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Research article

Heavy metal co-resistance with antibiotics amongst bacteria isolates from an open dumpsite soil

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ABSTRACT

Heavy metal co-resistance with antibiotics appears to be synergistic in bacterial isolates via similar mechanisms. This synergy has the potential to amplify antibiotics resistance genes in the environment which can be transferred into clinical settings. The aim of this study was to assess the co-resistance of heavy metals with antibiotics in bacteria from dumpsite in addition to physicochemical analysis. Sample collection, physicochemical analysis, and enumeration of total heterotrophic bacteria counts (THBC) were all carried out using standard existing protocols. Identified bacteria isolates were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion technique and the resulting multidrug resistant (MDR) isolates were subjected to heavy metal tolerance test using agar dilution technique with increasing concentrations (50, 100, 150, 200 and to 250 μg/ml) of our study heavy metals. THBC ranged from 6.68 to 7.92 × 10^5 cfu/g. Out of the 20 isolates subjected to antibiotics sensitivity, 50% (n = 10) showed multiple drug resistance and these were *B. subtilis, B. cereus, C. freundii, P. aeruginosa, Enterobacter* sp, and *E. coli* (n = 5). At the lowest concentration (50 μg/ml), all the MDR isolates tolerated all the heavy metals, but at 250 μg/ml, apart from cadmium and lead, all test isolates were 100% sensitive to chromium, vanadium and cobalt. The control isolate was only resistant to cobalt and chromium at 50 μg/ml, but sensitive to other heavy metals at all concentrations The level of co-resistance shown by these isolates is a call for concern.

1. Introduction

The therapeutic paradigm changed with the introduction of antibiotics into clinical use [1,2] which have continued to save lives around the globe [3]. Sadly, these gains are challenged globally with one of the greatest public health issues of our time, antibiotic resistance [3–5]. The range of infections caused by infectious agents and development of antimicrobial resistance have outpaced the development of newer antibiotics [3,4]. At an alarming rate, the spectrum of effective antibiotics to treat infectious diseases continue to narrow due to antibiotics resistance. The acquisition of resistance to antibiotics gives a microorganism a survival advantage over the sensitive ones [6]. The mechanisms of antibiotics resistance acquisition vary and include inactivation, the use of efflux pumps and even modification of targets [7]. The issues emanating from the misuse of antibiotics in medicine and agriculture are twofold: antibiotics pollution and abundance of antibiotics resistance genes (ARGs) [8]. Estimates indicates that antibiotics resistance presents with

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different economic burdens in different climes [9]. In the United States of America (USA), ARG is estimated to cost about $20 billion and $35 billion in direct and societal costs, respectively. In Europe, the cost is placed at £ 1.4 billion yearly while in developing countries, estimates are hard to come by as health care is largely privately funded [9]. More complicated is the fact that these ARGs have been shown to co-evolve with heavy metal resistance genes [10,11].

In trace amounts, heavy metals are needed by microbes for proper functioning, but at higher concentrations, they become toxic [12, 13]. Due to the ever-increasing human population, industrial activities continue to accumulate these metals in several ecosystems and as result, the autochthonous microbes have adapted means to handle these heavy metals [13]. Some of these techniques include the use of efflux pumps, complexing them and using them as an electron acceptor [14,15]. Studies have shown that, co-resistance of antibiotics resistance with heavy metals is via similar functional and structural strategies that are either plasmid or chromosomally borne [16,17]. Antibiotic resistance genes occur naturally in various environments in low abundance. However, studies have shown that their abundance increase in the presence of several pollutants such as heavy metals, crude oil and sewage [10,11,18]. Where this happens, these ARG can move from one microorganism to another via horizontal and vertical mechanisms triggering multi-drug resistance and compounding clinical outcomes of infectious diseases. Furthermore, these ecosystems can act as reservoirs for ARG crossing over from

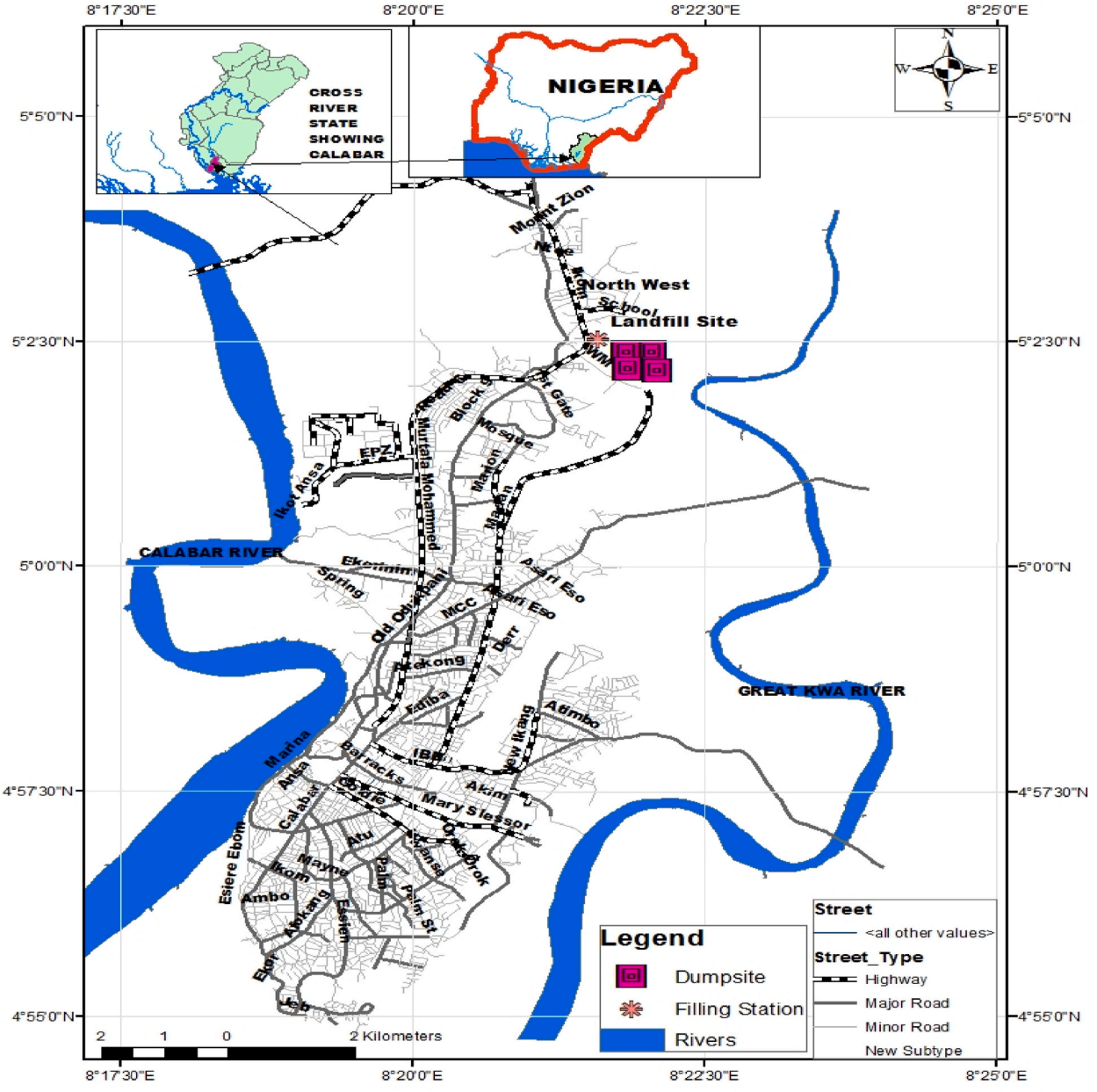

Fig. 1. Map of the study area.

environmental into clinical settings [11,19]. There are concerns that ecosystems polluted with metals such as open dumpsites used in the management of solid wastes in developing countries are becoming more common these days [20,21].

Solid wastes are often poorly handled in developing countries such as Nigeria and are managed using open dumpsites which are more common than the more sanitary landfills [21]. These solid wastes are composed of degradable and non-degradable fractions [19, 20]. Dumpsites receive large amounts of wastes daily from cities and thus, they are capable of accumulating heavy metals and as well as other pollutants [19,20,23–26]. These and other factors have the capacity to shape the microbial community in an open dumpsite soil, and its leachate [19]. The abundance of plasmid bearing bacteria have been shown to be comparatively higher in contaminated soils [17] and resistance plasmids in non-agricultural soils [27]. Heavy metals and antibiotics cross-resistance in impacted soil have been reported [10]. These environments could be reservoirs for antibiotic resistant genes and pathogens of humans [19,28] where these genes are turned on at low concentrations of antibiotics with potentials to be transferred to other hosts [28].

Antibiotics genes have been discovered in all kinds of environment even amongst the so-called pristine and controlled environments like compost thus supporting the fact that these genes have their origin in the environment [1,8,29,30]. Yet, the role of environmental factors in the spread of antimicrobial resistance is largely overlooked. Several studies exist that have established antibiotics and heavy metal co-resistance in polluted environments [26,31] but similar studies are few for dumpsite environment. The aim of this study was to evaluate co-resistance of heavy metal amongst multi-drug resistant bacteria isolates from an open dumpsite soil.

2. Materials and methods

2.1. Study site

Soil samples were collected from an open dumpsite popularly called Lemna dumpsite. The dumpsite is located on coordinates: 4°13′ E and 5°15′ E and 8°15 S and 8°21′S in Calabar Municipality, Capital City of Calabar, Cross River State, Nigeria. The study area is characterized by tropical wet and dry seasons, high annual rainfall of 3500–4000 mm, mangrove vegetation and an estimated run-off of 90%. The dumpsite receives huge quantity of wastes on a daily basis from domestic and industrial areas in the state. It covers a total area of approximately 3,265 m^2 and is less than a 1 km from a Ikot Effanga Mkpa stream, a source of untreated water for many households around the study site (see Fig. 1).

2.2. Collections of samples

Soil samples from three different locations (3 m apart from each point) within the dumpsite were collected in triplicates, totaling 9 samples as previously reported [26]. From each sampling point, 100 g of soil were collected using sterilized hand trowel from a depth of 0–15 cm after removing the top soil. The collected samples were first placed in oven sterilized aluminum sample plates and then into sterile plastic containers before transporting to the Laboratory for further analysis. Heavy metals (vanadium, chromium, nickel, lead, cobalt, cadmium and copper) and commercial antibiotic discs (Hardy Diagnostics, USA) were obtained from Globus Scientific Store in Calabar, Cross River State.

2.3. Analysis of physical and chemical parameters

Physicochemical parameters including pH, electrical conductivity, THC, total moisture, TOC, sodium, phosphorus, magnesium, potassium and calcium were evaluated as previously reported [32,33].

2.4. Heavy metals analysis

Collected soil samples were dried and passed through a 2 mm sieve. Exactly 5 g of the soil samples were digested with concentrated nitric acid and transferred to a 100 mL Teflon beaker. Thereafter, 10 mL of ultrapure concentrated HNO_3 (Merck) was added and the sample was heated to 100, 150, 210 and 280 °C on a hot plate for 0.5, 0.5,0.5 and 2 h with DK-20 heating digester. Finally, 2 mL of 1 N HNO_3 was added to the residue and the solution evaporated again on the hot plate, continuing until every sample was completely digested. After cooling, a further 10 mL of 1 N $HNO_3$was added. The solution was then diluted and filtered through a 0.45-l.0 mm nitrocellulose membrane filter. The concentration of heavy metals in the soil was determined using inductively coupled plasma atomic emission spectrometer (ICP, AES) (YobinYvon JY-24) [34]. International certified reference material (CRM 029) was used and analyzed at the beginning and end of each batch of samples to assess accuracy and precision of the analytical method. Heavy metal concentrations determined in the standard reference materials were compared with certified values. The instrument performance during analysis was monitored using internal standards. The calculated recovery capacities ranged from 90.2% to 108%, with regression coefficients (r^2) ranging from 0.91 to 0.97. The limits of detections were 0.052 (Pb), 0.018 (Cd), 0.015 (Mn), 0.025 (Ni), 1.790 (Zn), 0.079 mg/kg (Cr), 0.02 mg/kg (Fe) and 0.01 mg/kg (Co). Concentrations of heavy metals were expressed in mg/kg dry weight [35].

2.5. Enumeration of THBC

Total heterotrophic bacterial count (THBC) was enumerated as previously reported [36]. After serial dilution, exactly 0.1 mL of

desired dilution was used for enumeration on nutrient agar to which 50 μg/ml of Nystatin was added to inhibit fungal growth and plates incubated at 37 °C for 24 h and bacterial counts recorded after 24 h of incubation. The same volume was also plated out on Eosin Methylene Blue (EMB) agar and incubated for 37 °C for 24 h.

2.6. Purification of isolates and identification of isolates

Distinct colonies were picked from both agars and sub-cultured twice onto freshly prepared nutrient agar plates for purification. Pure isolates were characterized using Gram reaction and biochemical tests as reported previously [37].

2.7. Antibiotic susceptibility testing

This was done using the disc diffusion technique on freshly prepared Mueller Hinton Agar. The test antibiotics used in the study were Ciprofloxacin (30 μg), Streptomycin (30 μg), Septrin (15 μg), Gentamycin (15 μg), Amoxicillin (30 μg), Ceftriazone (10 μg), Ciprofloxacin (30 μg), Pefloxacin (10 μg), Gentamycin (30 μg) and Augmentin (10 μg) (Becton Dickinson, USA). The diameters of the zones of inhibition were interpreted as resistance and susceptible according to the NCCLS guideline [38].

2.8. Heavy metal resistant test

Isolates showing multiple drug resistance (resistance to atleast 2 antibiotics) were selected for heavy metal resistance test. This was done using as previously reported [26,39]. Briefly, a loopful of 12–16 h old bacteria culture in tryptic soy broth was inoculated on Mueller- Hinton Agar plates bearing different concentrations (50, 100, 150, 200 and 250 μg/ml) of the test heavy metal (chromium, vanadium, cobalt, cadmium and lead). Incubation was done at 37 °C for 24 h. After incubation, plates showing growth were regarded as resistant while those without growth were taken as sensitive. The most sensitive isolate from the sensitivity test was used as control against the heavy metals.

2.9. Data analysis

Replicate readings for the physicochemical parameters were analyzed using one way analysis of variance (ANOVA) with the significance set at $p < 0.05$.

3. Results

The results of the physicochemical and heavy metal analyses are presented in Tables 1 and 2. From Table 1, the parameters examined were pH, electrical conductivity, total hydrocarbon content (THC), total moisture, total organic carbon, phosphorus, magnesium, potassium, calcium, silt, clay, and sand. The pH values ranged from 6.50 to 7.60 and was highest in DS2. The EC values ranged from 13.20 to 16.60 μ/Scm^{-1} with DS3 having the highest value. The total hydrocarbon content (THC) was highest in DS2 with a value of 9.50 and least in DS3 with a value of 0.95 mg/kg. Total organic carbon (TOC) was highest in DS1 followed by DS3 and then DS2 with values of 5.0% 4.5% and 3.5%, respectively. Potassium values ranged from 0.50 to 0.90 mg/kg. Calcium on the other hand, gave much higher values with DS1 and DS2 having values of 8.50 and 8.00, respectively. The amounts of sand, slit and clay in the samples as presented in Table 1 shows that they vary significantly from one sample location to another. Across all the heavy metals examined in the dumpsite, DS2 recorded the highest values for all the evaluated heavy metals. The values were 22.67, 4.70, 0.80, 1.35, 5.05 and 0.37 mg/L, for Iron, zinc, cadmium, lead, manganese, nickel and chromium, respectively. Fe and Zn recorded the least values in DS3 while Cd had the least value of 0.20 mg/L in DS1. When compared to World Health Organization (WHO) [40] permissible standard, Fe, Cd and Pb were higher for all the study samples. Furthermore, the values of Ni in DS2 and DS3 were higher than those of

Table 1
Physicochemical analysis of the soil samples.

Parameters	DS1	DS2	DS3	p-value
pH	6.50 ± 0.21	7.60 ± 0.06	7.40 ± 0.02	<0.05
Electrical conductivity (EC) (μ/Scm^{-1})	14.30 ± 0.28	13.20 ± 0.61	16.60 ± 0.26	<0.05
Total hydrocarbon (THC) (mg/kg)	3.04 ± 0.25	9.50 ± 0.25	0.95 ± 0.22	<0.05
Total moisture (%)	0.71 ± 0.22	0.93 ± 0.23	0.76 ± 0.23	>0.05
Total organic carbon (TOC) (%)	5.00 ± 0.21	3.50 ± 0.24	4.50 ± 0.24	<0.05
Sodium (Na) (mg/kg)	1.05 ± 0.23	1.02 ± 0.00	1.03 ± 0.22	>0.05
Phosphorus (P) (mg/kg)	3.23 ± 0.22	1.23 ± 0.22	2.70 ± 0.23	<0.05
Magnesium (Mg) (mg/kg)	2.05 ± 0.22	2.20 ± 0.23	3.00 ± 0.25	<0.05
Potassium (K) (mg/kg)	0.50 ± 0.21	0.41 ± 0.22	0.90 ± 0.24	>0.05
Calcium (Ca) (mg/kg)	8.50 ± 0.25	8.00 ± 1.23	0.01 ± 0.21	<0.05
Silt (%)	85.00 ± 2.50	87.00 ± 3.21	65.00 ± 2.0	>0.05
Clay (%)	8.50 ± 1.21	8.70 ± 1.23	25.00 ± 2.21	<0.05
Sand (%)	85.50 ± 2.31	87.50 ± 2.10	58.50 ± 2.21	<0.05

Key: $p < 0.05$ = significant; $p > 0.05$ = Not significant.

Table 2
Heavy analysis of the soil samples.

Heavy metals (mg/L)	DS1	DS2	DS3	WHO [40]	p-value
Iron (Fe)	2.50 ± 0.22	2.67 ± 0.02	0.85 ± 0.06	0.30	<0.05
Zinc (Zn)	3.00 ± 0.02	4.70 ± 0.02	2.50 ± 0.02	15	<0.05
Cadmium (Cd)	0.20 ± 0.01	0.80 ± 0.01	0.21[a]0.02	0.001	<0.05
Lead (Pb)	1.00 ± 0.02	1.350 ± 0.02	BDL	0.04	>0.05
Manganese (Mn)	3.5 ± 0.05	5.05 ± 0.07	0.43 ± 0.20	1.5	<0.05
Nickel (Ni)	0.03 ± 0.20	0.37 ± 0.20	0.17 ± 0.02	0.05	<0.05
Chromium (Cr)	0.05 ± 0.02	0.09 ± 0.02	0.02 ± 0.02	0.05	<0.05
Cobalt (Co)	0.33 ± 0.03	1.33 ± 0.03	BDL	–	>0.05

Key: $p < 0.05$ = significant; $p > 0.05$ = Not significant; BDL = Below Detection Level.

WHO [40] but not DS1. Similar trend was also observed for Co whose values were higher than those of WHO [40]. However, the Cr values in DS2 were higher than WHO [40] acceptable limit, but not those of DS1 and DS3.

Table 3 shows the total heterotrophic counts for the various dumpsite samples. From the result, the microbial counts were 7.92×10^5, 6.91×10^5 and 6.68×10^5 for DS1, DS2 and DS3, respectively. The counts were lower on EMB and these were 2.84×10^4, 2.36×10^4 and 3.04×10^4, respectively. The bacterial isolates obtained in our study were *B. subtilis, B. cereus, S. marcescens Enterobacter* sp*, V. cholera, C. freundii, Yesinia Sp, Salmonella* sp and *Shigella* sp. In addition, *E. coli* was also obtained and were 10 in number. Some of these isolates especially *E. coli, P. aeruginosa; B. subtilis* and *B. cereus* were obtained in all three dumpsite samples (Table 4). In our study, 20 isolates were subjected to antibiotics sensitivity. A total of 50.00% (n = 10) showed multiple drug resistance, that is, were resistant to atleast two of the test antibiotics as shown in Tables 5 and 6. From Table 5, *Shigella* sp, *Salmonella* sp*, Vibrio cholera, S. marcescens* and *Yersinia* sp were 100% sensitive to the test antibiotics. *B. subtilis, B. cereus, C. freundii, Enterobacter* sp and *P. aeruginosa.* The test isolates in Table 5 were 100% susceptible to ceftriaxone and chloramphenicol while those in Table 6 only showed 100% susceptibility to ciprofloxacin.

Table 7 shows the heavy metal resistance profile of the multi-drug resistant isolates. The resistance profile of the multiple drug resistant isolates to the heavy metals showed that it was concentration dependent. Consistently, at 50 μg/ml, all the isolates showed high resistance that was 100, 90, 90, 100 and 100% for Cr, V, Cd, Co and Pb, respectively. At 100 μg/ml, resistance was 90, 70, 90, 90 and 90% respectively for Cr, V, Cd, Co and Pb, respectively. At 150 μg/ml, the isolates showed 60, 60, 80, 50 and 60% resistance, respectively to the test heavy metals. However, at 250 μg/ml, the isolates were most sensitive and apart from Cd and Pbthat the isolates showed 10 and 20% resistance, respectively, while to the rest of the heavy metals, the isolates were 100% sensitive. The control isolate was sensitive to the heavy metals at the lowest concentration, however, it showed resistance to Cr and Co. Unlike the test MDR, the control isolates showed 100% sensitivity to other concentrations.

4. Discussion

Dumpsites are usually rich with organic wastes in solid and liquid forms [21]. It is therefore not surprising that our soil sample was abundant with bacteria. Osazee et al. [41], reported lower bacteria counts in dumpsite and control soil samples which were 1.8×10^4 and 1.7×10^3 cfu/g, respectively. However, much higher THBC counts that ranged from 7.4×10^6 to 1.2×10^7 cfu/g for dumpsites in Bwari, Abuja were reported earlier [42]. Similar higher counts to our findings were obtained from a dumpsite from Port Harcourt by William and Hakam [43] who reported THBC that ranged from 4.4×10^7 and 1.2×10^8. These counts were within range of our THBC that ranged from 6.68 to 7.92×10^5 cfu/mg. In this study, *E. coli* had a prevalence of 50%. *Serratia* sp, *Klebsiella* sp, *Pseudomonas* sp and *Bacillus* have been reported as overlapping species in both polluted and pristine soil samples [26]. Furthermore, Osazee et al. [41] reported some similar isolates and these were *Bacillus* sp*, Pseudomonas* sp*, Aeromonas* sp*, Enterobacter* sp*, Klebsiella* sp*,* and *Staphylococcus* sp. Furthermore, William and Hakam [43] reported similar isolates in addition to *Streptococcus* and *Staphylococcus* species.

Dumpsites often usually accumulate pollutants that can alter the physicochemical parameters of the receiving environment as well as the microbial diversity and function. In an earlier study, a pH range of 6.7–7.6 was reported by Bassey et al. [21] for the same study site. In addition, they also reported electrical conductivity values that ranged from 14.30 to 15.6 μ/scm^{-1}. Both pH and electrical conductivity value were more agreeable to our findings. These were higher than our reported values. TOC, Na, P, Mg and Ca values reported in our study were more agreeable to those of previous studies by Bassey et al. [21] and Bassey et al. [22] for the same study site. However, our findings are lower than those previously reported by Adeyemi et al. [44] and Narty et al. [45] for dumpsite leachates and soil samples, respectively. In another earlier study, higher values of EC, Pb, Fe, Zn, Cr, Cu and TOC in Benin dumpsite than our study sites were reported with values that ranged from 164.00 to 540 μ/scm^{-1}, 0.025 to 0.015, 3.09 to 2.41, 1.99 to 1.47, 0.079 to

Table 3
Total heterotrophic bacteria counts (cfu/g).

Samples	Nutrient Agar	EMB Agar
DS1	7.92×10^5	2.84×10^4
DS2	6.91×10^5	2.36×10^4
DS3	6.68×10^5	3.04×10^4

Table 4
Microbial isolates identified using Bergey's manual.

S/N	Isolates
1.	*B. subtilis*
2.	*B. cereus*
3.	*S. marcescens*
4.	*C. freundii*
5.	*Enterobacter* sp
6.	*Vibrio cholera*
7.	Yersinia sp
8.	*P. aeruginosa*
9.	*Salmonella* sp
10.	*Shigella* sp
11.	*Escherichia coli*

Table 5
Antibiotics sensitivity of the various isolates.

Isolates	CPX	SXT	S	CN	CEP	OFX	AM	PEF	CH	AU
B. subtilis	21.20	17.30	–	18.50	17.50	–	16.80	18.20	20.60	–
B. cereus	–	21.70	19.30	–	18.40	19.70	15.60	–	18.60	19.40
S. marcescens	20.80	19.80	23.20	22.20	21.50	21.60	19.30	18.50	17.90	18.40
C. freundii	19.70	–	18.60	–	23.20	–	24.1	14.30	13.60	16.30
Enterobacter sp	18.10	–	24.30	–	19.50	20.40	–	19.35	21.45	–
Vibrio cholera	19.50	18.45	21.45	22.30	21.55	18.30	19.50	17.70	18.60	19.55
Yersinia sp	22.30	24.30	20.80	20.80	21.45	22.35	21.25	22.60	19.45	18.75
P. aeruginosa	19.65	–	–	–	18.35	19.65	–	18.45	21.20	–
Salmonella sp	21.45	25.30	20.10	19.85	18.50	17.50	16.50	18.60	17.85	21.60
Shigella sp	19.45	19.20	18.65	19.00	18.30	21.00	19.60	22.10	19.70	18.70

Key: CPX= Ciprofloxacin, SXT = Septrin, S= Streptomycin, CN = Gentamycin, CEP = Ceftriaxone, OFX= Ofloxacin, AM = Amoxicillin, PEF= Pefloxacin, CH=Chloramphenicol, and AU = Augmentin and "– "represents no inhibition (resistance).

Table 6
Antibiotics sensitivity of the various *E. coli* isolates.

Isolates	CPX	SXT	S	CN	CEP	OFX	AM	PEF	CH	AU
E. coli 1	20.60	23.10	22.00	23.20	26.00	22.40	26.40	26.00	24.00	22.00
E. coli 2	22.90	–	–	15.20	22.60	15.00	16.90	–	23.10	28.00
E. coli 3	26.00	25.00	27.00	26.00	23.00	17.00	26.00	26.00	24.00	21.00
E. coli 4	18.00	23.00	16.00	22.00	19.60	–	–	–	25.10	17.20
E. coli 5	21.10	24.00	20.90	24.80	17.60	18.80	23.20	26.00	19.20	25.80
E. coli 6	20.20	23.00	17.40	24.00	–	19.10	26.00	22.00	25.30	–
E. coli 7	24.00	25.00	26.20	27.10	16.00	23.20	25.1	23.10	–	–
E. coli 8	25.50	25.20	22.10	24.00	–	16.00	–	25.20	20.00	22.20
E. coli 9	24.00	26.20	24.80	24.20	14.60	26.00	26.70	26.20	24.9	24.10
E. coli 10	21.20	16.90	23.20	24.10	20.20	24.60	26.20	24.90	24.20	26.40

Key: CPX= Ciprofloxacin, SXT = Septrin, S= Streptomycin, CN = Gentamycin, CEP = Ceftriaxone, OFX= Ofloxacin, AM = Amoxicillin, PEF= Pefloxacin, CH=Chloramphenicol, and AU = Augmentin, and "– "represents no inhibition (resistance).

0.059, 0.305 to 0.233 and 1.05–1.56 mg/kg, respectively [40].

Antibiotics resistance is a complex global problem [46–49]. Resistances to antibiotics lower their effectiveness and increase economic burdens to individuals, public health and the society at large [50]. Antimicrobial drug resistance is driven by misuse and its genes can be transferred to other hosts in the environment [5,48]. In our study, 50% of the test isolates showed multidrug resistance to the test antibiotics and these are largely isolates that are often implicated in clinical settings in causing diseases in humans. They showed resistance to the beta lactamase, quinolones and aminoglycosides classes of antibiotics. It has been suggested that beta lactamase evolved millions of years ago implying that their presence even before the arrival of antibiotics in clinical usage [8,14] suggesting that bacterial resistance to antibiotics is evolutionary and seems inevitable and unstoppable. It is important the we understand how resistance genes spread from one bacterium to another [51] and from environment into clinical settings [19].

Resistance to antibiotics could be acquired via mutations which can be induced by very low concentrations of antibiotics in the environment or horizontal gene transfer of the resistance gene or just as seen in the Gram-negative bacteria whose outer membrane makes them resistant to several antibiotics [1,51]. This could be the reason different isolates showed resistance to several antibiotics such as perfloxacin, augmentin, amoxicillin, ceftriaxone, gentamycin, septrin, and streptomycin used in this study. ARG in Gram positive and negative isolates as observed in this study comes with high mortality and morbidity rates, and difficult to treat and

Table 7
Heavy metal resistance profile of the MDR isolates.

Isolates	Cr (μg/ml) 50	100	150	200	250	V (μg/ml) 50	100	150	200	250	Cd(μg/ml) 50	100	150	200	250	Co(μg/ml) 50	100	150	200	250	Pb (μg/ml) 50	100	150	200	250
E. coli 2	R	R	R	S	S	R	S	S	S	S	R	R	R	R	R	R	R	S	S	R	S	S	S	S
E. coli 4	R	R	S	S	S	R	R	R	R	S	R	R	R	R	S	R	R	S	S	S	R	R	R	R	S
E. coli 6	R	R	R	R	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	S
E. coli 7	R	R	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	R	R	R	R
E. coli 8	R	R	R	S	S	R	R	R	S	S	R	R	R	R	S	R	R	S	S	S	R	R	S	S	S
B. subtilis	R	S	S	S	S	R	S	S	S	S	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R
B. cereus	R	R	R	R	S	R	S	S	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	S
C. freundii	R	R	R	S	S	R	R	R	S	S	R	S	S	S	S	R	R	S	S	S	R	R	S	S	S
Enterobacter sp	R	R	R	S	S	R	R	R	S	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S
P. aeruginosa	R	R	R	S	S	R	R	R	S	S	R	R	R	R	S	R	R	R	R	S	R	R	R	R	S
Control isolate	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
Summary	Conc.	R (%)		S (%)		Conc.	R (%)		S (%)		Conc.	R (%)		S (%)		Conc.	R (%)		S (%)		Conc.	R (%)		S (%)	
	50	0		0		50	90		10		50	90		10		50	100		0		50	100		0	
	100	90		10		100	70		30		100	90		10		100	100		0		100	90		10	
	150	60		40		150	60		40		150	80		20		150	50		50		150	60		40	
	200	10		90		200	10		90		200	50		50		200	60		40		200	60		40	
	250	0		100		250	0		100		250	10		90		250	0		100		250	20		80	

Key: R = Resistant, S = Sensitive, V = vanadium, Cr = Chromium, Cd = Cadmium, Co = Cobalt and Pb = Lead, N/B: The control isolate is not included in the summary calculations.

manage infections [47].

Antibiotics resistance has reached a global dimension and it is no longer confined to clinics. Azam et al. [6] identified a pan drug resistant *E. coli* MRC11 that showed resistance to 20 out of 21 antibiotics in their study. Furthermore, they showed that the addition of certain heavy metals even at high concentration did not increase their susceptibility to these antibiotics. In our study, 5 out 10 of the *E. coli* isolates showed MDR to the test antibiotics, however, higher concentrations of the heavy metals were toxic to all the test isolates. The discharge of untreated municipal sewage and industrial wastes creates means of selection, multiplication and spread of resistance amongst bacteria [6]. Wesgate et al. [52] showed that exposure to low concentrations of triclosan but not to chlorohexidine, and hydrogen peroxide based biocidal agent drive ARGs. Apart from these pollutants, heavy metals resistance has been shown to be co-selected in environmental settings with that of antibiotics. In an earlier study, *S. aureus, E. coli* and *P. aeruginosa* were reported as the most resistant isolates to antibiotics and these isolates apart from *S. aureus* showed MDR in our study. Furthermore, these MDR in their study showed abundant to moderate growth with iron and zinc at higher concentrations [39]. Compared to our findings, *B. cereus, B. subtilis,* and *E. coli* all showed complete resistance to Pb. One isolate of *E. coli* showed complete resistance to Cd. The rest of the isolates in our study were sensitive at higher concentrations of the heavy metals.

Sources of these metals such as mercury, cadmium, copper, and zinc vary and they include solid wastes [20,21] as well as agriculture and aquaculture [11,35]. As these metals accumulate in the environment, at certain critical levels, they trigger co-selection mechanism with antibiotics resistance [53]. Chen et al. [54] investigated heavy metals and ARG in a copper tailing dam in China and found out that, genes coding for arsenic resistance and macrolides were the most abundant even though copper and lead where more abundant than arsenic in concentration. Furthermore, the abundance of the heavy metal resistance genes gave positive correlation with Cd suggesting that the metal plays an essential role in the selection of heavy metal resistance genes. Our study shows that the at highest concentration, 10% showed resistance to Cd while 20% showed resistance to lead.

Antibiotics resistance and tolerance of heavy metals in the environment are a growing global public health concern [19,55]. In an earlier study, a total of forty (40) aerobic bacteria isolated from sediment and water were subjected to various antibiotics including carbenicillin, gentamicin, kanamycin, chloramphenicol, and nalidixic acid [55]. Their results indicate that the isolates (37.50%) showed resistance to one or more antibiotics while 22% showed complete sensitivity. Compared to our findings, 50% of our isolates displayed multi-drug resistance to our test antibiotics. Furthermore, they subjected a total of 29 isolates to different concentrations of various heavy metals and found out that all their isolates were tolerant and able to grow at the various concentrations used in their study. In our study, the isolates were able to grow and tolerate all the concentrations used.

5. Conclusion

Antibiotics resistance genes have been found in all kinds of environment including those that are pristine and has the potential to cross into clinical settings with huge public health significance. In our study, we aimed to evaluate the co-resistance of heavy metals with antibiotics amongst isolates from dumpsite soil. Our results indicated the presence of heavy metals in concentrations higher than those permissible by the WHO. The bacterial isolates in our study were isolates that are frequently implicated in human infections in clinical settings. Isolates that showed multiple drug resistance were also able to tolerate higher concentrations of heavy metals utilized in the study. These findings suggest that pollutants like heavy metals could have their tolerance genes co-evolving with ARG in a synergistic manner. Furthermore, our MDR isolates showed heavy metal resistance that was dependent on the concentration of the heavy metals. These findings are a clear call for concern.

Author contribution statement

Uwem Edet, Ph.D: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ini Ubi Bassey, PhD; Akaninyene Joseph, PhD: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Declaration of interest's statement

The authors declare no competing interests.

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