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Susceptibility of Enterobacterales from Municipal Solid Wastes in Okada against Cephalosporins, Aminoglycosides, and Macrolides

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ABSTRACT

Municipal solid wastes represent important environmental reservoirs for antimicrobial-resistant bacteria and may facilitate the dissemination of resistance determinants within communities. This study investigated the in-vitro susceptibility of Enterobacterales recovered from municipal solid wastes in Okada, Edo State, Nigeria, to selected cephalosporins, aminoglycosides, and macrolides. Municipal solid-waste samples were collected from three selected dump sites in Okada Town and coded Dumpsite 1, Dumpsite 2, and Dumpsite 3. Samples were subjected to standard microbiological procedures for isolation and identification of Enterobacterales. Total coliform counts varied significantly among sampling locations, ranging from 8.75×10^5 to 1.70×10^7 CFU/g. The University Hostel recorded the highest mean concentration (7.14 ± 0.08 $\log_{10}$ CFU/g), followed by Dumpsite 3 (6.24 ± 0.05 $\log_{10}$ CFU/g) and Dumpsite 1 **(**cafeteria and food-service areas**)** (6.07 ± 0.12 $\log_{10}$ CFU/g) ($p < 0.05$). A total of 72 bacterial isolates were recovered, and their order of decreasing frequency (%) was; *Proteus* spp. (63.89%) > *Klebsiella* spp. (18.06%) > *Pseudomonas* spp. (8.33%) > *Escherichia* spp. (6.94%) > *Enterobacter* spp. (2.78%). Antimicrobial susceptibility test revealed pronounced resistance to several cephalosporins, with cefotaxime, ceftazidime, cefotaxime–clavulanic acid, ceftazidime–clavulanic acid, cefuroxime and ceftriaxone each recording 100% resistance. Resistance was comparatively lower to cefazolin (63.89%), amoxicillin–clavulanic acid (59.72%) and cefoxitin (52.78%). Meropenem showed greater activity, with 18.06% resistance, while gentamicin (5.56%), pefloxacin (2.78%) and ofloxacin (0%) exhibited the lowest resistance rates. MAR indices ranged from 0.52 to 0.67, with overall location-specific values of 0.56. The findings demonstrate substantial bacterial contamination and a high antimicrobial-resistance burden in municipal solid wastes, highlighting the need for improved waste management, environmental sanitation and antimicrobial-resistance surveillance in Okada.

Keywords: Okada, Municipal solid waste, Enterobacterales, Antimicrobial resistance, Cephalosporins, Macrolides, MAR index.

Introduction

Antimicrobial resistance (AMR) has become a major One Health challenge, extending beyond clinical settings to interconnected human, animal and environmental systems. The increasing prevalence of antimicrobial-resistant bacteria threatens effective treatment of bacterial infections, contributing to treatment failure, prolonged illness and increased transmission of antimicrobial-resistant pathogens (Jensen & Unemo, 2024). The problem is particularly important in low- and middle-income countries, where inadequate sanitation, poor waste management, limited surveillance and inappropriate antimicrobial use can promote the emergence and spread of resistance. Global evidence indicates that bacterial AMR already contributes substantially to mortality, with the burden expected to increase without effective interventions (Murray *et al*., 2022; Naghavi *et al*., 2024).

Municipal solid waste (MSW) represents an important environmental reservoir of AMR because it can receive human and animal waste, food residues, pharmaceutical products, plastics and other materials contaminated with resistant bacteria, antimicrobial residues and antimicrobial resistance genes (ARGs). Within landfill and open-dump environments, nutrients, moisture and other environmental conditions support bacterial survival and facilitate the persistence and horizontal transfer of resistance determinants.

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Mobile genetic elements and co-selective pressures, including heavy metals, may further enhance the maintenance and dissemination of resistance (Song *et al*., 2023; Anand *et al*., 2021). Consequently, MSW should be considered a dynamic component of the environmental AMR continuum rather than merely the endpoint of microbial contamination. Enterobacterales are particularly important organisms for environmental AMR surveillance because of their ecological versatility and clinical significance. Important members include *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Serratia* and *Morganella*. While some are normal gastrointestinal inhabitants, others are important opportunistic or pathogenic bacteria associated with urinary tract, bloodstream, gastrointestinal, wound and healthcare-associated infections. Their ability to acquire mobile resistance determinants, including extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases and carbapenemases, makes them valuable indicators of environmental resistance (Farkas et al. 2025).

The public-health importance of resistant Enterobacterales is demonstrated by the World Health Organization's classification of third-generation-cephalosporin-resistant and carbapenem-resistant Enterobacterales as critical-priority pathogens (WHO, 2024). Resistance to cephalosporins is particularly significant because it may indicate ESBL- or AmpC-mediated resistance. Assessment of responses to cephalosporins and amoxicillin-clavulanate can help characterize different β-lactam resistance phenotypes. Aminoglycosides provide additional information because resistance mechanisms may be linked to other resistance determinants on plasmids, integrons and related mobile elements (Rana et al., 2024). Although Enterobacterales often have limited intrinsic susceptibility to macrolides, their resistance patterns can also provide evidence of broader multidrug-resistance potential (Ma et al., 2024).

Researchers in Nigeria have increasingly demonstrated that waste-associated environments can harbour clinically significant resistant bacteria. Yusuf *et al*. (2023) recovered resistant *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa* and other organisms from sewage, sludge, food waste, plastics and soils associated with dumping sites. Adekanmbi *et al*. (2024) subsequently reported multidrug-resistant ESBL-producing *E. coli* from dumpsite leachate and adjacent surface water, demonstrating the potential movement of resistance from waste into environmental water systems. These findings are particularly relevant to Okada, Edo State, Nigeria, where municipal wastes originate from residential, commercial and institutional activities. Poor segregation and containment can create interfaces among waste, soil, drainage, water, animals, waste handlers and surrounding communities. However, environmental AMR surveillance remains geographically uneven, and information on Enterobacterales associated specifically with municipal solid waste in smaller or rapidly developing communities such as Okada remains limited.

Therefore, determining the in-vitro antimicrobial susceptibility of Enterobacterales isolated from municipal solid waste in Okada against cephalosporins, penicillin/β-lactamase-inhibitor combinations, aminoglycosides and macrolides is epidemiologically important. Such assessment can establish local resistance profiles, identify clinically relevant phenotypes and provide baseline evidence for environmental AMR surveillance. This would represent an important contribution to the growing body of environmental AMR research. Ultimately, the findings can support risk assessment, antimicrobial stewardship, improved waste-management practices and integrated One Health interventions aimed at limiting the environmental dissemination of antimicrobial resistance.

Materials and Methods

Study area

The study was conducted in Okada, Edo State, Nigeria, with sampling undertaken at three locations representing distinct municipal solid-waste environments: Dumpsite 1 (cafeteria and food-service areas), Dumpsite 2 and Dumpsite 3. The selected locations represented institutional, residential and community waste environments, respectively. These sites were selected to provide comparative information on the occurrence and antimicrobial-resistance characteristics of bacteria associated with municipal solid waste within the study area.

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Collection of municipal solid-waste samples

Municipal solid-waste samples were collected aseptically from the selected sampling locations using sterile sampling materials which included sterile disposable gloves, sterile stainless-steel forceps, sterile spatulas, and sterile sampling scoops. The samples consisted of representative portions of mixed municipal solid waste, including biodegradable/food waste, paper and cardboard materials, plastics, and other commonly encountered household and institutional waste components present at the designated sampling points. The samples were placed in appropriately labelled sterile containers and transported to the laboratory under chilled conditions (approximately 4°C). The collected samples were processed promptly upon arrival at the laboratory to minimize potential alterations in the resident bacterial population caused by microbial proliferation or loss during transportation and storage. For each sampling location, representative portions of mixed municipal solid waste, comprising readily observable biodegradable and non-biodegradable materials such as food residues, paper, plastics, and other household or institutional waste, were collected from different points within the designated sampling area and pooled to obtain representative composite samples. The samples were handled using aseptic procedures throughout collection and laboratory processing to minimize external contamination.

Isolation of bacterial isolates

The collected municipal solid-waste samples were processed for the isolation and enumeration of culturable bacteria using standard microbiological procedures (Public Health England, 2014). For microbiological enumeration, 25 g of each waste sample was aseptically weighed and introduced into a conical flask containing 225 mL of sterile 0.1% (w/v) peptone water, giving an initial 1:10 dilution. The mixture was agitated vigorously using a laboratory orbital shaker for approximately 3 min to facilitate the release and homogenization of microorganisms associated with the waste matrix. The homogenate was subsequently subjected to ten-fold serial dilution, with dilutions prepared up to 10^{-6}. Aliquots of 1 mL were aseptically dispensed from each dilution into duplicate sterile Petri plates.

Approximately 15 mL of molten MacConkey agar (HiMedia, India), maintained at a suitable pouring temperature, was added to the respective plates and mixed carefully with the inoculums by carefully rotating the plates in a circular motion in both clockwise and anticlockwise directions to ensure even distribution of the inoculum throughout the medium.

The plates were then allowed to solidify. Following complete solidification of the media, the MacConkey agar plates were incubated at 35°C for 24h. Following incubation, plates containing countable colonies were selected and the number of colonies was recorded for estimation of the bacterial population. Counts obtained on MacConkey agar were used to determine Total Coliform Counts (TCC).

Representative colonies exhibiting distinct morphological characteristics were selected and repeatedly subcultured to obtain pure cultures. Confirmed pure isolates were maintained on appropriately labeled agar slants and stored under refrigeration at 4°C until required for subsequent microbiological analyses.

Phenotypic identification of bacterial isolates

The bacterial isolates were identified using a combination of colonial morphology, cellular morphology, Gram's reaction and biochemical characteristics. Colony characteristics such as size, shape, elevation, pigmentation, texture and mucoid appearance were recorded following growth on the culture media. Gram staining was performed to differentiate Gram-positive and Gram-negative organisms and to determine cellular morphology.

Presumptive identification was subsequently performed using relevant biochemical reactions. The biochemical characteristics considered included urease, catalase, oxidase, indole, citrate utilization, methyl red, Voges–Proskauer, lactose fermentation, glucose fermentation, sucrose fermentation, gas production and hydrogen sulphide production, as applicable to the isolate.

The resulting phenotypic profiles were compared with standard diagnostic characteristics for the identification of the isolates (MacFaddin, 2000).

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Preparation of bacterial inoculum for antimicrobial susceptibility testing

Fresh, pure bacterial cultures were used for antimicrobial susceptibility testing. A representative colony from each isolate was transferred aseptically into sterile physiological diluents (0.85% sodium chloride) and adjusted to obtain a bacterial suspension of approximately 0.5 McFarland turbidity. The standardized suspension was used immediately for inoculation of Mueller–Hinton agar.

Antimicrobial susceptibility testing

The antimicrobial susceptibility profiles of the bacterial isolates were determined using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. A sterile swab was immersed in the standardised bacterial suspension and used to uniformly inoculate the entire surface of the Mueller–Hinton agar plate. The inoculated plates were allowed to stand briefly to permit absorption of excess inoculum before the antibiotic discs were applied aseptically. The antibiotic discs investigated comprised agents from several antimicrobial classes (HiMedia Laboratories Pvt. Ltd., India).

The cephalosporins tested included cefotaxime (30 µg), ceftazidime (30 µg), cefuroxime (30 µg) and ceftriaxone (30 µg)**.** Cefoxitin (30 µg) and meropenem (10 µg) were also included. β-lactam/β-lactamase-inhibitor combinations comprised cefotaxime–clavulanic acid (30/10 µg), ceftazidime–clavulanic acid (30/10 µg) and amoxicillin–clavulanic acid (20/10 µg). The aminoglycosides examined were gentamicin (10 µg) and streptomycin (10 µg), while the fluoroquinolones included ciprofloxacin (5 µg), pefloxacin (5 µg) and ofloxacin (5 µg)**.** The antibiotic discs were positioned with the aid of sterile forceps at appropriate distances on the inoculated Mueller–Hinton agar surface to prevent overlapping inhibition zones. The plates were thereafter, incubated under appropriate conditions, after which the diameters of the zones of inhibition surrounding each antibiotic disc were measured in millimeters with the aid of a transparent ruler. The susceptibility of each isolate was interpreted according to the applicable standard interpretive criteria used for the antimicrobial susceptibility testing (CLSI, 2024).

Determination of antimicrobial resistance

For each isolate, an antibiotic was classified as resisted when the measured inhibition-zone diameter fell within the resistance category specified by the interpretive standard used for the study (CLSI, 2024). The resistance data were subsequently organized according to bacterial identity and sampling location. The frequency and percentage of resistant isolates were calculated for each antibiotic and bacterial group. These data were used to determine the cumulative resistance burden.

Determination of multiple antibiotic resistance (MAR) index

The multiple antibiotic resistance index was calculated for individual bacterial groups and sampling locations using the conventional MAR index approach originally described by Krumperman (1983). The MAR index was calculated as:

$$\text{MAR} = \frac{\sum(AS)}{A \times n} \quad (1)$$

Where;

$\sum AS$ represents the cumulative number of antibiotic resistances recorded for the isolates within a bacterial group, A represents the total number of antibiotics tested and n represents the number of isolates examined.

For the present study, 15 antibiotics were included in the MAR calculation; therefore, A was equal to 15. The mean MAR index for each bacterial group was obtained by dividing the cumulative antibiotic resistance score by the product of the number of isolates and the total number of antibiotics tested. A MAR value greater than 0.20 was interpreted as indicating an isolate population associated with a high-risk source of antimicrobial contamination or exposure, following the conventional MAR interpretation of Krumperman (1983).

Statistical analysis

Statistical analyses were performed using Number Cruncher Statistical Software (NCSS), version 12. Descriptive statistical methods were employed to summarize bacterial counts and characterize their frequency distributions.

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Prior to inferential analysis, Levene's test was used to assess homogeneity of variance, while the Shapiro–Wilk test was applied to evaluate the normality of the data. Where the assumptions for parametric analysis were satisfied, one-way analysis of variance (ANOVA) was used to determine differences among groups. Statistical tests were considered significant at a probability level of $p < 0.05$.

Results

The total coliform counts obtained from municipal solid waste samples collected from Dumpsite 1, the Dumpsite 2 and the Dumpsite 3 are presented in Table 1. The mean total coliform concentrations were 6.24 ± 0.05 $\log_{10}$ CFU/g**,** 6.07 ± 0.12 $\log_{10}$ CFU/g, and **7**.14 ± 0.08 $\log_{10}$ CFU/g for Dumpsite 3, Dumpsite 1 and Dumpsite 2 respectively.

The Dumpsite 2 therefore had the highest mean total coliform burden, exceeding the Dumpsite 3 and Dumpsite 1 means by approximately 0.91 and 1.08 $\log_{10}$ CFU/g, respectively.

One-way analysis of variance (ANOVA) demonstrated a statistically significant difference in total coliform counts among the three sampling locations ($p < 0.05$). Tukey's honestly significant difference (HSD) post-hoc test showed that the Dumpsite 2 had significantly higher counts than both Dumpsite 3 ($p < 0.001$) and the Dumpsite 1 ($p < 0.05$). However, the difference between Dumpsite 3 and the Dumpsite 1 was not statistically significant ($p > 0.05$). Thus, the significant overall variation was principally attributable to the markedly elevated coliform burden in the Dumpsite 2 wastes.

Table 1: Total coliform counts in municipal solid wastes from sampling locations

Sampling Location	Coliform count (CFU/g) of municipal solid wastes			
	Minimum	Maximum	Mean ± SD (n = 4)	$\log_{10}$ Mean ± SD
Dumpsite 1	875,000	1,725,000	1,206,250 ± 367,069	6.07 ± 0.12
Dumpsite 2	11,000,000	17,000,000	14,125,000 ± 2,586,021	7.14 ± 0.08
Dumpsite 3	1,525,000	2,000,000	1,743,750 ± 204,507	6.24 ± 0.05

Key: n = number of samples; MSW: Municipal Solid Waste

The frequency of occurrence of the bacterial isolates in the various locations is presented in Table 2. The study identified five bacterial groups in municipal solid waste collected from Dumpsite 1, Dumpsite 2, and Dumpsite 3. *Proteus* spp. was the predominant isolate across all locations, occurring at 62.50%, 70.83%, and 58.33%, respectively. *Klebsiella* spp. followed, with frequencies of 25.00% at the Dumpsite 1, 8.33% at the Dumpsite 2, and 20.83% in Dumpsite 3. *Escherichia* spp. occurred less frequently, while *Pseudomonas* spp. was absent from Dumpsite 1 but occurred at 12.50% in both the Dumpsites 2 and 3. *Enterobacter* spp. was the least frequently recovered isolate, occurring at 4.17% in Dumpsites 2 and 3. Chi-square analysis showed no significant association between bacterial isolate distribution and sampling location ($\chi^2 = 7.904$, df = 8, $p = 0.44$). Cramér's V (0.23) indicated a small-to-moderate association, suggesting that observed differences may reflect environmental and waste-related factors.

Table 2: Frequency of Occurrence of Bacterial Isolate in various Location of Municipal Solid Waste

Bacteria Isolate	Frequency of Occurrence (n = 24 /%) in Location of MSW		
	Dumpsite 1	Dumpsite 2	Dumpsite 3
Proteus spp.	15 (62.50)	17 (70.83)	14 (58.33)
Klebsiella spp.	6 (25.00)	2 (8.33)	5 (20.83)
Escherichia spp.	3 (12.50)	1 (4.17)	1 (4.17)
Pseudomonas spp.	0	3 (12.50)	3 (12.50)
Enterobacter spp.	0	1 (4.17)	1 (4.17)

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The antimicrobial resistance profiles of 72 bacterial isolates recovered from municipal solid waste at Dumpsite 1, Dumpsite 2 and Dumpsite 3, as presented in Tables 3 to 5, demonstrated substantial resistance to several commonly used β-lactam antibiotics, while comparatively lower resistance was observed against aminoglycosides and fluoroquinolones. The isolates comprised *Proteus* spp. (46; 63.89%), *Klebsiella* spp. (13; 18.06%), *Pseudomonas* spp. (6; 8.33%), *Escherichia* spp. (5; 6.94%) and *Enterobacter* spp. (2; 2.78%). *Proteus* spp. was therefore the predominant bacterial group across the three sampling environments. At the Dumpsite 1, all 24 isolates showed complete resistance (100%) to cefotaxime, ceftazidime, cefotaxime–clavulanic acid, ceftazidime–clavulanic acid, cefuroxime and ceftriaxone. Resistance to cefazolin and amoxicillin–clavulanic acid was also considerable, occurring in 62.50% and 58.33% of isolates, respectively. Cefoxitin resistance was 50.00%, whereas meropenem resistance was considerably lower at 20.83%. The aminoglycosides demonstrated greater activity, with only 4.17% of isolates resistant to gentamicin and 20.83% to streptomycin. Similarly, fluoroquinolone resistance was low, with 8.33% resistance to both ciprofloxacin and pefloxacin and no resistance to ofloxacin.

Table 3: Antibiotic resistance prevalence of bacteria isolated from Dumpsite 1

Antibiotics	***Proteus* spp. n = 15; F (%)**	***Klebsiella* spp. n = 6; F (%)**	***Escherichia* spp. n = 3; F (%)**
Cefotaxime (30 µg)	15 (100.00)	6 (100.00)	3 (100.00)
Ceftazidime (30 µg)	15 (100.00)	6 (100.00)	3 (100.00)
Cefotaxime + Clavulanic acid (30/10 µg)	15 (100.00)	6 (100.00)	3 (100.00)
Ceftazidime + Clavulanic acid (30/10 µg)	15 (100.00)	6 (100.00)	3 (100.00)
Cefoxitin (30 µg)	9 (60.00)	2 (33.33)	1 (33.33)
Meropenem (10 µg)	3 (20.00)	2 (33.33)	0 (0.00)
Cefuroxime (30 µg)	15 (100.00)	6 (100.00)	3 (100.00)
Cefazolin (30 µg)	8 (53.33)	5 (83.33)	2 (66.67)
Ceftriaxone (30 µg)	15 (100.00)	6 (100.00)	3 (100.00)
Amoxicillin + Clavulanic acid (20/10 µg)	8 (53.33)	4 (66.67)	2 (66.67)
Gentamicin (10 µg)	1 (6.67)	0 (0.00)	0 (0.00)
Streptomycin (10 µg)	3 (20.00)	1 (16.67)	1 (33.33)
Ciprofloxacin (5 µg)	0 (0.00)	2 (33.33)	0 (0.00)
Pefloxacin (5 µg)	1 (6.67)	1 (16.67)	0 (0.00)
Ofloxacin (5 µg)	0 (0.00)	0 (0.00)	0 (0.00)

Key: n = total number of bacterial isolates examined; F = number of specific bacterial isolates resistant to tested antibiotics and % = percentage resistance of bacterial isolates to tested antibiotics.

A similar pattern occurred among the Dumpsite 2 isolates in Table 4. Complete resistance was recorded against cefotaxime, ceftazidime, their clavulanate combinations, cefuroxime and ceftriaxone. Cefoxitin Resistance occurred in 54.17% of isolates, while cefazolin and amoxicillin–clavulanic acid recorded resistance rates of 62.50% and 58.33%, respectively. Meropenem resistance remained relatively low at 20.83%. Gentamicin resistance was only 8.33%, while streptomycin resistance was 20.83%. Ciprofloxacin resistance was 12.50%, whereas neither pefloxacin nor ofloxacin showed resistant isolates. At Dumpsite 3, the same six antibiotics, cefotaxime, ceftazidime, cefotaxime–clavulanic acid, ceftazidime–clavulanic acid, cefuroxime and ceftriaxone, showed 100% resistance. Cefoxitin, cefazolin and amoxicillin–clavulanic acid showed resistance rates of 54.17%, 66.67% and 62.50%, respectively. Meropenem demonstrated greater activity, with only 12.50% resistance. Gentamicin resistance was 4.17%, while streptomycin and ciprofloxacin showed resistance rates of 25.00% and 12.50%, respectively. No resistance occurred against pefloxacin or ofloxacin. Across all 72 isolates, resistance was highest to cefazolin (63.89%), followed by amoxicillin–clavulanic acid (59.72%) and cefoxitin (52.78%). In contrast, meropenem resistance was only 18.06%, while gentamicin, pefloxacin and ofloxacin recorded 5.56%, 2.78% and 0%, respectively. Overall, the findings demonstrate a pronounced resistance burden among waste-associated Enterobacterales, particularly against cephalosporins, but comparatively preserved susceptibility to meropenem, aminoglycosides and fluoroquinolones.

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Table 4: Antibiotic resistance prevalence of bacteria isolated from Dumpsite 2

Antibiotics	*Proteus* spp. n = 17; F (%)	*Klebsiella* spp. n = 2; F (%)	*Escherichia* spp. n = 1; F (%)	*Pseudomonas* spp. n = 3; F (%)	*Enterobacter* spp. n = 1; F (%)
Cefotaxime (30 µg)	17 (100.00)	2 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Ceftazidime (30 µg)	17 (100.00)	2 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Cefotaxime + Clavulanic acid (30/10 µg)	17 (100.00)	2 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Ceftazidime + Clavulanic acid (30/10 µg)	17 (100.00)	2 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Cefoxitin (30 µg)	10 (58.82)	0 (0.00)	1 (100.00)	1 (33.33)	1 (100.00)
Meropenem (10 µg)	4 (23.53)	0 (0.00)	0 (0.00)	1 (33.33)	0 (0.00)
Cefuroxime (30 µg)	17 (100.00)	2 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Cefazolin (30 µg)	9 (52.94)	2 (100.00)	1 (100.00)	2 (66.67)	1 (100.00)
Ceftriaxone (30 µg)	17 (100.00)	2 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Amoxicillin + Clavulanic acid (20/10 µg)	9 (52.94)	2 (100.00)	1 (0.00)	2 (66.67)	1 (100.00)
Gentamicin (10 µg)	1 (5.88)	0 (0.00)	0 (0.00)	1 (33.33)	0 (0.00)
Streptomycin (10 µg)	3 (17.65)	1(50.00)	0 (0.00)	1 (33.33)	0 (0.00)
Ciprofloxacin (5 µg)	2 (11.77)	0 (0.00)	0 (0.00)	1 (33.33)	0 (0.00)
Pefloxacin (5 µg)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Ofloxacin (5 µg)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Table 5: Antibiotic resistance prevalence of bacteria isolated from Dumpsite 3

Antibiotics	*Proteus* spp. n = 14; F (%)	*Klebsiella* spp. n = 5; F (%)	*Escherichia* spp. n = 1; F (%)	*Pseudomonas* spp. n = 3; F (%)	*Enterobacter* spp. n = 1; F (%)
Cefotaxime (30 µg)	14 (100.00)	5 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Ceftazidime (30 µg)	14 (100.00)	5 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Cefotaxime + Clavulanic acid (30/10 µg)	14 (100.00)	5 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Ceftazidime + Clavulanic acid (30/10 µg)	14 (100.00)	5 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Cefoxitin (30 µg)	5 (35.71)	4 (80.00)	1 (100.00)	3 (100.00)	0 (0.00)
Meropenem (10 µg)	1 (7.14)	0 (0.00)	1 (100.00)	1 (33.33)	0 (0.00)
Cefuroxime (30 µg)	14 (100.00)	5 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Cefazolin (30 µg)	8 (57.14)	5 (100.00)	0 (0.00)	2 (66.67)	1 (100.00)
Ceftriaxone (30 µg)	14 (100.00)	5 (100.00)	1 (100.00)	3 (100.00)	1 (100.00)
Amoxicillin + Clavulanic acid (20/10 µg)	7 (50.00)	4 (80.00)	1 (100.00)	2 (66.67)	1 (100.00)
Gentamicin (10 µg)	0 (0.00)	0 (0.00)	0 (0.00)	1 (33.33)	0 (0.00)
Streptomycin (10 µg)	3 (21.40)	1 (20.00)	1 (100.00)	1 (33.33)	0 (0.00)
Ciprofloxacin (5 µg)	1 (7.14)	1 (20.00)	0 (0.00)	0 (0.00)	1 (100.00)
Pefloxacin (5 µg)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Ofloxacin (5 µg)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Key: n = total number of bacterial isolates examined; F = number of specific bacterial isolates resistant to tested antibiotics; % = percentage resistance of bacterial isolates to tested antibiotics.

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The multiple antibiotic resistance (MAR) index of the bacterial isolates recovered from the three sampling locations is presented in Table 6. A total of 72 bacterial isolates, comprising 24 isolates from each sampling location, were evaluated against 15 antibiotics.

At Dumpsite 1, 24 isolates were assessed, comprising 15 *Proteus* spp., six *Klebsiella* spp and three *Escherichia* spp. The corresponding ∑AS values were 125, 53 and 24, giving mean MAR indices of 0.56, 0.59 and 0.53, respectively. *Klebsiella* spp therefore recorded the highest species-specific MAR index, while *Escherichia* spp. had the lowest. When all 24 isolates were considered collectively, 202 antibiotic-resistance events were recorded, corresponding to an overall MAR index of 0.56.

At Dumpsite 2, the 24 isolates consisted of 17 *Proteus* spp., two *Klebsiella* spp., one *Escherichia* spp., three *Pseudomonas* spp. and one *Enterobacter* spp. Their respective ∑AS values were 140, 17, 9, 27 and 9, with MAR indices of 0.55, 0.57, 0.60, 0.60 and 0.60, respectively.

Thus, *Escherichia*, *Pseudomonas* and *Enterobacter* spp. exhibited the highest observed MAR index at this location, whereas *Proteus* spp. had the lowest. Collectively, the 24 isolates produced 202 resistance events, giving an overall MAR index of 0.56.

At Dumpsite 3, 24 isolates were similarly evaluated and comprised 14 *Proteus spp.*, five *Klebsiella spp.*, one *Escherichia* spp., three *Pseudomonas* spp. and one *Enterobacter* spp. The ∑AS values were 110, 46, 10, 28 and 9, respectively. The resulting MAR indices were 0.52 for *Proteus* spp., 0.61 for *Klebsiella* spp., 0.67 for *Escherichia* spp., 0.62 for *Pseudomonas* spp. and 0.60 for *Enterobacter* spp. The highest species-specific value recorded in the study was therefore 0.67 for *Escherichia* spp. at Dumpsite 3, while *Proteus* spp. had the lowest value at this location (0.52). The 24 isolates collectively accounted for 203 resistance events, corresponding to a calculated MAR index of 0.56. Taken together, the data demonstrate a consistently high MAR burden across all three environments, with no meaningful descriptive difference in the overall location-level MAR index.

Table 6: Multiple antibiotics resistance index

Bacteria Isolate	**MAR Index of bacteria in Location of MSW**											
	Dumpsite 1				**Dumpsite 2**				**Dumpsite 3**			
	n	**∑AS**	**A**	**Mean MAR Index**	**n**	**∑AS**	**A**	**Mean MAR Index**	**n**	**∑AS**	**A**	**Mean MAR Index**
Proteus spp.	15	125	15	0.56	17	140	15	0.55	14	110	15	0.52
Klebsiella spp.	6	53	15	0.59	2	17	15	0.57	5	46	15	0.61
Escherichia spp.	3	24	15	0.53	1	9	15	0.60	1	10	15	0.67
Pseudomonas spp.	0	0	0	0	3	27	15	0.60	3	28	15	0.62
Enterobacter spp.	0	0	0	0	1	9	15	0.60	1	9	15	0.60
All bacteria species	24	202	15	0.56	24	202	15	0.56	24	203	15	0.56

Key: ∑AS = Summation of the number of antibiotics to which specific bacterial isolates were resistant; A = Total number of antibiotics tested; n = Number of isolates examined.

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Discussion

The present study revealed that municipal solid waste (MSW) generated in Dumpsite 3, Dumpsite 1 and Dumpsite 2 carried substantial bacterial contamination, with location-specific differences. Mean concentrations ranged from 1.21×10^6 to 1.41×10^7 CFU/g, corresponding to approximately 6.07–7.14 $\log_{10}$ CFU/g. The Dumpsite 2 recorded the greatest bacterial burden, with a mean concentration of 1.41×10^7 CFU/g, while the Dumpsite 1 had the lowest mean value (1.21×10^6 CFU/g). The differences were significant ($p < 0.05$), with Dumpsite 2 differing from both other locations. The elevated contamination in Dumpsite 2 may reflect its residential setting, dense population, food remnants, biodegradable materials and moisture. These conditions support bacterial persistence and multiplication, consistent with observations that household waste supports diverse microorganisms (Kong *et al*., 2026). Waste age, storage time and management practices may further influence microbial abundance and diversity (Mandal *et al*., 2025). Although waste at Dumpsite 1 had the lowest count, its microbial burden remained considerable. Similar values in Dumpsite 1 and Dumpsite 3 may reflect comparable domestic and food-related waste streams. However, coliforms are broad Gram-negative groups and do not alone prove faecal contamination or identify pathogens. The high counts nevertheless demonstrate strong microbial activity within the waste environments. Ogunyemi *et al*. (2025a) identified more than 300 operational taxonomic units in solid-waste leachates from the Olusosun and Oke-Afa dumpsites in Lagos, while Ogunyemi *et al*. (2025b) reported diverse bacterial communities in Nigerian dumpsite leachates. Bassey *et al*. (2025) likewise observed greater microbial abundance in soil associated with the Lemna dumpsite than in non-dumpsite soil and recovered medically important genera including *Clostridium*, *Enterococcus* and *Pseudomonas*. Waste handlers and cleaners may repeatedly contact contaminated materials, while waste disturbance can release bioaerosols. Nwankwo *et al*. (2025) documented microbial colonization among municipal solid-waste handlers in Aba, Nigeria, highlighting occupational exposure. Rainfall and liquid movement through accumulated waste can also produce leachate that transports microorganisms into surrounding soil and water.

Nigerian investigations have reported bacterial contamination of dumpsite leachates and groundwater, including *E. coli* (Ogunyemi *et al*., 2025a; 2026). Future work should therefore examine leachate and other transmission pathways. Seventy-two bacterial isolates were recovered, with 24 isolates from each sampling location. All were Gram-negative rods, and the principal groups were *Proteus* spp., *Klebsiella* spp., *Escherichia* spp., *Pseudomonas* spp. and *Enterobacter* spp. *Proteus* spp. dominated, comprising 46/72 isolates (63.89%), followed by *Klebsiella* spp. (18.06%), *Pseudomonas* spp. (8.33%), *Escherichia* spp. (6.94%) and *Enterobacter* spp. (2.78%). These four groups represented 91.67% of isolates, demonstrating Enterobacterales predominance. This agrees with evidence that human-influenced environments harbour clinically and environmentally important bacteria (Larsson and Flach, 2022; Ferheen *et al*., 2024; Ekhosuehi *et al*., 2024; Liu *et al*., 2025). *Proteus* spp. were especially prominent in the waste of Dumpsite 2, where they represented 70.83% of isolates.

Their abundance may reflect biodegradable substrates and human or animal contamination. *Klebsiella* spp. were frequent at the Dumpsite 1 and Dumpsite 3 and are important because some members are opportunistic pathogens capable of persisting in varied environments (Elias *et al*., 2025). Di Cesare *et al*. (2024) demonstrated that *K. pneumoniae* can survive wastewater-treatment processes and enter receiving environments. Environmental *Klebsiella* populations may also carry resistance and virulence determinants (Ekhosuehi *et al*., 2024; Mabeo *et al*., 2025).

Escherichia spp., although less frequent, remain relevant because environmental *E. coli* may indicate faecal contamination and carry resistance determinants. *Pseudomonas* spp. occurred in Dumpsite 2 and Dumpsite 3 waste, while *Enterobacter* spp. were less common. Their coexistence illustrates ecological diversity and possible opportunities for maintenance and exchange of adaptive traits, including resistance determinants (Wang *et al*., 2022; Engobo *et al*., 2025).

Antimicrobial susceptibility testing revealed a pronounced resistance burden, particularly among β-lactam antibiotics. All isolates (100%) were resistant to cefotaxime, ceftazidime, cefuroxime and ceftriaxone, as well as cefotaxime–clavulanic acid and ceftazidime–clavulanic acid combinations.

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Resistance to third-generation cephalosporins is important because it is frequently associated with ESBL- and AmpC-mediated mechanisms. Nigerian studies have documented ESBL-, AmpC- and metallo-β-lactamase-producing Enterobacterales carrying blaTEM, blaSHV and blaCTX-M determinants (Akinjogunla *et al*., 2023). Adekanmbi *et al*. (2024) similarly recovered multidrug-resistant ESBL-producing *E. coli* from solid-waste dumpsite leachate and adjacent surface water. However, phenotypic resistance cannot by itself establish the presence of resistance genes. Resistance to cefazolin, amoxicillin–clavulanic acid and cefoxitin was also substantial, whereas meropenem resistance was lower.

Only 18.06% of isolates were resistant to meropenem, leaving 81.94% non-resistant. Ojo *et al*. (2024) reported a pooled meropenem resistance of 13.5% among carbapenem-resistant Enterobacterales in Nigeria. Meropenem activity is encouraging, but surveillance remains necessary because carbapenemase-producing Enterobacterales can persist through wastewater and downstream environments (Mollenkopf *et al*., 2025). Aminoglycosides and fluoroquinolones retained better activity. Gentamicin resistance was 5.56%, streptomycin resistance 22.22%, ciprofloxacin resistance 11.11%, and pefloxacin resistance 2.78%; no resistance occurred to ofloxacin. These profiles illustrate heterogeneous environmental resistance shaped by antimicrobial residues, co-selective contaminants, human-associated bacteria and mobile resistance elements.

The multiple antibiotic resistance (MAR) index confirmed considerable antimicrobial selection pressure. Overall values were approximately 0.56: 0.561 for the Dumpsite 1, 0.561 for the Dumpsite 2 and 0.564 for Dumpsite 3. All exceeded the conventional 0.20 threshold associated with environments exposed to substantial antimicrobial pressure (Krumperman, 1983). Species-specific MAR values ranged from 0.53–0.59 at Dumpsite 1, 0.55–0.60 at the Dumpsite 2 and 0.52–0.67 in Dumpsite 3, with the highest value recorded for *Escherichia* spp. This reinforces evidence that municipal waste systems can harbour resistant bacteria and antimicrobial-resistance genes. Overall, the study identifies Okada MSW as an environmental reservoir of bacterial contamination and antimicrobial resistance.

High bacterial loads, Enterobacterales predominance, β-lactam resistance and elevated MAR Indices indicate public-health significance. The results support One Health, recognizing circulation of resistant organisms and determinants among humans, animals and environmental compartments (Larsson and Flach, 2022; Yusuf *et al*., 2023; WHO, 2024; Rui *et al*., 2025).

Movement of students, staff, waste handlers, animals, wastewater and contaminated materials may connect university and community environments (Adekanmbi *et al*., 2024; Muhammad *et al*., 2026). Improved waste segregation, covered receptacles, prompt removal of organic waste, treatment and disposal, environmental monitoring and adequate personal protective equipment are therefore warranted.

These measures are consistent with WHO (2025), which emphasizes integrated waste-management systems, stronger governance and protection of populations exposed to poorly managed waste.

Conclusion

This study demonstrated substantial antimicrobial resistance among Enterobacterales recovered from municipal solid wastes in Okada, Edo State, Nigeria. The isolates exhibited variable but generally high resistance to cephalosporins, aminoglycosides, and macrolides, with multidrug-resistant phenotypes occurring frequently. The elevated multiple antibiotic resistance indices indicate considerable exposure to antimicrobial selective pressure and suggest that municipal solid wastes may serve as important reservoirs and dissemination points for resistant bacteria.

The findings underscore potential environmental and public-health risks associated with inadequate waste management and uncontrolled antimicrobial contamination.

Strengthened waste-management practices, antimicrobial stewardship, routine resistance surveillance, and improved environmental sanitation are therefore recommended to limit the spread of resistant Enterobacterales within the Okada community.

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