waste dumpsites and wash-off containing faecal matter particularly those of children and domestic animals passed or disposed of indiscriminately.

The majority of the isolates (84.1%) were resistant to at least one of the 12 tested antibiotics. This observation confirms earlier reports of the high prevalence of resistant *E. coli* in surface and well water samples. The majority of the isolates were resistant to ampicillin (62.9%) followed by tetracycline (47.5%) and streptomycin (40%). Similar studies by Adejuwon *et al*. [26] and Olowe *et al*. [27] found higher percentages of waterborne ampicillin and tetracycline-resistant *E. coli* (>70%). Our observation may not be unconnected with the indiscriminate use of these drugs due to their ease of availability [28]. Interestingly, meropenem and ceftazidime were the most effective agents tested. Meropenem is a relatively new antibiotic in this environment and its usage is restricted to complicated infections. It is not commonly prescribed as a first-line drug. Its relatively high cost and the fact that it is an injectable may have discouraged indiscriminate purchase over the counter and abuse.

Seventy-seven isolates were found to be multidrug-resistant which is consistent with previous findings. This finding may be due to indiscriminate use of antibiotics among the population as a result of self-medication and easy access to the drugs without prescription. The isolation of multidrug-resistant bacteria from sources that serve as drinking water in this community is of public health importance because it can easily serve as a reservoir through which antibiotic resistance characteristics can spread to other bacterial populations.

We identified five cefotaxime resistant isolates as ESBL producers by phenotypic evaluation. Previous studies have also reported ESBL production among waterborne isolates in varying proportions, albeit, not in this environment [22], [29]. ESBLs are enzymes capable of hydrolyzing penicillins, oxyimino-cephalosporins and monobactams. ESBLs can be transferred by mobile genetic elements or might be chromosomally mediated. Bacterial isolates carrying ESBL genes can serve as reservoirs for plasmid-borne antibiotic resistance genes in Gram-negative bacteria and are usually multi-drug-resistant. The existence of ESBL producers in well water is worrisome considering the effect of ESBLs on therapy. The emergence of ESBL producers may be associated with lingering impacts of antibiotics, which are used generally in humans and the food chain, producing limited antibiotic pressure in the environment [7].

Two isolates from different locations harbored cefotaximase (CTX-M) and temoniera (TEM) extended specrum beta-lactamase (ESBL) genes separately. The prevalence of CTX-M type β-lactamases in Enterobacteriaceae is on the rise and are currently more prevalent in some areas than the temoniera (TEM) and sulfhydryl variable (SHV) ESBL types. TEM and SHV forms have been found in hospital and community samples, such as livestock and well water [30]. CTX-M is a key public health concern due to its ability to be involved in hospital and community-acquired infections [18]. *E. coli* is usually responsible for producing CTX-M and appears to be a true community ESBL-producing pathogen [31].

Twenty-one isolates were resistant to ciprofloxacin which used to be the drug of last resort in this setting. Similar rates of resistance to ciprofloxacin have been reported in Nigeria [32]. Ciprofloxacin, a second-generation quinolone antibiotic is one of the most prescribed antibiotics globally. Like other quinolones, it acts on gyrase and topoisomerase IV enzymes which eventually affect DNA replication [33]. Quinolone resistance can be transmitted by plasmids among bacteria and four major plasmids mediated quinolone resistance (PMQR) genes (qnrs, qepA, oqxAB, aac (6′)-Ib-cr) are commonly implicated [34]. Four of the 21 ciprofloxacin-resistant isolates carried aac (6′)-Ib-cr/oqxAB. The aac (6′)-Ib-cr gene encodes resistance to fluoroquinolones and aminoglycosides. The gene has been detected in many locations, including Nigeria [32]. Its prevalence varies between species and has been reported to be more prevalent in *E. coli* than in other Gram-negative bacteria. A previous study in the study location among diarrheic children found the gene on a large self-transmissible plasmid in *E. coli* [35].

Besides, aac (6′)-Ib-cr was detected together with *oqxAB* another PMQR gene that encodes an efflux pump which confers resistance to quinoxaline–di-N-oxide olaquindox, used as a growth promoter in pig breeding. oqxAB expels flumequine, ciprofloxacin and norfloxacin from bacterial cells and increases the minimum inhibitory concentrations to these agents. Our finding is significant and it is the first report of oqxAB carrying *E. coli* from well water in this environment. The gene that codes for oqxAB has been detected in *E. coli* isolates from humans, animals, and the environment [36]. Previous reports have associated it with IS26 which was found on the 43-kb to 115-kb IncF transferable plasmid [36]. The detection of the

two plasmids medicated resistance genes in our study could undermine the therapeutic use of fluoroquinolones in the treatment of infectious diseases, and thus portends a great threat to public health, because *E. coli* can exchange its genes/elements with other organisms in the gut which could occur through ingestion of water that contains antibiotic resistant bacteria (ARB).

All the isolates that carried resistance genes harbored either class 1 or 2 integron or both. Antibiotic resistance genes are generally carried by mobile elements such as transposons, conjugative plasmids, or integrons [11]. Integrons play a significant role in the spread of resistance genes in water-borne pathogens. The detection of integrons in the isolates with resistance genes in our study agrees with previous reports of their prevalence in water-borne isolates and their link with multidrug resistance [37], [38].

4. CONCLUSION

This study has shown that most wells in the study area had coliform counts far above the World Health Organization standard, indicating that they are unsafe to drink. The presence of multidrug-resistant isolates in well water poses a serious risk to consumers since it may lead to outbreaks of untreatable water-borne diseases. The detection of resistance genes and mobile elements necessitates the need for enhanced surveillance programs that can provide essential knowledge on the persistence and mobility of resistance traits between the community and hospital environments.

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APPENDIX

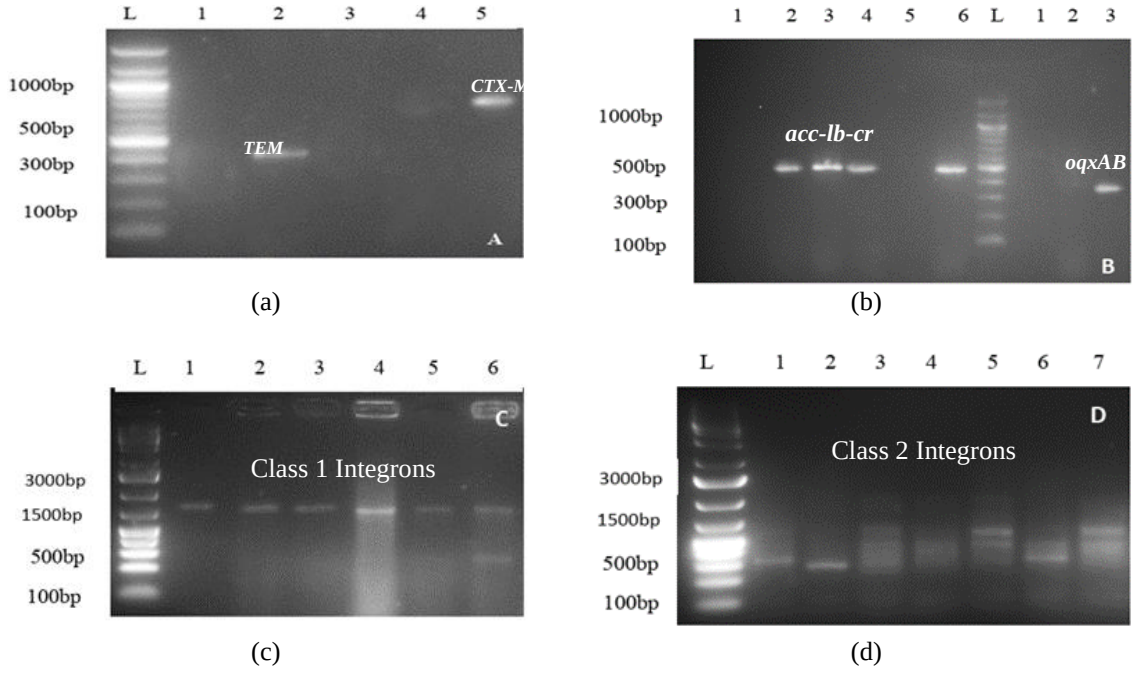

Figure 2. Gel pictures of amplified genes (a) Amplification of Extended Spectrum Beta-lactamase (ESBL) genes (CTX-M-874bp, SHV-293bp, TEM-403bp) in *Escherichia coli*. Lane L: 100bp Ladder; Lane 1: Isolate 139c; Lane 2: Isolate 79b (TEM); Lane 3: Isolate 98b; Lane 4: Isolate 116a (CTX-M); Lane 5: Isolate 116b (CTM-X), (b) Amplification of Plasmid Mediated Quinolone Resistant (PMQR) genes (oqxAB-313bp and acc-lb-cr-482bp). Lane 1: Isolate 35b; Lane 2: Isolate 180a (aac-lb-cr); Lane 3: 129b (aac-lb-cr); Lane 4: Isolate 130b (aac-lb-cr); Lane 5: Isolate 105C; Lane 6: Isolate 145b (aac-lb-cr); Lane L: 100bp Ladder; Lane 1: Isolate 130bp; Lane 2: Isolate 129b; Lane 3: Isolate 145b (oqxAB), (c) Amplification of Class 1 Integrons. Lane L: 1kb+ladder Lane 1: Positive control (042), Lane 2: isolate 116a; Lane 3: Isolate 79b; Lane 4: isolate 130b; Lane 5: Isolate 108a; Lane 6: Isolate 129b, (d) Amplification of Class 2 Integrons. Lane L: 1kb+ladder; Lane 1: Isolate 8a; Lane 2: Isolate 96c; Lane 3: Isolate 92b; Lane 4: Isolate 154; Lane 5: Isolate 102b; Lane 6: Isolate 129b; Lane 7: Isolate 145b