Spread of Multi-drug Resistant Aerosolised *Salmonella sp* and *Escherichiacoli* from Dumpsites in Built Environment of Abraka Town, Nigeria

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Abstract

Waste dumpsites are known to be hot spots for the development of multi-drug resistant (MDR) pathogenic microorganisms, which can be dispersed via aerosols. The dispersal of MDR *Salmonella sp* and *E. coli* in dumpsite-infested neighbourhoods of Abraka, a university town, was therefore investigated. Eosine Methylene Blue and Bismuth Sulphite agar plates were used to isolate airborne *E. coli* and *Salmonella sp*, respectively using settling plate technique. Air sampling was carried out at 3 neighbourhood dumpsites and at intervals of 50m up to 200m away from the dumpsites along the cardinal directions. The isolates were tested for antibiotics resistance using 10 antibiotics and those with multiple antibiotics resistance (MAR) index≥0.3 were considered MDR and subsequently subjected to plasmid curing, and re-tested for antibiotics resistance. The population of both organisms varied with cardinal directions and dumpsites, but generally declined as the distance from dumpsites increased (*E. coli*, 2.96-1.72 log cfu/m^3; *Salmonella sp*, 2.65-0.00 cfu/m^3). By overall assessment, mean MAR index for *E. coli* isolates was 0.39±0.01-0.64±0.04 and 0.37±0.02-0.68±0.03 for *Salmonella sp* isolates. MDR *E. coli* and *Salmonella sp* constituted 26.7-45.7 and 0.0-47.1% of the total population, respectively; and were encountered up to 200m away from the dumpsites at all directions although with decreasing numbers. There were significant reductions (P<0.05) in the MAR indexes of both organisms after curing thereby indicating plasmid involvement in the MDR. In conclusion, the aerosolised plasmid-encoded and chromosome-based genes in MDR organisms from dumpsites can be dispersed in neighbourhoods; and plasmids can spread antibiotics resistant genes by horizontal transfer and compound therapeutic options.

Keywords: **dumpsite; *E. coli*; *Salmonella*; multi-drug resistance; Abraka'**

Introduction

It is known that dumpsites are hot sports for the development of antibiotic resistance among microorganisms (Chung *et al.*,, 2018;Adekanmbi *et al*, 2021; Mokogwu and Ejechi, 2022). The occurrence of antibiotic resistant bacteria in dumpsites and surrounding air has been reported in several studies (Odeyemi, 2012; Waturu *et al.*, 2017; Borquaye *et al.*, 2019; Odum *et al.*,

2020; Olalemi *et al.,* 2020; Morgado-Gamero, 2021; Uwem *et al,* 2023). Dumpsites often contain domestic wastes, faecal matter, hospital wastes, biocides residue, industrial wastes, metallic wastes and urine, containing degraded and un-degraded antibiotics (Adekanmbi *et al.*, 2021; Nair 2021; Mokogwu and Ejechi, 2022) that predispose bacteria hitherto growing on biodegradable materials to develop antibiotic resistance or acquire resistance genes by horizontal transfer. Over 80% of the antibiotics used in developing countries are outside the hospital setting (Chang *et al.*,, 2015) hence antibiotics-related wastes reach dumpsites.

The dumpsite microorganisms are aerosolised or launched into the air by dumping activities, scavenging, air turbulence due to movements, wind action, rain water splashes and natural discharge of spores (Pepper and Gerba, 2015). The movement or dispersion of the bioaerosols is subsequently dependent on wind. Several diseases have been associated with exposure to bioaerosols. They include infectious diseases (e.g. influenza, pneumonia, tuberculosis, diarrhoea enteric fever) and non-infectious diseases (e.g allergic reactions, asthma, rhinitis). Those at risk are household and residents in the vicinity of dumpsites and workers in landfill sites (Schlosser, 2019).

Waste dumpsites litter many urban residential and non-residential built environments in Sub-Sahara Africa (SSA) due to severe failure of waste management policies and practices (Boadi *et al.,* 2005; Amegah and Agyei, 2017; Ferronato and Torretta, 2019). Most of the studies concerning pathogenic bacteria in dumpsites in Nigerian urban areas (Ambrose *et al.,* 2015; Akpeimeh *et al.,* 2019; Odum *et al.,* 2020; Olalemi *et al.,* 2020; Adekanmbi *et al.,* 2021; Mokogwu and Ejechi, 2022; Uwem *et al.,* 2023) have not included the

extent of dispersal of MDR bacteria from the dumpsites in built environment despite the public health implication of airborne pathogenic microorganisms.

Bioaerosols containing MDR bacteria can enter household through doors and windows and contaminate food and cooking utensils and cause infections. Worse still, resistance genes can be transferred to resident bacteria in homes and consequently reduce the efficacy of chemotherapy. Studies on airborne MDR bacteria have focused more on hospital indoor air (Mbim *et al.,* 2016; Iroha *et al* 2020; Onifade *et al.,* 2020), immediate vicinity of dumpsites (Odeyemi, 2012) or other environments like restaurant (Chimbekujwo *et al.*, 2022) and kitchen (Ejechi and Ochei, 2017). *Salmonella sp* is the causative agent of typhoid fever while *E. coli* is an indicator of potential faecal pollution. Thus they can be useful indicators of the extent of dispersal of other MDR pathogens from dumpsites in a tropical built environment. The investigation was therefore undertaken to ascertain the extent of spread of *Salmonella sp* and *E. coli* in three neighbourhoods of the in Abraka a university town.

Materials and Methods

Source of bioaerosols samples

Abraka is a University town located in Ethiope East Local Government of Delta State, Nigeria. The population of Abraka town is about 20,000 (NPC, 2006) and is characterised by waste dumpsites in nooks and crannies of the town. Waste dumpsites in three neighbourhoods were selected for the study. The bioaerosols sampling points were, the dumpsite air, and the air at 50, 100, 150 and 200m away from the dumpsites along the four cardinal directions. Sampling took place at 7.30pm-8.00pm when it was expected that turbulence due to human activity would have reduced since the collections were by settle plate technique.

Isolation and enumeration

Plates containing selective media Eosine Methylene Blue agar (EMB) and Bismuth Sulphite Agar (BSA) were used for the isolation and enumeration of *E. coli* and *Salmonella sp*, respectively. They were exposed at the designated sampling locations for 30 minutes. Thereafter triplicate plates per location were retrieved and taken to the laboratory and incubated at room temperature (37^{o}C) for 24-48 hours. Distinct colonies were counted, sub-cultured and stored in agar slants at 4 ^{o}C for subsequent tests (Cheesbrough, 2018).

Antibiotics resistance tests

Mueller-Hinton agar-based disc diffusion method (CLSI, 2011) was used for determining the resistance of the isolates to 10 antibiotics (Nitrofurantoin, 300 µg; Amoxicilin clavulanate, 30 µg; gentamicin, 10 µg; levofloxacin, 5µg; cefuroxime, 30 µg, ampiclox, 30 µg; cefotaxime, 30 µg; imipenem/cilastatin, 10µg; ofloxacin, 5 µg; and cefexime, 5 µg). The interpretation of the zones of inhibition was measured as stipulated by CSLI (2011). The multiple antibiotics resistance (MAR) index was subsequently calculated as: Number of antibiotic resistant ÷ total number of antibiotics used.

Plasmid curing

Isolates with MAR index of 0.3 and above were subjected to plasmid curing by the sodium dodecyl sulphate technique. The cured isolates were subsequently tested for antibiotics resistance as before (Buckner, Ciusa, and Piddock., 2018)

Data Analysis

The population of the isolates was calculated with the Omeliansky formula (Awad and Mawla, 2012): N=5a x 104(bt)-1. N=cfu/m3; a=number of colonies per Petri dish, b=dish square cm, t=exposure time in minutes. The MAR index before and after curing, was compared by *t* test.

Results

Figure 1 presents the population of *E. coli* in the air of each of the three dumpsites and the air up to 200m away at all cardinal directions. The population of the *E. coli* isolates declined along the cardinal directions up to the last point sampled. The population varied with cardinal directions and dumpsites (Figure 1). The same trend was observed with *Salmonella sp* although the population was markedly lower than that of *E. coli* (Figure 2). In addition, unlike *E. coli*, *Salmonella sp* was not encountered at 200m away from the 3 dumpsites along some cardinal directions (Figure 2).

The number of *E. coli and Salmonella sp* isolates associated with each of the MAR indexes declined with increasing index as shown in Figure 3. There were no isolates with MAR indexes 0.8-1.0 and 0.9-1.0 for *Salmonella sp* and *E. coli*, respectively. The trend was similar with respect to the three dumpsites (Figure 3). Isolates with MAR index of 0.3 and above (taken as MDR) were generally less than 50% for both *E. coli* and *Salmonella sp*

MDR isolates of *E. coli* were found at all distances from the dumpsite although with declining numbers as distance from the dumpsites increased (Table 1). An identical trend was encountered with *Salmonella sp* MDR isolates except that they were not found at 200m away from dumpsites 2 and 3 (Table 1). Plasmid curing significantly reduced the MAR indexes across all sampling points and dumpsites as shown in Table 2.

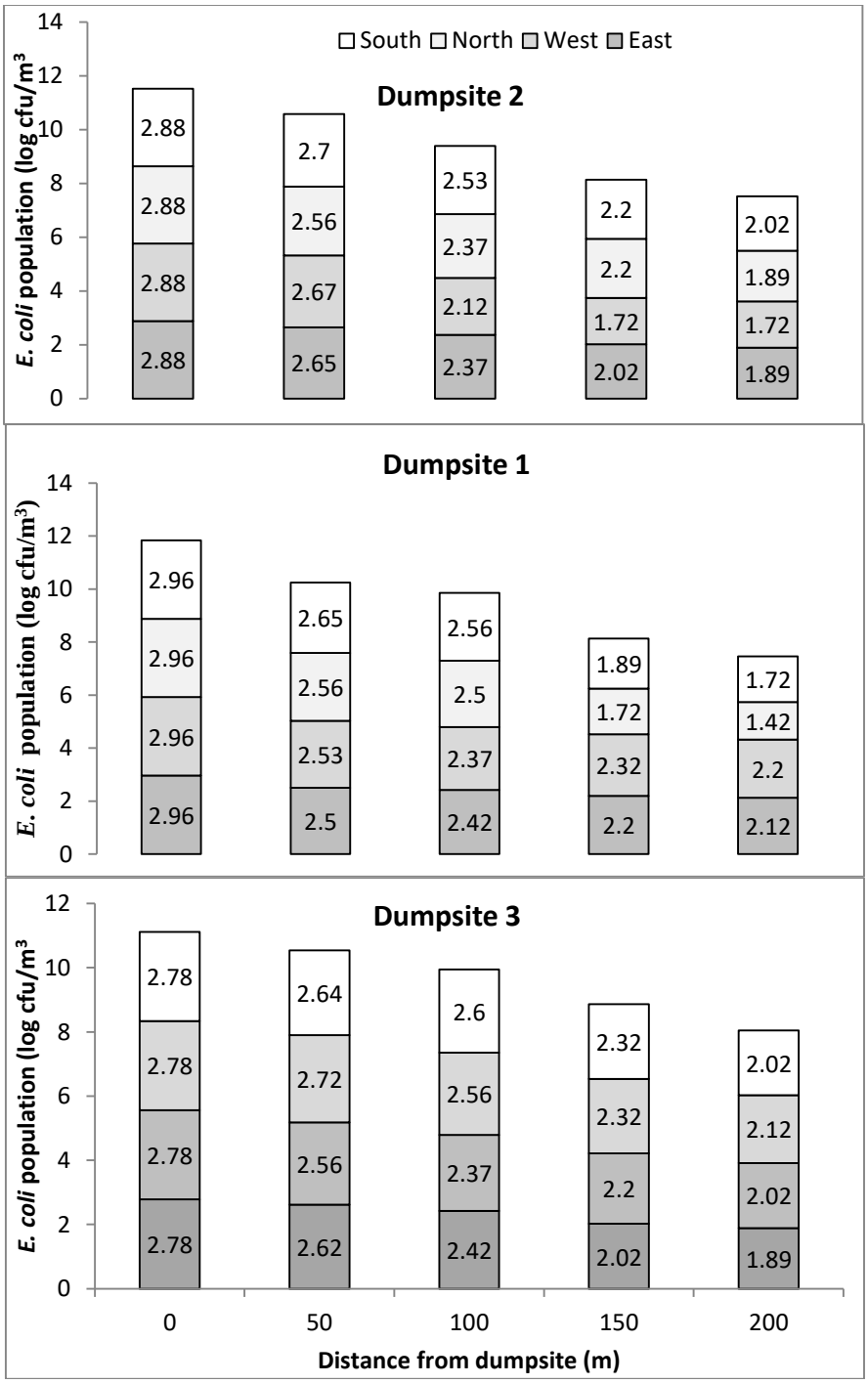

Figure 1 Airborne *Escherichia coli* population at dumpsites and distances away along cardinal directions

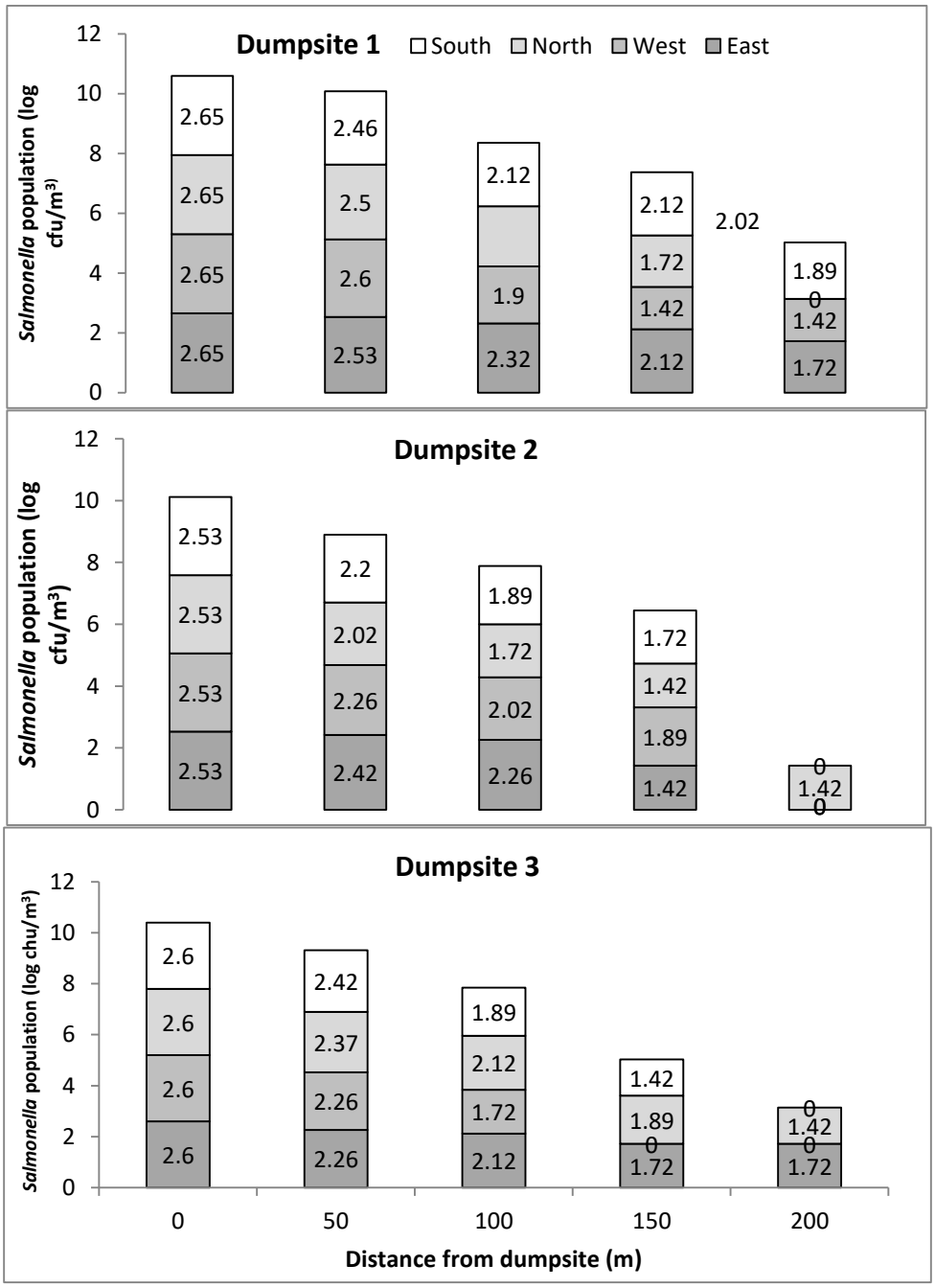

Figure 2 Airborne *Salmonella sp* population at dumpsites and distances away along cardinal directions

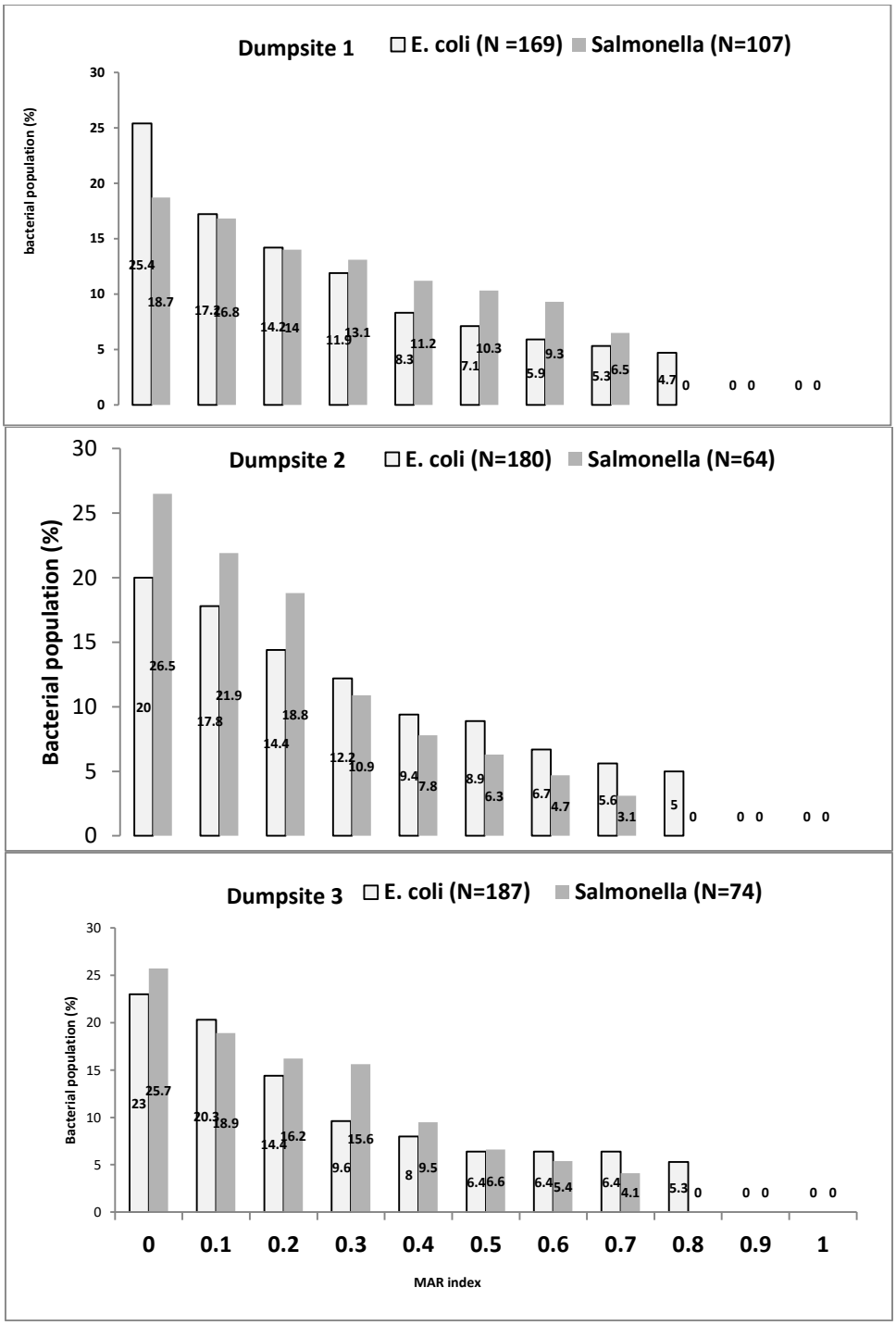

Figure 3 Trends in the multiple antibiotics resistance (MAR) indexes of aerosolised *E. coli* and *Salmonella sp*

Dumpsites	*Distance (m)	*E. coli*			*Salmonella sp*		
		Total isolates	MDR isolates		Total isolates	MDR isolates	
		*N	*n*	%	*N	*n*	%
1	0 (dumpsite)	35	16	45.7	17	8	47.1
	50	56	25	44.6	51	19	37.3
	100	45	15	33.3	20	7	35.0
	150	19	6	31.6	13	4	30.8
	200	14	4	28.6	6	2	33.3
2	0 (dumpsite)	46	21	45.6	13	6	46.2
	50	69	30	43.5	27	12	44.4
	100	35	11	31.4	16	6	37.5
	150	18	5	27.8	7	2	28.6
	200	12	3	25.0	1	0	0.0
3	0 (dumpsite)	23	10	43.5	15	4	26.7
	50	67	29	43.3	33	8	24.2
	100	55	18	32.7	15	4	26.7
	150	26	8	30.8	6	1	16.7
	200	15	4	26.7	5	0	0.0

Table 1 The spread of aerosolized multi-drug resistant (MDR) isolates from dumpsites

*From dumpsite; MDR=isolates with multiple antibiotics resistant (MAR) index ≥0.3. *N=Total from all cardinal points

Table 2. Effect of plasmid curing on the MAR index of dispersed isolates

Dumpsites	[a]Distance (m)	Mean MAR index±SD			
		[b]*E. coli*		[b]*Salmonella sp*	
		Before curing	After curing	Before curing	After curing
1	0	0.64±0.04	0.20±0.01	0.68±0.03	0.44±0.02
	50	0.53±0.02	0.34±0.01	0.49±0.02	0.40±0.02
	100	0.50±0.01	0.42±0.02	0.37±0.02	0.30±0.01
	150	0.52±0.02	0.30±0.02	0.40±0.02	0.25±0.01
	200	0.48±0.02	0.40±0.02	0.44±0.03	0.34±0.01
2	0	0.68±0.05	0.30±0.01	0.57±0.03	0.40±0.02
	50	0.65±0.04	0.30±0.01	0.53±0.03	0.35±0.02
	100	0.54±0.03	0.20±0.01	0.50±0.03	0.42±0.02
	150	0.51±0.02	0.30±0.02	0.48±0.02	0.32±0.01
	200	0.47±0.02	0.10±0.01	[c]None	[c]None
3	0	0.56±0.03	0.20±0.01	0.60±0.04	0.26±0.01
	50	0.52±0.02	0.20±0.01	0.45±0.02	0.30±0.02
	100	0.45±0.01	0.35±0.01	0.42±0.02	0.28±0.01
	150	0.39±0.01	0.15±0.01	0.40±0.02	0.25±0.01
	200	0.43±0.01	0.22±0.01	[c]None	[c]None

[a]From dumpsite; [b]Significant differences occurred after curing at all locations (t-test, $P<0.05$)'
[c]No MDR isolate

Discussion

The finding of MDR *E. coli and Salmonella sp* in the air at all directions and up to 200m away from dumpsites in the neighbourhoods substantiates the well known propensity of bioaerosols to "travel" some distances away from source. Although microorganisms have been reported to travel great distances (>1000 km) especially when carried by dusty winds (Mazar *et al.,* 2016), it cannot be so in a built environment because the buildings can restrain wind movement. The dispersal of both organisms in all directions as indicated by the trend in the cardinal points can be explained by meteorological conditions and wind deflection in all directions that is usually associated with buildings (Gao *et al.,* 2012). This deflection can also be caused by vegetation and vehicular traffic. This was the rationale for taking samples along the cardinal points and not just upwind and downwind directions. The deduction from the interpretation of the role of buildings and trees in built environment is that it limits the spread of bioaerosols which should be an advantage. However, it should be noted that this seeming advantage can be nullified by the presence of dumpsites in nooks and crannies of neighbourhoods which is typical of urban Nigerian settings.

Although microorganisms in aerosols can come from other sources in the neighbourhood, dumpsite as the source of these MDR bacteria is most probable because of the fact that they are hot spots for the development of antibiotics resistance (Odeyemi, 2012; Waturu *et al.,* 2017; Borquaye*et al.,* 2019; Odum *et al.,* 2020; Olalemi *et al.,* 2020; Morgado-Gamero,

2021; Adekanmbi *et al.,* 2021; Nair 2021; Mokogwu and Ejechi, 2022; Uwem *et al,* 2023). Besides MDR *E. coli* and *Salmonella sp* were isolated from the air over the dumpsites and their presence was not unexpected because they are known to be present in dumpsites (Nyandjou *et al.,* 2018; Obumneme *et al.,* 2019; Adekanmbi *et al.,* 2021; Nair, 2021; Kapali *et al.,* 2023). Dumpsites are receptors of faecal matter and domestic, poultry and hospital wastes that are associated with enteric bacteria (Gerba *et al.,* 2011; Nair, 2021). These pathogens are aerosolized by wind and human activities as bioaerosols and their spread and survival depend on wind, gravity settling, desiccation (drying), temperature fluctuations, and exposure to ultraviolet (UV) radiation (Pepper and Gerba, 2015).

The study revealed that *E. coli* and *Salmonella sp* isolates were able to spread in the neighbourhoods up to 200m in all directions despite the above stated militating factors which may have caused reduction in numbers with increasing distance. This population decreasing trend of microorganisms from sources such as waste dumpsites or landfills is well known (Vilavert *et al.,* 2012;Nair, 2021). However, the finding of *E. coli* and *Salmonella sp* isolates up to 200m away from dumpsites indicated the danger of the presence of dumpsites within built environment or neighbourhoods. The endemic nature of *Salmonella sp* infections in Nigeria (Akinyemi *et al.,* 2018) can partly be explained by the spread of airborne *Salmonella* sp. from the dumpsites located in nooks and crannies of neighbourhoods. The dispersal of *E. coli* an indicator organism, suggests the potential presence of other pathogens in the dispersed bioaerosols from dumpsites.

The health implication of the spread of airborne pathogens from waste disposal sources has been reported. For example

residents within 150-200m of composting plant complained of respiratory diseases (Herr *et al.,* 2003) while those within 50-100m of dumpsites complained of odour and health challenges (Shammi *et al.,* 2023). Nair (2021) reported that workers in landfill sites take home microorganisms impinged on their dresses and disseminate infection. Children playing near dumpsites in urban neighbourhoods, adults frequently passing by dumpsites and scavengers may also transport microorganisms to their homes by similar modes. The spread of MDR *E. coli* and *Salmonella sp* from dumpsites in the neigbourhood as the findings revealed is potentially hazardous to the residents because of limited chemotherapy options in the event of an outbreak of bacterial infections.

The involvement of plasmids in the antibiotics resistance of the *E. coli* and *Salmonella sp* isolates was indicated by the significant reductions of MAR indexes after plasmid curing. The inference is that horizontal transfer and spread of resistant genes would further compound the efficacy of treatment. This should be worrisome to public health agencies hence solutions to the indiscriminate dumping of wastes in most urban neighbourhoods in Nigeria and sub-Sahara Africa should be found. Built environments unlike the open field, are likely to confine bioaerosols by providing surfaces for impingement of microorganism on walls, windows and surrounding tree canopies. This hypothesis is the subject of an on-going investigation.

Conclusion

It can be concluded that the presence of numerous dumpsites in Abraka town neighbourhoods which is typical of urban areas in Nigeria should be of concern to public health agencies because they are sources of aerosolised infectious agents.

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