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Antibiotic susceptibility patterns of heavy metal−tolerant Gram−negative bacteria isolated from a major dumpsite in Ibadan, Nigeria

Blessing Ifeoma Nwadike✉, Jemimah Barwa

Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan 200284, Nigeria

ABSTRACT

Objective: To determine the antibiotic susceptibility patterns of heavy metal-tolerant Gram-negative bacteria isolated from a major dumpsite in Ibadan, Nigeria.

Methods: Gram-negative bacteria were isolated using MacConkey and eosin methylene blue agar and presumptive identification to species level was done using conventional methods. The isolates were screened for their tolerance to heavy metals. Antimicrobial susceptibility was tested by Kirby-Bauer disc diffusion method with the following antibiotics: tetracycline (10 µg), cotrimoxazole (25 µg), gentamicin (10 µg), cefuroxime (30 µg), chloramphenicol (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), ceftazidime (30 µg), and meropenem (10 µg). The zones of growth inhibition were interpreted based on the Clinical Laboratory Scientific Institute. Identification of the most metal-tolerant isolates was done using 16S rRNA.

Results: A total of 79 Gram-negative bacteria were isolated, 31 of which tolerated a minimum of three heavy metals (Pb at 10 350 µg/mL; Cu at 750 µg/mL; Cr at 750 µg/mL in the wet season and Pb at 2 350 µg/mL; Cu at 450 µg/mL; Cr at 300 µg/mL in the dry season). All 31 isolates showed varying degrees of multiple antibiotic resistance indexes. The isolates showed the highest resistance to cefuroxime and meropenem (100%) and the least resistance to chloramphenicol (51.6%). The most metal-tolerant isolates were identified as *Aeromonas hydrophila* and *Pseudomonas aeruginosa*.

Conclusions: This study revealed that the waste dumpsite contains significant amounts of heavy metals and antibiotic-resistant bacteria which may pose serious public health risks if they enter the food chain. The study highlighted the health risks of using untreated dumpsite soils for vegetable farming due to pathogen and heavy metal transfer, and it is unsuitable for agricultural use.

KEYWORDS: Heavy metals; Dumpsites; Antibiotic resistance; Gram-negative bacteria

1. Introduction

A dump is defined as "a site used to dispose of solid wastes without environmental controls"[1]. In many African countries, open dumping is favoured over landfills as the primary means of solid waste disposal. People living close to dumpsites are in danger of contracting diseases associated with dumps. As the global population grows and the demand for essential resources such as food increases, the volume of waste generated by households daily continues to rise. Cortez and Ching[2] reported higher concentrations of heavy metals like copper, zinc, lead and cadmium in dumpsite soils in comparison to its counterpart agricultural land. Similarly, Akmal and Jamil[3] demonstrated that irregular waste disposal sites in the vicinity of residences cause illness. Heavy metals

Significance

This study provides a novel exploration of the relationship between heavy metal tolerance and antibiotic resistance in bacteria isolated from municipal dumpsite soil in Ibadan, where such interactions have been largely unexamined. This research examines their co-selection mechanisms within the same bacterial isolates. Understanding this connection is essential for addressing the growing public health threat posed by multidrug-resistant bacteria in environments with significant industrial and domestic waste accumulation.

✉To whom correspondence may be addressed. E-mail: blessing.nwadike123@gmail.com; okolieblessing@yahoo.com

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are defined as metals that naturally occur in the Earth's crust and have a specific gravity greater than 4.5 g/cm³. The most common heavy metals found in the environment include arsenic (As), lead (Pb), mercury (Hg), nickel (Ni), chromium (Cr), copper (Cu), zinc (Zn), and cadmium (Cd)[4]. Heavy metals are harmful and can remain in the soil for many years. Populations residing in proximity to dumpsites often cultivate vegetables and other arable crops there due to the nutrient-rich environment. Olayiwola *et al*.[5] demonstrated the accumulation of heavy metals such as lead, zinc, iron, nickel, and cadmium in cultivated plants grown in dumpsite soils. Microorganisms that are resistant to heavy metals play a role in preserving antibiotic-resistant genes. The most common mechanisms of tolerance are metal sorption, mineralisation, absorption and accumulation, extracellular precipitation, enzymatic reduction or oxidation to a less toxic form, and heavy metal outflow from the cell[6]. González and Ghneim-Herrera[7] highlighted five major mechanisms by which bacteria tolerate the presence of heavy metals. Bacteria make use of external defenses like the cell wall, plasma membrane, or capsule to hinder the penetration of metal ions into the cell. They could also export toxic metals from the cytoplasm through active transport. Extracellular sequestration entails the gathering of metal ions by cellular elements positioned in the periplasm or outer membrane, or by forming insoluble compounds through metal ion complexation. On the other hand, intracellular sequestration relies on accumulating metals in non-bioavailable states within the cytoplasm, aiming to shield essential cellular components from exposure. Lastly, they could tolerate heavy metals through the re-oxidation of metal ions. Microbes that are tolerant to heavy metals can be employed in bioremediation, effectively accumulating and neutralizing heavy metals in contaminated environments[8]. There is a correlation between metal tolerance and antibiotic resistance because the genes responsible for both traits are likely to be closely linked on the same plasmid[9].

According to Antimicrobial Resistance Collaborators[10], antimicrobial resistance (AMR) stands as a paramount global concern for public health and development. In 2019 alone, bacterial AMR is believed to have directly caused 1.27 million deaths worldwide and played a role in an additional 4.95 million deaths. Genes associated with antibiotics have been found in various environments, including supposedly untouched and regulated environments such as compost, indicating their environmental origins[11].

Within the environment, antimicrobial residues exert a range of negative impacts across different trophic levels of the ecosystem. Antibiotics persist in the environment as antibiotic residues present in manure, which, when applied to farmland, can leach into the soil and groundwater[12]. Many antibiotics are not readily broken down by natural microbial processes, further contributing to their prolonged activity in the environment[13]. In the long term, antibiotic residues in soil can disrupt the delicate balance of microbial communities, affecting nutrient cycling, plant growth, and crop yields. They can also impair plant health by causing physiological disturbances. Additionally, when antibiotic residues enter the food chain, they can contaminate food crops, posing potential risks to human and animal health[14]. In aquatic environments, antibiotic contamination can directly harm aquatic organisms, alter microbial community composition and diversity, and facilitate the spread of antibiotic resistance genes[15,16]. Another significant concern is the emergence of antibiotic-resistant bacteria in the environment, leading to prolonged illnesses, higher healthcare costs, and increased mortality rates[17]. Even at concentrations below levels that inhibit growth, antibiotics can act as signalling molecules, triggering changes in bacterial gene expression, gene transfer, virulence, biofilm formation, quorum sensing, and the regulation of gene activity. Resistance genes carried on plasmids can enhance bacterial survival through mechanisms such as efflux pumps, which actively remove antibiotics and heavy metals from the cell[18]. In addition, co-resistance occurs when genes encoding resistance to antibiotics and heavy metals are located on the same plasmid, allowing bacteria exposed to heavy metals to develop antibiotic resistance. Cross-resistance further complicates treatment by enabling a single resistance mechanism, such as an efflux pump, to confer resistance to multiple antimicrobial agents[19,20]. Continued exposure of bacterial cells to pharmaceuticals also enhances the horizontal transfer of drug resistance genes (known as HGT-horizontal gene transfer) among microbes, frequently involving bacteria from diverse evolutionary backgrounds, including non-pathogenic and pathogenic strains. These substances can be taken up by plants, disrupting vital physiological functions like photosynthesis, germination, and growth. Ultimately, these residues pose risks to human health through contaminated water and agricultural produce consumption, potentially causing cancer, allergic reactions, or disruptions to the natural intestinal microflora[21].

Antibiotic resistance can arise through mutations triggered by minimal antibiotic concentrations in the environment, horizontal transfer of resistance genes, or, as observed in Gram-negative bacteria, through their outer membrane, which confers resistance to multiple antibiotics[22,23]. Previous studies have identified several bacterial species, such as *Pseudomonas*, *Bacillus*, and *Enterobacter* that exhibit both antibiotic resistance and heavy metal tolerance[6,9,11]. Among these, *Pseudomonas* species are widely recognized for their adaptability and ability to survive in contaminated environments due to their efficient efflux pump systems and biofilm formation[24]. *Bacillus* species, known for their spore-forming ability, can withstand harsh environmental conditions, including exposure to toxic heavy metals and antibiotics[25]. *Enterobacter* species, commonly found in soil and water, exhibit significant resistance to both heavy metals and antibiotics, making them a concern in environmental and clinical settings[26]. Therefore, there is need to determine if heavy metal-tolerant Gram-negative bacteria can show resistance to a panel of antibiotics.

2. Methods

2.1. Description of the study area

The study site was Lapite dumpsite in Akinyele Local Government

Area, Ibadan, Oyo State, Nigeria. The dumpsite is delimited by longitudes 3.91160°E and 3.91464°E and latitudes 7.5825°N and 7.57032°N (Supplementary Figure 1). Every day, tonnes of waste accumulated from different areas in Ibadan and its surrounding areas are deposited onto the dumpsite, forming substantial piles of waste with varying compositions, reaching heights of up to 3.0 meters above ground level. Dumping activities at the site are unrestricted, resulting in the scattering of industrial, agricultural, domestic, and medical waste, including used syringes, across the dumping area. It was observed that food plants such as maize, cassava, banana, and tomato were grown around the dumpsite (Supplementary Figure 2).

2.2. Soil sample collection

Each soil sample (250 g) was collected in a separate 500 mL jar from the dumpsite in August (marked by heavy rainfall) and November (onset of the dry season), 2023. Samples were collected from three distinct locations (in duplicates) within the dumpsite, each situated 50 meters apart for both periods. The samples were aseptically transported in sterile jars to the Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, for analysis.

2.3. Laboratory analysis

2.3.1. Physicochemical and heavy metal analysis of soil samples

The samples were digested according to the method described by Environmental Protection Agency[27]. The digested samples were analyzed for metals (Pb, Cd, Cr, Hg, and Cu) using an atomic absorption spectrophotometer (Pye Unican model SP-2900). The pH, organic matter, moisture content (%), macronutrients (carbon, nitrogen, phosphorus, potassium, calcium, magnesium, and sodium), and heavy metal (Pb, Cd, Cr, Hg, and Cu) content of the soil samples were analyzed.

2.3.1.1. Soil pH determination

The pH of the soil was determined using the 2.5:1 soil-to-water suspension method. Twenty grams (20±0.1 g) of air-dried, sieved soil was weighed into a 100 mL beaker, and 50 mL of deionized water was added. The mixture was stirred for 10 minand then allowed to stand for 30 min to reach equilibrium. Subsequently, the suspension was stirred again for 2 min, and the pH was measured using a calibrated pH meter (standardized with buffer solutions at pH 4.0 and 7.0).

2.3.1.2. Organic carbon determination (Walkley–Black method)

The organic carbon content of the soil samples was determined using the Walkley-Black Oxidation method. A sample of 0.1 to 0.5 g of air-dried, ground (60-mesh) soil was weighed into a block digester tube, and 5 mL of 1 N potassium dichromate ($K_2Cr_2O_7$) solution was added, followed by 7.5 mL of concentrated sulfuric acid (H_2SO_4). The mixture was placed in a preheated block digester at 145 °C-155 °C and digested for 30 min. After cooling to room temperature, the digest was transferred quantitatively into a 100 mL conical flask, and 0.3 mL of ferroin indicator solution was added. The solution was then titrated against 0.2 M ferrous ammonium sulfate solution while stirring with a magnetic stirrer to ensure proper mixing. The endpoint was determined by a colour change from greenish to brown, and the titration volume was recorded.

The percentage of organic carbon was calculated based on the amount of potassium dichromate reduced by the organic matter in the soil. The formula used for this calculation is:

Total organic carbon (%) = (Volume of dichromate added – Volume of ferrous ammonium sulfate used) × Normality of ferrous ammonium sulfate × 0.003 × 100/Mass of soil sample

A correction factor of 1.33 was applied to account for incomplete oxidation, ensuring an accurate estimation of the total organic carbon content.

2.3.1.3. Moisture content determination

Moisture content was measured using the oven-drying method. Ten grams of fresh soil were weighed into a pre-weighed crucible and placed in an oven set at 105 °C for 24 h. The sample was removed, cooled in a desiccator, and weighed again. The percentage of moisture content was calculated as:

Moisture content (%) = (Initial weight of the soil – Dry weight of the soil) × 100/Initial weight of the soil

2.3.1.4 Macronutrient extraction and analysis

Macronutrients were extracted using the ammonium acetate extraction method. Five grams of soil were mixed with 50 mL of 1 N ammonium acetate (NH_4CH_3COO) solution in a shaker for 30 minutes. The mixture was filtered through Whatman No. 42 filter paper, and the filtrate was analyzed for macronutrient content using a Buck Scientific Atomic Absorption Spectrophotometer (Model 210/211 VGP).

2.3.1.5 Total nitrogen determination

Total nitrogen was determined using the Kjeldahl Distillation method. A sample of 1 g of soil was placed in a digestion flask, and 10 mL of concentrated sulfuric acid (H_2SO_4) along with a catalyst mixture (potassium sulfate and copper sulfate) was added. The sample was heated at 400 °C in a digestion block until the solution turned clear. After cooling, 50 mL of distilled water was added, and the solution was transferred to a distillation unit where 40% sodium hydroxide (NaOH) solution was introduced. The released ammonia

was absorbed in boric acid solution and titrated against 0.02 N hydrochloric acid (HCl) until a pink endpoint was reached. The nitrogen content was calculated based on the volume of acid used.

2.3.2. Isolation of bacterial isolates

Microbiological analysis of the obtained soil samples was done using the pour plate method. A stock solution was prepared for serial dilution by dispensing ten grams of the soil sample into 100 mL of distilled water and shaken thoroughly. One milliliter of the solution was transferred aseptically into Petri dishes containing nutrient agar, eosin methylene blue agar and MacConkey agar and gently swirled. The Petri plates were left to sit at room temperature, inverted and incubated at 37 °C for 18-24 h. After the period of incubation, presumptive Gram-negative bacteria colonies were selected. All distinct colonies were subcultured to obtain pure cultures[28]. The Gram-negative isolates were identified using conventional methods[29].

2.3.3. Determination of heavy metal–tolerant bacteria

The tolerance of the isolates to Cd, Pb, Cr, Hg, and Cu was determined by the agar dilution method[30], which were added in the form of cadmium sulphate, lead acetate, chromium sulphate, mercuric chloride and copper (II) sulphate salts, respectively. The initial concentration of these metal salts in nutrient agar plates was 50 µg/mL, and bacterial growth was observed by streaking on the different plates. The metal concentrations were incrementally increased by 50 µg/mL on fresh agar plates, and the Minimum Inhibitory Concentration (MIC) was recorded as the lowest concentration at which the isolates failed to grow.

2.3.4. Antibiotic susceptibility testing of the isolates

The antibiotic susceptibility profile of isolates that tolerated up to three heavy metals was assessed using the Kirby-Bauer disc diffusion method[31]. Standardized volumes of the isolates were swabbed onto freshly prepared Mueller-Hinton agar. Antibiotic discs containing tetracycline (10 µg), cotrimoxazole (25 µg), gentamicin (10 µg), cefuroxime (30 µg), chloramphenicol (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), ceftazidime (30 µg), and meropenem (10 µg) were aseptically placed on the inoculated media. The Petri dishes were incubated at 37 °C for 24 h, after which the diameters of inhibition zones were measured and interpreted according to standard guidelines[32, 33].

2.4. Molecular identification of isolates

Two isolates that demonstrated tolerance to four heavy metals and resistance to all 11 antibiotics were selected for further analysis of their molecular identity using 16S rRNA sequencing. The polymerase chain reaction (PCR) mixture consisted of 10 µL of 5× GoTaq colorless reaction, 3 µL of 25 mM $MgCl_2$, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each of the 27F (5'- AGA GTT TGA TCM TGG CTC AG-3') and 1525R (5′-AAGGAGGTGATCCAGCC-3′) primers, and 0.3 units of Taq DNA polymerase (Promega, USA), made up to 42 µL with sterile distilled water. A 8 µL DNA template was added to complete the reaction mix[34]. The DNA of the isolates was extracted using the protocol[35]. The integrity of the amplified 1.5 kb gene fragment was verified on a 1% agarose gel ran to confirm application. The amplified fragments were sequenced using a Genetic Analyzer 3130 xl sequencer (Applied Biosystems), according to the manufacturers' instructions, with the BigDye Terminator v3.1 Cycle Sequencing Kit. All genetic analysis were performed using Bio Edit software and MEGA 6 software.

2.5. Ethical statement

Ethical approval for the study was waived because it did not involve any human subjects and vertebrate animals. Sampling of the environmental materials was conducted in accordance with institutional guidelines and local environmental regulations.

Table 1. Physicochemical parameters of the dumpsite soil samples.

Samples	Wet Season			Mean±SD	Dry Season			Mean±SD
	1	2	3		4	5	6	
Moisture content (%)	10.00	15.20	14.60	13.367±2.182	9.70	10.00	10.20	9.967±0.205
pH	7.64	8.01	8.87	8.173±0.515	9.29	8.02	8.15	8.487±0.571
Organic carbon (%)	11.18	12.04	9.63	10.950±1.000	6.08	8.96	7.52	7.520±1.176
Organic matter (%)	19.27	20.76	16.61	18.880±1.717	10.48	15.45	12.47	12.800±2.042
Total Nitrogen (%)	1.06	1.31	1.10	1.157±0.110	0.67	0.99	0.83	0.830±0.131
Total phosphate (%)	0.15	0.20	0.16	0.169±0.020	0.01	0.03	0.03	0.004±0.002
Calcium (%)	0.40	0.45	0.46	0.437±0.029	0.71	1.43	1.53	1.221±0.365
Magnesium (%)	0.13	0.11	0.13	0.123±0.010	1.12	0.31	0.40	0.609±0.363
Potassium (%)	0.19	0.13	0.17	0.161±0.025	1.92	0.34	0.45	0.904±0.718
Sodium (%)	0.02	0.03	0.03	0.025±0.002	0.72	0.35	0.48	0.517±0.153

3. Results

The physicochemical analysis of the samples is presented in Table 1. The pH values ranged from 7.64 to 9.29, and was highest in sample 4, which was collected during the dry season. This pH range falls within the slightly alkaline to strongly alkaline category, suggesting potential contamination from various waste sources at the dumpsite. Given that the site receives industrial, agricultural, domestic, and medical waste, alkaline substances such as ash, cement, lime-based fertilizers, pharmaceutical residues, and ammonia from decomposing organic matter may contribute to elevated pH levels. These alkaline conditions can significantly impact soil chemistry, particularly the mobility and bioavailability of metals. Seasonal variations also played a role, as the highest pH was recorded during the dry season, when reduced rainfall and moisture content may have led to the accumulation and concentration of alkaline substances in the soil. The organic matter ranged from 10.48%-20.76% with sample 2 (wet season) having the highest value. The moisture content was highest (15.20%) in sample 2, obtained during the wet season, and the least (9.70%) in sample 4, obtained during the dry season.

The heavy metal composition of the soil samples obtained in this study is shown in Table 2. The Cd concentration in wet-season samples (3.17 mg/kg and 12.67 mg/kg) and Cr in a dry-season sample (109.50 mg/kg) were above the permissible limit set by World Health Organization (as cited by Osmani, *et al*)[36] and National Environmental Standards and Regulation Enforcement Agency (NESREA)[37]. Some of the samples had no trace of Cd or Pb. The Total Heterotrophic Bacteria Count (THBC) of the isolates from the dry season were comparatively higher (1.1×10^7-1.1×10^8 CFU/g) when compared to the samples from the wet season (7.1×10^6 - 2.5×10^7 CFU/g) (Table 3).

Out of 79 isolates obtained, 31 were observed to tolerate up to three heavy metals. The 10 most predominant bacterial species are *Pseudomonas* spp., *Aeromonas* spp., *Proteus vulgaris*, *Morganella morganii*, *Klebsiella oxytoca*, *Proteus* spp., *Serratia liquefaciens*, *Enterobacter* sp., *Citrobacter* sp. and *Erwinia* sp. But two isolates, *Pseudomonas aeruginosa* (S70) and *Aeromonas hydrophila* (S43), tolerated the highest number of metals (four metals). *Pseudomonas aeruginosa* exhibits multiple resistance mechanisms that contribute to its tolerance to heavy metals and antibiotics. This resistance can be attributed to a co-regulation mechanism between antibiotic influx and heavy metal efflux. *Pseudomonas* are also known to harbour metal resistance mechanisms such as enzyme production, loss of outer membrane proteins, target mutations and mutlidrug efflux systems.

The isolates obtained during the wet season had higher Minimum Inhibitory Concentrations (MICs) (Pb at 10350 µg/mL; Cu at 750 µg/mL; Cr at 750 µg/mL) in comparison with those obtained during the dry season (Pb at 2350 µg/mL; Cu at 450 µg/mL; Cr at 300 µg/mL).

The antibiotic resistance profiles of the 31 isolates showed that all the isolates were resistant to cefuroxime and meropenem (100%), while chloramphenicol had the lowest (51.6%) resistance pattern (Table 4).

The molecular identities of the two isolates that tolerated up to four heavy metals, as determined by 16S rRNA gene sequence analysis is shown in Table 5. The identities of the isolates were confirmed to be *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. The phylogenetic tree inferring the degree of relatedness between the 16S rDNA sequence of the isolates with other closely related 16S rDNA sequences is presented in Figure 1.

Table 2. Comparison of heavy metal composition in the soil samples.

Soil samples	Wet season			Dry season			Permissible limit	Permissible limit
	1	2	3	4	5	6	WHO[36]	NESREA[37]
Cd (mg/kg)	0	3.17	12.67	0	0	0	0.8	3
Pb (mg/kg)	0	18.25	0	0	0	0	85	164
Cu (mg/kg)	3.03	1.02	0.53	1.01	2.77	6.03	36	100
Cr (mg/kg)	67.16	41	43.83	109.50	53	63.25	100	100
Hg (mg/kg)	0.04	0.02	0.02	0.1	0.05	0.05	-	4

Table 3. Total heterotrophic bacteria count of the isolates in the soil samples.

Season	Sample	THC (CFU/g)	Log_{10} (CFU/g)	Mean ± SD
Wet	1	7.1×10^6	6.9	7.06±0.24
	2	8.6×10^6	6.9	
	3	2.5×10^7	7.4	
Dry	4	1.1×10^8	8.0	7.53±0.40
	5	1.1×10^7	7.0	
	6	3.3×10^7	7.5	

Table 4. Antibiotics susceptibility profile of the bacterial isolates (n=31).

Antibiotics	Resistant (%)	Intermediate (%)	Susceptible (%)
Tetracycline	28 (90.3)	2 (6.5)	1 (3.2)
Cotrimoxazole	25 (80.6)	4 (12.9)	2 (6.5)
Gentamicin	29 (93.5)	0 (0.0)	2 (6.5)
Cefuroxime	31 (100)	0 (0.0)	0 (0.0)
Chloramphenicol	16 (51.6)	5 (16.2)	10 (32.3)
Ceftriaxone	26 (83.9)	4 (12.9)	1 (3.2)
Cefotaxime	29 (93.5)	0 (0.0)	2 (6.5)
Ciprofloxacin	22 (71.0)	3 (9.7)	6 (19.4)
Amikacin	27 (87.1)	1 (3.2)	3 (9.7)
Ceftazidime	30 (96.8)	1 (3.2)	0 (0.0)
Meropenem	31 (100)	0 (0.0)	0 (0.0)

4. Discussion

The moisture content levels, organic carbon, organic matter, and nitrogen were significantly lower in samples collected during the dry season due to lower rainfall and humidity, and higher atmospheric temperature at the time of sampling. According to World Weather and Climate Information (2024)[38], weather conditions varied between the two sampling periods. In August (wet season), when the first set of samples was collected, the average precipitation was 201 mm, with a maximum temperature of 29 °C and humidity at 88%. In November (dry season), when the second set of samples

Table 5. Molecular identities of selected isolates.

Sample code	Scientific name	Max score	Total score	Query cover (%)	E value	Percentage identity (%)	Accession
S43	*Aeromonas hydrophila*	2172	2172	99	0	99.58	PP759396
S70	*Pseudomonas aeruginosa*	2185	2185	99	0	99.67	PP759394

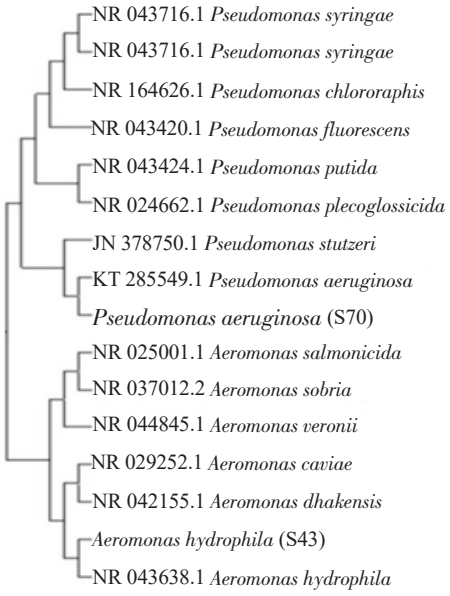


Figure 1. Neighbour-joining tree showing phylogenetic relationships of *Aeromonas hydrophila* (S43) and *Pseudomonas aeruginosa* (S70) with related species.

was collected, the average precipitation dropped to 43 mm, while the maximum temperature increased to 33 °C, and humidity decreased to 80%. These variations significantly influenced the distribution and activity of the microbial community. Higher temperature is associated with increased bacterial growth rates and horizontal gene transfer which are factors contributing to antibiotic resistance and heavy metal tolerance[39].

The permissible limit of Cd in the soil samples in this study exceeded the acceptable limit recommended by WHO and NESREA. The extreme values can be attributed to the disposal of domestic, commercial, and industrial garbage, which often contains cadmium-containing materials such as batteries, pigments, and alloys[40, 41]. The Cr level found in one of the soil samples was also above the permissible limit, possibly due to the presence of waste materials from automobiles, electronic waste, polythene, and plastic materials[42].

Dumpsites are usually rich in solid and liquid organic wastes[43]. It is, therefore, not surprising that the soil samples were abundant in bacteria, with a THBC ranging from 1.08×10^8 to 7.1×10^6 CFU/g. The high bacterial counts obtained in this study are similar to those obtained in Nairobi, Kenya by Waturu *et al*.[44], who studied the antimicrobial resistance encountered in garbage collection areas and dumpsites in Nairobi and obtained a THBC that ranged from 1.11×10^{11} to 8.2×10^{10} CFU/g.

The MICs of metals were higher in the isolates obtained during the wet season (Pb at 10 350 µg/mL; Cu at 750 µg/mL; Cr at750 µg/mL) compared to those obtained during the dry season (Pb at 2350 µg/mL; Cu at 450 µg/mL; Cr at 300 µg/mL). The observed significant differences in MICs between the seasons can be attributed to variations in environmental conditions that influence microbial growth. The isolates demonstrated greater tolerance during the wet season, likely due to favourable conditions such as higher soil moisture, organic matter, organic carbon, and nitrogen levels, which support microbial activity. In contrast, reduced soil moisture during the dry season lowers microbial activity[45]. Rainfall distribution plays a key role by altering soil water and nutrient availability, with water shortages proving detrimental to the survival of soil microorganisms. This was proven in a study by Umair *et al*.[46] who showed that microbial communities that live in dry soils become active and successively shift their compositions during the wet season. In their study, the average relative abundance of some bacterial groups was considerably higher during the wet season compared to the dry season. Li *et al*.[47] also confirmed how precipitation influences soil microbial communities due to its impact on soil water availability. The amount of precipitation also indirectly affects plant productivity, carbon allocation, and litter input, all of which play crucial roles in shaping soil microbial populations. The high tolerance of the isolates to heavy metals can be attributed to their exposure to the soil, as the metal concentrations in some of the soil samples exceeded the permissible limits set by WHO and

NESREA. This aligns with Ibrahim *et al*.[48] who pointed out that microorganisms develop a tolerance to pollutants when regularly exposed to them. Some metal ions are useful to bacteria in small amounts because they can serve as cofactors for enzymes involved in metabolic processes, among other benefits[49]. However, when they exceed a certain threshold, they become toxic, while some heavy metals, such as lead and mercury, are very harmful to microorganisms even at very low concentrations[50]. The heavy metals selected for this study were lead, mercury, chromium, cadmium, and copper, as they are among the most common heavy metals that induce human poisoning[50,51]. The isolates in this study showed tolerance to all these metals in varying concentrations. The threshold for toxicity varies depending on the specific heavy metal and the bacterial species involved.

The identification of multiple antibiotic-resistant bacterial isolates from the sampled dumpsite underscores the role of such environments as reservoirs for antimicrobial-resistant microorganisms, as highlighted by previous studies[52]. The high resistance levels observed in most isolates to the antibiotics tested align with the growing global issue of MDR in bacteria. This finding emphasizes the significant public health threat posed by these organisms. Indeed, bacterial AMR is a major global health problem. It poses the largest threat to human health, underscoring the urgency of addressing such reservoirs of resistance[10]. Gram-negative bacteria are among the top antibiotic-resistant pathogens emphasized by the WHO, posing a serious threat to human health. Just like the findings of Parmanik[53] who recorded that the critical group of multi-drug-resistant bacteria includes *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and Enterobacteriaceae, this study's most resistant bacteria include *Pseudomonas aeruginosa* and a member of the Enterobacteriaceae family. The isolates that tolerated the most metals and resisted multiple antibiotics were identified as *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. This finding is also consistent with the study of Nguyen *et al*.[54], who identified *Pseudomonas aeruginosa* as having resistance to several heavy metals and antibiotic classes and Joseph *et al*.[55] who identified members of the Enterobacteriaceae.

Cefuroxime and meropenem exhibited the highest (100%) resistance pattern, although this is unconventional because resistance to meropenem is uncommon in most bacteria. Meropenem has a broad spectrum of activity against most common Intensive Care Unit (ICU) pathogens in *in vitro* studies and, in particular, was more active than most other agents (including imipenem) against Enterobacteriaceae[56]. Meropenem is also highly resistant to hydrolysis by most of the serine-based β-lactamases produced by Gram-negative bacteria. Despite the proven efficacy of meropenem, the 100% resistance observed in this study is similar to the findings of Adesoji *et al*.[57] and Hassuna *et al*.[58], who recorded a 100% and 96.5% resistance of *Pseudomonas aeruginosa* to cefuroxime and meropenem respectively. The high resistance to both antibiotics in this study contrasts the findings of Sampah *et al*.[59] who recorded lower resistance levels: 58.3% resistance to cefuroxime and 2.6% to meropenem. Chloramphenicol had the least resistance, and this is linked to its broad-spectrum antibiotic properties and limited use, reducing the likelihood of resistance development[60].

The high antibiotic resistance pattern of the isolates to the antibiotics used can be attributed to the fact that the study dumpsite, being a significant city dumpsite, has received various waste materials, including some from hospitals and pharmacies. Hospital effluents are a significant source of antibiotics, containing a complex mixture of pharmaceuticals, illegal drugs, their metabolites, and various susceptible and antibiotic-resistant microorganisms. Such a mixture can increase selective pressure on bacteria, thereby contributing to the development and dissemination of antimicrobial resistance[61]. This aligns with the findings of Larsson and Flach[62], who pointed out that prolonged exposure to antibiotics results in the selection for antibiotic resistance.

According to Nguyen *et al*.[54] and Zhu *et al*.[63], the presence of antibiotics or heavy metals in an environment can select for tolerance to both. Therefore, there is a correlation between metal tolerance and antibiotic resistance in bacteria because there is a likelihood that the plasmid encoding for heavy metal tolerance may also encode for antibiotic resistance.

The presence of multiple antibiotic resistant bacteria in this study is a concerning threat as it may pose risks to humans when it enters the food chain and exacerbate the health conditions of individuals already suffering from superficial infections.

5. Conclusions

This study highlights the potential health risks associated with using dumpsite soils for growing vegetables without proper treatment. The presence of pathogens and heavy metals in these soils suggests a risk of contamination, which could impact food safety and human health. While this study did not directly assess contaminant transfer to crops, previous research indicates that such transfer is possible, particularly for vegetables consumed with minimal processing.

To mitigate these risks, dumpsite soils intended for agricultural use should be managed through appropriate control measures, including the application of mulch, capping with clean soil, and the establishment of vegetative covers. Soil amendments and bioremediation strategies should also be explored to reduce contaminant levels and improve soil quality.

Future research should focus on directly assessing the transfer of heavy metals and microbial contaminants from soil to crops under different environmental conditions. Further studies should also investigate the long-term impact of dumpsite contamination on agricultural productivity and determine the optimal distance between dumpsites and farmlands to minimize the risk of contaminants entering the food chain.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Nwadike BI and Barwa J conceptualized the study and designed the experimental frame work. Barwa J performed the laboratory experiments and Nwadike BI supervised data collection and analysis. Barwa J was involved in preliminary draft and Nwadike BI in critical review of manuscript. Nwadike BI and Barwa J read, revised and approved the final manuscript for publication.

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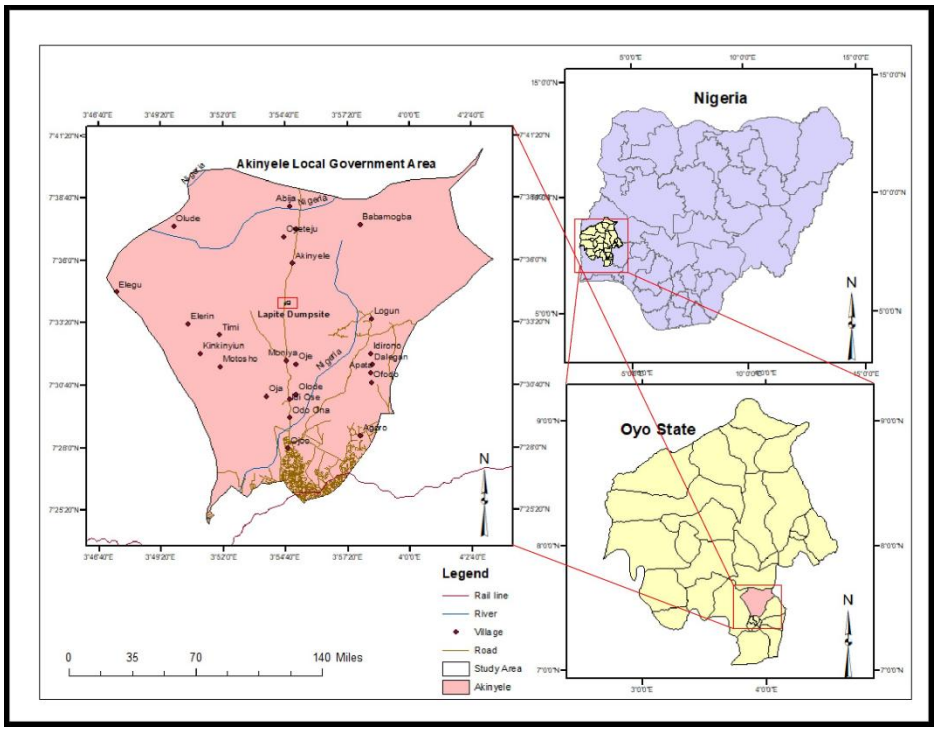
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Supplementary Figure 1. The location of Lapite dumpsite.



Supplementary Figure 2. Food crops grown around Lapite dumpsite.