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Prevalence of Antibiotic Resistance *Bacillus* and *Klebsiella* Species from Leachate in Dumpsites around Rivers State University, Port Harcourt

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ABSTRACT

Leachates from waste dumpsites are due to the decomposition of waste, it produces pungent odors and are recognized as important sources of environmental pollution and may serve as reservoirs for pathogenic microorganisms capable of contaminating surrounding soil and water resources. This study investigated the prevalence and antibiogram of *Bacillus* and *Klebsiella* species isolated from leachate around dumpsites in Rivers State University, Port Harcourt, Nigeria. Leachate samples were collected from five locations: Road A, Sciences, 7 Days Adventist, and Deeper Life (in RSU) and Mile 3 market (close to RSU). Bacteriological characteristics, antibiotic susceptibility patterns, and resistance genes were determined using standard microbiological, and molecular procedures. Total Coliform Count, and Fecal Coliform Counts ranged from 1.42–2.29 × 10^{10}, 1.16–1.57 × 10^{8}, and 3.2–6.9 × 10^{7} CFU/mL, respectively. *Bacillus* prevalence was overall 87.5%, while *Klebsiella* was over 82.5%. Both *Bacillus* and *Klebsiella* species exhibited considerable resistance to ceftazidime, cefuroxime, cloxacillin, and Augmentin, whereas 100% susceptibility to ofloxacin was recorded. Molecular analysis identified *Klebsiella ornithinolytica*, *K. oxytoca*, *K. pneumoniae*, *Bacillus cereus*, *B. mycoides*, and *B. subtilis*. The findings in this study revealed that dumpsite leachate may provide an environmental reservoir for antibiotic-resistant bacteria however the application of ofloxacin and cefuroxime could be adopted in the treatment as they were resulted in 100% susceptibility.

Keywords: Waste Dumpsites, Leachate, Antibiotic Resistance, Antibiogram

Introduction

Leachate generated by indiscriminate waste disposal and may contain elevated concentrations of nutrients, organic matter, heavy metals, and gases capable of contaminating surrounding soil and groundwater systems. It is also characterized the production of organic acids and temperature above 70-80 ºC with pungent odour which propels the pollutant fast through the soil into the aquifers to cause pollution of water resources (Ogbonna et al., 2010; Okpara et al., 2021; Olagbemiro et al., 2025). Leachate could be defined as a mixture of percolated rainwater, water produced by the biodegradation of waste, and the inherent water in waste, which contains large amounts of dissolved organic matter (DOM), salts, heavy metal ions, and other organic compounds (e.g., chlorinated aliphatics and pesticides) (Liu et al. 2015).

Landfill leachate has been generally found toxic, presenting potential threats to the surrounding environment and ecosystems (Baderna et al. 2019). Leachate DOM can affect microbial activity, because membrane fouling, decrease the effluent quality of the coagulation process, interact with organic contaminants, and change the fate of heavy metals in their transport behavior, stability, and bioavailability (Bouaouda et al., 2023).

Municipal solid waste dumpsites receive a wide variety of wastes generated from domestic, commercial, industrial, agricultural, and healthcare activities. In many developing countries, including Nigeria, waste management systems remain inadequate due to rapid urbanization, population growth, insufficient infrastructure, limited financial resources, and weak regulatory enforcement.

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Consequently, open dumping remains a common waste disposal practice despite its associated environmental and public health consequences (Bandala et al., 2021).

Dumpsites typically contain heterogeneous mixtures of biodegradable and non-biodegradable materials, including food wastes, plastics, electronic wastes, industrial residues, animal wastes, hospital wastes, sewage sludge, pharmaceutical products, and chemical contaminants (Mondragón-Quiguanas *et al.,* 2022) and their composition depend on waste characteristics such as landfill age, climatic conditions, microbial activity, moisture content, temperature, and management practices (Bhalla *et al.,* 2012). Studies have reported the isolation of several bacterial genera including *Bacillus* and *Klebsiella* are commonly isolated from landfill leachates and dumpsite environments in which they demonstrated varying degrees of antibiotic resistance (Remmas *et al.,* 2023).

Antimicrobial resistance occurs when microorganisms develop the ability to survive exposure to antimicrobial agents that were previously effective against them and resistant bacterial infections are associated with increased morbidity, mortality, prolonged hospitalization, treatment failure, and escalating healthcare costs (Adegoke et al*.,* 2022). Environmental matrices such as soil, wastewater, sewage systems, rivers, groundwater, agricultural lands, sediments, and landfill environments serve as reservoirs where resistance genes can persist and spread among microbial populations. Furthermore, previous studies have demonstrated that environmental ecosystems play a critical role in the emergence, maintenance, and dissemination of resistant microorganisms and antibiotic resistance genes (ARGs) (Larsson & Flach, 2022).

Previous findings in Nigeria have isolated antibiotic-resistant *Bacillus* and *Klebsiella* species from dumpsite soils (Odum *et al.* (2020) and have also reported antibiotic contamination in waste-associated environmental matrices (Ajibola et al., 2022). These findings highlight the potential role of waste disposal environments in the dissemination of resistant microorganisms nevertheless, there is paucity of information regarding the prevalence and antibiotic susceptibility patterns of *Bacillus* and *Klebsiella* species in dumpsite leachates within Rivers State hence the need for this study.

Materials and Methods

Study Area

The study was conducted around the main campus of Rivers State University, Port Harcourt, Nigeria. It is situated within the Niger Delta region, approximately between latitude 4°47′N and 4°52′N and longitude 6°55′E and 7°03′E. Several active and abandoned dumpsites are located within and around the university. These dumpsites receive waste from institutions, residential and commercial centers. The continuous accumulation and decomposition of organic waste materials, combined with rainfall infiltration, result in the formation of leachate (Olagbemiro et al., 2025). Figure 1 shows the map of the study area around Rivers State University.

Sample Collection

A total of forty (40) Leachate samples were collected from five selected dumpsites namely Road A, Mile 3, Deeper life, Science and Seventh Day Adventist around Rivers State University following standard environmental sampling procedures described by APHA (2023). Prior to collection, sampling containers were appropriately labeled with the sampling location, sample identification code, date, and time of collection. Appropriate personal protective equipment, including hand gloves and protective clothing, was worn during sample collection, the samples were collected using 750ml sterile plastic container placed in an ice chest containing ice packs and transported to the Microbiology laboratory of Rivers State University for analysis within 2 hours of collection.

Bacteriological Analyses of the Water Samples

The bacterial population of the leachate samples from the five locations was enumerated using standard microbiological methods as described by Prescott *et al.* (2011) and adopted Ogbonna *et al.,* 2021. All glassware used during the course of this study was carefully washed with detergent and rinsed with water. Culture media (nutrient agar and MacConkey agar) prepared to manufactures specification were sterilized in an autoclave at 121^0C at 15 psi for 15 minutes. Normal saline was used as diluent at a concentration of 0.85% (0.85g in 100ml), sodium chloride (Cheesbrough, 2006).

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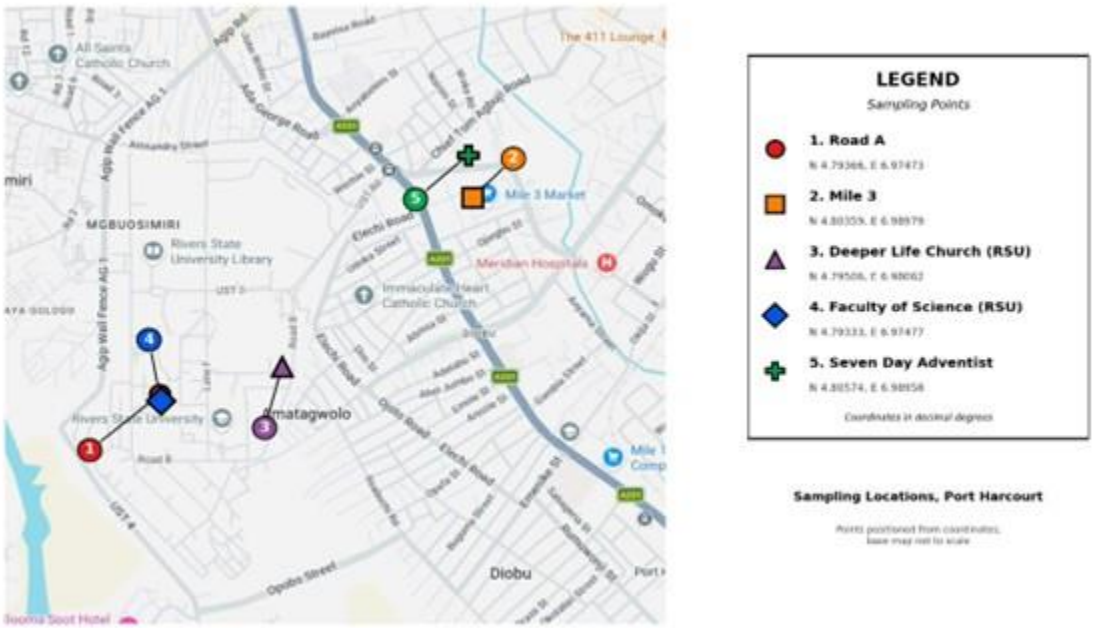


Figure 1: Map of the Study Area Showing the Waste Dump Sites

Microbial Enumeration, Purification and Maintenance of isolates

Adopting the method by Popoola et al. (2024), the total heterotrophic bacterial count (THBC) and total coliform count (TCC) were determined by counting the colonial growth on the cultured plates and the cfu/ml (colony forming unit) were determined. The different isolates of the cultures were purified by subculturing on the freshly prepared nutrient agar plates based on their different cultural morphological characteristics. After the growth of the purified isolates, they were maintained on the slant media in the bijou bottles and stored at lower temperature 4 °C for further analyses.

Identification of Bacterial Isolates

Pure bacterial isolates were identified using standard microbiological methods described by Cheesbrough (2006). The isolates were subjected to a series of biochemical tests, including catalase, oxidase, indole, methyl red, Voges–Proskauer, citrate utilization, sugar fermentation, and triple sugar iron agar (TSI) tests. The results obtained from the biochemical tests were used for the identification of the bacterial isolates. The identities of the isolates were confirmed by reference to *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994).

Antibiotic Susceptibility Testing

In vitro sensitivity pattern of the identified isolates of *Bacillus* and *Klebsiella* species were studied by Kirby-Bauer disc diffusion method using numbers of antibiotic discs. The inoculums were prepared by transferring colonies from the pure culture to broth (normal saline) and matched with 0.5 McFarland (containing approximately, 3.0×10^{8}CFU/ml of cells). The standardized inoculums were then inoculated onto Mueller Hinton (MHA) agar plate by soaking with sterile swap stick. The discs were placed aseptically on the surface of the agar plate. The plates were incubated at 37ºC for 24hours for development of inhibition zone. The diameters of zone of inhibition were measured and the interpretation was carried out according to Clinical and Laboratory Standards Institute chart (Zimmermann et al., 2012).

Molecular Techniques

Extraction of DNA

DNA extraction is a phenomenon by which DNA is separated from proteins, membranes and other cellular materials contained in the cell. Boiling method was used for the extraction process. A 24 hours old pure culture of the isolates was put in Luria-Bertani (LB) Broth and incubated at 37ºC.

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About 0.5ml of an overnight broth culture of the isolates in Luria Bertani (LB) was put into properly labelled Eppendorf tubes and filling to mark with normal saline and was centrifuged at 14000rpm for 3 min and the supernatant was decanted leaving the DNA at the base. This process was repeated 3 times. The cells were re-suspended in 500ul of normal saline and heated at 95ºC for 20 min. The heated bacterial suspension was cooled on ice (About 10minutes) and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro-centrifuge tube and stored at -20ºC for other down-stream reactions (Bell *et al.,* 1998).

DNA Quantification

DNA quantification is a phenomenon carried out to determine the concentration of DNA as well as its purity. The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The Beer Lambert's principle which is used to evaluate the quality and quantity of the genomic DNA is used by the Nanodrop spectrophotometer. The Nanodrop spectrophotometer was connected to a computer with Nanodrop software installed. The software of the equipment was lunched by double clicking on the Nanodrop icon. The sample pedestals were properly cleaned. The equipment was initialized using 2µl of sterile distilled water and blanked using 2µl of Normal saline. About 2µl of the extracted DNA of the individual isolates of fungi and bacteria was loaded onto the lower pedestal to measure the concentration of the sample, and the upper pedestal was brought down to make contact with the DNA on the lower pedestal. Then, DNA concentration was measured by clicking the "measure" button displayed on the computer screen (Olsen and Morrow, 2012).

16S rRNA Amplification: The 16srRNA Amplification was carried out using an ABI 9700 Applied Biosystems Thermal Cycler and method described by Srinivasan *et al*. (2015). The 16s rRNA region of the rRNA gene of the bacterial isolates were amplified using the forward primer; 27F:5'-AGAGTTTGATCMTGGCTCAG-3' and Reverse primer; 1492R:5'-CGGTTACCTTGTTACGACTT-3'primers at a final volume of 50 micro litres for 35 cycles. The PCR mix includes: (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template, Buffer 1X and water.

The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light trans-illuminator for a 1500bp amplicons (Srinivasan *et al*., 2015).

DNA Sequencing

Sequencing of the amplified product was carried out using the Big-Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ulBigDye® terminator v1.1/v3.1, 2.25ul of 5 x Big Dye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows; 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4minutes (Srinivasan *et al*., 2015).

Phylogenetic Analysis

Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN prior to the edition of the obtained sequences using the bioinformatics algorithm Trace edit. MAFFT were used to align these sequences. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969).

Results

Table 1 presents the mean bacterial populations of the leachate samples in terms of total heterotrophic bacteria (THB), total coliform counts (TCC) and faecal coliform counts (FCC). The total heterotrophic bacterial population ranged from $1.42 \pm 0.85 \times 10^{10}$ CFU/mL at Deeper Life to $2.29 \pm 0.22 \times 10^{10}$ CFU/mL at 7 Days Adventist. The overall difference in total heterotrophic bacterial counts among the locations was statistically significant ($P = 0.05$). Total coliform counts ranged from $1.16 \pm 0.36 \times 10^{8}$ CFU/mL at 7 Days Adventist to $1.57 \pm 0.10 \times 10^{8}$ CFU/mL at Mile 3.

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However, the variation in total coliform counts among the locations was not statistically significant (P = 0.05). Faecal coliform counts ranged from 3.2 ± 0.4 × 10^7 CFU/mL at Mile 3 to 6.9 ± 0.65 × 10^7 CFU/mL at 7 Days Adventist.

Despite the observed variation, there was no statistically significant difference in faecal coliform counts among the study locations (P = 0.05). The results generally indicate a high bacterial population in the leachate samples across all sampled locations.

Table 1: Bacterial Population (Mean ± SD) of Leachate from Various Locations around Rivers State University

Location	Bacterial Population (CFU/ml) of Leachate		
	Total Heterotrophic Bacteria ($\times10^{10}$)	Total Coliform ($\times10^8$)	Faecal Coliform ($\times10^7$)
Road A	1.52 ± 0.85[a]	1.36±0.10 [a]	64±0.70 [a]
Sciences	1.78 ± 0.36 [a]	128±0.70 [a]	6.2±0.85 [a]
Mile 3	1.79 ± 0.57 [a]	1.57±0.10 [a]	3.2±0.4 [a]
7 Days Adventist	2.29 ± 0.22[ab]	1.16±0.36 [a]	6.9±0.65 [a]
Deeper life	1.42 ± 0.85 [a]	1.43±0.85 [a]	5.5±0.74 [a]
P. Value	0.007	0.523	0.331

Note: *Means with similar superscripts within the group showed no significant difference (P>0.05).

The prevalence of *Bacillus* and *Klebsiella* species across the study locations is presented in Table 2.

Table 2: Prevalence of *Bacillus* and *Klebsiella* Species across the Study Locations

Location	No. of Samples	Prevalence	
		Bacillus **Positive n (%)**	***Klebsiella*** **Positive n (%)**
Road A	8	8 (100.0)	8 (100.0)
Sciences	8	8 (100.0)	8 (100.0)
Mile 3	8	6 (75.0)	5 (62.5)
7 Days Adventist	8	8 (100.0)	8 (100.0)
Deeper Life	8	5 (62.5)	4 (50.0)
Total	40	35 (87.5)	33(82.5)

A total of 40 samples were examined, comprising eight samples from each location. *Bacillus* species were detected in all samples from Road A, Sciences, and 7 Days Adventist, representing a prevalence of 100.0% at each location. Mile 3 recorded a prevalence of 75.0%, while Deeper Life recorded the lowest prevalence of 62.5%.

Overall, *Bacillus* species were isolated in 35 of the 40 samples, representing an overall prevalence of 87.5%. Similarly, *Klebsiella* species were isolated in all samples from Road A, Sciences and 7 Days Adventist, representing 100.0% prevalence at each location. Mile 3 recorded a prevalence of 62.5%, whereas Deeper Life recorded the lowest prevalence of 50.0%. Overall, *Klebsiella* species were detected in 33 out of the 40 samples, representing an overall prevalence of 82.5% (Fig. 2).

Table 3 presents the antibiotic sensitivity pattern of *Klebsiella* spp. isolated from leachate samples collected from Road A, Science, Mile 3, 7 Days Adventist and Deeper Life locations. The isolates demonstrated a high level of resistance to several antibiotics. All isolates were completely susceptible (100%) to ofloxacin (OFL), indicating its high effectiveness against the *Klebsiella* isolates. Gentamicin (GEN) showed relatively high activity, with susceptibility ranging from 75% to 100% across the locations. Augmentin (AUG) showed complete resistance in most locations, while ceftriaxone (CPR) demonstrated variable activity, with susceptibility ranging from 0% to 5s0%. Nitrofurantoin (NIT) was effective against isolates from Science, 7 Days Adventist and Deeper Life but showed complete resistance among Road A isolates. Overall, the results indicate multidrug resistance among the *Klebsiella* isolates.

Table 4 shows the antibiotic sensitivity pattern of *Bacillus* spp. isolated from the various leachate sampling locations. All isolates exhibited complete resistance to cefuroxime (CRX) and cloxacillin (CXM). Resistance to ceftazidime (CAZ) and AUG was also predominantly high. Conversely, all isolates showed complete susceptibility to OFL. GEN and CPR demonstrated moderate to high susceptibility, while erythromycin (ERY) showed marked variation among locations.

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Table 3: Antibiotic Sensitivity Pattern *of Klebsiella* sp. Isolated in Leachate from Various Locations

Anti-biotic	Road A Isolates (N=8)			Science Isolates (N=8)			Mile 3 Isolates (N=5)			7Days Adventist Isolates (N=8)			Deeper Life Isolates (N=4)		
	R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
CAZ	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)	7(87.5)	1(12.5)	0(0)	4(100)	0(0)	0(0)
CRX	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)	8(100)	0(0)	0(0)	4(100)	0(0)	0(0)
GEN	0(0)	0(0)	8(100)	0(0)	2(25)	6(75)	0(10)	2(40)	3(60)	0(0)	2(25)	6(75)	0(0)	0(0)	4(100)
CXM	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)	8(100)	0(0)	0(0)	4(100)	0(0)	0(0)
OFL	0(0)	0(0)	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)	8(100)	0(0)	0(0)	4(100)
AUG	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	4(80)	1(20)	0(0)	8(100)	0(0)	0(0)	4(100)	0(0)	0(0)
CPR	0(0)	0(0)	8(100)	7(87.5)	1(12.5)	0(0)	0(10)	0(0)	5(100)	0(0)	2(25)	6(75)	0(0)	1(25)	3(75)
NIT	4(50)	0(0)	4(50)	0(0)	0(0)	8(100)	0(0)	1(20)	4(80)	0(0)	0(0)	8(100)	0(0)	0(0)	4(100)

Key: CAZ= Ceftazidime (30µg) **CRX**= Cefuroxime (30µg), **GEN**= Gentamicin (10µg), **CXM** = Cloxacillin (5µg), **OFL**= Ofloxacin (5µg), **AUG**= Augmentin (30µg), **CPR**= Ceftriaxone (30µg) **NIT**= Nitrofurantoin (30µg).

Table 4: Antibiotic Sensitivity Pattern *Bacillus spp.* Isolated in Leachate from Various Locations

Anti-biotic	Road A Isolates (N=8)			Science Isolates (N=8)			Mile 3 Isolates (N=6)			7Days Adventist solates (N=8)			Deeper Life Isolates (N=5)		
	R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
CAZ	7(87.5)	1(12.5)	0(0)	8(100)	0(0)	0(0)	6(100)	0(0)	0(0)	7(87.5)	1(12.5)	0(0)	5(100)	0(0)	0(0)
CRX	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	6(100)	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)
GEN	0(0)	2(25)	6(75)	0(0)	2(25)	6(75)	0(100)	0(0)	6(100)	0(0)	2(25)	6(75)	0(0)	0(30)	5(70)
CXM	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	6(100)	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)
OFL	0(0)	0(0)	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	6(100)	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)
AUG	8(100)	0(0)	0(0)	8(100)	0(0)	0(0)	6(100)	0(0)	0(0)	8(100)	0(0)	0(0)	5(80)	0(0)	0(0)
CPR	0(0)	2(25)	6(75)	7(87.5)	1(12.5)	0(0)	0(0)	0(0)	6(100)	0(0)	2(25)	6(75)	0(0)	1(20)	4(70)
ERY	8(100)	0(0)	0(0)	6(75)	2(25)	0(0)	4(50)	0(0)	4(50)	0(0)	0(0)	8(100)	1(10)	0(0)	4(90)

KEY: CAZ= Ceftazidime (30µg) CRX= Cefuroxime (30µg), GEN= Gentamicin (10µg), CXM = Cloxacillin (5µg), OFL= Ofloxacin (5µg), AUG= Augmentin (30µg), CPR= Ceftriaxone (30µg) ERY= Erythromycin (30µg).

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Figure 2 presents the spatial variation in the antibiotic resistance pattern of *Klebsiella* spp. isolated from leachate samples collected from the different study locations. The isolates exhibited considerable variation in their resistance patterns across the sampling locations. Generally, high levels of resistance were observed against ceftazidime (CAZ), cefuroxime (CRX), cloxacillin (CXM), and Augmentin (AUG). Complete resistance to CRX and CXM was consistently observed across all locations, indicating widespread resistance to these antibiotics.

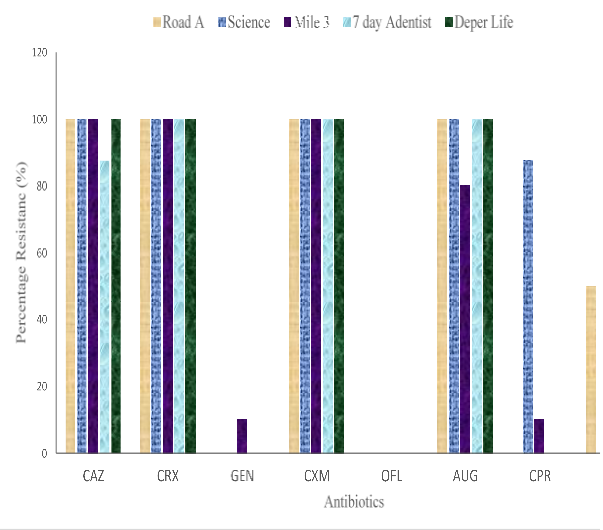


Figure 2: Spatial Variation of Resistance Pattern of *Klebsiella* Species

Resistance to CAZ was also high, although slight spatial variation was observed, particularly among isolates from 7 Days Adventist. In contrast, all *Klebsiella* isolates demonstrated complete susceptibility to ofloxacin (OFL) across the study locations. The response to gentamicin (GEN), ceftriaxone (CPR), and nitrofurantoin (NIT) varied spatially, with differences in resistance and susceptibility observed among locations.

Figure 3 illustrates the spatial variation in the antibiotic resistance pattern of *Bacillus* spp. isolated from leachate samples from the different sampling locations. The isolates generally exhibited high resistance to ceftazidime (CAZ), cefuroxime (CRX), cloxacillin (CXM), and Augmentin (AUG).

Complete resistance to CRX and CXM was consistently recorded across all locations.

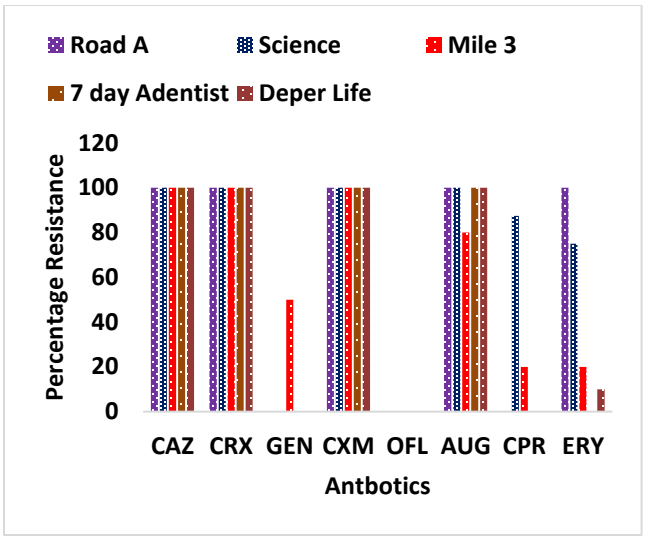


Figure 3: Spatial Variation of Resistance Pattern of *Bacillus* Species

Plate 1 presents the agarose gel electrophoresis image result showing the amplification of the 16S rRNA gene of the selected bacterial isolates.

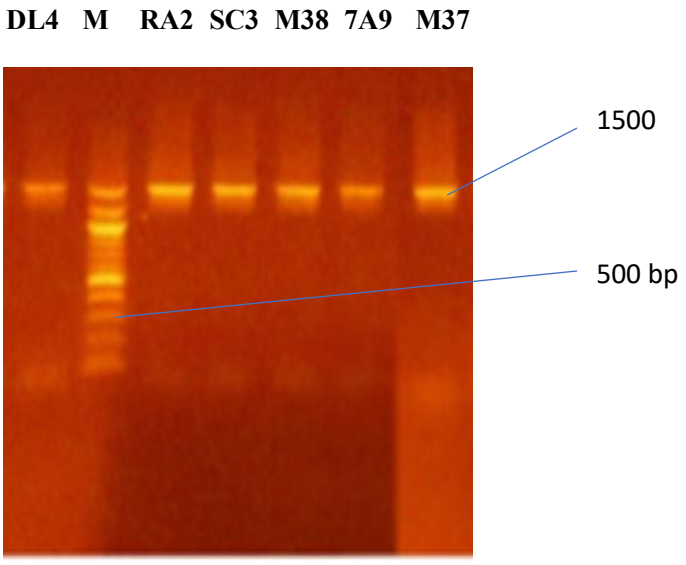


Plate 1: Agarose Gel Electrophoresis showing the Amplified 16S rRNA Gene of the bacteria Isolates at 1500bp

Distinct DNA bands were observed at approximately 1500 bp, corresponding to the expected size of the bacterial 16S rRNA gene. The molecular size markers ranging between 500 bp and 1500 bp served as references for estimating the sizes of the amplified DNA fragments.

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The successful amplification of the 16S rRNA gene confirms the presence of bacterial genomic DNA suitable for molecular identification and further genetic analysis.

Figure 4 presents the phylogenetic tree showing the evolutionary relationships among the selected bacterial isolates.

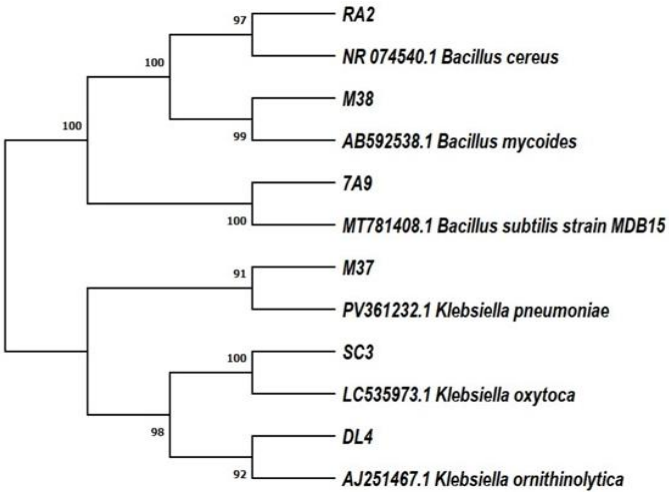


Figure 4: Phylogenetic Tree showing Evolutionary relationships of taxa

The phylogenetic analysis revealed varying degrees of genetic relatedness among the identified *Klebsiella* and *Bacillus* species. The isolates were grouped according to their genetic similarities, with closely related taxa clustering together, while genetically distinct isolates were separated into different branches.

The identified organisms included *Klebsiella ornithinolytica* (DL4), *Klebsiella oxytoca* (SC3), *Klebsiella pneumoniae* (M37), *Bacillus cereus* (RA2), *Bacillus mycoides* (M38), and *Bacillus subtilis* (7A9). The phylogenetic relationships support the molecular identification of the isolates and demonstrate the genetic diversity of bacteria associated with the leachate samples.

Discussion

The study showed a high bacterial population of leachate with high populations of total heterotrophic bacteria, total coliforms and faecal coliforms, recorded from every dumpsite sampled in this study.

Only the heterotrophic population showed a significant difference between sites, with the 7 Days Adventist dumpsite supporting the largest population, whereas coliform and faecal coliform populations were broadly similar across sites. A high heterotrophic population is expected in a warm, wet and nutrient-rich matrix that supports heterotrophic growth. The relatively similar total coliform and faecal coliform populations across the studied locations sites may indicate widespread faecal contamination and comparable opportunities for the persistence of these indicator organisms. Similar to the findings in this study, Akudo (2022), in a study of municipal solid-waste leachate from Iguomo and St. Saviour dumpsites in Benin City, also reported coliform counts. In contrast, the populations recorded in this study are considerably larger than those reported by other Nigerian researchers working on dumpsites in Akure and on dumpsites in Oredo, Benin City (Olalemi et al., 2020). The presence of coliform organisms indicates faecal and anthropogenic contamination and suggests that the leachate can serve as a reservoir and vehicle for microorganisms of public-health significance. This is particularly important where untreated leachate drains into surface water, soil or groundwater. Studies in Nigeria have demonstrated that contaminants originating from dumpsite leachate can migrate into surrounding water systems (Badmus et al., 2022).

In relation to the prevalence of *Bacillus* and *Klebsiella*, both genera were very widely distributed. *Bacillus* was isolated from most of the samples and *Klebsiella* from a slightly smaller majority. Three dumpsites yielded both genera from every sample, and the other two sites yielded lower prevalently, with the lowest prevalence at Deeper Life. The dominance of *Bacillus* is understandable as it forms endospores that survive desiccation, heat, low pH and metal stress, it produces extracellular enzymes that degrade the complex polymers in refuse, and it is ubiquitous in soil, which is the main cover material at open dumps (Akinsemolu et al., 2024).

Nigerian dumpsite studies report the same dominance. *Bacillus* and *Pseudomonas* dominated the bacteria of dumpsite soils in Benin City *Bacillus subtilis* was recovered at dumpsites in Akure and *Bacillus* and *Klebsiella* were among the airborne bacteria at refuse dumps on a university campus in Benin City (Olalemi *et al.,* 2020).

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Klebsiella is a copiotrophic, facultatively anaerobic member of the faecal and soil flora. It is capsulated, forms biofilm and is metabolically versatile, which lets it persist and multiply in nutrient-rich, low-oxygen leachate (Ogbonna *et al.,* 2023). Its high prevalence in this study agrees with the faecal indication results and with its recovery from dumpsite environments.

The notable feature is that the same three sites yielded both genera from every sample, while the remaining two sites were lower and similar to each other. Two explanations are offered, and the present data cannot separate them. The first is that the three sites with complete prevalence also carried the heaviest bacterial loads and, at least for two of them, the highest lead and copper, which is consistent with metal selection for tolerant genera. The second is that site-level factors that were not measured, such as the age and moisture of the waste, the proportion of faecal or clinical material, and the timing of sampling relative to rainfall, are responsible. Mile 3 is of particular interest because it had the greatest organic and ammonia load yet lower prevalence of both genera. High free ammonia can inhibit many bacteria, and intense competition in a very nutrient-rich matrix may also reduce recovery. These are hypotheses and should be presented as such. With so few sites, a formal test of association between metals, bacterial load and prevalence should be reported if the interpretation is to be argued and not merely proposed.

The *Klebsiella* isolates were uniformly susceptible to the fluoroquinolone tested and largely susceptible to the aminoglycoside, but they were resistant to the cephalosporins, to cloxacillin and, with few exceptions, to Augmentin. Ceftriaxone and nitrofurantoin behaved variably from site to site.

Resistance is therefore concentrated in the β-lactam class and is almost absent for fluoroquinolones and aminoglycosides. This profile agrees with those reported from Nigerian clinical and environmental settings. Bacteria from dumpsites in Benin City were highly sensitive to fluoroquinolones and aminoglycosides but strongly resistant to β-lactams (Egwuatu and Orkeh, 2019) Airborne bacteria from refuse dumps in the same city were most susceptible to ofloxacin, ciprofloxacin and gentamicin and least susceptible to ampicillin and ampiclox, and *Klebsiella* had the highest multiple antibiotic resistance index among them (Ogbonna & Azuonwu, 2019).

In Port Harcourt, uropathogenic *Klebsiella* showed extensive β-lactam resistance and ESBL-producing *Klebsiella* has been documented in hospitals in Ile-Ife and Lagos (Egwuatu and Orkeh, 2019). The similarity between leachate isolates and clinical isolates is epidemiologically meaningful. It indicates that the same resistance determinants circulate between the human population and its waste, and that dumpsite leachate receives and amplifies those (Ogbonna & Azuonwu, 2019).

Resistance of *Klebsiella* to it therefore reflects intrinsic resistance, not acquisition, and should be described that way. In addition, *K. pneumoniae* carries a chromosomal SHV-1 penicillinase that confers natural resistance to aminopenicillins, and *K. oxytoca* carries the chromosomal K1 (OXY) enzyme, whose overproduction can additionally lower susceptibility to ceftriaxone and cefuroxime (Bush and Jacoby, 2010).

Acquired β-lactamases**;** Resistance to the extended-spectrum cephalosporins is the classical phenotype of extended-spectrum β-lactamase (ESBL) production, particularly by CTX-M enzymes, which have spread worldwide and dominate in Nigeria (Paterson and Bonomo, 2005; Cantón et al., 2012). Selective pressure:β-Lactams are the most widely used antibiotics in Nigeria, and they are frequently obtained without prescription, taken incompletely, and sometimes of doubtful quality. Residues and resistant faecal bacteria from households, informal clinics, patent medicine shops and pharmacies reach dumpsites through discarded packaging, expired drugs and faecal waste. Because the β-lactams are the most heavily used, resistance to them is the most heavily enriched.

Susceptibility to the fluoroquinolone and aminoglycoside; these agents are used less often than β-lactams in the community, and the corresponding resistance genes were not screened here. Plasmid-mediated quinolone and aminoglycoside resistance are nonetheless increasingly co-carried with *bla*CTX-M on the same plasmids, so this susceptibility should not be regarded as permanent. It shows that these drugs remain the most dependable options and that they deserve protection through stewardship (Cantón et al., 2012). In relation to site variation, ceftriaxone worked against the isolates from some dumpsites but failed against those from others, and nitrofurantoin resistance appeared at only one dumpsite.

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Because the same genera were sampled within one city, these differences point to differing local inputs, for example a waste stream at one site containing more cephalosporin-exposed bacteria or antibiotic residues, and a nitrofurantoin-exposed urinary or faecal source at another. The small number of isolates tested per site means that these site contrasts are indicative and not statistically established.

The resistance of *Klebsiella* is dominated by a single antibiotic class with limited extension to others, so the description "multidrug resistance" in the results should be aligned with the Magiorakos et al. (2012) definition, which requires non-susceptibility in at least three antimicrobial classes.

The *Bacillus* isolates resisted cefuroxime and cloxacillin, were mostly resistant to ceftazidime and Augmentin, were fully susceptible to the fluoroquinolone, were moderately to highly susceptible to the aminoglycoside and ceftriaxone, and varied in their response to erythromycin. Resistance to β-lactams is expected, because members of the *B. cereus* group intrinsically produce β-lactamases (a penicillinase and a metallo-β-lactamase) and are generally resistant to penicillins and most cephalosporins, while fluoroquinolones, aminoglycosides, vancomycin and clindamycin remain the usual therapeutic options (Bottone, 2010).

The resistance of leachate *Bacillus* to these agents is therefore partly inherent to the genus and partly acquired. The variable response to erythromycin reflects local differences in macrolide exposure and in the macrolide resistance genes carried by soil *Bacillus* and co-resident bacteria. Comparably high β-lactam resistance with retained fluoroquinolone activity has been reported in dumpsite bacteria from Benin City.

Because *Bacillus* is not generally an enteric pathogen, its main importance is as a reservoir and vector of resistance genes in the environmental resistome, although *B. cereus* can cause food poisoning and opportunistic wound and eye infections (Bottone, 2010).

The resistance pattern was constant for the core agents (resistance to cefuroxime and cloxacillin everywhere, susceptibility to the fluoroquinolone everywhere) and variable for ceftriaxone, the aminoglycoside, nitrofurantoin and erythromycin.

A constant core pattern across all locations suggests a common, regionally dominant resistance reservoir, which is expected where the human population and its antibiotic consumption are shared. Variation in the other agents indicates local drivers, such as the proportion of faecal and clinical waste, metal content and occasional disposal of expired antibiotics. The sites with the heaviest metal loads were not simply the sites with the most resistant isolates, which indicates that co-selection by metals is only one contributor and that the human source of the resistant bacteria is at least as important.

Amplification of the 16S rRNA gene produced products of the expected size, and sequencing assigned the representative isolates to *Klebsiella ornithinolytica*, *K. oxytoca*, *K. pneumoniae*, *Bacillus cereus*, *B. mycoides* and *B. subtilis*. This confirms the presumptive biochemical identities and shows species-level diversity within each genus. Two points need care. *K. ornithinolytica* is now classified as *Raoultella ornithinolytica* (Drancourt et al., 2001), and many databases and papers use that name. In addition, *B. cereus* and *B. mycoides* belong to the closely related *B. cereus* group, which 16S rRNA sequencing alone cannot reliably separate, so these identifications should be described as members of the *B. cereus* group unless supported by a second marker such as *gyrB* or *rpoB*. The recovery of clinically important species such as *K. pneumoniae* and *K. oxytoca* from leachate near a university community is of public health significance, because these species cause urinary tract, wound and bloodstream infections.

Conclusion

This study has demonstrated substantial microbial contamination. Total heterotrophic bacterial counts, total coliform and faecal coliform counts were high across all sampled locations. *Bacillus* spp. and *Klebsiella* spp. were highly prevalent, with overall prevalence of 87.5% and 82.5%, respectively. The antibiotic susceptibility investigation revealed an important multidrug-resistance pattern among the isolates. Both *Bacillus* and *Klebsiella* demonstrated 100% resistance to ceftriaxone and cefuroxime, while ofloxacin showed 100% susceptibility among the tested isolates. High resistance was also observed to ceftazidime and amoxicillin-clavulanate, whereas susceptibility to gentamicin and ciprofloxacin varied according to species and sampling location.

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The findings in this study revealed that dumpsite leachate may provide an environmental reservoir for antibiotic-resistant bacteria however the application of ofloxacin and cefuroxime could be adopted in the treatment as they were resulted in 100% susceptibility.

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