JOURNAL OF SCIENCE, TECHNOLOGY AND EDUCATION (JSTE)

A PUBLICATION OF THE
DEPARTMENT OF SCIENCE,
TECHNOLOGY & MATHEMATICS
EDUCATION (STME),
NASARAWA STATE UNIVERSITY, KEFFI

VOLUME
9

ISSN: 2651-5539

JOURNAL OF SCIENCE, TECHNOLOGY AND EDUCATION (JSTE) VOL. 9. NO. 16

JSTE PUBLICATIONS, 2024 www.nsukjste.com/

ISSN: 2651-5539

ANTIBIOGRAM AND DETECTION OF HIGH-MOLECULAR-WEIGHT-PLASMIDS IN HEAVY-METAL RESISTANT BACTERIA ISOLATED FROM WOOD-WASTE SITES (SAW DUST DEPOSITS FROM WOOD INDUSTRY) IN DELTA STATE

Ogbe K.[a,b], Igere, B. E. [a,b*], Egbule O. S.[a,b], Owhe-Urheghe U. B. [a,b]

[a] Department of Microbiology Delta State University, Abraka, Delta State, Nigeria
[b] Biotechnology and Emerging Environmental Infections Pathogens Research Group (BEEIPREG), Department of Microbiology, (Biotechnology Unit) Delta State University, Abraka, Delta State, Nigeria.
Corresponding Email: bright-igere@delsu.edu.ng

Citation: Ogbe K., Igere, B. E., Egbule O. S., Owhe-Urheghe U. B. (2025). Antibiogram and Detection of High-Molecular-weight-Plasmids in Heavy-Metal Resistant Bacteria Isolated from wood-waste Sites (Saw Dust Deposits from Wood Industry) in Delta State. *Journal of Science, Technology, and Education (JSTE)*; www.nsukjste.com/ *9*(16), 208-223.

Abstract

The menace of plasmids continues to arouse research interest especially as it is harbored by diverse bacterial strains in unpredictable nexus. This study was designed to investigate the antibiogram and presence of High-Molecular-Weight-plasmids (HMW-Plasmids) in heavy metal resistant bacteria in saw-dust dump sites in some wood industries in Sapele, Delta State, Nigeria. A total of ten (10) samples were collected from about 10cm depth in different locations of sawmills in Sapele, Delta State. Plasmid analysis and antibiotic-heavy metal resistance was tested by subjecting recovered isolates to the following concentration of the heavy metals: 0.302mM, 0.604mM, 0.906mM and 1.2mM of Pb; 0.515mM, 1.03mM, 1.54mM and 2.06mM of Cr and 0.435mM, 0.87mM, 1.304mM and 1.74mM of Cd for both pre- and post-curing respectively. A total of seven (7) bacteria species comprising of four (4) Gram positive (*Staphylococcus aureus*, *Bacillus cereus*, *Cellulomonas* sp. and *Micrococcus* sp.) and three (3) Gram negative bacteria (*Salmonella* sp., *Pseudomonas aeruginosa* and *Escherichia coli*) species were isolated. The heavy metals resistance of the isolates was observed to decrease with increasing concentration of the heavy metals. A total of sixteen (16) multi drug resistances (MDR – resistance to three (3) or more classes of antibiotics) of the isolates were also observed. However, after the isolates were cured of plasmids, the isolates became susceptible to all the tested concentrations of Pb, Cr and Cd. Antibiotics resistance still persist in some of the isolates (*Pseudomonas* sp., *Bacillus* sp. and *Salmonella* sp.), but these observed responses were far less compared to those before the isolates were cured of plasmids, indicating that the antibiotics and heavy metal resistance observed among strains was plasmid mediated. The isolation of antibiotics-heavy metal resistant *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Micrococcus* sp., *Salmonella* sp., *Staphylococcus aureus* and *Cellulomonas* sp. is of utmost health importance as these bacteria may pose serious threat to public health, especially to those working in the wood processing industries in Delta State, Nigeria. It is therefore suggested that, efforts should be made by all workers in these wood industries to put on inter-personal protective equipment's during work hours. The government should also develop policy on the management of such wood wastes release to void spread of such strains in diverse nexus

Keywords: High-Molecular-Weight-plasmids; sawdust dump site; heavy metal; bacterial strains

Introduction

Bacteria have evolved adaptive mechanisms that enable them to survive in environments contaminated with diverse chemical and contagious toxic heavy metals. This resistance is often attributed to prolonged exposure in polluted habitats, including those contaminated by industrial and anthropogenic activities (Ayangbenro & Babalola, 2017; Igere *et al.,* 2020a; Singh *et al.,* 2021). Heavy metals such as chromium (Cr), nickel (Ni), copper (Cu), cadmium (Cd), and lead (Pb) are non-biodegradable and persist in the environment, where they can accumulate to toxic levels in microbial cells, disrupting vital processes such as enzyme activity and membrane integrity (Adedayo *et al*., 2020; Zhang *et al*., 2022).

Although trace quantities of some metals (e.g., Cu and Zn) are essential for bacterial metabolism, elevated concentrations are toxic. Bacteria residing in metal-polluted soils and water bodies often develop resistance via chromosomal mutations and horizontal gene transfer, frequently mediated by plasmids that carry metal resistance genes (MRGs) (Bharagava *et al*., 2020; Onwosi *et al.,* 2022). These plasmid-borne genes facilitate survival under metal stress and make such microbes promising candidates for bioremediation (Akinola & Onibere, 2021 Khan *et al.,* 2023).

Plasmid curing is a method employed to establish the role of plasmids in metal resistance. This involves the use of agents such as acridine orange, ethidium bromide, ultraviolet radiation, or elevated temperatures to eliminate plasmids from bacterial cells (Liu et al., 2019; Eze et al., 2021). If resistance traits are lost after curing, it implies a plasmid-mediated mechanism. Interestingly, metal resistance is often linked to antibiotic resistance through co-selection mechanisms, whereby resistance genes for both stressors are co-located on the same genetic elements (Seiler & Berendonk, 2012; Ashraf *et al.,* 2022). This phenomenon has significant public health implications, especially in agricultural and industrial settings, where metals and antibiotics are concurrently present (Zhu *et al*., 2013; Igbinosa *et al*., 2020).

Sawdust, a by-product of timber processing, comprises fine wood particles generated during cutting, milling, and sanding. It is widely available and has numerous industrial applications ranging from fuel and insulation to soil amendment and water treatment (Akinwekomi *et al*., 2020). Due to its porosity and chemical composition, sawdust exhibits adsorptive properties, particularly for heavy metals like lead and cadmium (Tan *et al*., 2019; Okoye & Ogbonna, 2021). Furthermore, sawdust can serve as a niche for diverse microbial populations, including those capable of degrading xenobiotics or tolerating toxic environments (Nassauer *et al*., 2011; Hassan *et al*., 2023).

Given its ecological and industrial relevance, this study investigates the presence of plasmids in heavy metal-resistant bacteria isolated from sawdust deposits in wood industries in Delta State, Nigeria. The study also assesses the correlation between plasmid presence and resistance to both antibiotics and heavy metals.

Materials and Methods

A total of ten (10) samples were collected from five wood industries (sawmills) in Sapele, Delta State, Nigeria. Samples were collected using sterile black polythene bag and was immediately transported to department of Microbiology Laboratory, Delta State University for analysis.

The samples were subjected to a 10-fold serial dilution. Using sterile pipette, ninety millilitres of distilled water was transferred into 10 sterile test tubes. Ten millilitre of the liquefied sample was pipetted into the first test tube containing ninety millilitres of sterile water (10^{-1} dilution). The serial dilution continued until 10^{-10} was reached for the sample (Cheesbrough, 2004) .

One millilitre of the diluent from 10^{-3} was pipetted into sterile petri dishes and while maintaining appropriate aseptic measure, the molten agar was dispensed into the petri dishes containing the diluent and allowed to solidify. The process is known as pour plate method. Once solidified, the plates were incubated at 37 °C for 24hrs for bacteria growth. Isolates from culture after 24hrs of incubation at 37°C were identified using cultural characteristics and biochemical tests reported by Cheesbrough (2004). The isolates were identified by subjecting them to gram staining and biochemical methods. Some of the isolates that was identified are *Escherichia* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Salmonella* sp., *Staphylococcus* sp. and *Cellulomonas* sp. (Igere *et al*., 2021;2023; 2024)

Plasmid Curing of Bacteria Isolates

Plasmid curing was performed to determine the role of plasmids in the resistance characteristics of the bacterial isolates. The curing agent used was acridine orange, prepared by dissolving 1 g of acridine orange in 10 ml of ascorbic acid and suspending the mixture in 990 ml of distilled water. The solution was shaken vigorously to ensure homogeneity. This method followed the protocol described by (Cheesbrough, 2009), with minor modifications for increased efficacy.

The bacterial isolates were first suspended in 5 ml of normal saline and gently shaken to obtain uniform suspension. After resting for 2–3 minutes, 3 ml of the suspension was aseptically transferred into test tubes containing 2 ml of the acridine orange solution. The mixture was then placed in a shaker incubator for 2–4 hours to enhance interaction between the cells and the curing agent. Following this step, the cultures were incubated at 37 °C for 18–24 hours. The principle behind plasmid curing is the ability of intercalating agents like acridine orange to disrupt plasmid replication without affecting chromosomal DNA. Once the plasmids are removed, any corresponding loss in antibiotic or heavy metal resistance can be assessed by subjecting the cured isolates to susceptibility testing. This provides evidence on whether the resistance traits are plasmid-mediated.

Recent studies have supported the effectiveness of acridine orange in plasmid elimination. For instance, (El-Naggar *et al*., 2020) demonstrated that treatment with acridine orange led to successful loss of resistance plasmids in multidrug-resistant *Escherichia coli* strains isolated from wastewater. Similarly, (Adeyemo *et al*., 2021) conducted plasmid curing of soil bacteria in Nigeria and established a significant reduction in heavy metal resistance after treatment, indicating plasmid-borne resistance traits.

This procedure remains critical in assessing the environmental and clinical implications of mobile genetic elements. The ability to cure plasmids and monitor phenotypic changes provides insight into resistance mechanisms, as plasmids are often responsible for horizontal gene transfer and the spread of resistance genes in environmental bacteria (Al-Ani *et al*., 2022; Nnaji *et al.,* 2023).

Determining the antimicrobial activity of the isolates

The test revealed that the bacteria isolates showed resistance to some antibiotics, while some bacteria isolates were resistance to all the tested antibiotics, where we have organism like *Pseudomonas* sp. Details of antibiotics resistance will be represented in tables for the gram-positive isolates (*Staphylococcus* sp., *Bacillus* sp., *Cellulomonas* sp. and *Micrococcus* sp.) and gram-negative isolates (*Salmonella* sp., *Pseudomonas* sp. and *Escherichia* sp.) alongside with the multi-resistance profile before and after plasmid curing for organisms obtained from saw dust dump sites. (Igere *et al.,* 2020b; CLSI*,* 2015).

Determining the heavy metal activity of the isolates

Test for the resistance of the isolates to varying concentrations (0.302mM, 0.604mM, 0.906mM and 1.2mM for Lead (Pb); 0.515mM, 1.03mM, 1.54mM and 2.06mM for Chromium (Cr) and 0.435mM, 0.87mM, 1.304mM and 1.74mM for Cadmium (Cd) showed high level of heavy metal resistance. Although, *Escherichia* sp. was completely inhibited at the highest concentration of the heavy metals (1.2mM for Pb, 2.06mM for Cr and 1.74 for Cd), the other isolates (except *Micrococcus* sp.) still showed significant resistance most notably *Pseudomonas* sp. and *Bacillus* sp.

Statistical analysis

Results of the study were presented in tables and figures which are developed by applying excel spread sheet while antibiotic-heavy metal resistance patterns were measured and recorded in percentages, as multiple antibiotic resistance (MAR) index for both before and after plasmid analysis was recorded and assessed using excel spread sheet.

Results

Table 1: Mean Total Heterotrophic Bacterial Counts for Sampled Wood-Dust in Sapele

Months	**Total Heterotrophic Bacterial Counts (x 10^{-5}cfu/ml)**				
	A	**B**	**C**	**D**	**E**
AUG, 2024	10.02 ± 0.17	22.89± 1.27	11.04± 0.12	10.96±0.42	3.40±0.04
SEPT 2024	11.05 ±0.05	19.45±0.63	10.40±0.01	11.02±0.06	3.60±0.11
Oct, 2024	10.64 ±0.19	24.85±0.35	10.38 ±0.06	13.00±0.22	4.70±0.02

KEYS:
Sapele Nexus

Tables 1 shows the total heterotrophic bacterial count of recovered strains in saw-dust samples taken from dump sites in some wood industries in Sapele, Delta State, Nigeria during study period (2024). A highest mean total heterotrophic count during the sample period (24.85±0.35) in site B was recorded, whereas site E had the lowest colonial count (4.70±0.02). Site A, C and D had a mean total heterotrophic count similar in range and number (10.64 ±0.19), (10.38 ±0.06) and. (13.00±0.22)

Table 2: Cultural Morphology and Biochemical Characterisation of Bacteria Isolates

Shape	**Gram**	**Catalase**	**Oxidase**	**Citrate**	**Indole**	**Urease**	**Motility**	**Glucose**	**Sucrose**	**Lactose**	**Acid**	**Gas**	**H_2S**	**Tentative Bacteria Genera**
Rod	-	+	+	-	-	-	+	-	-	-	+	+	-	*Pseudomonas aeruginosa*
Rod	+	+	+	+	-	-	+	+	+	-	+	-	-	*Bacillus cereus*
Rod	-	+	-	-	+	-	+	+	-	+	+	+	-	*Escherichia coli.*
Cocci	+	+	-	+	+	+	-	+	+	+	+	-	-	*Staphylococcus aureus* .
Rod	-	+	-	-	-	-	+	+	-	-	+	-	+	*Salmonella* sp.
Rod	+	+	-	-	+	+	+	+	+	-	+	+	-	*Cellulomonas* sp.
Cocci	+	-	-	-	-	+	-	+	-	-	+	+	-	*Micrococcus* sp.

A total of twenty-two bacteria strains among seven (7) bacteria species comprising of four (4) Gram positive (*Staphylococcus aureus*, *Bacillus cereus*, *Cellulomonas* sp. and

Micrococcus sp.) and three (3) Gram negative bacteria (*Salmonella* sp., *Pseudomonas aeruginosa* and *Escherichia coli*) species were isolated (Tables 2).

Table 3: Prevalence of the Identified Bacteria Species in the Study area

Bacteria Isolates	Numbers of Isolates	Rate of Occurrence
Pseudomonas aeruginosa	5	22.73%
Bacillus cereus	3	13.64%
Escherichia coli	6	27.27%
Staphylococcus aureus	2	9.09%
***Salmonella* sp.**	2	9.09%
***Cellulomonas* sp.**	3	13.64%
***Micrococcus* sp.**	1	4.55%
Total	**22**	**100%**

Table 3:shows the prevalence of the Identified Bacteria Species in the Study area as among the total of twenty-two bacteria strains *Bacillus cereus*, was most prevalent of four (4) Gram positive and *Escherichia coli* was most prevalent of three (3) Gram negative bacteria species (Tables 3).

Table 4a: Antibiotics Zone of Inhibition (diameter in mm) of Gram-Positive Isolates Tested

ISOLATES (No.)	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
***Staphylococcus aureus* (2)**	26	18	10	10	10	16	22	22	24	26
***Bacillus* sp. (3)**	22	16	0	10	10	12	26	18	14	12
***Cellulomonas* sp. (3)**	26	18	10	10	10	14	26	18	16	20
***Micrococcus* sp. (1)**	0	11	10	10	10	10	30	16	16	16
Total (9)										

KEY: PEF = Pefloxacin, CN = Gentamycin, APX = Amplicox, Z = Zinnacef, AM = Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin.

Table 4b: Interpretation of Inhibition Zone for Gram-Positive Isolates to Tested Antibiotics

ISOLATES (No.)	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
***Staphylococcus aureus* (2)**	S	S	R	R	R	S	S	S	S	S
***Bacillus* sp. (3)**	S	S	R	R	R	R	S	S	S	R
***Cellulomonas* sp. (3)**	S	S	R	R	R	S	S	S	S	S
***Micrococcus* sp. (1)**	R	R	R	R	R	R	S	S	S	S
Total (9)										

KEY: S = Sensitive, R = Resistance

The Table 4a show antibiotics zone of inhibition (diameter in mm) of Gram-positive isolates tested while Table 4b: shows interpretation of inhibition zone for Gram-positive isolates to tested antibiotics

Table 5a: Antibiotics Zone of Inhibition (diameter in mm) of Gram-negative Isolates Tested

ISOLATES (No.)	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
***Escherichia coli* (6)**	10	18	22	20	10	10	18	22	24	10
***Salmonella* sp. (2)**	20	20	22	24	20	14	20	28	24	22
***Pseudomonas aeruginosa* (5)**	10	12	10	10	12	10	10	10	12	10
Total (13)										

KEY: PEF = Pefloxacin, CN = Gentamycin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Trimethoprim-sulphamethaxazole, CH = Chloramphenicol, SP = Sparfloxacin, AM = Amoxacillin, AU = Amoxicillin/clavulanate, OFX = Ofloxacin.

Table 5b: Interpretation of Inhibition Zone for Gram-negative Isolates to Tested

ISOLATES (No.)	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
***Escherichia coli* (6)**	S	S	R	R	R	S	S	S	R	S
***Salmonella* sp. (2)**	S	S	S	S	S	S	S	S	S	S
***Pseudomonas aeruginosa*. (5)**	R	R	R	R	R	R	R	R	R	R
Total (13)										

KEY: S = Sensitive, R = Resistance

The Table 5a shows: antibiotics zone of inhibition (diameter in mm) of Gram-negative isolates tested while Table 5b: shows interpretation of inhibition zone for Gram-negative isolates to tested

Table 6: Multi drug resistance profile

ISOLATES (NO)	MDR NO (%)
***Bacillus cereus* (3)**	3(100.0)
***Staphylococcus aureus* (2)**	2(100.0)
***Cellulomonas* sp. (3)**	3(100.0)
***Micrococcus* sp. (1)**	1(100.0)
***Escherichia coli* (6)**	2(33.33)
***Pseudomonas* sp. (5)**	5(100.0)
TOTAL = 20	16(80)

Isolates were observed to be resistant to more than six (6) antibiotics indicating multi-drug resistances (MDR – resistance to three (3) or more classes of antibiotics) of the isolates (Table 6).

Table 7a: Resistance of Isolates to Varying Concentrations of Lead (Pb)

Isolates	Metal concentration (mM)			
	0.302	0.604	0.906	1.2
Escherichia coli	R	R	I	S
Cellulomonas sp.	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R
Micrococcus sp.	R	R	I	S
Staphylococcus aureus	R	R	I	S
Salmonella sp.	R	R	R	R
Bacillus cereus	R	R	R	R

KEY: R = Resistant; I = Intermediate; S = Sensitive

Table 7b: Resistance of Isolates to Varying Concentrations of Chromium (Cr)

Isolates	Metal concentration (mM)			
	0.515	1.03	1.54	2.06
Escherichia coli	R	R	I	S
Cellulomonas sp.	R	R	R	S
Pseudomonas aeruginosa	R	R	R	R
Micrococcus sp.	R	R	I	S
Staphylococcus aureus	R	R	R	I
Salmonella sp.	R	R	R	I
Bacillus cereus	R	R	R	R

KEY: R = Resistant; I = Intermediate; S = Sensitive

Table 7c: Resistance of Isolates to Varying Concentrations of Cadmium (Cd)

Isolates	**Metal concentration (mM)**			
	0.435	0.87	1.304	1.74
Escherichia coli	R	R	S	S
***Cellulomonas* sp.**	R	R	I	I
Pseudomonas aeruginosa	R	R	R	I
***Micrococcus* sp.**	R	R	I	I
Staphylococcus aureus	R	R	I	I
***Salmonella* sp.**	R	R	R	R
Bacillus cereus	R	R	R	I

KEY: R = Resistant; I = Intermediate; S = Sensitive

The Table 7a: shows resistance of isolates to varying concentrations of Lead (Pb), Table 7b: shows resistance of isolates to varying concentrations of Chromium (Cr) and Table 7c: shows resistance of isolates to varying concentrations of Cadmium (Cd). However, after the isolates were cured of plasmids, the isolates became susceptible to all the tested concentrations of Pb, Cr and Cd. After the isolates were cured of plasmid, the resistance to antibiotics was again tested to ascertain the level of antibiotics resistance. Results obtained revealed that antibiotics resistance showed by the isolates were plasmid mediated as most of the isolates became significantly susceptible to the tested antibiotics.

After the isolates were cured of plasmid, their resistance to the three metals (Pb, Cr and Cd) was again tested using the same concentrations as before and it shows that all the isolates lost their resistance capability, indicating that the resistance to the heavy metals were plasmids born.

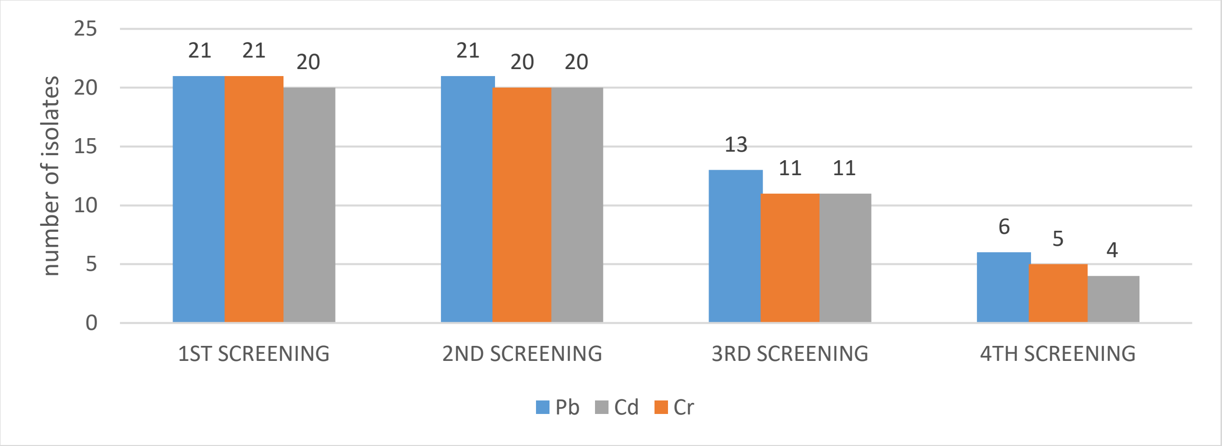

Figure 1: Number of resistant bacteria isolates in the 1st, 2nd, 3rd and 4th screenings using different concentration of heavy metals. 5th and 7th screening was after plasmid curing

Discussion

Microbial ubiquity has recently found relevance in sawdust particles as related environmental waste release investigators continues to explore diverse environmental milieu. Sawdust particles in many wood-processing industries have been shown to harbor diverse bacterial strains with some of them acquiring extra-chromosomal nucleic acids (plasmid-bearing bacteria). Some of these plasmid-born strains harbor heavy metals resistant markers, both of which may be hazardous when contract with humans occurs. Such exposure possesses the potential of eliciting significant biological public health implications. This study focused on detecting plasmids in heavy-metal-resistant bacterial species isolated from sawdust deposits in selected wood industries in Delta State, Nigeria.

A total of twenty-two (22) bacterial isolates were recovered from sampled sawdust from wood industrial waste dump sites. The strains were shown to be multidrug-resistant (MDR) isolates—defined as resistant to three or more classes of antibiotics—were identified in this study. *Pseudomonas aeruginosa* showed resistance to all antibiotics tested. The high resistance of *Pseudomonas aeruginosa* may be attributed to its possession of multiple resistance mechanisms, including low outer membrane permeability, production of antibiotic-inactivating enzymes, and a wide range of efflux pumps and porins that actively exclude antimicrobial agents. These findings are consistent with earlier observations by Walsh, (2003); and Lambert, (2002) and are further corroborated by (Ahmed *et al.* 2021), who observed that *Pseudomonas aeruginosa* isolates from

Nigerian hospital environments exhibited multidrug resistance due to similar adaptive mechanisms.

The detection of resistant isolates such as *Pseudomonas aeruginosa*, *Bacillus sp.*, and *Salmonella sp.* in sawdust poses a serious public health concern. Sawdust is often used for various domestic and agricultural purposes—including its use as cooking fuel, animal bedding, and mulch—thus increasing the risk of human and animal exposure to hazardous contaminants. Eze and Akinbobola (2020) reported similar findings in their study of bacterial load in sawdust from Nigerian markets, highlighting the role of these particles in the transmission of pathogenic bacteria and toxic substances.

Heavy metals such as lead (Pb), chromium (Cr), and cadmium (Cd), which are known to be toxic and carcinogenic, were also detected. These metals are often dispersed through air from dumpsites and can accumulate in the atmosphere. Upon inhalation or contact, they may disrupt biological functions and accumulate in organs such as the brain, liver, and kidneys. This aligns with the findings of (Jaishankar *et al.,*2014), who documented the toxicological profiles of heavy metals and their implications on organ function, including mental disorders and immune dysfunction.

As heavy metal concentrations increased from 0.302 mM to 1.2 mM for Pb, 0.515 mM to 2.06 mM for Cr, and 0.435 mM to 1.74 mM for Cd, bacterial resistance declined significantly. For instance, *Escherichia coli.* showed complete inhibition at the highest concentration of each metal. Similarly, *Micrococcus sp.* exhibited minimal growth at high Cd concentration and complete inhibition at elevated levels of Pb and Cr. These results correspond with those of (Dinu *et al*., 2011), who reported a decrease in bacterial survival rates with increased heavy metal concentrations in environmental samples.

Interestingly, some isolates retained resistance even at higher concentrations of heavy metals. Molecular analysis revealed that heavy metal resistance genes were plasmid-borne. This finding is alarming due to the plasmid's ability to horizontally transfer resistance traits between bacterial species. This observation supports the reports of (Smith & Romesberg, 2007; Boucher *et al*., 2009), who independently demonstrated the widespread presence of plasmids in bacteria and their role in disseminating resistance genes. In Nigeria, (Olowe *et al*., 2022) observed similar plasmid-mediated heavy metal and antibiotic resistance in *Enterobacteriaceae* isolated from contaminated river sediments, confirming that plasmids remain key drivers of environmental resistance propagation.

Conclusion

The diversity of bacterial strains isolated from selected study wood dust mill and their potential environmental implications in the metropolitan studied nexus indicates that such sites are possible reservoir of the strains. Such resistant plasmids to both heavy metal and antibiotics remain potential threat to environmental wellness and public health and may have been horizontally acquired from other environmental strains. This finding further underscores the public health risk associated with sawdust contact and/or use and exposure in Delta State. The presence of plasmid-harboring, multidrug-resistant, and heavy-metal-tolerant bacteria in sawdust necessitates stricter monitoring and regulation of sawdust disposal and use in wood-processing environments.

Acknowledgement

Special appreciation to all the post graduate student of the department of microobiology and biotechnology for their regular encouragement in continuing with studies. We will not fail to mention the support of the laboratory staff of the department. The study was funded by individual funds

Conflict of interest

Authors declared non

References

Adedayo, M. R., Adebayo, A. O. and Aladejana, A. V. (2020). Heavy metal tolerance and biosorption potential of bacteria isolated from industrial effluent. *African Journal of Environmental Science and Technology,* ***14***(4), 108–118.

Adeyemo, A. O., Akinlabi, B. A. and Fawole, O. B. (2021). Plasmid curing of heavy metal resistant bacteria isolated from auto-mechanic workshop soils in Lagos, Nigeria. *Nigerian Journal of Microbiology*, **35**(2), 104–112.

Ahmed, M., Bello, M. and Yusuf, U. (2021). Multidrug-resistant *Pseudomonas aeruginosa* isolates from clinical and environmental sources in Nigeria. *African Journal of Clinical and Experimental Microbiology*, **22**(1), 45–52.

Akinola, M. O. and Onibere, B. J. (2021). Plasmid-mediated resistance in bacteria from metal-contaminated soil. *Journal of Applied Sciences and Environmental Management,* ***25***(1), 69–76.

Akinwekomi, V. A., Oseni, S. O. and Oladipo, I. C. (2020). Utilization of wood sawdust for the removal of lead (Pb2+) from aqueous solutions. *Journal of Environmental Chemical Engineering,* ***8***(5), 104-109.

Al-Ani, L. A., Jasim, S. A. and Al-Jebouri, M. M. (2022). Evaluation of acridine orange as a curing agent for plasmid-mediated antibiotic resistance in uropathogenic *E. coli*. *Iranian Journal of Microbiology*, **14**(4), 307–315. https://doi.org/10.18502/ijm.v14i4.10361

Ashraf, M. A., Zahir, Z. A., Asghar, H. N. and Ashraf, S. (2022). Metal resistance and antibiotic resistance in bacteria from tannery effluents. *Journal of Hazardous Materials, 432*, 128-657.

Ayangbenro, A. S. and Babalola, O. O. (2017). A new strategy for heavy metal polluted environments: A review of microbial biosorbents and biosorption processes. *Environmental Sustainability, 1*, 1–16. https://doi.org/10.1007/s42398-017-0006-y

Bharagava, R. N., Raj, A., Yadav, S. and Saxena, G. (2020). Bioremediation of heavy metals by microorganisms. In R. N. Bharagava (Ed.), *Environmental pollutants and their bioremediation approaches* (**pp**. 1–25). CRC Press.

Boucher, Y., Labbate, M., Koenig, J. E. and Stokes, H. W. (2009). Integrons: Mobilizable platforms that promote bacterial adaptation. *Nature Reviews Microbiology*, **7**(8), 589–599.

Cheesbrough, M. (2009). *District Laboratory Practice in Tropical Countries* (2nd ed.). Cambridge University Press.

Dinu, L. D., Bucur, L. and Petrovici, A. (2011). Heavy metal resistance of bacteria from industrial waste. *Romanian Biotechnological Letters*, **16**(3), 6172–6178.

El-Naggar, N. E., Saber, W. I. and Abd El-Hamid, M. I. (2020). Plasmid curing of multidrug-resistant *E. coli* isolates using intercalating agents. *Scientific Reports*, **10**(1), 20844. https://doi.org/10.1038/s41598-020-77890-7

Eze, M. O. and Akinbobola, A. (2020). Microbial characterization of sawdust in open Nigerian markets: Implications for public health. *Journal of Environmental Science and Public Health*, **4**(2), 147–156.

Eze, M. O., Chukwu, E. E. and Orji, M. U. (2021). Evaluation of antibiotic and heavy metal resistance in bacteria isolated from abattoir effluent in Enugu, Nigeria. *African Journal of Clinical and Experimental Microbiology,* ***22***(1), 47–55. https://doi.org/10.4314/ajcem.v22i1.6

Hassan, I. A., Ibrahim, D. and Olayinka, O. A. (2023). Sawdust as a natural adsorbent for heavy metal removal from contaminated water: A review. *Environmental Advances, 13*, 100322.

Igbinosa, I. H., Igbinosa, E. O. and Okoh, A. I. (2020). Antibiotic and heavy metal co-resistance among bacteria isolated from wastewater. *Environmental Science and Pollution Research, 27*, 35414–35427.

Igere BE Okoh AI, Nwodo UU, Non-serogroup O1/O139 agglutinable *Vibrio cholerae*: a phylogenetically and genealogically neglected yet emerging potential pathogen of clinical relevance, Arch. Microbiol. 204 (6) (2022d) 323, doi:10.1007/s00203-022-02866-1.

Igere BE, Okoh AI, and Nwodo UU. 2022a. Lethality of resistant/virulent environmental vibrio cholerae in wastewater release: An evidence of emerging virulent/antibiotic-resistant-bacteria contaminants of public health concern. Environmental Challenges. 1;7:100504

Igere, B.E., Onohuean, H. and IGBINOSA, E.O., (2023). Polymyxin sensitivity/resistance cosmopolitan status, epidemiology and prevalence among O1/O139 and non-O1/non-O139 Vibrio cholerae: A meta-analysis. *Infectious Medicine*.

Igere BE, Ehwarieme AD, Okolie EC & Gxalo O. (2021) Exogenous and cell-free nucleic acids in water bodies: A penchant for emergence of pandemic and other particulate nucleic acid associated hazards of public health concern in water nexus *Nigerian Journal of Science and Environment*, Vol.19 (2)

Igere BE, Okoh AI, and Nwodo UU. 2022b. Atypical and dual biotypes variant of virulent SA-NAG-Vibrio cholerae: an evidence of emerging/evolving patho-significant strain in municipal domestic water sources. Annals of Microbiology. 72(1):1-13.

Igere BE**,** Okoh AI, Nwodo UU. (2020b). Antibiotic susceptibility testing (AST) Reports: A basis for environmental/epidemiological surveillance and infection control amongst environmental *Vibrio cholerae*., *Int. J. Environ. Res. Public Health.* 17, 5685; doi:10.3390/ijerph17165685.

Igere BE, Onohuean H, Nwodo UU, (2022e) Modern knowledge-scape possess petite influence on the factual persistence of resistance determinants (ARGs/MGEs): a map and assessment of discharged wastewater and water bodies, Heliyon 8 (12) e12253, doi:10.1016/j.heliyon.2022.e12253.

Igere BE, Onohuean H, Nwodo UU, (2022f) Water bodies are potential hub for spatio-allotment of cell-free nucleic acid and pandemic: a pentadecadal (1969-2021) critical review on particulate cell-free DNA reservoirs in water nexus, Bull. Natl. Res. Cent. 46 (1) 56, doi:10.1186/s42269-022-00750-y.

Igere BE,, Onohuean H., and Gxalo O. (2022c).. Occurrence of New Delhi Metallo-Beta-Lactamase 1 Producing Enterococcus Species in Oghara Water Nexus: An Emerging Environmental Implications of Resistance Dynamics. *Microbiology Insights*, *15*, 11786361221133731.

Igere, B.E., Okoh, A.I., Nwodo, U.U. (2020a). Wastewater treatment plants and release: The vase of Odin for emerging bacterial contaminants, resistance and determinant of environmental wellness, *Emerg. Contam.* https://doi.org/10.1016/j.emcon.2020 .05.003.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B. and Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, **7**(2), 60–72.

Khan, M. A., Sajid, M. and Ahmad, N. (2023). Microbial resistance to heavy metals: Mechanisms and biotechnological strategies for remediation. *Environmental Pollution, 311*, 120013.

Lambert, P. A. (2002). Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *Journal of the Royal Society of Medicine*, **95**(Suppl 41), 22–26.

Liu, H., Wang, J., Liu, Y. and Liu, J. (2019). Plasmid-mediated resistance mechanisms of heavy metals in bacteria: A review. *Journal of Hazardous Materials, 365*, 665–673.

Nassauer, S., Whitaker, D. and Wang, L. (2011). The evolving uses of cellulose in the food and packaging industries. *Industrial Biotechnology, 7*(2), 90–93.

Nnaji, C. C., Eze, M. O. and Ajibade, G. A. (2023). Molecular assessment of plasmid-borne antibiotic resistance in *Klebsiella* and *Proteus* species from hospital effluents in South-East Nigeria. *African Journal of Clinical and Experimental Microbiology*, **24**(1), 11–19.

Okoye, B. C. and Ogbonna, C. A. (2021). Adsorptive performance of sawdust for the removal of cadmium and lead from contaminated water. *Global Journal of Environmental Science and Technology, 9*(1), 10–21.

Olowe, O. A., Adekunle, A. A. and Ajayi, A. O. (2022). Plasmid-mediated resistance in bacteria isolated from polluted Nigerian rivers. *Nigerian Journal of Microbiology*, **36**(1), 75–83.

Onwosi, C. O., Igbokwe, N. A. and Ekwealor, I. A. (2022). Bacterial plasmid-mediated tolerance to heavy metals from contaminated soil in Nigeria. *Archives of Environmental Protection, 48*(2), 63–72.

Seiler, C. and Berendonk, T. U. (2012). Heavy metal-driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology, 3*, 399. https://doi.org/10.3389/fmicb.2012.00399

Singh, J. S., Abhilash, P. C., Singh, H. B. and Singh, R. P. (2021). Genomic basis of metal resistance in bacteria and the application of omics tools for the management of metal-contaminated sites. *Frontiers in Microbiology, 12*, 650032.

Smith, P. A. and Romesberg, F. E. (2007). Combating bacteria and drug resistance by inhibiting mechanisms of persistence and adaptation. *Nature Chemical Biology*, **3**(9), 549–556.

Tan, C. X., Nancharaiah, Y. V., van Hullebusch, E. D. and Lens, P. N. (2019). Sawdust for the removal of toxic metals from aqueous solutions: A review. *Bioresource Technology Reports, 7*, 100-206.

Walsh, C. (2003). *Antibiotics: Actions, origins, resistance*. ASM Press.

Zhang, Y., Cheng, M., Yang, J. and Liu, C. (2022). Bacterial resistance to heavy metals and antibiotics in contaminated environments: Molecular insights and biotechnological applications. *Science of The Total Environment, 834*, 155-202.