

Research Article

Prevalence of Antibiotics and Cr, Cd, Cu Metals Co-resistant Coliforms in Refuse Dumpsites and Air of Surrounding Buildings in a Nigerian Urban Setting

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Abstract: Prevalence of co-resistance to antibiotics and Cr⁶⁺, Cd²⁺, and Cu²⁺ among coliforms from dumpsites and surrounding buildings' indoor and outdoor air was investigated in Warri town, in the Niger Delta region of Nigeria. A total of 1,465 coliforms were isolated with McConkey and Eosin Methylene Blue Agar, and 38.8% had multiple antibiotics resistance (MAR) index ≥ 0.3 based on nine antibiotics. The prevalence of coliform bacteria with a MAR index of ≥ 0.3 varied (20.0 - 69.2%) and was lower in indoor and outdoor air farther away from the dumpsites. All dumpsite isolates were resistant to Cr and Cd but not to Cu, while resistance varied (48.5 - 100%) among indoor and outdoor air isolates. Plasmid-encoded resistance was indicated when the mean MAR (0.48 ± 0.04 - 0.5 ± 0.04) and multiple heavy metal resistance (MHMR) indexes (0.66 ± 0.00) declined to 0.11 ± 0.01 - 0.36 ± 0.03 and 0.33 ± 0.00 , respectively, after curing. The differences in MAR indexes of isolates from the four dumpsite locations and between indoor and outdoor air isolates were only significant ($P < 0.05$) after curing, unlike the MHMR index, where there was no significant difference before and after curing. The prevalence of metals and antibiotics co-resistance, which was based on the concomitant reduction in MAR and MHMR indexes after curing, was higher in isolates from dumpsites than in indoor and outdoor air (32.1 - 38.1 vs 0.0 - 16.8%). The role of plasmid-encoded genes in the co-resistance phenomenon was therefore indicated. Dumpsites as selective pressure spots for the emergence of resistance genes in bacteria should be of public health concern in countries characterised by indiscriminate dumping of wastes, as this can result in potential spread to houses via bioaerosols.

Keywords: antimicrobial resistance, bioaerosols, indicator bacteria, municipal wastes

1. Introduction

The presence of microorganisms that are resistant to antibiotics in the environment is of public health concern because of the risk of the spread of genes that are associated with the resistance. The occurrence of antibiotic-resistant microorganisms in water or soil contaminated with wastes has been linked with the presence of heavy metals in a phenomenon termed co-resistance. Indeed, reports show that heavy metals can induce antibiotic resistance in microorganisms [1-4]. Thus, the presence of substantial concentrations of heavy metals in the environment is therefore a public health concern.

The widespread presence of heavy metals in the environment is attributable to natural and anthropogenic sources

[5-7]. The increasing concentration of these metals in the environment exposes microorganisms to their toxic effects, thereby leading to the emergence of resistant mechanisms that tend to operate in an identical pattern of resistance to antibiotics [8]. These mechanisms have been reported in several studies [9-14]. However, metals have been present for a long period of time in the biosphere before the discovery and use of antibiotics. Hence, it is reasonable to deduce that metals are associated with the induction of antibiotic resistance in microorganisms.

The occurrence of microorganisms that are co-resistant to metals and antibiotics has been investigated mainly in water bodies, sewage, soils, aquaculture, food-producing animals, and many industrial effluents [1, 15-23]. Urban waste or refuse dumpsites and the associated bioaerosols have not been sufficiently investigated as potential sources of metal-induced microbial resistance to antibiotics in Sub-Saharan Africa (SSA). Most of the available reports on dumpsites focus on either antibiotic-resistant bacteria [24-26] or metal concentrations [27-32]. Wastes (including agricultural and hospital wastes) are indiscriminately dumped in many urban areas in SSA due to poor waste management [33-35]. For example, it is common to see waste dumpsites near households, markets, on roadsides, and in drainages (gutters) in many urban areas of Nigeria.

There are few reports on the co-occurrence of heavy metals and antibiotics resistance in bacteria in urban dumpsites and polluted soil in some Nigerian towns [36-39]. However, none of these studies considered the prevalence of metal or antibiotics co-resistance, different dumpsites such as gutters, markets, and the air quality of residential areas or shops located near the dumpsites. The prevalence of plasmid-mediated co-resistance genes was also not investigated. The danger of plasmid-borne genes is that the horizontal transfer of resistance genes to pathogens is accelerated and may spread through homes and shops via aerosols or rodents and insects that obtain food from wastes at dumpsites [40, 41]. Thus, the healthcare challenges posed by the difficulties of treating infections caused by microorganisms that are resistant to antibiotics become an impetus for ascertaining the prevalence of bacterial resistance to antibiotics and metals in neighbourhood dumpsites.

It is known from literature sources [29-32] that several metals are found in refuse dumpsites. The concentrations of metals in the environment are often elevated above their naturally occurring levels due to ingress from anthropogenic sources. Several reports [42-46] identified anthropogenic sources that increase metal concentrations in the environment; they included some of the discarded materials that are often found in municipal dumpsites in many Nigerian urban neighbourhoods, including Warri. By visual observation, the dumpsite materials can be identified as textiles, paper, leather (footwear), timber products, agricultural wastes, biocides, and left-over food. Others include batteries, electrical and electronic wastes, automobile brake pads and linings, metallic containers, polythene packaging bags, polyvinyl chloride (PVC) wastes, and faecal matter. Most of these dumpsite materials tend to contain greater sources of Cr, Cd, and Cu than other metals, as can be inferred from the above-cited reports [42-46]. Any metal that the concentration is most likely to be frequently elevated by anthropogenic sources can be adjudged as good “candidates” for testing resistance to metals by microorganisms from dumpsites. This was the rationale for selecting Cr, Cd, and Cu for the investigation.

The objective of the study was therefore to: (1) ascertain the extent of occurrence of multiple antibiotics and heavy metals (Cr, Cd, and Cu) resistance amongst coliform bacteria from dumpsites in four areas (residential, markets, roadsides, and gutters) and in the indoor and outdoor air quality of houses and shops surrounding dumpsites; (2) determine the differences in multiple antibiotics and heavy metal resistance indexes between coliform isolates from the four refuse dumpsite locations; and (3) determine the prevalence of plasmid-mediated co-resistance to antibiotics and heavy metals amongst the coliform isolates. Coliform bacteria were considered useful for the study because of their role as an indicator of the likely presence of enteric pathogenic bacteria.

2. Materials and methods

2.1 Source of samples

Municipal waste or refuse dumpsites located in the city of Warri, in the oil-producing Niger Delta region of Nigeria, were the sources of samples for the study. The locations of the 12 dumpsites selected for the investigation are shown in Figure 1, which was derived from the Global Positioning System (GPS) coordinates obtained with GPSMAP 76CSx (Garmin Ltd., USA) and Smart GPS location software from Google for comparison. The selection was guided

by the need to select three dumpsites from each of the following locations: roadsides, residential areas, market or shop premises, and drainage or gutters. The dumpsites generally do not have defined boundaries but can range from 10 to 50 m in length and 5 to 20 m in width. Warri is located in the tropical rainforest belt of Nigeria, with a warm climate all year round (30 to 35 °C). The collection of samples was retrieved during the dry season. Although rain usually falls throughout the year, it is markedly lower during the dry season (November to March). Hence, the movement of bioaerosols will be limited during the dry season as rain (wet deposition) can greatly affect the movement of bioaerosols from dumpsites to buildings.

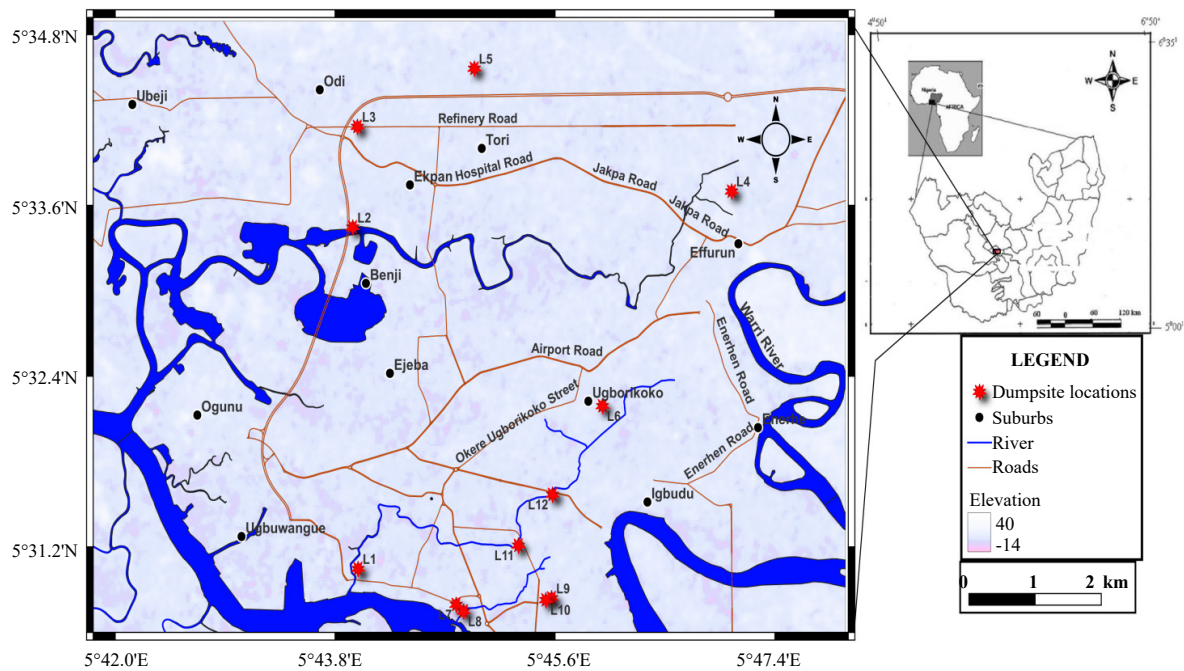


Figure 1. Map of the city of Warri showing the locations of the 12 dumpsites where the samples were collected

2.2 Collection of refuse dump soil samples

A sterile trowel was used to collect soil samples at a depth of 2 to 10 cm in triplicates from the edge and middle of the refuse dumps after removing the surface wastes to gain access to the soil beneath. The soil samples were placed in polythene bags that were previously sterilised by overnight immersion in 75% ethanol and subsequently transported to the laboratory for analyses. The sites were visited three times for sampling at fortnightly intervals from December to January (2020 to 2021) when there was no rainfall. This period is usually characterised by frequent winds that are more intense than the winds of the wet season due to the presence of the North-East Trade wind, otherwise known as Harmattan. The Harmattan season occurs in Nigeria and other West African countries at this time of the year. It is therefore expected that there will be a greater spread of bioaerosols during this season.

2.3 Isolation and identification of coliform bacteria from soil samples and air

McConkey (MA) and Eosine Methylene Blue (EMB) agar plates were inoculated with a 0.1 mL aliquot of serially diluted soil samples and incubated at room temperature (30 ± 2 °C) for 24 to 48 hours. The MA and EMB Petri dishes were also exposed overnight in the kitchens and sitting rooms of 10 randomly selected houses located approximately 60 and 120 m away from the edge of refuse dumpsites. Another set of plates was exposed outdoor of houses 10 m away from the walls of the buildings for the purpose of sampling outdoor air. The procedure was repeated for indoor and outdoor air of shops located 60 and 120 m away from market refuse dumpsites. All the plates were incubated at room temperature for 24 to 48 hours as before. Emerging reddish or pink colonies on MA indicate coliform bacteria and were

subcultured on fresh MA plates for purification. Colonies with metallic sheen against the light indicate *Escherichia coli*, and they were also subcultured on fresh EMB plates for purification. The pure isolates were subsequently identified by standard microbiological protocol (cultural, morphological, and biochemical tests) with the aid of Bergey's Manual of Systematic Bacteriology [47].

2.4 Antibiotic susceptibility test

The disk diffusion method on Mueller-Hinton agar was used to determine the susceptibility of the coliform bacterial isolates [48]. The antibiotics used were: ceftazidime, 30 µg; amoxicillin/clavulanic acid, 30 µg; cefuroxime, 30 µg; gentamycin, 10 µg; ofloxacin, 5 µg; cefotaxime, 30 µg; ampicillin, 30 µg; erythromycin, 30 µg; and cloxacilin, 5 µg. The multiple antibiotics resistance (MAR) index of each of the identified coliform bacterium from the different sources was determined by dividing the number of antibiotics resisted by the total number of antibiotics used [49]. Only isolates with a MAR index of ≥ 0.3 were used for subsequent tests.

2.5 Heavy metal resistance test

The isolates were tested for resistance to three heavy metals (Cd^{2+} , Cu^{2+} , and Cr^{6+}) using solutions of cadmium chloride, copper sulphate, and potassium dichromate that were prepared with deionised water and sterilised with autoclave at 121 °C for 15 minutes. The resistance of the isolates to the metals was determined in nutrient agar plates incorporating: Cr^{6+} , 100 µg/mL; Cd^{2+} , 100 µg/mL; and Cu^{2+} , 600 µg/mL. The concentrations were confirmed with an atomic absorption spectrometer (Perkin Elmer, Model 3100). The plates were subsequently inoculated with 1 mL of normal saline suspension containing 10^3 bacterial cells and incubated at 30 °C for 24 to 48 hours. Bacteria that grew at these concentrations were considered resistant, following the lead of Allen et al. [50] and Lee et al. [51]. The multiple heavy metal resistance (MHMR) indexes were calculated with three (the number of heavy metals) by the same procedure used for the MAR index.

2.6 Plasmid curing

The isolates with MAR index of ≥ 0.3 were subjected to plasmid curing by the sodium dodecyl sulphate method [52]. The protocol for antibiotics and metal resistance tests was also repeated, and the MAR and MHMR indexes were subsequently calculated as before. The plasmid was deemed to be involved in the resistance to the tested antibiotics and heavy metals if a reduction in MAR and MHMR index values occurred.

2.7 Data analyses

The prevalence of coliform isolates with MAR index of ≥ 0.3 was obtained by subtracting the number of isolates with MAR index of ≥ 0.3 from the total number of isolates and dividing by the total number of isolates before multiplying with 100 to convert to percentage. A similar procedure was used to determine the prevalence of heavy metal resistance. A one-way analysis of variance (ANOVA) was used to analyse the differences in the MAR and MHMR indexes of the total coliforms from the four dumpsite locations before and after curing. The differences in the MAR and MHMR indexes of houses' and shops' indoor and outdoor air total coliforms were analysed by a *t*-test. The indoor and outdoor air total coliforms were also compared by *t*-test on the basis of the two distances (60 m and 120 m) from the refuse dumpsites. The prevalence of plasmid-mediated co-resistance to antibiotics and metals among the coliforms was determined by calculating the percentage of the number that showed concomitant reductions in the MAR and MHMR indexes after curing. Pearson correlation statistics were used to analyse the association between the MAR and MHMR indexes of all coliform bacterial isolates and also the association between the MAR and MHMR indexes of dumpsites coliforms and buildings' airborne coliforms.

3. Results

A total of 1,465 coliform bacteria were isolated from the refuse dumpsites and indoor and outdoor air samples, but only 569 (38.8%) had MAR index values that were ≥ 0.3 by overall assessment (Table 1). A MAR index of 0.3 was used as the minimum for selecting the isolates because it indicates a high risk of antibiotic-contaminated discharges into the environment [53]. There were variations in the prevalence of coliform bacteria with MAR ≥ 0.3 both by the source of the coliform bacteria and the individual species (Table 1). The prevalence of the coliforms (MAR ≥ 0.3) was higher in the indoor and outdoor air of houses and shops closer to the refuse dumpsites than in those farther away (Table 1). Although there were few exceptions (e.g., *Klebsiella*), the prevalence of specific coliform species with a MAR index ≥ 0.3 was not markedly different across the four dumpsite locations (Table 1). In relation to heavy metals, the prevalence of resistance to Cr and Cd was 100% for all the isolates from the refuse dumpsites but markedly lower with respect to isolates from indoor and outdoor air (Table 2). As observed with MAR, indoor air and outdoor air isolates from houses closer to dumpsites had a higher prevalence of resistance than those located farther away (Table 2). There was no result presented for the prevalence of Cu in Table 2 because no isolate was resistant to Cu.

Table 1. Prevalence of multiple antibiotics resistance coliform bacteria with MAR index ≥ 0.3

		<i>E. coli</i>		<i>Klebsiella</i>		<i>Enterobacter</i>		<i>Citrobacter</i>	
		<i>N</i>	[n (%)]	<i>N</i>	[n (%)]	<i>N</i>	[n (%)]	<i>N</i>	[n (%)]
Roadside*	00	32	17 (53.1)	46	31 (67.4)	41	11 (26.8)	37	14 (37.8)
Markets*	00	38	22 (57.9)	51	23 (45.1)	56	15 (26.8)	39	14 (35.9)
Gutters*	00	34	15 (44.1)	43	19 (44.2)	42	12 (28.6)	43	11 (25.6)
Residential areas*	00	42	23 (54.8)	52	36 (69.2)	49	17 (34.7)	46	16 (34.8)
Indoor air of household	60	24	09 (37.5)	25	11 (44.0)	32	08 (25.0)	32	10 (31.3)
	120	18	05 (27.8)	17	09 (52.9)	25	08 (32.0)	21	05 (23.8)
Outdoor air of household	60	26	11 (42.3)	22	10 (45.5)	36	11 (30.6)	28	08 (28.6)
	120	12	04 (33.3)	11	04 (36.4)	22	07 (31.8)	18	05 (27.8)
Indoor air of shops	60	21	11 (52.4)	28	13 (46.4)	41	12 (29.3)	29	09 (31.0)
	120	15	03 (20.0)	17	10 (58.8)	31	07 (22.6)	17	04 (23.5)
Outdoor air of shops	60	24	13 (54.2)	29	14 (48.3)	38	10 (26.3)	32	12 (37.5)
Total (air)	120	13	03 (23.1)	18	11 (61.1)	31	09 (29.0)	21	07 (33.3)
Total (overall)	N/A	299	136 (45.5)	359	191 (53.2)	444	127 (28.6)	363	115 (31.7)

Note: *N* = total number of isolates; and * = refuse dumpsites

Table 2. Prevalence of heavy metal resistance amongst coliform bacteria with MAR index ≥ 0.3

	Proximity of houses or shops located to refuse dump (m)	N	<i>E. coli</i>		<i>Klebsiella</i>		<i>Enterobacter</i>		<i>Citrobacter</i>				
			% resistant to:		% resistant to:		% resistant to:		% resistant to:				
			Cr	Cd	Cr	Cd	Cr	Cd	Cr	Cd			
Roadside*	NA	32	100	100	46	100	100	41	100	100	37	100	100
Market premises*	NA	38	100	100	51	100	100	56	100	100	39	100	100
Gutter*	NA	34	100	100	43	100	100	42	100	100	43	100	100
Residential area*	NA	42	100	100	52	100	100	49	100	100	46	100	100
House indoor air	60	24	96.4	100	25	100	78.4	32	75.0	100	32	100	100
	120	18	68.4	58.6	17	58.6	68.0	25	68.0	80.0	21	76.2	80.9
House outdoor air	60	26	92.5	95.5	22	95.5	85.5	36	61.1	94.4	28	100	100
	120	12	75.6	64.5	11	64.5	74.6	22	90.9	81.8	18	83.3	77.7
Shop indoor air	60	21	93.5	100	28	100	100	41	87.8	100	29	100	100
	120	15	85.5	65.5	17	69.3	69.6	31	64.5	83.9	17	82.4	58.8
Shop outdoor air	60	24	100	98.6	29	89.8	100	38	55.3	100	32	100	100
	N/A	13	68.8	58.2	18	57.5	84.8	31	48.4	77.4	21	71.4	61.9

Note: None of the isolates resisted Cu hence it was excluded from the table. NA = not applicable; and * = refuse dump and location

The mean index values for MAR and MHMR of the total coliforms isolated from refuse dumps, indoor and outdoor air are presented in Table 3. Before curing, the mean MAR index of the coliforms from all sources tended to be clustered at the mid-point of the scale because the range stood at 0.48 - 0.54 (Table 3). However, curing markedly reduced it to 0.11 - 0.36 (Table 3). The statistical comparison of the MAR indexes of coliforms isolated from the four refuse dumpsites showed that the differences were significant only after curing (Table 3). A similar trend was observed when the MAR indexes of indoor and outdoor airborne coliforms were compared (Table 3). Unlike MAR, the mean MHMR index of all the isolates was found to be the same before curing (Table 3). It was also reduced after curing, but again to the same value for all the isolates, as shown in Table 3. Thus, there were no differences in the MHMR indexes of coliforms from the dumpsites, and between indoor and outdoor airborne coliforms before and after curing (Table 3).

Table 3. MAR and MHMR indexes of total coliform bacteria isolated from refuse dumpsites, indoor and outdoor air of houses and shops within the vicinity of refuse dumpsites

Source of coliform bacteria	Mean MAR index ± SD		Mean MHMR ± SD		
	Before curing	After curing	Before curing	After curing	
Refuse dumps	Roadside	0.48 ± 0.04	0.11 ± 0.01	0.66 ± 0.00	0.33 ± 0.00
	Market	0.52 ± 0.04	0.22 ± 0.01	0.66 ± 0.00	0.33 ± 0.00
	Gutters	0.49 ± 0.05	0.22 ± 0.01	0.66 ± 0.00	0.33 ± 0.00
	Residential area	0.52 ± 0.05	0.11 ± 0.01	0.66 ± 0.00	0.33 ± 0.00
	<i>P</i> (ANOVA)	> 0.05	< 0.05	> 0.05	> 0.05
Houses	Indoor air	0.54 ± 0.04	0.24 ± 0.01	0.66 ± 0.00	0.33 ± 0.00
	Outdoor air	0.52 ± 0.03	0.36 ± 0.03	0.66 ± 0.00	0.33 ± 0.00
	<i>P</i> (<i>t</i> -test)	> 0.05	< 0.05	> 0.05	> 0.05
Shopss	Indoor air	0.54 ± 0.04	0.33 ± 0.02	0.66 ± 0.00	0.33 ± 0.00
	Outdoor air	0.48 ± 0.04	0.22 ± 0.01	0.66 ± 0.00	0.33 ± 0.00
	<i>P</i> (<i>t</i> -test)	> 0.05	< 0.05	> 0.05	> 0.05

Note: SD = standard deviation

The results of the statistical comparison of the MAR and MHMR indexes of the coliforms from indoor and outdoor air on the basis of proximity to refuse dumpsites can be seen in Table 4. It showed that the index value of coliforms isolated from indoor and outdoor air of houses and shops closer to the refuse dumpsites was significantly higher than that of houses or shops farther away before curing (Table 4). This trend was reversed after curing (Table 4). With respect to the MHMR index, no significant difference was encountered due to distance before and after curing (Table 4). As shown in Figure 2, there were no marked differences in the prevalence of antibiotics and heavy metal co-resistance of the coliform bacteria isolated from the four refuse dumpsite locations. Compared to isolates from refuse dumps, the prevalence of antibiotics and metal co-resistance among indoor and outdoor air coliforms was markedly lower and tended to decline with distance from the dumpsites (Figure 2). Although the coefficient varied, there were significant correlations between the MAR and MHMR indexes of the coliform bacterial isolates except for the isolates from buildings that are at a greater distance from dumpsites (Table 5). The correlation between the MAR or MHMR indexes of dumpsite coliforms and nearby buildings' airborne coliforms was generally significant (Table 6). The number of significant correlations and their strength declined as the distance of the airborne source of the coliforms from the dumpsites increased (Table 6).

Table 4. Statistical analyses of the differences in MAR and MHMR indexes of total coliform bacteria isolated from the air of houses and shops on the basis of distance from refuse dumpsites

Index	Sampling locations	Curing	Distance from refuse dumpsites (m)		Sign. diff. (<i>P</i>)
			60	120	
MAR (M ± SD)	House	Before curing	0.54 ± 0.04	0.22 ± 0.02	< 0.0
		After curing	0.12 ± 0.01	0.22 ± 0.02	< 0.05
	Shop	Before curing	0.44 ± 0.04	0.36 ± 0.02	< 0.05
		After curing	0.11 ± 0.06	0.36 ± 0.02	< 0.05
MHMR (M ± SD)	House	Before curing	0.66 ± 0.00	0.66 ± 0.00	> 0.05
		After curing	0.33 ± 0.00	0.33 ± 0.00	< 0.05
	Shop	Before curing	0.66 ± 0.00	0.66 ± 0.00	> 0.05
		After curing	0.33 ± 0.00	0.33 ± 0.00	> 0.05

Note: Sign. diff. = significant difference

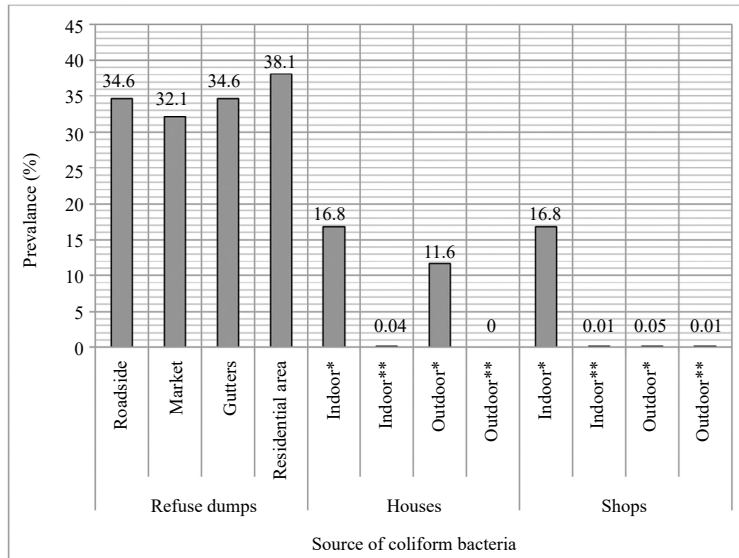


Figure 2. Prevalence of plasmid-mediated antibiotics and heavy metal co-resistance among total coliform bacteria. Note: * = 60 m from refuse dumpsites; and ** = 120 m from refuse dumpsites

Table 5. Correlation of MAR and MHMR indexes in coliform bacterial isolates

Source of coliform bacteria		Distance from dumpsite (m)	Correlation coefficient (<i>r</i>)
Dumpsites	Roadside	NA	0.47*
	Market	NA	0.42*
	Gutters	NA	0.50**
	Residential area	NA	0.58**
Buildings	Indoor air	60	0.41*
		120	0.25
	Outdoor air	60	0.46*
		120	0.48*

Note: NA = not applicable; * = $P < 0.05$; and ** = $P < 0.01$

Table 6. Correlation of MAR or MHMR indexes of dumpsites and buildings' air coliform bacteria

Index	Dumpsite coliforms	Correlation coefficient (<i>r</i>)			
		Indoor air coliforms		Outdoor air coliforms	
		^a 60 m	^a 120 m	^a 60 m	^a 120 m
MAR	Roadside	0.31*	0.25*	0.38*	0.23*
	Market	0.32*	0.18	0.35*	0.16
	Gutters/drainage	0.33*	0.19	0.45*	0.20
	Residential area	0.38*	0.26*	0.52**	0.27*
MHMR	Roadside	0.29*	0.14	0.22	0.15
	Market	0.25*	0.18	0.30*	0.16
	Gutters/drainage	0.28*	0.20*	0.36*	0.27*
	Residential area	0.30*	0.25*	0.38*	0.28*

Note: ^a = distance from dumpsite; * = $P < 0.05$; and ** = $P < 0.01$

4. Discussion

The finding demonstrates that between 30 and 40% of the coliform bacterial isolates had a MAR index higher than 0.2. This suggests a substantial discharge of antibiotics into the refuse dumpsites or the induction of resistance to antibiotics. The antibiotics may have originated from human or animal discharges in either metabolised or un-degraded form [54, 55]. Open defaecation and urination are common practises in many Nigerian urban neighbourhoods, and these may contain undegraded antibiotics excreted from the urine of those on medication. Agricultural wastes, which usually contain prophylactic antibiotics, are also often disposed of in refuse dumpsites in Warri town. All these factors bring to existence a spot for selective pressure that may result in the emergence of metal and antibiotic co-resistant microorganisms. Although antibiotic-resistant bacteria have been isolated in many dumpsites from some developing countries, including SSA [25, 26, 30, 56-58], prevalence has not been thoroughly investigated. This is against the background of severely limited data on antimicrobial resistance and surveillance in Africa [59, 60].

The high level of resistance to Cr and Cd among the coliform bacterial isolates can be attributed to frequent exposure because these metals are commonly present in municipal wastes. The major sources of Cd in refuse dumpsites include expired batteries, plastics where Cd has been used as stabilisers, and disposed metallic materials are usually coated with Cd to resist corrosion [7]. A similar explanation can be advanced for the resistance of the coliform isolates to Cr because the metal reaches the soil and refuse dumpsites from several sources: wood preservatives, leather wastes, porcelain and ceramic wastes, textiles, chrome plating, cement, and paints [61]. It was unexpected that all the isolates would be sensitive to Cu because it has been utilised as a biocide since ancient times [62]. A plausible explanation is that the Cu concentration in the soil matrix may actually be low or mostly present in forms that are limited in the context of exposure to microorganisms. For example, it is known that Cu binds strongly to organic matter in soil with attendant limited mobility [7]. In addition, electrical and electronic materials that are the major sources of Cu wastes in refuse dumps are not biodegradable to release Cu ions into the soil matrix for substantial contact with microorganisms. It can also be attributed to its antagonistic mechanisms because some reports have indicated that microorganisms that are able to tolerate Cu are few due to its non-specific and multi-target killing mechanisms [62, 63].

The finding that the prevalence of Cr/Cd/antibiotics co-resistant coliform bacteria was substantial (34 - 38%) suggests that there was a co-selection of resistance genes. This inference is supported by the results of the correlation analyses. Several reports have indeed indicated that this co-selection phenomenon has often been triggered by the presence of metals [4, 64-69]. This is an alarming discovery as the potential spread to residential areas via bioaerosols is indicated by the presence of resistant coliforms in the indoor and outdoor air of nearby homes and shops. It is impossible to compare the prevalence level to those of similar studies on refuse dumpsites, sewages, sediments, or water bodies in Nigeria [22, 36-39, 70, 71] and other SSA countries: Madagascar [55]; Ghana [25]; and the Democratic Republic of Congo [4, 72], because the prevalence and potential airborne spread were not given attention. The involvement of plasmid-encoded genes and the potential horizontal spread were also not considered. The reduction of the MAR index was due to curing indicated loss of plasmid-encoded resistance genes to some of the test antibiotics. With respect to the metals, all the isolates were resistant to two of the three tested metals, which gave a resistance index value of 0.66 for all the isolates. Therefore, the reduction to 0.33 after curing indicated a loss of resistance to either Cr or Cd. The role of plasmids in encoding resistance to metals and antibiotics is well known [1, 8, 21, 73, 74] and it usually leads to a reduction of the range of antibiotics and heavy metals resisted by microorganisms [8, 74, 75]. Thus, this study highlights the epidemiological dimension of the antibiotics resistance induced by metals in dumpsites in urban neighbourhoods.

The statistical indication that the locations of refuse dumpsites did not have a marked influence on the MAR or MHMR indexes of the coliform bacteria suggests that there may be no major difference in the type of waste at the four sites. Thus, an identical selective pressure is likely to operate in these municipal waste dumpsites irrespective of location. The absence of a functional waste removal system in many urban areas of Nigeria [76] compels many residents to dump waste in gutters, drains, or on roadsides. The MAR and MHMR indexes of indoor and outdoor airborne coliform bacteria were not significantly different because they may have originated from the same refuse dumpsites.

The occurrence of coliform bacteria with co-resistance to antibiotics and heavy metals in the air of outdoor and indoor buildings close to dumpsites can be attributed to wind action promoted by scavenging activities. Indeed, some reports showed that airborne microorganisms can get as far as 1000 m or more from the edge of waste dumps [77, 78]. It was expected that the MAR and MHMR index values of the indoor and outdoor isolates would decline as the distance from the dumpsites increased because of precipitation and sedimentation, and the likely presence of airborne coliforms

that may have originated from sources outside the dumpsites. This inference is supported by the fact that the MAR and MHMR index values of indoor and outdoor isolates from shops or houses located far away (approximately 120 m) from refuse dumps did not change after curing, unlike shops or houses located closer (approximately 60 m).

The study was conducted during the Harmattan season because the spread of antibiotic-resistant bacteria from dumpsites to human habitations is likely due to the Harmattan wind, and this is crucial in the context of public health. While wind can launch or levitate particles and microorganisms into the air, rainfall causes depositions that tend to “clean” the air [79]. Although it can be argued that the rainfall (wet season) can cause a marked increase in the population of antibiotic-resistant bacteria in the dumpsites due to higher moisture conditions, it is of less public health concern because of the dispersal limitations of the season. However, proper launching of the organisms into the air would resume after the wet season, and the dispersal pattern encountered in the previous dry season is likely to be repeated due to the same seasonal effect. Microorganisms obtain moisture for survival in dumpsites during the dry season from the usual low rainfall that occurs in a tropical environment.

4.1 Implication of findings and recommendation

Three major public health issues emerge as the outcome of this investigation. The first is the occurrence of plasmid-encoded antibiotic-resistant coliform bacteria in dumpsites close to human habitation. The presence of coliform bacteria warns of the inherent danger of the likely presence of pathogenic bacteria of faecal origin, which can acquire the plasmids by horizontal transfer. Secondly, the dumpsites contain substances with the potential to elevate the concentrations of heavy metals and subsequently induce metal resistance in bacteria, which can in turn trigger antibiotics resistance. Thirdly, nearby human habitation, especially food and water, becomes exposed to airborne coliform bacteria carrying plasmid-borne antibiotic resistance genes. The potential for spreading the genes to other bacteria by horizontal transfer cannot be ignored. These problems can be eliminated or mitigated by mass media campaigns on the health risks of dumpsites and appropriate waste management undertaken by the public health agency, municipal, or local government authorities. This can be achieved by encouraging the patronage of commercial waste collectors and/or designating waste collection points where large waste collection bins are positioned in neighbourhoods. The municipal government agents can evacuate the waste at specified intervals. Routine public health inspection of the neighbourhood environment with appropriate penalties for defaulters can induce compliance and make the measures sustainable.

5. Conclusion

Municipal waste dumpsites in differing locations were shown to be potential “breeding” sites for antimicrobial resistance in urban neighbourhoods and markets. The occurrence of antibiotics/Cr/Cd co-resistant coliform bacteria in the dumpsites suggests the metal induction of antimicrobial resistance. The presence of antibiotics and metal-resistant coliforms in the indoor and outdoor air of nearby buildings indicated that antimicrobial resistance can emanate from dumpsites and reach buildings via aerosols and perhaps via rodents and insects. The finding that plasmid-encoded genes were implicated in the development of the resistance warns of the potential horizontal transfer of such genes to pathogenic bacteria in homes. Thus, the substantial prevalence of metals and/or antibiotics co-resistant coliforms in dumpsites located in the nooks and corners of typical Nigerian urban settlements and their egress to homes has epidemiological implications. This finding can be considered a contribution towards narrowing the gap in the surveillance data on antimicrobial resistance in SSA.

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Conflict of interest

The authors declare that there is no conflict of interest.

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