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Study on Antibiotic Resistance Profiles Exhibited by *Vibrio* Species Isolated from Landfill Soils in Zaria Metropolis, Northern Nigeria

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Abstract

Antibiotic resistance in bacteria presents a major risk to public health and the environment. Infections caused by Vibrio species continue to be a serious public health concern. This study investigated the occurrence and antibiotic resistance profiles of Vibrio species isolates from landfill soils in Zaria Metropolis. A total of one hundred and twenty (120) soil samples were collected from designated landfills in four locations in Sabon-Gari, Samaru, Tudun-Wada, and Zaria City. Vibrio species were isolated using Thiosulphate citrate bile salt sucrose (TCBS) agar. Bacteriological analysis of the soil samples revealed 9(7.50%) Vibrio species isolates with Vibrio cholerae non-O1 exhibiting the highest prevalence of 4 (3.33%) among all isolates. Using the Kirby-Bauer method, the isolates were tested for susceptibility against ten commonly used antibiotics belonging to three different classes. The highest resistance was to Ampicillin (88.89%), while all the isolates (100%) showed susceptibility to Gentamicin and Chloramphenicol. Five isolates (55.56%) were Multidrug Resistance (MDR). The highest Multidrug Resistance (MDR, 60%) was observed in Vibrio cholerae non-O1. The isolate resistant to the highest number of antibiotics was obtained from the Tudun-Wada sample location. Three isolates (33.33%) showed resistance to 4 antibiotics, while 2 isolates, 1(11.11%) each were resistant to 5 and 6 antibiotics. The isolates were Multiple Antibiotic Resistance (MAR) to 4-6 antibiotics, and four different phenotypic resistance profiles were observed among them. The origin and varying levels of resistance to multiple antibiotics indicated could be traced to the faecal constituent of the waste in landfills produced by people or animals that have been treated indiscriminately with various antibiotics or items containing residual antimicrobial agents disposed of in dump soils, highlighting the potential environmental reservoirs of antibiotic resistance and call for further research to understand the implications for public health and environmental management.

Keywords: *Vibrio species, Antimicrobial Resistance, Multidrug Resistance, Public Health and Environmental Management, Zaria Metropolis*

INTRODUCTION

Wastes in landfills (waste dumpsites) with no proper waste handling methods are a source of pathogens in the soil, contributing to community-acquired infection. Zaria Metropolis is a dense urban city with many economic and social activities such as markets, institutions, and industries. This has created a steady growth in population and human activities, resulting in the indiscriminate littering and dumping of wastes, which constitute a daily nuisance in many of our communities. These wastes serve as reservoirs for diverse microbial communities due to their accumulated materials, including domestic, industrial, and medical waste. It has been reported that environments offering an amalgamation of organic matter, contaminants,

and pharmaceutical residues create an ecological niche conducive to the proliferation and dissemination of antibiotic-resistant bacteria (Oviasogie *et al.*, 2010; Osunwoke and Kuforiji, 2012). *Vibrio*, a Gram-negative bacteria, stands out among the myriad bacterial species that can persist in these environments due to their adaptive potential and clinical significance (Smith *et al.*, 2023).

About 57 to 85% of the waste generated worldwide is disposed of in landfills devoid of effective treatment, and about 5.3 million people, which include 4 million children, die each year from waste-related diseases (Adekanle *et al.*, 2014; Nyandjou *et al.*, 2018).

Antibiotic resistance has become a pressing health issue, necessitating a thorough understanding of its prevalence, mechanisms, and sources. The emergence of antibiotic resistance in environmental bacteria, particularly *Vibrio species*, is a growing concern due to its potential impact on human health (Hossain *et al.*, 2012). Understanding the antibiotic resistance profiles of *Vibrio* isolates from landfill soils is imperative, as these bacteria may act as potential vectors for antibiotic resistance dissemination in both environmental and clinical settings.

This study aimed to investigate the antibiotic resistance profiles of *Vibrio* isolates obtained from soils in landfills, shedding light on the potential environmental reservoirs and pathways of antibiotic resistance dissemination.

MATERIALS AND METHODS

Samples Collection

Soil samples were collected from soils in landfills in Zaria Metropolis. The locations were Samaru, Sabon-Gari, Tudun-Wada, and Zaria City. The study area lies between the latitudes 11°07'N to 11°51'N and longitudes 7°43'E to 7°45'E. At each sampling site, surface debris was removed, and soil was dug to a depth of 15cm using a hand trowel (Isirima *et al.*, 2005). The soil was then scooped into a clean, low-density polythene bag and transported in cool boxes to the Bacteriology Laboratory in the Department of Microbiology, Ahmadu Bello University, Zaria, for analysis. Samples were stored at 4°C if not analysed immediately.

Samples Processing

Isolation and Biochemical identification of *Vibrio species*

Twenty-five grams (25 g) of soil was suspended in 225 ml of sterile distilled water and mixed using a flame-sterilized glass rod. Serial dilutions were made from soil dilution. 1ml soil dilution were enriched in 9ml alkaline peptone water (pH 8.6) and incubated at 37°C for 8hrs. A loopful of the enriched peptone water culture (taken from the surface) was streaked on Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar (Difco). The TCBS agar was melted, brought to 45°C, and poured into sterile petri dishes. After solidifying, plates were inoculated and incubated at 35°C

for 24 to 48 hours and at 37°C for 24h. Three to five yellow or greyish yellow (sucrose-fermenting) and green or blue-green (non-sucrose fermenting) isolated colonies ranging from 2-3mm in size on agar plates were randomly picked from each sample and subsequently subcultured on fresh TCBS agar plates. The pure isolates were subjected to Gram staining and oxidase test. Only Gram-negative oxidase-positive isolates were selected for biochemical identification using the API 20 NE kit. The strips were then read, and the final identification was secured using API lab plus software (BioMerieux, Marcy l'Etoile, France).

Serological identification of *Vibrio cholerae* using the *Vibrio cholerae* Antisera

The serological type of *Vibrio cholerae* is based on its O somatic antigens. A certain amount (3 colonies) of overnight bacterial growth on Nutrient agar at 37°C was suspended in 0.5 ml physiological saline and antigenic suspension. A drop of antiserum and physiological saline (30ul) as control was placed onto a cleaned glass slide partitioned into several parts with a glass pencil. The antigenic suspension was placed onto the serum, and the physiological saline was placed on the glass slide. The reagents were mixed by tilting the glass slide back and forth for 1 minute to see if there was agglutination, which was grossly observed with light through the slide. Only strong agglutination observed within 1 minute in the reaction with each serum was regarded as positive. Delayed or weak agglutination was regarded as negative.

Because serotyping with live cells may be impossible for some strains of *Vibrio cholerae* O1, all the test organisms with negative polyvalent sera were retested by heating antigen suspension as follows. Bacterial growth was suspended in 3ml physiological saline and heated to 121°C for 15 mins. The heated solution was centrifuged at 900rpm for 20 mins, the supernatant was discarded, and the precipitate was then suspended with 0.5ml physiological saline and used as a heated cell suspension. Polyvalent sera that showed negative results with the heated antigen suspension were identified as *Vibrio cholerae* non-O1.

Antibiotic susceptibility test

Bacterial susceptibility to antimicrobial agents was performed by the disk diffusion method on Mueller-Hinton Agar (Oxoid), using guidelines established by Clinical Laboratory Standards Institute guidelines (CLSI, 2014). A total of 10 antibiotic discs (Mast Diagnostics, Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) which includes Ampicillin 10 µg, Amoxicillin 10µg, Cephalexim 30 µg, Tetracycline 10 µg, Erythromycin 15 µg, Chloramphenicol 30 µg, Ofloxacin 10µg, Gentamicin 10 µg, Norfloxacin 10 µg and Sulphamethoxazole 25 µg were employed. The resistance or susceptibility profile of the isolates was determined by measuring the inhibitory zone and then compared with the interpretative chart to determine the sensitivity of the isolates to the antibiotics. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate, and resistant categories according to the interpretation standard of the CLSI (CLSI, 2014).

Multiple antibiotic resistance (MAR) index

The multiple antibiotic resistance (MAR) index, when applied to a single isolate, is defined as [a/b], where [a] represents the number of antibiotics to which the isolates were resistant and [b] represents the number of antibiotics to which the isolates were exposed. MAR index higher than 0.2 identifies organisms that originate from high-risk sources of contamination, where antibiotics are often used. Overall, the multi-drug resistance of the

Vibrio species was exemplified by resistance to at least four antibiotics tested (Ezekiel *et al.*, 2011).

RESULTS

Table 1 shows the occurrence of *Vibrio* species isolates from soil in landfills at various sampling locations in Zaria Metropolis. *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholerae* non-O1 recorded prevalence values of 7.50% in all samples analyzed, with *Vibrio cholerae* non-O1 having the highest prevalence value 4(3.33 %). A high incidence of *Vibrio* species 4(13.33%) was observed in samples taken from the Tudun-Wada location. Antimicrobial susceptibility pattern results of the various isolates, as presented in Table 2, show the susceptibility of all isolates to Gentamicin (100%) and Chloramphenicol (100%). Resistance of isolates was observed on Ampicillin (88. 89%), followed by Amoxicillin (77.78%), Tetracycline (66.67%), and Sulphamethoxazole (55.56%). However, Erythromycin and Ciprofloxacin displayed better better-performing index with 11.11% isolate resistance. Table 3 shows the Antibiotic resistance profiles of *Vibrio species* isolates. Five isolates 5(55.56%) were Multidrug-resistant. The isolates were MAR to 4-6 antibiotics with *Vibrio cholerae* non-O1 having the highest phenotypic resistance profiles (AMP, AML, CL, E, TE, SXT). Percentage Multidrug Resistance of *Vibrio species* isolates, as presented in Figure 1, show that 11.11% of the isolates were MAR to five and 6 antibiotics each while 33. 33% were MAR to 4 antibiotics.

Table 1: Occurrence of *Vibrio species* isolates from soil in landfills at various sampling locations in Zaria Metropolis

Sampling Location	No. of Samples analyzed	<i>Vibrio parahaemolyticus</i> Number (%)	<i>Vibrio vulnificus</i> Number (%)	<i>Vibrio cholerae</i> non-O1 Number (%)	Total No. of isolates/location Number (%)
Sabon-Gari	30	1(3.33)	0(0.00)	1(3.33)	2 (6.67)
Samaru	30	0(0.00)	1(3.33)	0 (0.00)	1(3.33)
Tudun-Wada	30	1(3.33)	1(3.33)	2 (6.67)	4(13.33)
Zaria city	30	1(3.33)	0(0.00)	1(3.33)	2 (6.67)
Total	120	3(2.50)	2 (1.67)	4(3.33)	9 (7.50)

Table 2: Antimicrobial susceptibility patterns of *Vibrio* species isolated from soil in landfills in Zaria Metropolis.

Antibiotics Tested	Disc potency (µg)	<i>Vibrio parahaemolyticus</i> (N=3)			<i>Vibrio vulnificus</i> (N=2)			<i>Vibrio cholerae</i> non-O1 (N=4)		
		R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Group I- Inhibitors of cell wall synthesis										
Ampicillin	10	3(100)	0(0.0)	0(0.0)	1(50.0)	1(50.0)	0(0.0)	4(100)	0(0.0)	0(0.0)
Amoxicillin	10	3(100)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	1(50.0)	3(75.5)	0(0.0)	1(25.0)
Cephalexin	30	1(33.3)	1(33.3)	1(33.3)	0(0.0)	1(50.0)	1(50.0)	1(25.0)	1(25.0)	2(50.0)
Group II - Inhibitors of protein synthesis										
Gentamicin	30	0(0.0)	0(0.0)	3(100)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)	4(100)
Erythromycin	15	0(0.0)	1(33.3)	2(66.7)	0(0.0)	1(50.0)	1(50.0)	1(25.0)	1(25.0)	2(50.0)
Chloramphenicol	30	0(0.0)	0(0.0)	3(100)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)	4(100)
Tetracycline	10	2(66.7)	0(0.0)	1(33.3)	1(50.0)	1(50.0)	0(0.0)	3(75.5)	0(0.0)	1(25.5)
Group III - Inhibitors of nucleic acid synthesis										
Ciprofloxacin	10	1(33.3)	0(0.0)	2(66.7)	0(0.0)	1(50.0)	1(50.0)	0(0.0)	1(25.0)	3(75.0)
Norfloxacin	10	0(0.0)	1(33.3)	2(66.7)	1(50.0)	1(50.0)	0(0.0)	1(25.0)	0(0.0)	3(75.0)
Sulphamethoxazole	25	2(66.7)	0(0.0)	1(33.3)	1(50.0)	0(0.0)	1(50.0)	2(50.0)	1(25.0)	1(25.0)

KEY: Ampicillin- AMP, Amoxicillin -AML, Cephalexin -CL, Gentamicin-CN, Erythromycin-E, Chloramphenicol -C, Tetracycline -TE, Ciprofloxacin-CIP, Norfloxacin -NOR, Sulphamethoxazole-SXT, N: Number of isolates tested, R: Resistant, I: Intermediate, S: Susceptible

Table 3: Antibiotic resistance profiles of *Vibrio* species isolated from soil in landfills at various sampling locations

Isolate Code	<i>Vibrio</i> species isolates	MARI	Antimicrobial Resistance Patterns	Resistance classification
SG 04	<i>Vibrio cholerae</i> non-O1	0.4	AMP, AML, TE, NOR	MDR
SG 21	<i>Vibrio parahaemolyticus</i>	0.5	AMP, AML, CL, TE, SXT	MDR
SA 13	<i>Vibrio vulnificus</i>	0.3	AML, TE, SXT	NMDR
TW 02	<i>Vibrio parahaemolyticus</i>	0.4	AMP, AML, TE, SXT	MDR
TW 09	<i>Vibrio vulnificus</i>	0.2	AMP, NOR	NMDR
TW 17	<i>Vibrio cholerae</i> non-O1	0.1	AMP	NMDR
TW 26	<i>Vibrio cholerae</i> non-O1	0.6	AMP, AML, CL, E, TE, SXT	MDR
ZC 12	<i>Vibrio parahaemolyticus</i>	0.3	AMP, AML, CIP	NMDR
ZC 23	<i>Vibrio cholerae</i> non-O1	0.4	AMP, AML, TE, SXT	MDR

KEY: SG = Sabon-Gari, SA = Samaru, TW = Tudun-Wada, ZC = Zaria city, MDR - Multidrug resistance, NMDR- Non Multidrug resistance. MARI = Multiple Antibiotic Resistance Index, Ampicillin- AMP, Amoxicillin -AML, Cephalexin -CL, Gentamicin-CN, Erythromycin-E, Chloramphenicol -C, Tetracycline -TE, Ciprofloxacin-CIP, Norfloxacin -NOR, Sulphamethoxazole-SXT, N: Number of isolates tested, R: Resistant, I: Intermediate, S: Susceptible

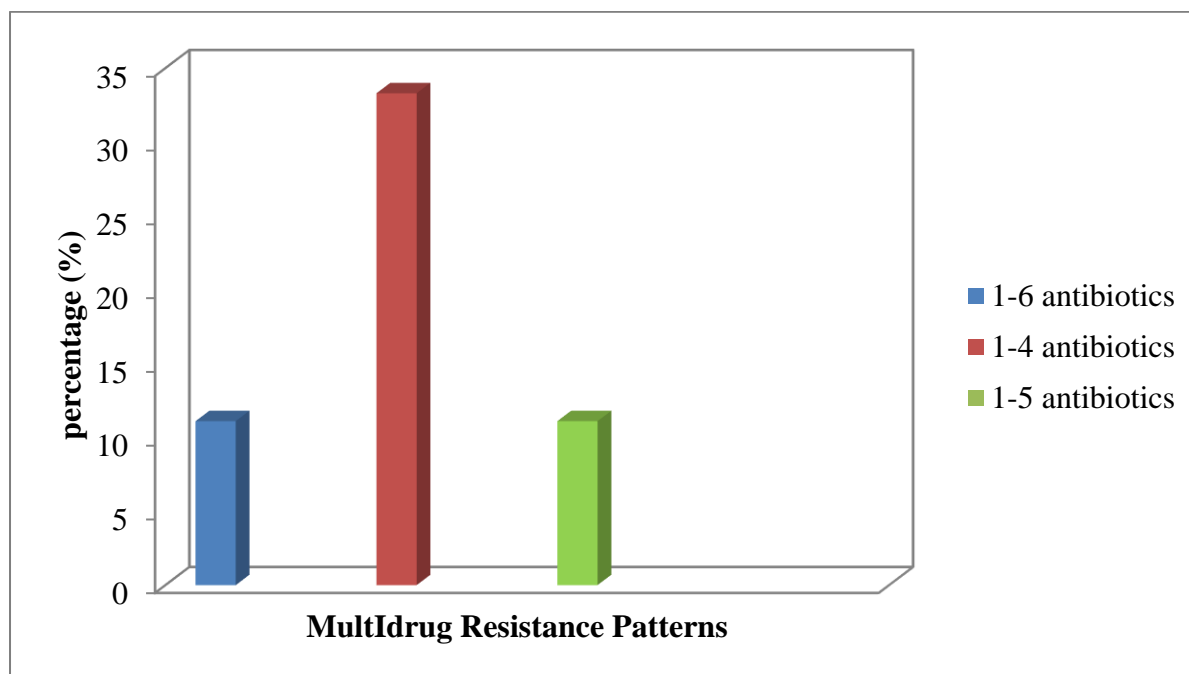


Figure 1: Percentage of Multidrug Resistance of *Vibrio* species isolates

DISCUSSION

The study revealed a prevalence of *Vibrio* species, including *Vibrio parahaemolyticus* (2.50%), *Vibrio vulnificus* (1.67%) and *Vibrio cholerae* non-O1 (3.33%), with an overall prevalence of 7.50%. This finding aligns with previous research indicating the widespread distribution of *Vibrio* species in various environmental niches, including soil and water (Odeyemi *et al.*, 2020). The occurrence of these pathogenic Gram-negative bacteria isolated and identified suggests a significant sanitary risk, especially to waste workers, scavengers, and those living close to the landfills. Their presence may be due to fecal contamination of landfills by humans or animals. Moreover, identifying *Vibrio cholerae* non-O1 as the most prevalent species (3.33%) underscores its significance as a potential environmental pathogen, even outside of epidemic contexts (Elmahdi *et al.*, 2016). Other researchers like Nyandjou *et al.* (2018), and Ossei and Ducar, (2008) also observed that most *Vibrio cholerae* strains in the environmental reservoir belong to the non-O1/non-O139 serogroups. The pathogen has an extraordinary capability to adapt to different environments, although often associated with fecal pollution.

There was variation in the incidence of *Vibrio* species among sampling locations, with the Tudun-Wada location showing a notably high incidence of 13.33%. This suggests that environmental factors or waste composition in

that area may favor the proliferation of *Vibrio* species. Bier *et al.* (2015) also reported that proximity to anthropogenic activities and waste disposal practices could contribute to higher *Vibrio* species concentrations in specific areas.

All isolates showed 100% susceptibility to Gentamicin and Chloramphenicol, indicating that these antibiotics remain effective against the *Vibrio* species in the study area. This aligns with previous studies where the MDR bacteria obtained from environmental samples showed 0% resistance to Chloramphenicol, Cefotaxime, and Gentamicin (Olukosi *et al.*, 2016). However, resistance was observed in varying degrees against other antibiotics tested, with the highest resistance observed against Ampicillin (88.9%), followed by Amoxicillin (77.78%), Tetracycline (66.67%), and Sulphamethoxazole (55.56%). The marked resistance of *Vibrio* strains to the inhibitors of cell wall synthesis (β -lactams) and Tetracycline, as shown in the present study, agrees with the findings of Ash *et al.* (2002), Gregory *et al.* (2013) working on rivers in the United States who reported over 80% resistance of their isolates to Ampicillin (10 μ g) and 86% resistance to Tetracycline (10 μ g). Similarities in resistance among isolates from both environmental sources indicate a possible infiltration of pathogens from landfill soils to water sources. These antibiotics are commonly used to prevent diseases in human beings and animals. Therefore, *Vibrio* species entering into the soil with human or animal faeces with antibiotic-resistant plasmids might have

contributed to the prevalence of gene resistance in the landfill soil environment.

The isolates were 66.7% susceptible to Ciprofloxacin. High susceptibility levels to the newer, more valuable antimicrobial compounds, such as phenicols, aminoglycosides, and fluoroquinolones (Ciprofloxacin), could yield good therapeutic results in treating infections caused by MDR *Vibrio species*.

The observed high resistance of the isolates to some of the antibiotics in this study is not surprising as factors that could promote or encourage the development of antibiotic resistance, such as non-adherence to treatment strategies, sub-standard drugs, over-the-counter sale of potent antibiotics and self-medication, are present in developing countries including Nigeria. Therefore, monitoring antibiotic resistance among *Vibrio species* isolates from landfill soils is important to detect emerging resistant pathogens and antibiotic resistance trends.

Identifying multidrug-resistant *Vibrio* isolates, with 55.56% exhibiting resistance to 4-6 antibiotics (Figure 1), underscores the complexity of antimicrobial resistance dynamics in environmental reservoirs. The presence of *Vibrio cholerae* non-O1 with the highest phenotypic resistance profile (AMP, AML, CL, E, TE, SXT) aligns with the findings of Lazarus *et al.* (2015), who reported that a specific *Vibrio species* could have the potential to acquire and disseminate resistance genes, posing significant challenges for antibiotic stewardship efforts. Moreover, the percentage of isolates displaying multidrug resistance further emphasizes the urgent need for integrated surveillance and intervention strategies to mitigate the spread of MDR *Vibrio* strains (Baker-Austin *et al.*, 2017).

Multidrug resistance is a cause for concern in both clinical and veterinary medicine because it limits the therapeutic options available for treatment.

CONCLUSION

The prevalence of *Vibrio species* isolated from soil in landfills in Zaria was 7.5%. There was variation in the incidence of *Vibrio species* among sampling locations, with the Tudun-Wada location showing a notably high incidence of 13.33%. All isolates showed susceptibility to Gentamicin and Chloramphenicol. However, resistance was observed in varying degrees against other antibiotics tested, with the highest

resistance observed against Ampicillin (88.89%), followed by Amoxicillin (77.78%), Tetracycline (66.67%), and Sulphamethoxazole (55.56%). Erythromycin and Ciprofloxacin displayed lower resistance rates (11.11%). *Vibrio cholerae* non-O1 demonstrated the highest levels of drug resistance, particularly to Ampicillin (100%) and Tetracycline (75.5%), and exhibited MDR of 60%. Five isolates (55.56%) were identified as multidrug-resistant. These MDR isolates showed resistance to 4-6 antibiotics, with *Vibrio cholerae* non-O1 exhibiting the highest phenotypic resistance profile, including resistance to Ampicillin, Amoxicillin, Chloramphenicol, Erythromycin, Tetracycline, and Sulphamethoxazole. The percentage of isolates displaying multidrug resistance was 11.11% for resistance to five and six antibiotics each and 33.33% for resistance to four antibiotics. This finding suggests a concerning level of antimicrobial resistance within these *Vibrio* populations, highlighting the need for further investigation and potential intervention strategies to mitigate the spread of resistant strains.

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