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Leachate emanating from a municipal solid waste dumpsite is a hotspot of Extended Spectrum β-Lactamase (ESBL)-producing *Escherichia coli* harboring CTX-M β-lactamase

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ABSTRACT

Background: Antibiotic resistance constitutes a key challenge to global public health and is on an alarming rise. In Nigeria, this quagmire has been extensively studied in clinical settings but not in the environmental sector, particularly in landfill leachates. **Aim:** We aimed to assess the susceptibility profile of *Escherichia coli* from leachate obtained from Lapite dumpsite, to determine the potential of dumpsite leachate as a source of ESBL-producing *E. coli*. **Methods:** Cefotaxime-resistant *E. coli* was isolated on cefotaxime-enriched chromogenic medium and identified via PCR using the *uidA* gene. The confirmed isolates from the pool were assessed for susceptibility to antibiotics using the disc-diffusion method, while the phenotypic detection of ESBL production was done using the double disc synergy test. Detection of ESBL genes was done using primer-specific PCR. **Results:** Twenty-three ESBL-producing *E. coli* were obtained, and they showed 100% resistance to cefotaxime and cefpodoxime, with 60.87% resisting imipenem (a carbapenem). However, none of the isolates was resistant to amoxicillin-clavulanate. The resistance to other antibiotics tested was: gentamicin (4.35%), ciprofloxacin (17.39%), nalidixic acid (26.09%), ceftazidime (26.09%) and chloramphenicol (30.43%). Seventy-four percent of the isolates had multiple antibiotic resistance index (MARI) greater than 0.3, with 30.4% (n=7/23) exhibiting multidrug resistance phenotypes. All the isolates carried $bla_{\text{CTX-M}}$, but none harboured bla_{SHV} or bla_{TEM}. **Conclusion:** Landfill leachates could contribute to the evolution of antibiotic-resistant ESBL-producing *E. coli* harbouring ESBL genes. There should be effective precautionary methods in place to prevent dumpsite leachates from flowing into adjoining environment to safeguard public and environmental health.

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Introduction

Our ability to treat clinical infections using conventional antibiotics is being progressively eroded by the increase in the abundance of antibiotic-resistant bacteria. With the current challenge of few antibiotics in the pipeline and the increasing adaptive capability of bacteria to withstand antibiotic insults, antibiotic resistance represents a significant public health. One way to manage and curtail antibiotic resistance will be to identify the major hotspots favouring their emergence. The role of hospitals and agricultural settings in the global rise of antibiotic resistance cases has been extensively studied, however, the contribution of the environment as a source of resistance development is gradually gathering momentum and increasingly being studied [1, 2]. This has necessitated the need to identify potential hotspots within the environment that may serve as 'cooking pots' for resistance emergence and as conduits for their dissemination [3]. One such site that is gaining notoriety and environmental relevance as a hotspot of antibiotic-resistant bacteria and their determinants is the municipal solid waste dumpsite and landfill.

The disposal of Municipal Solid Waste (MSW) directly at landfills or in open dump areas without segregation and treatment is an important environmental issue [4]. Municipal solid waste contains items such as personal care products; unused pharmaceuticals; biocides; heavy metals; sanitary tissue papers from households, clinics, and hospitals; waste from industrial, agricultural and faecal materials [5, 6]. The continuous discharge of antimicrobial agents and other chemicals into dumpsites can accumulate selective pressure on the resident bacterial population, leading to the selection of resistant strains. Degraded pharmaceutical agents and toxic wastes are washed down into leachates that drain and spill over to surrounding environments such as water bodies.

The presence of antimicrobial residues in leachates is an ugly scenario that portends a substantial threat to public health, as shown in the erstwhile study of **Chung *et al.*** [7], who reported that leachates from MSW dumpsites contained unacceptably high levels of human antibiotics. Chung and colleagues emphasised that ciprofloxacin, erythromycin, and trimethoprim were well above the "Predicted No Effects Concentration" (PNEC), a concentration high enough to select and enrich antibiotic-resistant bacteria (ARB) in landfill leachates.

This situation is even more critical in Nigeria due to the indiscriminate use of antibiotics in personal care products, biocides and other household agents, and their disposal into MSW dumpsites. The cumulative effect of this practice is the long-term interaction between bacteria and residual chemical agents (such as biocides, metals, and residual antibiotics), which provides a thriving condition to drive the evolution and selection of bacteria with enhanced or high levels of resistance to conventional antibiotics. Residual antibiotics and resistant bacteria present in leachates are known to pollute surrounding environments (such as surface and groundwater bodies) due to runoff and leaching [6, 8-9], thereby exposing receiving water bodies that are heavily reliant on by the general populace to the dangers of antibiotic-resistant bacteria.

Ibadan, a city in Southwest Nigeria where this study was carried out, is characterized by the indiscriminate and illegal disposal of wastes from several sources into dumpsites. Dumpsite operations are not adequately monitored or regulated. The inappropriate siting of dumpsites close to residential areas exposes residents to potentially serious health problems. One major dumpsite that is worth investigating is the Lapite dumpsite. Lapite dumpsite falls within the category of dumpsites that are not being properly managed. Leachates from the site flow directly into the environment, gaining access to water bodies, subsequently leading to pollution challenges which could culminate in serious health challenges [10].

The role of MSW dumpsites and landfills in Nigeria as hotspots of antibiotic resistance and their determinants is currently less understood. We previously revealed in our study carried out at the Awotan MSW dumpsite in Southwest Nigeria that leachates could serve as important reservoirs and potential conduits of antibiotic-resistant Gram-negative enteric bacteria harbouring extended spectrum β-lactamase (ESBL) genes in Nigeria [11]. Piggybacking on the results of our previous study, we hypothesize that other dumpsites at different locations in Ibadan, such as Lapite, can harbour resistant bacteria carrying resistance genotypes, especially those harbouring ESBL genes. This study, which was carried out at Lapite dumpsites, is part of our ongoing investigation and

aimed to obtain a clearer picture of the public health risk posed by MSW dumpsites as reservoirs of antibiotic-resistant bacteria in Ibadan, Nigeria.

Methods

Description of the study area and collection of samples

This cross-sectional study was conducted at the Lapite MSW dumpsite (Latitude 7.376736, and Longitude 3.939786), which is situated within an area of 20 hectares in Moniya area of Ibadan. Ibadan is a densely populated city in the South-western part of Nigeria. Lapite MSW dumpsite was established in 1998 and receives close to 9,000 tonnes of solid waste annually. It is being managed by the Oyo State Government under the Oyo State Waste Management Authority (OYOWMA). Wastes from commercial, hospital, electrical, household, industrial, and other sources are commonly disposed into the dumpsite. The nearest settlement to the dumpsite is approximately two kilometres; thus, leachate may threaten the groundwater quality and the microbial diversity of the area. Leachates from the dumpsite pile up in pools at the base of the wastes and three designated points approximately one kilometre apart were selected for sample collection. Leachates from the three points were collected in sterilized, pre-cleaned sample bottles for four months (November 2022 – February 2023) and transported to the laboratory on ice for subsequent analysis. A total of 36 leachate samples were collected over the four-month sampling period. There was no need for ethical approval, as no samples from human or animal subjects were collected. The location of the Lapite dumpsite in Southwest Nigeria is shown in **Figure (1).** An overview of the Lapite municipal solid waste dumpsite during one of the clearing processes by the Oyo State Waste Management (OYOWMA) is shown in **Figure (2).**

Isolation and identification of cefotaxime-resistant *Escherichia coli*

Approximately 2 ml of the leachate sample was discharged into a test tube containing brain heart infusion broth (3 ml) containing cefotaxime (6 µg/ml). The set-up was incubated overnight at 35±2°C. A loopful of the culture was streaked onto already prepared CHROMagar™ *E. coli* (CHROMagar, France) and incubated. Colonies of *Escherichia coli* with a bluish appearance on the chromogenic medium were picked, subcultured, and stored until pending further work. The presumptive organisms were characterized using conventional methods and by targeting a housekeeping gene (*uidA*), which is responsible for β-glucuronidase production in *E. coli* [12]. The confirmed isolates were selected for antibiotic susceptibility testing and ESBL phenotyping.

ESBL phenotyping and antibiotic susceptibility testing

The double disc synergy test (DDST) was used for the phenotypic detection of ESBL production in the confirmed isolates, while the susceptibility of the isolates to nine selected antibiotics was assessed by the Kirby-Bauer disc diffusion assay [13]. The tested antibiotics which were cefotaxime (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), ceftazidime (30 µg), amoxicillin/clavulanate (30 µg), imipenem (10 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), gentamicin (10 µg), azithromycin (15 µg), and cefpodoxime (30 µg) were purchased from Oxoid, United Kingdom. The choice of antibiotics and the interpretation of the data were done using the CLSI [13] guidelines.

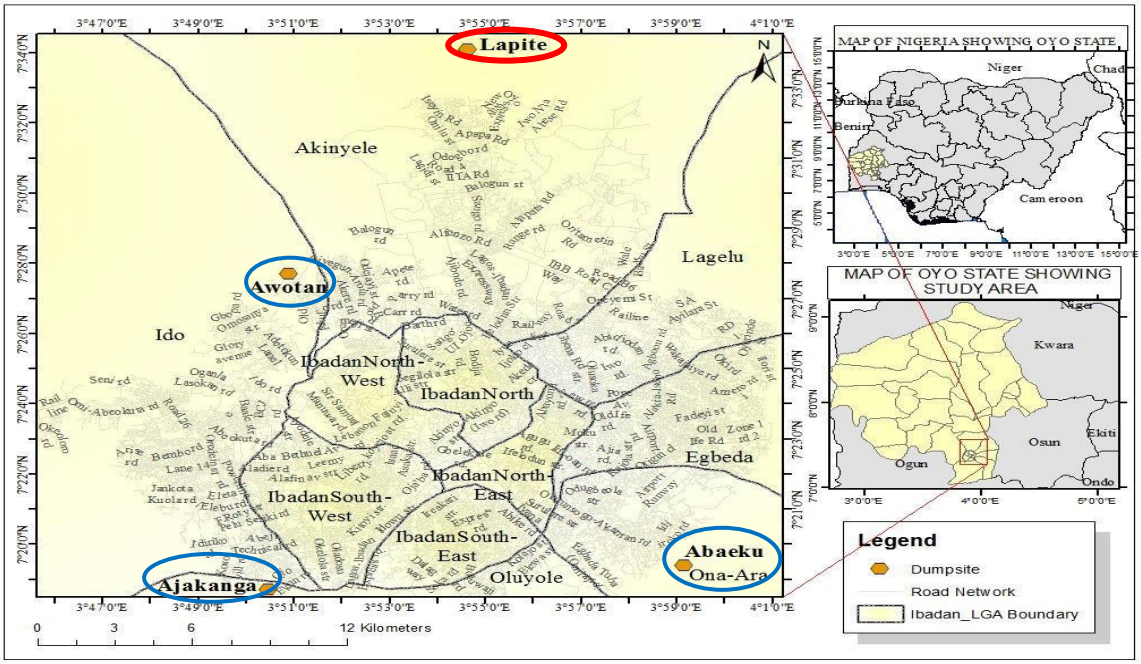
Detection of ESBL genes (*bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, and *bla*$_{\text{SHV}}$)

The *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genes were amplified using a previously described PCR protocol [14, 15]. Briefly, the amplification conditions included an initial denaturation step at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, primer annealing at 50°C for 30 seconds, extension at 72°C for 90 seconds (30 cycles), and a final extension at 72°C for 10 minutes. The amplification condition for *bla*$_{\text{CTX-M}}$ is as follows: initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, primer annealing at 56°C for 1 min, extension at 72°C for 60 s (30 cycles) and terminal extension at 72°C for 10 min. Amplicons were electrophoresed on a 1.5% agarose gel. A multidrug-resistant *E. coli* ALC08 carrying *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ reported by **Adekanmbi *et al.*** [16] was used as the positive control. The sequence of the oligonucleotide primers used in this study are given in **Table (1).**

Table 1. Oligonucleotide primers used in this study.

Target Gene	Primer sequence	Expected Amplicon size (bp)	Reference
uidA	AAAACGGCAAGAAAAAGCAG ACGCGTGGTTACAGTCTTGCG	147	[12]
$bla_{\text{CTX-M}}$	TTTGCGATGTGCAGTACCAGTAA CGATATCGTTGGTGGTGCCATA	543	[14]
bla_{TEM}	GAGTATTCAACATTTTCGT ACCAATGCTTAATCAGTGA	857	[15]
bla_{SHV}	TCGCCTGTGTATTATCTCCC CGCAGATAAATCACCACAATG	768	[15]

Figure 1. Location of major dumpsites in Ibadan, Southwest Nigeria (Google Map).



(Note**:** The Lapite dumpsite where this study was carried out is in the **RED** ring. The other municipal solid waste dumpsites in Ibadan, Oyo state are highlighted in the **BLUE** rings**).**

Figure 2. Overview of the Lapite municipal solid waste dumpsite during one of the clearing operations (Source: Oyo State Waste Management Authority).

Results

Isolation of *Escherichia coli* strains with the ESBL phenotype

Here, 23 confirmed and non-repetitive ESBL-producing *E. coli* strains were obtained from the pool of cefotaxime-resistant bacteria isolated from dumpsite leachates over a four-month sampling period. Eight ESBL-producing *Escherichia coli* were obtained from sampling points A and B, while seven ESBL producers were recovered from sampling point C, as shown in **Table (2).**

Susceptibility to the selected panel of antibiotics

The ESBL-producing *E. coli* isolates showed complete resistance to cefotaxime and cefpodoxime, while 60.87% and 30.43% of the isolates were resistant to imipenem and chloramphenicol respectively. The resistance of the isolates to the other antibiotics was: ceftazidime (26.09%), nalidixic acid (26.09%), ciprofloxacin (17.39%) and gentamicin (4.35%). None of the ESBL-producing isolates was resistant to amoxicillin-clavulanate, as shown in **Figure (3).**

Detection of ESBL genes

All the ESBL-producing *E. coli* carried $bla_{\text{CTX-M}}$ (cefotaximase) and devoid of bla_{TEM} and bla_{SHV}. A representative gel picture showing the detection of $bla_{\text{CTX-M}}$ in the isolates is shown in **Figure (4). Table 3** shows the multiple antibiotic resistance index (MARI) and possession of ESBL genes by isolates recovered from the Lapite dumpsite leachate.

Table 2. Frequency of occurrence of ESBL-producing *E. coli* in the Lapite dumpsite leachates.

Sampling point	Frequency of ESBL-producing *E. coli* obtained	% ESBL-producing *E. coli* obtained (%)
A	8	34.8
B	8	34.8
C	7	30.4

Table 3. MARI and Occurrence of ESBL genes by cefotaxime-resistant *E. coli* found in Lapite dumpsite leachate.

Isolate	MARI	$bla_{\text{CTX-M}}$	bla_{TEM}	bla_{SHV}
E. coli L1a	0.22	+	-	-
E. coli L1b	0.22	+	-	-
E. coli L1c	0.22	+	-	-
E. coli L1d	0.33	+	-	-
E. coli L1e	0.33	+	-	-
E. coli L1f	0.44	+	-	-
E. coli L1g	0.33	+	-	-
E. coli L1h	0.44	+	-	-
E. coli L2a	0.33	+	-	-
E. coli L2b	0.44	+	-	-
E. coli L2e	0.88	+	-	-
E. coli L2f	0.22	+	-	-
E. coli L2g	0.55	+	-	-
E. coli L2h	0.22	+	-	-
E. coli L2i	0.33	+	-	-
E. coli L2j	0.33	+	-	-
E. coli L3d	0.33	+	-	-
E. coli L3e	0.33	+	-	-
E. coli L3f	0.44	+	-	-
E. coli L3g	0.77	+	-	-
E. coli L3h	0.22	+	-	-
E. coli L3i	0.77	+	-	-
E. coli L3k	0.77	+	-	-

KEY: +: Present: +; -: Absent

. **Figure 3.** Resistance of the ESBL-producing *E. coli* from Lapite dumpsite leachate to a panel of antibiotics.

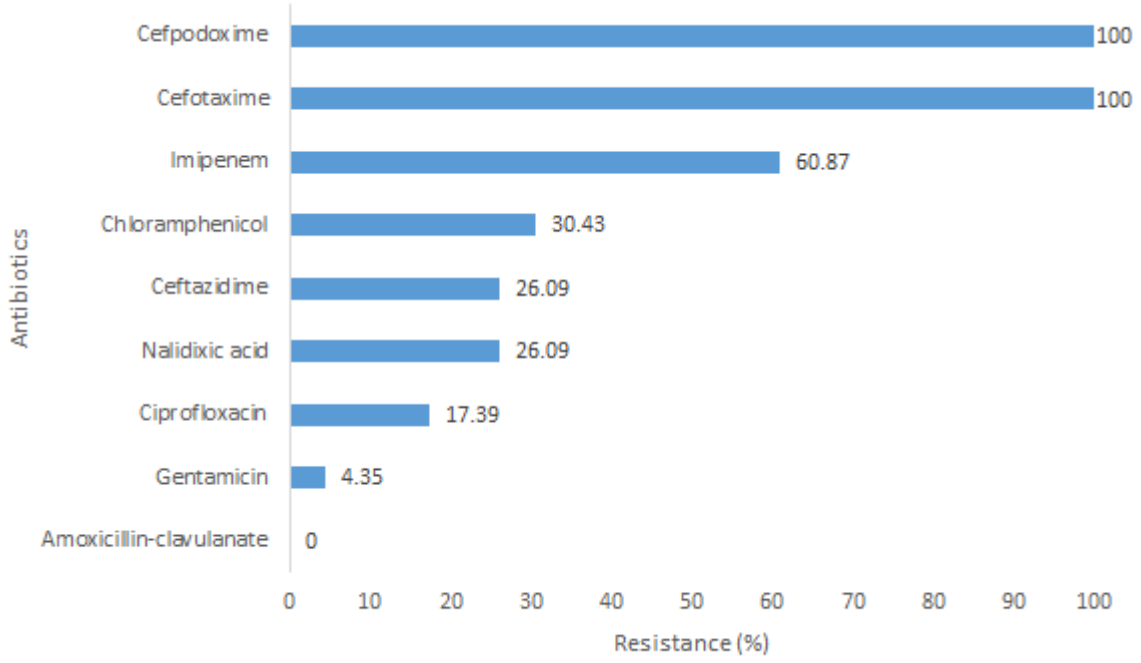
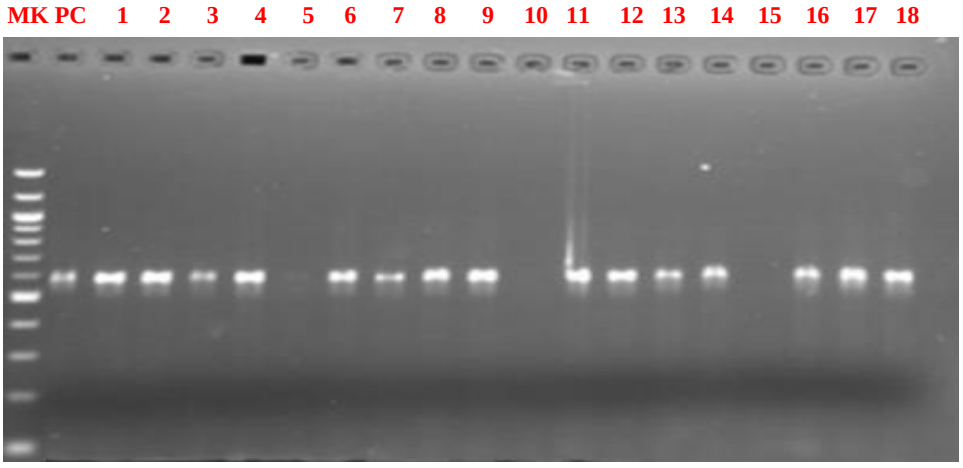


Figure 4. Representative gel picture showing the detection of *bla*$_{CTX-M}$ in some of the ESBL-producing *E. coli*.



KEY: MK: DNA ladder (100bp), PC: Positive control, Note that Lanes 5, 10 and 15 are empty.

Discussion

In Nigeria, domestic, industrial, and pharmaceutical wastes are usually collected and deposited on landfill dumpsites. The interaction between the various components of the waste could lead to the generation of leachates containing deleterious chemicals, biocidal agents, and toxic substances, which could exert pressure on the leachate microflora. Different bacterial genera carrying resistance genotypes have been isolated from leachates [17-19].

We purposively sampled leachates oozing out of Lapite municipal solid waste dumpsite (one of the notorious dumpsites in the Ibadan metropolis), for the isolation of ESBL-producing enteric bacteria, with particular emphasis on *E. coli*. ESBL genes have been described in different matrices, including humans, animals, and even the environment, but how dumpsite leachates contribute to the possible emergence and dissemination of these genes has been investigated by a few studies [11, 20]. We prioritized ESBL-producing *E. coli* to highlight the role of dumpsite

leachates as reservoirs of antibiotic resistance determinants. CHROMagar™ *E. coli* was used for the isolation of the bacterium because the chromogenic medium has high selective and discriminatory power [21]. *Escherichia coli* (n=23) isolated from the leachates were preselected using cefotaxime (a third-generation cephalosporin) as a strategy to determine whether they were ESBL producers because the emergence and spread of ESBL-producing bacteria is one of the foremost public health issues [22, 23].

The assessment of antibiotic resistance phenotypes in the identified organisms from the dumpsite leachate revealed a high degree of antibiotic resistance. Seventy-four percent (n=17/23) exhibited MARI above 0.3, indicating a high level of multidrug resistance in the *E. coli* emanating from the leachate. The resistance of more than twenty percent of the isolates (n=5/23) to three or more classes of antibiotics suggests that the leachate is a breeding reservoir of antibiotic-resistant bugs as suggested by **Athanasakopoulou *et al.*** [24]. This current observation agrees with our previous reports on leachates from the Awotan dumpsite in Ibadan, where MDR *E. coli* were also obtained [11]. It should be noted that the Awotan dumpsite is located in the same city and geographical location as in the present study. The level of resistance to antibiotics by the isolates could be attributed to the abuse and improper disposal of antibiotics, which could have made the dumpsite an enrichment reservoir for antibiotic-resistant bacterial strains. The deposition of antibiotic-embedded materials and heavy metals in dumpsites is capable of exposing bacteria to selective pressure and results in horizontal transfer of resistance, turning dumpsites into reservoirs for the proliferation and spread of antibiotic resistance determinants [17, 25].

Moreover, the convergence of MDR and ESBL phenotypes in isolated organisms can amplify their risk of infection and associated treatment costs. It is more worrisome that the isolates were resistant to the third-generation cephalosporins (cefotaxime and cefpodoxime) tested in this study because these agents are largely affordable to patients in developing countries and are thus widely used in clinical practice. Moreover, 60.87% of the ESBL-producing *E. coli* (n=14) were resistant to imipenem, which is among the last-resort antibiotics for the treatment of infections caused by ESBL- or AmpC-producing bacteria, as well as patients with records of multidrug-resistant bacterial infections [26]. Efflux pump modifications in the cell outer membrane configuration and AmpC-type β-lactamase overexpression have been linked to imipenem resistance [27, 28]. The detection of imipenem-resistant, MDR and ESBL-producing *E. coli* in dumpsite leachates constitutes a clear threat to public health and calls for immediate action.

The increased resistance to cefotaxime and cefpodoxime in this study could be attributed to the carriage of $bla_{\text{CTX-M}}$ gene by the isolates. The detection of $bla_{\text{CTX-M}}$ is consistent with the predominance of the gene, especially $bla_{\text{CTX-M-15,}}$ in *E. coli* isolates of human origin as reported in different geographical regions [29]. The presence of $bla_{\text{CTX-M}}$ in this study is in agreement with most studies that have reported the wide occurrence of the gene and its dominance globally [30-32]. Several factors have been responsible for the rapid emergence and increasing prevalence of $bla_{\text{CTX-M}}$ globally, including plasmid dissemination and the clonal spread of bacteria carrying the gene. In addition, the selective pressure posed by the misuse and excessive use of extended spectrum cephalosporins have also played a major part in the epidemiological success of the $bla_{\text{CTX-M}}$ [32-34].

Conclusions

Leachates from dumpsites are a budding hub of ESBL-producing *E. coli* with multidrug resistance phenotypes, and carrying a high burden of $bla_{\text{CTX-M}}$. Put together, our findings strongly suggest that policymakers should prioritize the health and well-being of the governed by ensuring that dumpsites are located far from residential areas and that waste materials from homes, agricultural farms, healthcare centres, and other waste-generating institutions are properly disposed of. We recommend continuous examination of leachates and their stoppage from entering groundwater to prevent outbreaks of disease in the regions where dumpsites are located. We also suggest that leachates from dumpsites should be treated by siting an appropriate leachate treatment plant close to the vicinity of dumpsites.

List of abbreviations

ESBL: Extended Spectrum β-Lactamase
MSW: Municipal Solid Waste
ARB: Antibiotic-Resistant Bacteria

MDR: Multidrug Resistant
PNEC: Predicted No Effects Concentration
CLSI: Clinical and Laboratory Standard Institute
IITA: International Institute of Tropical Agriculture
OYOWMA: Oyo State Waste Management Authority
NPC: National Population Commission

Declarations

Ethics approval and consent to participate:
Not applicable.

Consent for publication:
Not applicable.

Availability of data and materials
My manuscript has no associated data.

Competing interest
There is no competing interest as regard this study.

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Authors' contributions
"AOA developed the original idea and the protocol with HAS. AOA and AGR wrote the preliminary draft. AOA, OCA and AVO analysed the data. AOA, AGR, OCA, DJA, AVO, OAB, TAA, BFT and HAS performed the laboratory experiments and were involved in the collection of the data. All the authors read and approved the final manuscript."

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