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Solid waste dumpsite leachate and contiguous surface water contain multidrug-resistant ESBL-producing *Escherichia coli* carrying Extended Spectrum β-Lactamase (ESBL) genes

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Abstract

Dumpsites generate leachates containing bacteria that may carry antibiotic resistance genes, such as extended spectrum β-lactamase (ESBL). However, the contribution of dumpsite leachates in the environmental spread of ESBL genes has not been investigated in greater detail. This study aimed to quantify the impact of Ajakanga dumpsite leachate on the spread of ESBL genes through surface water. The susceptibility of *Escherichia coli* isolated from dumpsite leachate and the accompanying surface water to selected antibiotics was assessed by the standardized disc diffusion method. The isolates were evaluated for phenotypic ESBL production using the double disc synergy test (DDST). The detection of ESBL genes in the isolates was carried out using a primer-specific polymerase chain reaction (PCR). *Escherichia coli* isolates from leachate ($n=26/32$) and surface water ($n=9/12$) expressed ESBL phenotype. The ESBL-producing isolates showed the highest level of resistance to the 3rd generation cephalosporin antibiotics: cefotaxime (100%), cefpodoxime (97%), ceftazidime (97%), with low resistance observed to imipenem (6%) and azithromycin (3%). All the isolates were multidrug-resistant, showing resistance to three or more classes of antibiotics. All the ESBL-producing *E. coli* obtained carried bla_{CTX-M}, 21/35 (60%) carried bla_{TEM} while none of the isolates bore bla_{SHV}. This study found that ESBL-producing *Escherichia coli* from dumpsite leachate and nearby surface water had identical resistance signatures indicating the relatedness of the isolates, and that dumpsite leachate could contribute to the transfer of ESBL-producing bacteria and their genes to receiving

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surface water. This study has necessitated the need for a review of the guidelines and operational procedures of dumpsites to forestall a potential public health challenge.

Keywords Ajakanga dumpsite, Solid waste, Dumpsite leachate, Surface water, Extended Spectrum β-Lactamase (ESBL), *Escherichia coli*

Introduction

Globally, dumpsites are widely accepted as means for the disposal and management of municipal solid waste (MSW) because they can reduce the effect of MSW on the surrounding environment [1]. In developing countries, dumpsites are usually located in the city suburbs to prevent the chance of direct human interactions, even though the wastes are mostly discharged at the dumpsites without treatment [2]. However, the increasing global population and urbanization have expanded the frontiers of human habitation close to these dumpsites [3].

The corollary to this development is that dumpsites originally designed to manage MSW have transformed into a subject of concern in environmental management discourse due to their unplanned effects on environmental degradation and groundwater pollution. The deposition of industrial and pharmaceutical wastes into dumpsites has increased the prospect of groundwater and surface water contamination [4]. One of the major contemporary contaminants emanating from dumpsites is leachate [5]. Leachates contain metals and toxic substances such as biocides, and antibiotic residues [6]. Exposure of bacteria to these toxic agents can initiate the emergence of antibiotic resistance [6] and the spread of antibiotic resistance genes (ARGs) [7]. This makes dumpsite leachate an essential source of pathogens and ARGs in the environment [8].

In Ibadan, South-west Nigeria, information available more than a decade ago indicated that the residents generated about 485,860,260 kg of solid waste per annum [9] with barely one-tenth of the wastes being evacuated by both the public and private waste collectors [9]. A fraction of the overall waste collected is land-filled into randomly selected sites (dumpsites) with little or no consideration for urban expansion because nearly all the dumpsites are presently operated close to residential areas. One major dumpsite worthy of investigation is the improperly-managed Ajakanga dumpsite located in Ibadan, Nigeria. The ineffective management of municipal solid wastes at the site has resulted in the direct flow of leachates from the site into the environment and comes with the possibility of exacerbating pollution challenges faced by residents in the area with the likelihood of human infections. Previous studies conducted on the leachate from Ajakanga dumpsite showed elevated physicochemical parameters of surrounding soil and groundwater [10], it is however unknown whether the leachates harbor antibiotic-resistant bacteria (ARB).

While dumpsite leachates are possible reservoirs of ARB, investigations are required to show evidence of the environmental spread of ESBL genes through leachates. In this study, isolates recovered from dumpsite leachates from the Ajakanga dumpsite and adjoining surface water were assessed for antibiotic resistance, and ESBL phenotype and genotype, within a cross-sectional framework. This study aimed to quantify the potential impact of dumpsite leachate on the emergence and spread of ESBL in South-western Nigeria.

We project that a cross-sectional study of dumpsite leachate and the leachate-receiving surface water could highlight the likelihood of the environmental spread of ESBL-producing *E. coli* to the immediate surroundings. The ESBL-producing *E. coli* was prioritized in this study because this group of bacteria was recommended by the Tricycle protocol for antimicrobial resistance surveillance in the human, animal, and environmental sectors [11]. We focused on ESBL-producing *E. coli* due to their ability to harbor resistance genes, posing a major challenge for infection treatment [12].

Materials and methods

Description of the study site and sample collection

Samples analysed in this study were collected from Ajakanga dumpsite that lies between Latitudes 7^{0} 18.70'N and 7^{0} 18.90'N and Longitudes 3^{0} 50'E and 3^{0} 51' E in Oluyole municipality, Ibadan, a city in the South-western part of Nigeria [10]. Ajakanga dumpsite is operated by the Oyo State Waste Management Authority (OYOWMA) with an estimated area of 10.034 ha. The dumpsite was opened for use in 1996 to receive wastes from sources that include commercial, hospital, electronic, household, industrial, and unclassifiable sources [13]. At the base of the dumpsite, there was an observable accumulation of leachate which drains to the adjacent surface water. Four sampling points were identified and sampling was conducted at intervals of two weeks for four months. A total of 64 samples (32 leachate and 32 surface water) were collected throughout the sampling period. At each sampling point, about 250 mL of the leachate sample was allowed to drain into already-sterilized sampling bottles whereas an equal amount of contiguous surface water was aseptically collected into sterile pre-cleaned sample bottles. Upon collection, samples were transported to the Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan

in ice packs for analysis within two hours of collection. The location of the Ajakanga dumpsite is shown in Fig. 1.

Isolation and identification of cefotaxime-resistant *Escherichia coli*

Bacterial isolates used in this study were obtained on chromogenic medium using the streak plate method as previously described [14]. Briefly, a 3mL aliquot of leachate and surface water samples were separately dispensed into test tubes that contained Brain Heart Infusion broth (Becton, Dickinson and Company, France) to which 6 µg/mL of cefotaxime was incorporated. First, the setup was incubated overnight at 35±2ºC, and then a loopful of the broth was inoculated on CHROMagar™ *E. coli* (CHROMagar, France) using the streak plate method with further incubation. Colonies that yielded blue coloration on the medium were randomly selected, purified, and stored in 15% glycerol stock and agar slope for further studies. To avoid clonality and duplication of isolates in our sampling, we picked an isolate per plate per sampling point for each sampling round. Presumptively identified *E. coli* isolates were cultured in Luria Bertani broth (HiMedia Laboratories, India) on a rotary shaker overnight at 35±2ºC for DNA extraction. The genomic DNA was extracted using the Promega Wizard® Genomic DNA Purification Kit as specified by the manufacturer. The isolates were characterized using the 147 base pair (bp) housekeeping gene (*uidA*), that encodes β-glucuronidase in *E. coli* [15]. The primer sequences for *uidA* are as follows: Forward: 5′-AAAACGGCAAGAAAAAGCAG-3′ and Reverse: 5′-ACGCGTGGTTACAGTCTTGCG-3′. Confirmed *E. coli* isolates were selected for phenotypic detection of extended spectrum β-lactamase (ESBL) production and antibiotic susceptibility testing.

Phenotypic detection of ESBL production and susceptibility to antibiotics

The phenotypic detection of ESBL production in the isolates was performed using the double disc synergy test (DDST). In contrast, the isolates' antibiotic resistance pattern to a carefully selected panel of ten antibiotics was carried out using the Kirby-Bauer disc diffusion method [16]. The tested antibiotics were imipenem (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), ceftazidime (30 µg), cefpodoxime (30 µg), cefotaxime (30 µg), amoxicillin/clavulanate (30 µg) and azithromycin (15 µg) procured from Oxoid Ltd., Basingstoke, United Kingdom.

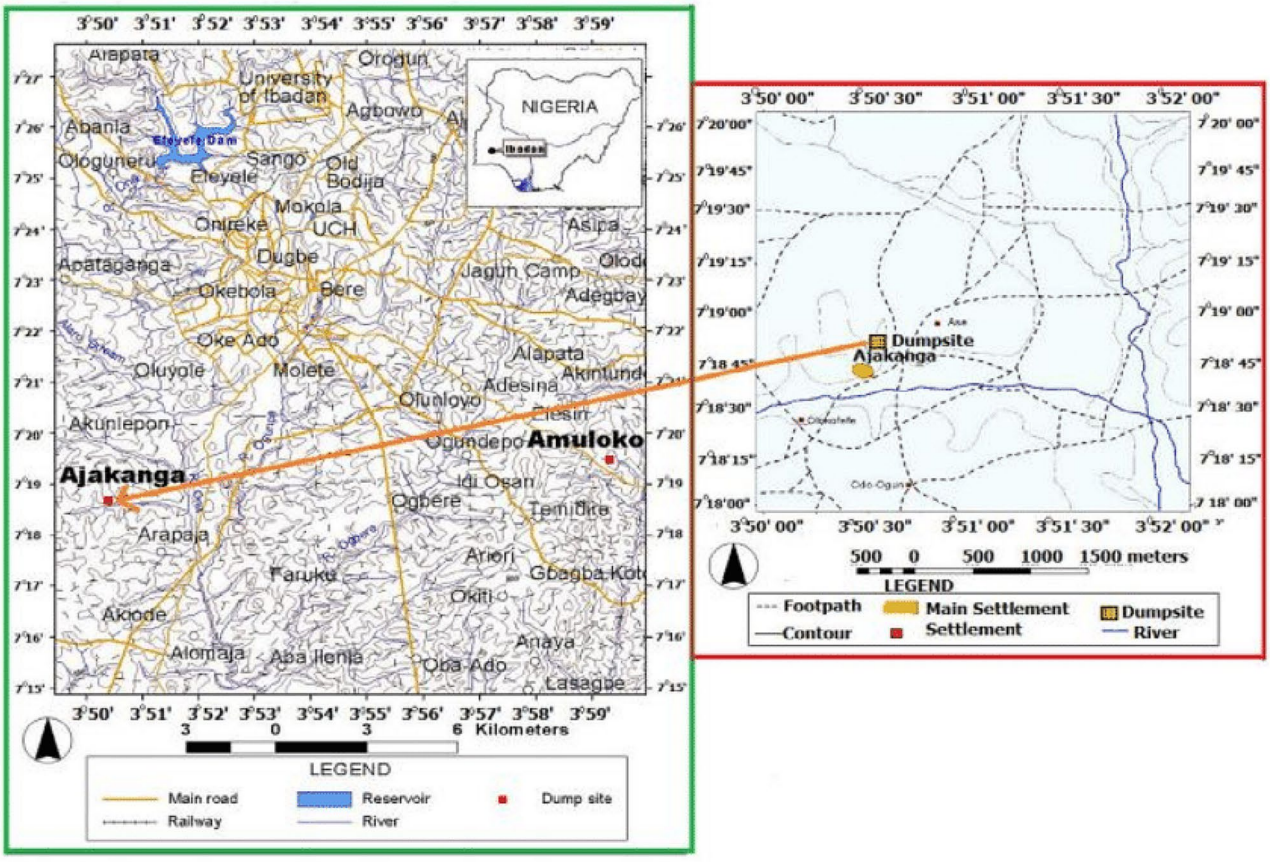

Fig. 1 Geographical Map of Ibadan showing Ajakanga area (Extracted from Nigerian Geological Survey Agency, Ibadan Sheet No. 59, 1980)

The choice of the selected antibiotics was informed based on the recommendations of CLSI [16]. The result of the antibiotic susceptibility testing (AST) was interpreted following the standard guidelines of the CLSI [16]. Each isolate was assessed for multiple antibiotic resistance index (MARI) using the formula:

$$MARI = a/b$$

where a=number of antibiotics the isolate showed resistance to and b=number of antibiotics the isolate was tested against [17].

Detection of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes

Multiplex PCR was used for the amplification of bla_{SHV} (768 bp) and bla_{TEM} (857 bp) genes as described by Maynard et al. [18] using the following PCR conditions: initial denaturation step at 94°C for 5 min, denaturation at 94°C for 30 s, primer annealing at 50°C for 30 s, extension at 72°C for 90 s (30 cycles) and terminal extension at 72°C for 10 min. The primer sequences for the two genes are as follows: (bla_{TEM}: Forward: 5′-GAGTATTCAACATT TTCGT-3′ and Reverse: 5′-ACCAATGCTTAATCAGT GA-3′) and (bla_{SHV}: Forward: 5′-TCGCCTGTGTATTA TCTCCC-3′ and Reverse: 5′-CGCAGATAAATCACCA CAATG-3′). The amplification of bla_{CTX-M} (543 bp) was performed using single PCR [19], with the following conditions: initial denaturation step at 94°C for 5 min, denaturation at 94°C for 30 s, primer annealing at 56 ^{0}C for 1 min, extension at 72°C for 60 s (30 cycles) and terminal extension at 72°C for 10 min. The primer sequences for bla_{CTX-M} are as follows: Forward: 5′-TTTGCGATG TGCAGTACCAGTAA-3′ and Reverse: 5′-CGATATCG TTGGTGGTGCCATA-3′. Amplicons were resolved on 1% agarose gel electrophoresis. A multidrug-resistant *E. coli* ALC08 isolated from abattoir leachate, carrying bla_{CTX-M}, bla_{SHV} and bla_{TEM} as reported by Adekanmbi et al. [14] was used as the positive control.

Results

Recovery of isolates and detection of ESBL-producing *Escherichia coli*

The sampling yielded an equal number of samples for leachate (n=32) and surface water (n=32) totaling 64. A total of 44 *E. coli* isolates were identified from the four-month sampling period, with 32 obtained from leachate while 12 isolates were recovered from the receiving surface water. Overall, 35 ESBL-producing *E. coli* were recovered from the sampling, with 26 isolates obtained from the dumpsite leachate and nine isolates from the surface water (Table 1).

Resistance to antibiotics and antibiotypes of the isolates

The resistance of the isolates to a panel of antibiotics is shown in Fig. 2. All the ESBL-producing *E. coli* isolates were resistant to cefotaxime. However, 97% of the isolates resisted ceftazidime and cefpodoxime, compared to 66% and 74% that were respectively resistant to amoxicillin-clavulanate and trimethoprim-sulfamethoxazole. The resistance pattern of the rest of the isolates varied from gentamicin (34%), and tetracycline (40%), to nalidixic acid (51%). Imipenem (a carbapenem) and azithromycin (a macrolide) were the most potent antibiotics against the isolates, with 6% and 3% respectively of the ESBL-producing *E. coli* showing resistance to them. The antibiotypes of the ESBL-producing *Escherichia coli* are presented in Fig. 3.

Detection of ESBL genes

As shown in Table 2, all the 35 ESBL-producing *E. coli* isolates carried bla_{CTX-M} which encodes the cefotaximases while 60% (n=21) carried bla_{TEM}. However, bla_{SHV} gene was not detected in any of the ESBL-producing *E. coli* isolates obtained.

Relationship between MARI and incidence of ESBL genes in the *E. coli* obtained in this study

Figure 4 shows the relationship between MARI and the incidence of ESBL genes in the isolates obtained in this study. Worthy of note is the fact that all the isolates with MARI between 0.4 and 0.7 carried bla_{TEM}, while those with MARI of 0.3 and 0.8 respectively did not carry the gene. All the isolates irrespective of the MARI value carried bla_{CTX-M}.

Discussion

The current study is a part of our investigation on the role of leachates from dumpsites in South-west Nigeria in the dissemination and spread of ESBL-producing bacteria and their genes. It is also a follow-up to our previous publications on leachate from Awotan dumpsite reputed as a hotspot of multidrug-resistant *Enterobacteriaceae* carrying both extended spectrum and AmpC β-lactamase genes [20]. The current study showed that leachates from municipal solid waste dumpsites are budding reservoirs for the proliferation and spread of ESBL-producing bacteria and their genes as previously shown [20]. Moreover, this study provides additional information on the

Table 1 Recovery of ESBL-producing *E. coli* from Ajakanga dumpsite leachate and receiving surface water

Sample	No. of *E. coli* obtained	No. of ESBL-producing *E. coli*	Proportion of ESBL-producing *E. coli* (%)
Dumpsite leachate (DL)	32	26	81.3
Receiving surface water (SW)	12	9	75.0

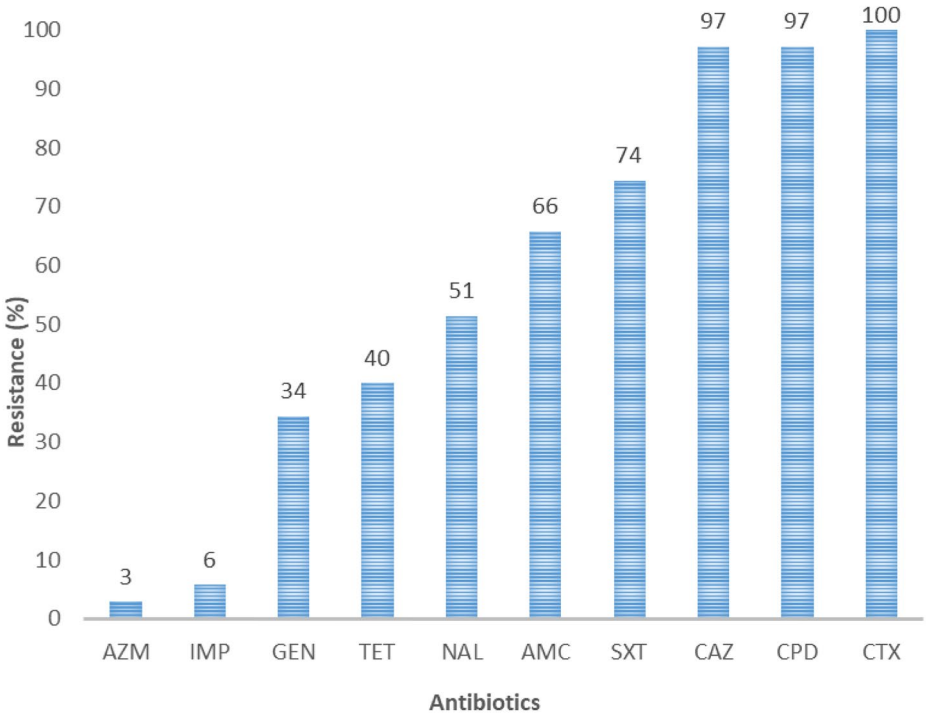

Fig. 2 Resistance of the ESBL-producing bacteria to a panel of antibiotics. KEY: AMC: Amoxicillin-clavulanate; CTX: Cefotaxime; CPD: Cefpodoxime; CAZ: Ceftazidime; GEN: Gentamicin; IMP: Imipenem; NAL: Nalidixic acid; TET: Tetracycline; AZM: Azithromycin; SXT: Trimethoprim-sulfamethoxazole **Note:** The values have been rounded off to the nearest whole number

contribution of non-healthcare sources to the proliferation of antibiotic resistance.

In this study, the role of dumpsite leachate in the environmental spread of ESBL-producing *E. coli* into the surrounding water ecosystem was investigated to gain insight into the dangers posed to humans. Firstly, we report the proportion of *E. coli* isolates obtained from the leachates (81.3%) and receiving surface water (75.0%) that were ESBL producers. This report parallels our previous finding on the prevalence of cefotaxime-resistant *E. coli* isolates from leachate generated at Awotan municipal solid waste dumpsite located in the same region where this study was carried out as reported by Adekanmbi et al. [20]. This observation was consequential because Ajakanga dumpsite was not partitioned nor separated from having contact with the surrounding environmental media. Our findings square with the report of Adelowo et al. [21] who isolated *E. coli* harboring $bla_{\text{CTX-M-15}}$ from wetlands polluted with human feces in the same settings. Taken together, these observations showed a gradual accumulation of ESBL-producing *E. coli* in environmental matrices in the region.

The resistance pattern of the isolates obtained makes the situation worse because the majority of the clinically relevant antibiotics used to counter bacterial infection were practically resisted by these isolates, a view also shared by Popoola et al. [22]. Different solid wastes including pharmaceutical leftovers are often deposited at the dumpsite [20]. These pharmaceutical wastes may have enriched the resident bacterial population for AMR phenotype and genotype as revealed by the isolates' resistance to tested antibiotics and their possession of resistance genes. This is in concordance with the findings of Focardi et al. [6] who recently reported significant changes in the composition and function of the bacterial community upon exposure to leachates and metals [6]. The findings on the prevalence of ESBL-producing *E. coli* may not be accidental after all, because wastes were disposed of at the dumpsite without sorting, segregation, and treatment. All the isolates obtained in this study passed the criterion for classification as multidrug resistant, showing phenotypic resistance to three or more different classes of antibiotics.

The first sulfhydryl variable enzyme (SHV-1) was identified in 1970 in a strain of *E. coli* [23]. The spectrum of activity of this initial enzyme was against penicillin and first-generation cephalosporins [24]. As a result of amino acid alterations that caused some configurational changes in their active sites, the hydrolytic activity of the SHV enzymes has evolved to an extended spectrum from the initially known narrow spectrum. This has led to the high ubiquity of these enzymes in so many compartments

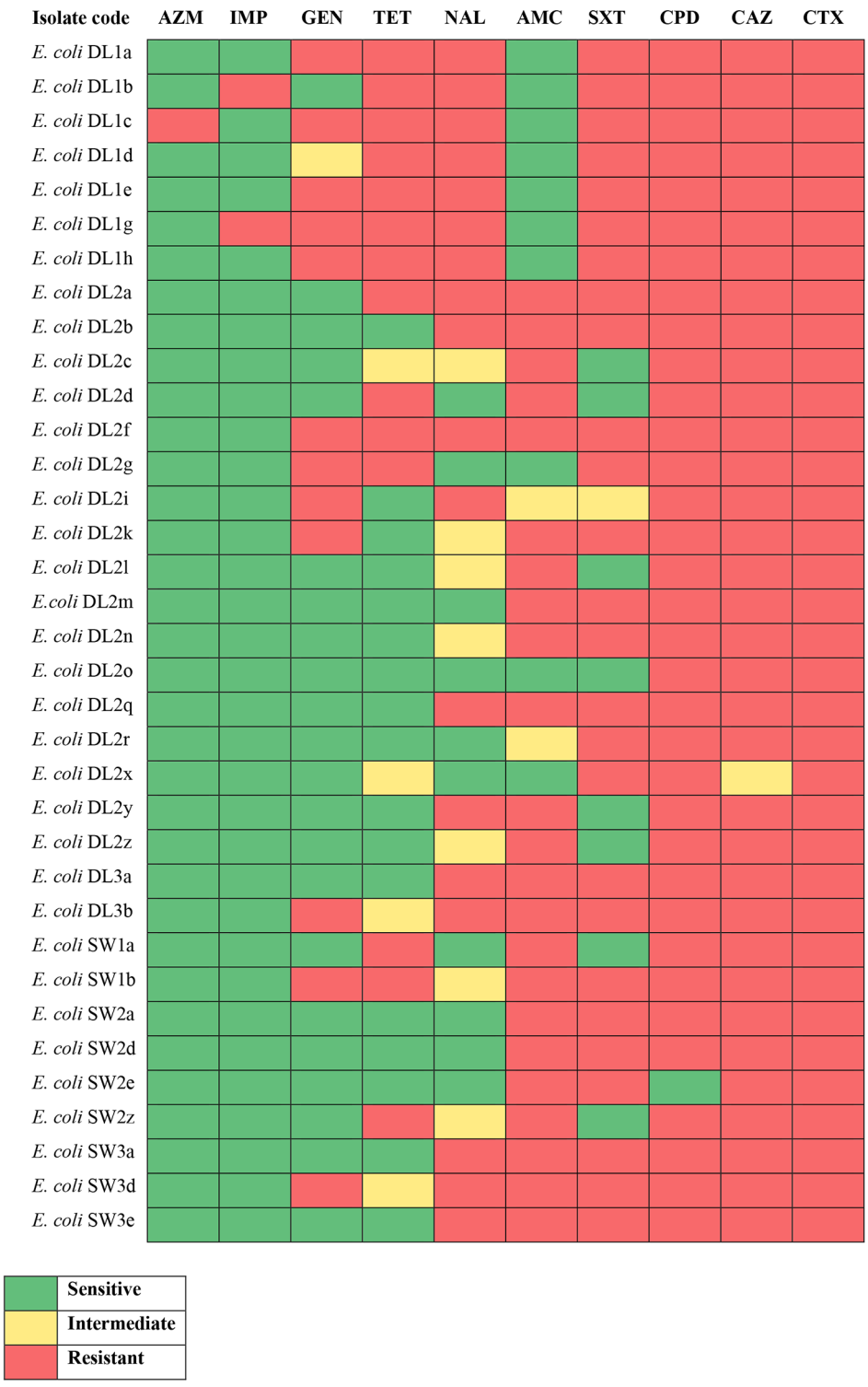

Fig. 3 Heat map showing the antibiotypes of the ESBL-producing *E. coli* from Ajakanga dumpsite leachate and receiving surface water. KEY: AMC: amoxicillin-clavulanate; CTX: cefotaxime; CPD: cefpodoxime; CAZ: ceftazidime; GEN: gentamicin; IMP: imipenem; NAL: nalidixic acid; TET: tetracycline; AZM: azithromycin; SXT: trimethoprim-sulfamethoxazole

Table 2 Incidence of ESBL genes in *E. coli* isolated from dumpsite leachate and receiving surface water

Isolate	CTX-M β-Lactamase	TEM β-Lactamase	SHV β-Lactamase
E. coli DL1a	+	+	-
E. coli DL1b	+	-	-
E. coli DL1c	+	-	-
E. coli DL1d	+	-	-
E. coli DL1e	+	+	-
E. coli DL1g	+	-	-
E. coli DL1h	+	+	-
E. coli DL2a	+	+	-
E. coli DL2b	+	+	-
*E. coli*DL2c	+	+	-
E. coli DL2d	+	+	-
E. coli DL2f	+	-	-
E. coli DL2g	+	+	-
E. coli DL2i	+	+	-
E. coli DL2k	+	-	-
E. coli DL2L	+	+	-
E. coli DL2m	+	-	-
E. coli DL2n	+	+	-
E. coli DL2o	+	-	-
E. coli DL2q	+	-	-
E. coli DL2r	+	+	-
E. coli DL2x	+	-	-
E. coli DL2y	+	+	-
E. coli DL2z	+	+	-
E. coli DL3a	+	+	-
E. coli DL3b	+	+	-
E. coli SW1a	+	-	-
E. coli SW1b	+	+	-
E. coli SW2a	+	+	-
E. coli SW2d	+	+	-
E. coli SW2e	+	-	-
E. coli SW2z	+	+	-
E. coli SW3a	+	-	-
E. coli SW3d	+	-	-
E. coli SW3e	+	+	-

Note: DA: Dumpsite Leachate; SW: Surface Water; +: Present; -: Absent

(human, animal, and environment) thereby suggesting an ecological migration. SHV comprises so many allelic variants which include those that are ESBL and non-ESBL [25]. In this study, none of the ESBL-producing *E. coli* carried bla_{SHV}. This observation is discordant with the findings of Adekanmbi et al. [20], who reported the carriage of bla_{SHV} by enteric bacteria isolated from leachate of a municipal solid waste dumpsite at Awotan in the same region as this study. In their study, they reported that bla_{SHV} was the least occurring of the ESBL genes detected with 31.7% of the total organisms in their study carrying the gene.

In this study, 60% (21/35) of the ESBL producers obtained carried $bla_{TEM,}$ a determinant responsible for mediating resistance to some β-lactam-based antibiotics including penicillin, ampicillin, and the first-generation cephalosporins. The carriage of bla_{TEM} by *E. coli* from dumpsite leachate is not a new phenomenon. In a study by Adekanmbi and his co-workers on leachate from Awotan Municipal Solid waste dumpsite, it was reported that 17 of 20 *E. coli* obtained totaling 85% carried bla_{TEM} [20]. The relatively few studies on dumpsite leachate all around the globe make it difficult to have a comparison on the carriage of ESBL genes from the source environment.

CTX-M β-lactamase has been the most widely occurring β-lactamase since the first case was reported in the 1980s [26]. They have over the years outnumbered the other ESBLs, with one of the factors favoring their spread being the extensive use of extended spectrum cephalosporins and other antibiotics that can co-select for CTX-M-producing strains, especially in veterinary practices

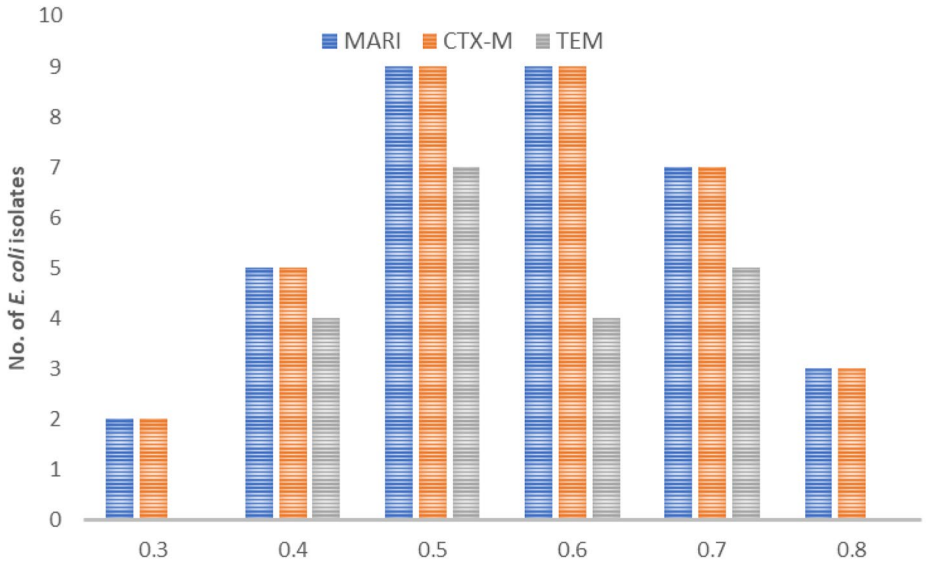

Fig. 4 MARI and incidence of ESBL genes in ESBL-producing *E. coli* isolated from Ajakanga dumpsite leachate and receiving surface water. **Note:** None of the isolates carried the bla_{SHV}, thus the gene was not included in the figure

[27, 28]. In this study, all the isolates obtained from the leachate and receiving surface water carried bla_{CTX-M} which mediates resistance to cefotaxime. This gene has been reported to be predominant in *Enterobacterales* and corroborated the findings of other studies on the high frequency of occurrence of the gene in several reservoirs [29–32].

In ESBL-producing bacteria, the co-occurrence of resistance determinants against cephalosporins, aminoglycosides, tetracycline, sulfonamides, and quinolones provide ESBL genes an advantage for maintenance due to co-selection processes [33, 34]. The high level of antibiotic resistance shown by the ESBL-producing *E. coli* in this study is most likely because they are equipped with genetic antimicrobial resistance armament that supports the resistance phenotypes. This development is a public health threat to residents of Ibadan, where the natural drainage pattern permits the seepage of organisms into shallow hand-dug wells used by several residents owing to poor municipal water infrastructural facilities [35] and this will increase the chance of human infection by drug-resistant ESBL-producing *E. coli* isolates.

We observed that 94.29% (n=33/35) of the *E. coli* isolates have a MARI between 0.4 and 0.8. Isolates with MARI ≥0.2 constitute risk on their own and those with MARI≥0.4 suggest the origin of the isolates to be of high antimicrobial usage [36, 37]. This implies that antibiotics accumulate in the dumpsite leachates and the contiguous surface water altogether. The detection of multidrug-resistant ESBL-producing *E. coli* with rooftop MARI necessitates proper surveillance programs to monitor antimicrobial resistance determinants [38] particularly in leachates.

Conclusion

Practical steps are required to avert public health emergencies by way of sorting, removing, and proper disposal of hospital and pharmaceutical wastes to curtail exposure of bacteria to antibiotic residues that can impose selective pressure on bacterial populations. Results presented in this study are expected to spur public policy on the provision of adequate infrastructural resources for waste management sufficient enough to prevent the emergence and dissemination of ARGs into the water ecosystem in this region. Measures that include regulation of the over-the-counter purchase of 3rd generation cephalosporin are also suggested. The procedure for the operationalization of dumpsites including sorting, segregation, and treatment of wastes should be reviewed. Wastes with a greater propensity to select for antibiotic resistance such as hospital and pharmaceutical wastes should be properly treated before being deposited at the dumpsites.

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Author contributions
AOA developed the original idea and the protocol. AOA, AGR, DJA, OCA, KAB, EPF, AVO and AdOA performed the experiments and were involved in the collection of data. AOA and AGR wrote the preliminary draft and analyzed the data with the other authors. All authors read, revised, and approved the manuscript for publication.

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Data availability
"All the data generated or analyzed during the execution of this study are included in this published article".

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval
Not applicable.

Consent to participate
Not applicable.

Consent for publication
Not applicable.

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