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ORIGINAL ARTICLE

Assessment of multidrug-resistant phenotypes and detection of ampicillin-resistant determinants among *Escherichia coli* isolates of groundwater origin: case study—Osogbo, Southwest Nigeria

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Abstract
Multidrug resistance in groundwater contaminants is becoming a major health concern in developing countries, especially the ampicillin-resistant *Escherichia coli*. This study aimed at profiling antibiotic resistance and possible detection of ampicillin-resistant genes in *Escherichia coli* obtained from forty groundwater samples (20 each of boreholes and wells) in Osogbo metropolis, Southwest Nigeria. Grab sampling was done using 1L sterile plastic bottles, isolation of *E. coli* was performed using pour plate technique on eosin methylene blue agar and their identity confirmed by polymerase chain reaction (PCR) using *uid*A gene. Antibiotics susceptibility test of the isolates to ten commercially available antibiotics was done following Kirby–Bauer disc diffusion technique. Multiple antibiotic resistant phenotypes (MARPs) and indexing (MARI) were estimated accordingly. The possible presence of Ctx-M, SHV, ampC, and TEM-H resistant genes in all ampicillin-resistant *E. coli* were checked for using PCR. All the 55 presumptive *E. coli* isolates, 35 from 10 boreholes and 20 from 7 wells, were *uid*A positive. Overall, 50 (91%) of the *E. coli* were resistant to ampicillin, followed by trimethoprim and ertapenem 43 (78%), doxycycline 40 (73%), ceftazidime 38 (69%), and tetracycline 37 (67%). Of the 55 *E. coli* isolates, only 1 was resistant to 2 drugs (AK-AMP), others were multi-resistant, ranging from 4 to 9 drugs, with the highest MARP (9) being AMP–CAZ–ETP–S–AK–DO–TE–W–C. MARI also between 0.4 and 0.9, above the 0.2 acceptable limit. Exactly 24 (48%) of the phenotypically ampicillin-resistant *E. coli* isolates harboured TEM-H only. The existence of multidrug-resistant *E. coli* and TEM-H resistant gene in the groundwater pose a huge threat to all and sundry who rely heavily on this source of water for diverse purposes. Hence, adequate monitoring and antimicrobial resistance surveillance of groundwater bodies is advocated to safeguard public health.

Keywords Groundwater · *Escherichia coli* · Multidrug resistance · Public health · Nigeria

Introduction

Water is a prerequisite for living. Its essentiality in the overall well-being of man cannot be overemphasized. Among diverse water sources, groundwater (borehole and hand-dug well) is considered the ideal candidate for drinking owing to its perceived cleanness and safety (Edokpayi et al. 2018a, b). It is presumed that the different earth's strata filter out contaminants in surface water during percolation before reaching the underground (Keesari et al. 2015). Unfortunately, groundwaters are now recognised to be prone to microbial contamination. Over two decades ago, outbreaks of waterborne diseases were reported in some nations of the world following ingestion of contaminated groundwaters (Anderson and Bohan, 2001; Paruch et al. 2015; Vignesh et al. 2015). The location, type and degree of human activities, and environmental condition in and around groundwater play a significant role in influencing groundwater quality (Olasoji et al. 2019).

Pollution of groundwater arises when boreholes are drilled, and wells dug without adhering strictly to hygienic standards and/or consulting relevant regulatory agencies. Previous studies observed bacteria, including *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus*,

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Enterococci sp., somatic coliphages, species of *Salmonella*, *Shigella*, and *Klebsiella*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, and *Aeromonas hydrophila* in groundwater supplies (Potgieter et al. 2006; Aydin 2007; Olaitan et al. 2013; Llopis-González et al. 2014; Owolabi et al. 2014; Keesari et al. 2015; Onyango et al. 2018; Odiyo and Makungo, 2018; Alsalme et al. 2021).

Microbiological assessment is vital to identifying contamination of water with pathogens capable of compromising human health and provides an avenue to avert potential health hazards including morbidity and mortality (Stein et al. 2010; Titilawo et al. 2020). *Escherichia coli* is commonly regarded as the indicator organism of microbial water contamination (USGS 2018). Albeit, some pathogenic strains of *E. coli* are responsible for several morbidities and mortalities in all ages (Kaper et al. 2004). *E. coli* is mostly significant owing to its ability to transmit water-related diseases such as diarrhoea, the second cause of mortality amidst children below 5 years of age (Chissaque et al. 2018). It accounts for approximately 9% of 5.6 million childhood deaths and 88% of all reported cases of diarrhoea in Black Africa and South Asia countries (Liu et al 2016; Chissaque et al. 2018).

Antimicrobials are key in reducing illnesses and deaths linked with veterinary and human infectious diseases. For *E. coli* infections, ampicillin has been the drug of choice for treatment but recently, its resistance rate has increased (Alanazi et al. 2018). In general, ampicillin prevents the synthesis of bacterial cell wall during replication in bacteria, however, resistant strains encode β-lactamase, changes the target protein in cell wall, reduces the permeability of outer membrane, and increases the expression of drug efflux pump (Kapoor et al. 2017).

The excessive and indiscriminate usage of antimicrobial in therapy, growth promotion, and prophylaxis in animal and human medicine are dominant drivers of the development and communicability of resistance traits among disease-causing and normal floral bacteria (Dadgostar 2019). In addition, complex socioeconomic and behavioural patterns traceable to individuals promote transmission of drug resistant microorganisms (Okeke et al. 1999; Medina and Pieper 2016). Aquatic milieu is known to play a key role in the dissemination of microbes among humans, animals and the environment (Amaya et al. 2012).

Poor water availability and inaccessibility to portable water by the people of Osogbo, Osun State, Nigeria is a great challenge. Available data reveal that about 844 million people do not have access to basic water service, portable water is not available for 2.1 billion people worldwide and the Goal 6 of sustainable development goals (SDGs) targeted at providing adequate supply and access to safe clean water by the year 2030 is gradually becoming a mirage (UN 2018). Thus, continuous supply of potable water remains a major challenge to millions of people around the world, especially in developing countries.

Osogbo township is blessed with abundance of natural water bodies but activities around them limit their usage for drinking purposes. Hence, majority of the inhabitants rely heavily on boreholes or hand-dug well for sustenance. Unfortunately, the water sources are rarely treated or untreated, exposed, or handled unhygienically. This is the scenario in areas, where they depend on a single source for daily living. In addition, some groundwaters are found near contaminated environments, including, municipal dumpsites, sewerage systems, amongst others, all of which pose health risks to human beings.

Reports on microbial assessments of groundwater used by the inhabitants of Osogbo have been documented (Olowe et al. 2005; Owolabi et al. 2014), with emphasis on presumptive identification of microorganisms in the water. To our knowledge, the antimicrobial susceptibility of PCR-confirmed *E. coli* from boreholes and hand-dug wells in Osogbo, Osun State Nigeria has not been investigated, let alone the detection of resistance determinants. The current study, therefore, elucidated the drug resistance pattern and ampicillin resistant genes in PCR-confirmed *E. coli* obtained from selected boreholes and hand-dug wells within Osogbo metropolis, Osun State, Nigeria.

Methodology

Sample collection

Forty water samples, comprising 20 each of boreholes and wells, were collected across Osogbo metropolis, Nigeria over a 3-month sampling period (September–November 2020). The geographic coordinates of the sampling locations were documented (Fig. 1). Water samples from borehole outlets were collected into a germ-free plastic of 1 L capacity. With the aid of a clean rope, sterilized plastic bottles were suspended into hand-dug wells, pulled up when filled and immediately covered. All samples were transported to the laboratory on ice and analysed within 6 h.

Isolation of *Escherichia coli* from the groundwater samples

Pour plate technique was employed for isolation of *E. coli*. Aliquot of water sample (1 ml) was dispensed into sterile Petri dishes in duplicates. To the Petri dishes, 20 ml of eosin methylene blue agar was dispensed, mixed gently, allowed to gel, and incubated at 37 °C for 18–24 h. Distinctive metallic-sheen colonies observed were enumerated as appropriate. The colonies were purified by streaking on nutrient agar

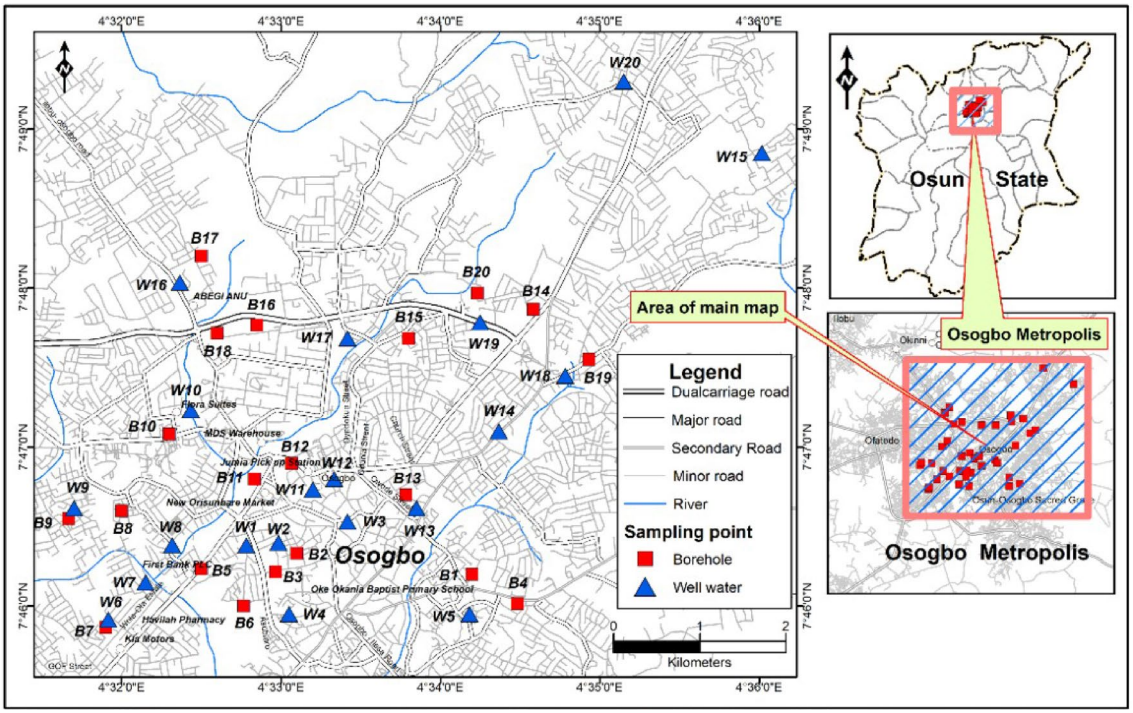

Fig. 1 Map showing groundwater sample locations across Osogbo metropolis

plates until pure cultures were obtained and stored on agar slants until when needed.

Molecular identification of the *E. coli* isolates

DNA extraction

Genomic DNA extraction of the *E. coli* isolates was done by boiling method (Maugeri et al. 2004; Torres et al. 2005). Briefly, distinct 18–24 h old *E. coli* colonies on nutrient agar plates were aseptically picked and resuspended in 200 µl sterile distilled water, boiled at 100 °C for 10 min, and centrifuged at 12,000 rpm for 10 min. The supernatant containing DNA was carefully removed with a sterile pipette into germ-free Eppendorf tubes and kept at − 80 °C for PCR amplification.

PCR-based confirmation of *E. coli* isolates

The *uid*A gene (F: AAAACGGCAAGAAAAAGCAG; R: ACGCGTGGTTAACAGTCTTGCG) of the 55 *E. coli* DNA extracts was amplified using thermocycler. Briefly, PCR mixture (25 µl) consisting of 12.5 µl of PCR master mix (Thermo Scientific), 0.5 µl each of primer (Inqaba Biotech, SA), 5 µl of template DNA, and 6.5 µl of PCR grade water was amplified using SimpliAmp Thermal Cycler (Applied Biosystems, Life Technologies, Singapore). Controls, both positive and negative were also included. Cycling condition, i.e., initial denaturation at 94 °C for 5 min followed by 35 cycles of 30 s denaturation at 95 °C; annealing at 58 °C for 1 min; extension at 72 °C for 1 min, and a final extension step for 5 min at 72 °C, as previously described (Titilawo et al. 2015a).

Gel electrophoresis

Amplicons (5 µl) were loaded in a gel electrophoresis tank containing 1% SeaKem LE agarose gel (Lonza, USA) dissolved in 1X TAE electrophoresis buffer-stained ethidium bromide (Sigma-Aldrich, USA), and run at 100 V for 1 h. Viewing was done using the Bio-Rad ChemDoc (Hercules, USA).

Antimicrobial susceptibility testing

Disc diffusion assay (Kirby-Bauer et al. 1966) was employed in estimating the susceptibility pattern of the isolates to ten commercially available antibiotic discs, belonging to seven classes, i.e., aminoglycosides–amikacin (30 µg), streptomycin (10 µg), carbapenem–ertapenem (10 µg), sulfonamides–trimethoprim (5 µg), phenicols–chloramphenicol (30 µg); tetracyclines–tetracycline (10 µg), penicillins–ampicillin (10 µg), tetracyclines–doxycycline (30 µg), cephalosporins–ceftazidime (30 µg), quinolones–ofloxacin (5 µg). *Escherichia coli* isolates

(18–24 h) were suspended into physiological saline. Exactly 100 µl of 0.5 McFarland standard bacteria solution was inoculated and spread on Muller Hinton agar plates and allowed to dry. Antibiotic discs were gently placed on the plates using a disc dispenser (Oxoid), and incubated at 37 °C for 24 h. The diameter of zone of inhibition was measured and recorded in the nearest millimetre. Isolates were considered as susceptible (*S*), intermediate (*I*) and resistant (*R*) to test drugs based on the diameter of inhibition interpretation guideline of the Clinical and Laboratory Standards Institute (CLSI 2019). The frequency of antibiotic-resistant isolates was calculated using the equation: $(X/Y) \times 100$. Where '*X*' is the sum of isolates resistant to a drug and '*Y*' is the sum of isolates from the sample.

Multiple antibiotic resistant phenotypes and indexing of the isolates

Multiple antibiotic-resistant phenotypes (MARPs) for each sampling site were generated for isolates exhibiting resistance to three or more antibiotics according to Wose et al. (2010). Multiple antibiotic resistance indexes (MARI) for each location were also estimated from the expression:

$$\text{MARI} = x/y,$$

where '*x*' represents the number of antimicrobial agents to which the isolate was resistant and '*y*' is the total sum of antimicrobial agent tested against one isolate (Blasco et al. 2008; Titilawo et al. 2015a).

Also, the antibiotic resistance index (ARI) for each sampling location was evaluated using the formula:

$$\text{ARI} = A/N(Y),$$

where '*A*' is the total number of resistant isolates recorded, '*N*' is the number of isolates and '*Y*' represents the total number of antibiotics tested (Tandra and Sudha 2014; Titilawo et al. 2015b).

Detection of ampicillin resistance determinants

PCR amplification and gel electrophoresis procedures (described above) were employed for the identification of resistant determinants among *E. coli* resistant to ampicillin. The details of primers used are shown in Table 1.

Results

Escherichia coli counts from the groundwater samples

A total of 55 distinct colonies showing characteristic greenish metallic sheen on EMB agar and presumptively identified as *E. coli* were obtained from the groundwater samples (Table 2). Borehole water had a higher mean count (35) from 10 samples compared to well water with 20 colonies recovered from 7 samples. Interestingly, not one *E. coli* was recovered from 10 and 13 borehole and well water samples, respectively (Table 2).

Molecular confirmation of the recovered *E. coli* isolates

All the 55 phenotypically identified *E. coli* isolates yielded 147 bp on gel electrophoresis after PCR amplification of the *uid*A gene. The gel electrophoresis profile of representative PCR-confirmed *E. coli* is shown in Fig. 2.

Antibiotic resistance patterns of the *E. coli* isolates

Resistance of the isolates to the different test antibiotics ranged from 20% to 91%. Exactly 50 (91%) of the *E. coli* showed resistance to ampicillin, followed by trimethoprim and ertapenem 43 (78%), doxycycline 40 (73%), ceftazidime 38 (69%), tetracycline 37 (67%), streptomycin 22 (40%) and amikacin 18 (33%) (Fig. 3).

Susceptibilities of the isolates were in the order: ofloxacin 43 (78%), chloramphenicol 34 (62%), amikacin 33 (60%),

Table 1 Primers used for PCR detection of ampicillin resistance genes

Primer	Oligonucleotide sequence (5′–3′)	Product size (bp)	Annealing Temp (°C)	References
TEM-H	F: CCCCGAAGAACGTTTTC R: ATCAGCAATAAAACCAGC	516	52	Colom et al. (2003)
CTX-M	F: CGATGTGCAGTACCAGTAA R: TTAGTGACCAGAATCAGCGG	585	57	Saladin et al. (2002)
ampC	F: TGGCGTATCGGGTCAATGT R: CTCCACGGGCCAGTTGAG	503	55	Zhu et al. (2013)
SHV	F: AGGATTGACTGCCTTTTTG R: ATTTGCTGATTTCGCTCG	392	56	Colom et al. (2003)

Table 2 Average amounts of *E. coli* from the borehole and well water samples

Borehole		Well water	
Sample code	Mean count	Sample code	Mean count
BW1	07	WW1	–
BW2	–	WW2	01
BW3	01	WW3	–
BW4	–	WW4	–
BW5	05	WW5	03
BW6	04	WW6	–
BW7	11	WW7	–
BW8	–	WW8	–
BW9	01	WW9	05
BW10	01	WW10	02
BW11	03	WW11	05
BW12	01	WW12	01
BW13	–	WW13	03
BW14	01	WW14	–
BW15	–	WW15	–
BW16	–	WW16	–
BW17	–	WW17	–
BW18	–	WW18	–
BW19	–	WW19	–
BW20	–	WW20	–
Total	35	Total	20
Grand total = 55			

BW, Borehole water; WW, Well water; '–' Nil

streptomycin 25 (45%) and tetracycline 16 (29%), trimethoprim 12 (22%), ceftazidime and ertapenem 9 (16%), doxycycline 8 (15%) and ampicillin 5 (9%), respectively (Fig. 3).

Multiple antibiotic resistance phenotypes (MARPs) of the *E. coli* isolates

The MARPs generated for the *E. coli* isolates are presented in Table 3. Only 1 of the 55 *E. coli* isolates was resistant to 2 drugs (AK-AMP), while the remaining 54 were multi-resistant, ranging from 4 to 9 drugs (Table 3). None was resistant to the 10 drugs involved in the study. When this was expressed in terms of frequency, 2%, 11%, 13%, 21%, 23%, and 30% of the isolates exhibited multiple antibiotic resistance to 9, 5, 8, 7, 4, and 6 antimicrobials, respectively. The highest MARP (9) obtained across the sampling sites was AMP–CAZ–ETP–S–AK–DO–TE–W–C (Table 3). In the same vein, antibiotic resistance index (ARI) ranged from 0.08 to 0.80, whereas multiple antibiotic resistance index (MARI) was from 0.4 to 0.9 (Table 4).

Detection of ampicillin-resistant genes

All ampicillin-resistant *E. coli* (50) were examined for Ctx-M, SHV, ampC, and TEM-H resistance genes. Surprisingly, only 24 (48%) of the phenotypic ampicillin-resistant *E. coli* isolates harboured TEM-H (516 bp) and other genes were not detected. Figure 4 shows the representative gel picture of TEM-H amplification.

Discussion

Unsafe water is implicated in several waterborne illnesses (Nanfack et al. 2014), with contaminated groundwater fast becoming an emerging public health concern (Li et al. 2021). The current study elucidated the antibiotic resistance profile and ampicillin-resistant gene in PCR-confirmed *E. coli* isolates from selected boreholes and hand-dug wells within Osogbo metropolis, Osun State, Nigeria.

Escherichia coli is a specific indicator for faecal water pollution, and the presence of other waterborne pathogens (Takal and Quaye-Ballard 2018). In this study, *E. coli* was detected in 17 groundwater sources suggesting contamination with faecal material. More *E. coli* was recovered from borehole (35) compared to well water (20) (Table 2). This signifies the extent of pollution relating either to natural and/or anthropogenic activities around the water sources. The presumptive 55 *E. coli* isolates were *uid*A positive after PCR amplification. Earlier investigations also isolated *E. coli* from groundwater sources in India (Sharma et al. 2017), Indonesia (Dayanti et al. 2018), Nigeria (Titilawo

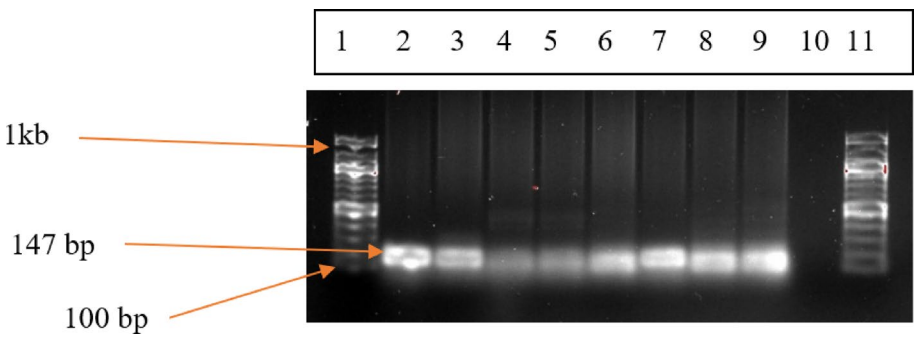

Fig. 2 PCR-amplified *uid*A gene of *E. coli* isolates after gel electrophoresis. Lane 1, DNA ladder; lanes 2–9, Positive isolates; lane 10, Negative control; lane 11, DNA ladder

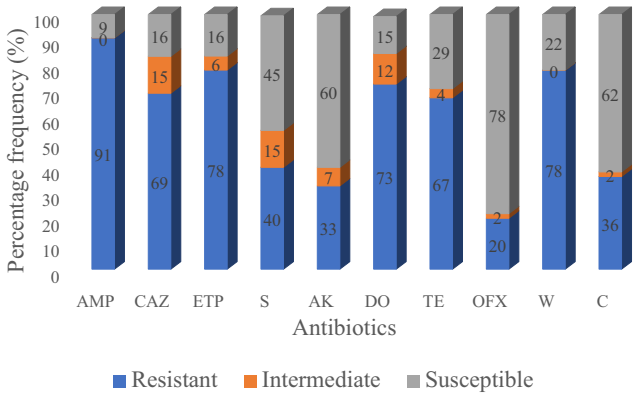

Fig. 3 Antibiotic sensitivity pattern for recovered *E. coli* isolates. AMP, Ampicillin; CAZ, Ceftazidime; ETP, Ertapenem; S, Streptomycin; AK, Amikacin; DO, Doxycycline; TE, Tetracycline; OFX, Ofloxacin; W, Trimethoprim; C, Chloramphenicol

et al. 2020), and Ghana (Takal and Quaye-Ballard 2018). Diseases such as diarrhoea, cholera, dysentery, typhoid, polio, and deaths have been associated with consumption of contaminated (WHO 2021). According to World Health Organization, water for drinking purposes should be devoid of *E. coli* (WHO 2011).

In the current investigation, the incidence of *E. coli* in some borehole and well water is not surprising as the water sources were unhygienically located and/or handled by users. Groundwater contamination occurs through seepage, fractured rock, holes and cracks, macropores root systems, and animal burrows (Romijn 2002; Mokuolu et al. 2017). Some of the water sources were sited close to contaminated flowing river, gutters, toilets facilities/sewerage systems, dumpsite, rarely or not treated, since construction and some of the wells were uncovered. The behaviour of users also determines the level of contamination of drinking water sources (Abdellah et al. 2012). During this study, some of the well water dependents were seen using dirty drawers to collect water, especially the shallow ones. This is unsafe and could transfer microbes on the bucket surface into the water.

The attainment of sustainable development goals (SDGs) associated with well-being, food security, safe water, and cleanliness is facing severe opposition by the widely acknowledged resistance to antimicrobial agents (Interagency Coordination Group on Antimicrobial Resistance 2019). This work reveals that resistance of our *E. coli* isolates to ampicillin was 50 (91%), trimethoprim and ertapenem 43 (78%), doxycycline 40 (73%), ceftazidime 38 (69%), tetracycline 37 (67%), streptomycin 22 (40%) and amikacin 18 (33%). The highest prevalence of ampicillin-resistant *E. coli* observed in this study agrees with Aslan et al. (2018), Praveenkumarreddy et al. (2020), and Singh et al. (2020). This is particularly worrisome, because ampicillin is the choice drug for treating livestock and human infections due to its effectiveness and low toxicity (FAO 2016; Pacifici 2021). Resistance to this drug, therefore, has possible consequences on increasing infections and deaths in humans and animals (Monger et al., 2021). Other researchers also reported high resistances to trimethoprim, ertapenem, doxycycline, ceftazidime, tetracycline, and streptomycin in aquatic environments, clinical and veterinary samples (Brolund et al. 2010; Hyle et al. 2010; Tadesse et al. 2012; Olorunmola et al. 2013; Mulder et al. 2019; Jahantigh et al. 2020; Mapanguy et al. 2021).

High-level susceptibility of the isolates to ofloxacin, chloramphenicol, and amikacin may be due to decreased exposure arising from low use of the antibiotics. In addition, the intravenous route of administration of ofloxacin and amikacin possibly restricts their indiscriminate use (Cheesbrough 2000). Significantly reduced resistances to fluoroquinolone and aminoglycoside antibiotics have been documented elsewhere (Titilawo et al. 2015a; Singh et al. 2020) Hence, the antimicrobials can be effective for treating *E. coli* infections.

In this work, antibiogram presented 100% resistance to ≥ 2 antimicrobials (Table 3). This finding is alarming and a serious health concern. Multiple drug resistance is a signal of abuse or excessive use of an antimicrobials for treatment of clinical and veterinary bacterial infections (Ramesh et al. 2010; Titilawo et al. 2015a). Previous investigation reported 75% multi-resistant *E. coli* from groundwater samples in India (Sharma et al. 2017), 46.9% from drinking water sources in Tanzania (Lyimo et al. 2016), and 100%, 90%, and 83% in soil, irrigated vegetable and wastewater, respectively, in Nsukka, Southeast Nigeria (Chigor et al. 2020). Olowe et al. (2008) also noted > 90% drug-resistant *E. coli* isolates to three or more commonly used antibiotics from clinical samples in Osogbo, Southwest Nigeria.

Multidrug-resistant *Escherichia coli* is a major threat to sustainable healthy living and livestock management. Altogether, the MDR *E. coli* in our investigation were not sensitive to a range of four to nine drugs, and mostly to ampicillin, ceftazidime, ertapenem, doxycycline and tetracycline. Previous finding of Lateef et al. (2005) revealed that a significant number of *E. coli* recovered from hospital, edible, and effluent samples in Southwest Nigeria were also not susceptible to different antibiotics, ranging between two and seven drugs, and mostly cotrimoxazole, tetracycline, and amoxicillin. Likewise, Titilawo et al. (2015a) noted *E. coli* resistance to five to nine drugs, majorly sulphamethoxazole, gentamycin, amoxycillin and ampicillin. Resistance of our isolates to ceftazidime and ertapenem is a public health concern as the drugs are known to be a viable therapeutic option to treat *E. coli* infections due to their effectiveness and low toxicity profile (Lartigue et al. 2007; Yuan et al. 2016). Ampicillin and tetracycline are commonly implicated in the therapy of airway and gastrointestinal infections, mastitis, etc. in humans and animals (Okeke et al. 1995; Hart and

Table 3 Pattern of antibiotics multiple antibiotics resistance phenotypes of *E. coli* isolates

Number of antimicrobials	Resistance profile	Frequency
Borehole		
Sampling site BW1 ($n=7$)		
6	AMP–AK–DO–TE–OFX–W	2
	AMP–ETP–DO–TE–W–C	1
	AMP–CAZ–AK–DO–TE–C	1
7	AMP–ETP–AK–DO–OFX–W–C	2
Sampling site BW3 ($n=1$)		
7	AMP–CAZ–ETP–AK–DO–OFX–W	1
Sampling site BW5 ($n=5$)		
4	AMP–CAZ–DO–C	1
5	AMP–CAZ–ETP–DO–W	1
7	AMP–CAZ–ETP–AK–DO–OFX–W	1
8	AMP–CAZ–ETP–S–DO–TE–W–C	2
Sampling site BW6 ($n=4$)		
5	CAZ–ETP–S–DO–W	1
8	AMP–CAZ–ETP–S–DO–TE–W–C	2
9	AMP–CAZ–ETP–S–AK–DO–TE–W–C	1
Sampling site BW7 ($n=11$)		
4	AMP–CAZ–ETP–W	1
	AMP–AK–DO–OFX	1
	AMP–ETP–S–TE	2
	AMP–ETP–TE–W	1
5	AMP–ETP–S–DO–TE	2
6	AMP–CAZ–AK–DO–OFX–W	1
	AMP–CAZ–ETP–S–TE–W	2
Sampling site BW9 ($n=1$)		
6	AMP–CAZ–ETP–DO–TE–W	1
Sampling site BW10 ($n=1$)		
5	AMP–CAZ–AK–DO–W	1
Sampling site BW11 ($n=3$)		
7	AMP–CAZ–ETP–S–DO–TE–W	1
8	CAZ–ETP–S–DO–TE–OFX–W–C	1
	AMP–CAZ–ETP–S–DO–TE–W–C	1
Sampling site BW12 ($n=1$)		
6	AMP–CAZ–ETP–TE–W–C	1
Sampling site BW14 ($n=1$)		
7	AMP–CAZ–ETP–S–DO–TE–W	1
Well water		
Sampling site WW2 ($n=1$)		
8	AMP–ETP–AK–DO–TE–OFX–W–C	1
Sampling site WW5 ($n=3$)		
4	AMP–CAZ–ETP–W	1
5	AMP–CAZ–ETP–DO–TE	1
7	AMP–CAZ–ETP–S–TE–W–C	1
Sampling site WW9 ($n=5$)		
4	AMP–AK–DO–W	1
5	AMP–AK–DO–OFX–W	1
6	AMP–CAZ–AK–DO–OFX–W	2
	AMP–CAZ–ETP–DO–TE–W	1
Sampling site WW10 ($n=2$)		
6	AMP–ETP–DO–TE–W–C	1

Table 3 (continued)

Number of antimicrobials	Resistance profile	Frequency
7	CAZ–ETP–S–AK–TE–W–C	1
Sampling site WW11 ($n=4$)		
4	AMP–CAZ–ETP–W	1
	AMP–CAZ–ETP–C	1
6	CAZ–ETP–S–DO–TE–W	1
7	AMP–CAZ–ETP–S–AK–DO–TE	1
	AMP–CAZ–ETP–S–DO–TE–W	1
Sampling site WW12 ($n=1$)		
4	CAZ–ETP–DO–W	1
Sampling site WW 13 ($n=3$)		
6	AMP–CAZ–ETP–DO–TE–W	1
	AMP–CAZ–ETP–TE–W–C	1
7	AMP–CAZ–ETP–DO–TE–W–C	1
Total		55

BW, Borehole water; WW, Well water; AMP, Ampicillin; CAZ, Ceftazidime; ETP, Ertapenem; S, Streptomycin; AK, Amikacin; DO, Doxycycline; TE, Tetracycline; OFX, Ofloxacin; W, Trimethoprim; C, Chloramphenicol; *n*, Number of isolates

Table 4 Predominant antibiotic resistance pattern of *E. coli* isolates across the sampling locations

Sample	Sample location	AMP	CAZ	ETP	S	AK	DO	TE	OFX	W	C	TOTAL	*ARI*	MARI
Borehole water	BW1	7	1	3	0	6	6	5	4	5	4	41	0.13	0.9
	BW3	1	1	1	0	1	1	0	1	1	0	07	0.70	0.8
	BW5	5	5	4	3	0	5	3	0	4	3	32	0.16	0.8
	BW6	3	4	4	4	1	4	3	0	4	3	30	0.23	0.9
	BW7	11	4	8	6	3	4	7	2	5	0	50	0.08	0.9
	BW9	1	1	1	0	0	1	1	0	1	0	06	0.60	0.6
	BW10	1	1	0	0	1	1	0	0	1	0	05	0.50	0.5
	BW11	2	3	3	3	0	3	3	1	3	2	23	0.30	0.9
	BW12	1	1	1	0	0	0	1	0	1	1	06	0.60	0.6
	BW14	1	1	1	1	0	1	1	0	1	0	07	0.70	0.7
Well water	WW2	1	0	1	0	1	1	1	1	1	1	08	0.80	0.8
	WW5	3	3	3	1	0	1	2	0	2	1	16	0.27	0.8
	WW9	5	3	2	0	3	5	2	2	5	0	27	0.16	0.8
	WW10	1	1	2	1	1	1	2	0	2	2	13	0.45	0.9
	WW11	4	5	5	3	0	3	3	0	3	1	27	0.20	0.8
	WW12	0	1	1	0	0	1	0	0	1	0	04	0.40	0.4
	WW13	3	3	3	0	0	2	3	0	3	2	19	0.23	0.7

BW, Borehole water; WW, Well water; AMP, Ampicillin; CAZ, Ceftazidime; ETP, Ertapenem; S, Streptomycin; AK, Amikacin; DO, Doxycycline; TE, Tetracycline; OFX, Ofloxacin; W, Trimethoprim; C, Chloramphenicol

Ariuki 1998; Hidron et al. 2008; Willems and Schaik 2009; Zhang et al. 2012). The increased ineffectiveness of tetracyclines in the current work can be attributed to the availability and accessibility of the drug for self-prescription and medication in both humans and livestock farms in Nigeria (Chigor et al. 2010; Olatoye 2010).

Usually, drug-resistant bacteria spread to the environment via excreta, and water environments are recognised reservoirs and transmission paths for the spreading of antibiotic resistance (Karkman et al. 2018). In the natural environment, antimicrobials provide selective pressure which alters the behaviour and fitness of a bacterial, advancing antibiotic resistance (Amarasiri et al. 2020). Oftentimes, environments harbouring antibiotic-resistant bacteria signal the possibility of antimicrobial contamination of that area (Gunaseelan and Ruban 2011).

Location of the boreholes close to channels of polluted waterbody earlier reported to harbour MDR bacterial

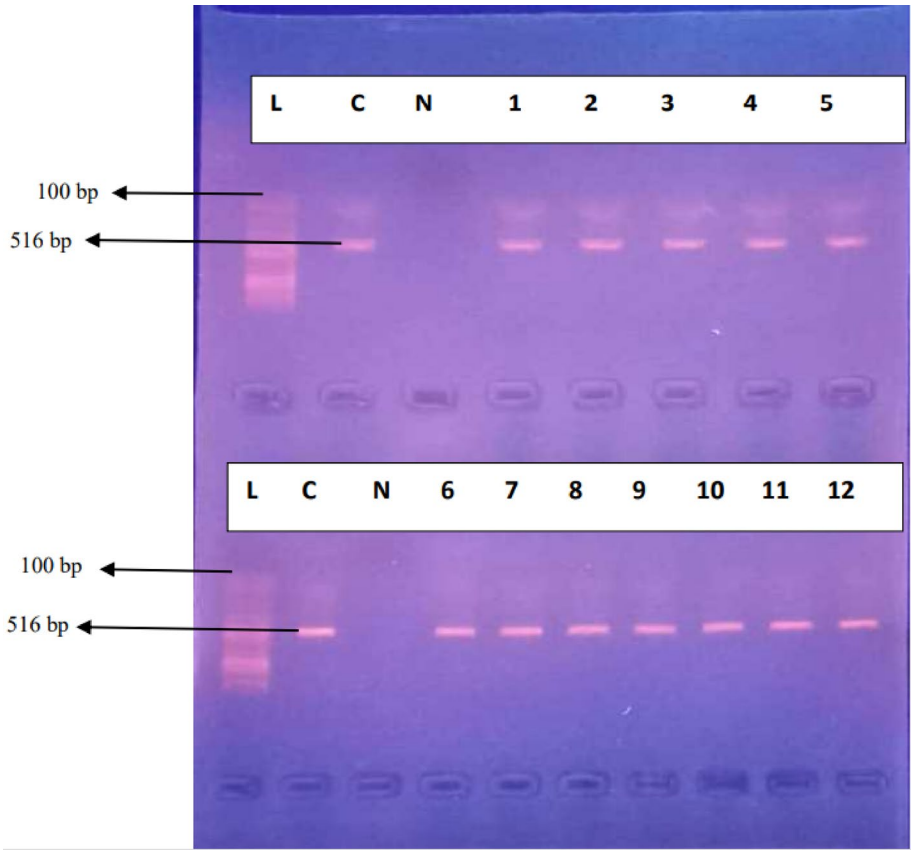

Fig. 4 Gel electrophoresis representative for TEM-H gene. L, DNA ladder; C, Positive control; N, Negative control; '1–12', *E. coli* isolates

(Titilawo et al. 2020), toilet facilities, dumpsites, and heavy anthropogenic activities around the groundwaters may be the source of contamination with MDR *E. coli*. In addition, free-range animals and their wastes also sighted around W5 during sampling could be a source of contamination. Animals are often administered antibiotics during rearing and their faeces contains relatively high levels of antimicrobial resistant bacteria (He et al. 2020). All these possibly influenced a higher than the safe limit for MARI (0.2) as observed in this study. MARI between 0.4 and 0.9 signifies high-risk origin of the isolates, where antimicrobials are frequently employed for treatment. Other studies reported MARI value greater than 0.2 in surface waters of Osun state Nigeria (Titilawo et al. 2015a), water purification and supply lines in North–West Province of South Africa (Ateba et al. 2020), raw meats, ready-to-eat meat, and their related sample in Ghana (Adzitey et al. 2021).

Intrinsic mechanism of Enterobacteriaceae to confer resistance to beta-lactam antibiotics through inactivation of beta-lactam ring present a huge challenge to public health concern (Bush and Jacoby 2010; Bush 2018; Matloko et al. 2021). The detection and prevalence (24; 48%) of TEM-H gene in the *E. coli* suggests that the gene is responsible for the phenotypic resistance noticed in the isolates. Nonetheless, the null detection of ampC, CTX-M, and SHV indicates that the isolates did not habour the genes or they are plasmid-encoded in Assawatheptawee et al. (2017) and Titilawo et al. (2015a) detected ampC gene in *E. coli* from aquatic environments in Northern Thailand and Southwestern Nigeria, respectively. Generally in *E. coli*, ampC gene encoded in the chromosome is constitutively low or poorly expressed but non-inducible, and same determinants located on mobile genetic plasmid favours overproduction of beta-lactamases and are easily transmitted within and between different bacterial species and hosts (Hanson and Sanders 1999; Haenni et al. 2014).

Conclusion

The quality of groundwater, an important resource for drinking in Osogbo metropolis is currently threatened by contamination with multidrug-resistant microorganisms. The study investigated the prevalence of multidrug-resistant *E. coli* from 40 groundwater sources (20 each of borehole and well water), and detected ampicillin-resistant gene in phenotypic resistant isolates. A total of 17 groundwater samples were contaminated by *E. coli*, and borehole waters recorded higher counts compared to well water. This suggests unhygienic location and poor sanitary practices around the water sources. Interestingly, all the 55 (100%) isolates were confirmed *E. coli* using *uid*A gene and showed

resistance to more than one antimicrobial, the most resistant being to ampicillin 53 (91%). Multiple antibiotic-resistant phenotypes and indices estimated indicate elevated levels of drug resistant *E. coli* in the groundwater samples and their occurrence can jeopardize human health especially in households and neighbourhoods that depend on the waters to meet their daily needs. The detection of TEM-H gene in the isolates poses a huge threat to public health, because the resistant gene is easily transferred horizontally within and between bacterial species and hosts. Thus, there is a need to regularly treat the groundwaters, prior consumption to reduce the risk of *E. coli* infection. Proper location and construction, and hygienic practices around groundwaters should be encouraged. The challenge of antibiotic resistance observed herein can be reduced by creating more awareness together with enforcement of law and policy that restrict illicit prescription and dispensing of antimicrobials. In addition, well-planned inspection programmes targeted at monitoring groundwater associated antimicrobial resistance patterns are essential. Further study on microbial water quality and profiling of antibiotic resistance aimed at mapping various waterborne diseases in Osogbo metropolis, Nigeria is recommended.

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Declarations

Competing interest The authors declare no competing interests.

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