

FUDMA Journal of Sciences (FJS) ISSN online: 2616-1370 ISSN print: 2645 - 2944 Vol. 4 No. 2, June, 2020, pp 639 – 644 DOI: <u>https://doi.org/10.33003/fjs-2020-0402-196</u>



## MULTIDRUG RESISTANT BACTERIA IN DUMPSITES WITHIN ABRAKA, DELTA STATE, NIGERIA

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## ABSTRACT

This study investigated the prevalence of antibiotic resistant bacteria in soils obtained from dumpsites in Abraka, Delta State, Nigeria. Four (4) dumpsites were studied by collection of soil samples from each dumpsite. Isolates were obtained using the standard pour-plate method and pure cultures identified based on their cultural and biochemical characteristics. These were then subjected to antibiotic susceptibility test using the agar-disc diffusion method. A total of 61 isolates were obtained and identified to belong to nine (9) different genera. The isolates were Escherichia spp., Klebsiella spp., Staphylococcus spp., Bacillus spp., Pseudomonas spp., Proteus spp., Micrococcus spp., Streptococcus spp. and Neisseria spp. Among the isolates obtained, Escherichia spp. had the highest occurrence (26.23%) and Neisseria spp. the lowest occurrence frequency of 1.63%. Pefloxacin was the least effective antibiotic as 96% and 76.67% of gram-positive and gram-negative organisms respectively, were resistant to the drug. All the isolates exhibited multidrug tolerance and a multiple antibiotic resistance (MAR) index of 0.8 was recorded for 31.15% of the isolates tested. The implication of these findings is a possible spread of persistent bacterial infections among the resident population. Proper waste management and siting of dumpsites in locations distant from residential areas will help in preventing the public health hazards associated with dumpsites.

Keywords: Antibiotic susceptibility, Bacteria, Prevalence, Dumpsite soil, Public health

## INTRODUCTION

The indiscriminate disposal of wastes and improper management of waste disposal sites are some of the major environmental challenges in Nigeria. The dense human population and industrialization of urban areas can be said to influence the rate at which these dumpsites occur; an important cause of environmental pollution. A vast variety of waste are constantly generated in Nigeria and all over the globe (Oviasogie and Agbonlahor, 2013). Some of the major types of wastes found in domestic dumpsites are food-wastes, agricultural wastes, human wastes (faeces and urine), and medical wastes. Some of these wastes are hazardous to human health. Waste treatment and disposal sites have been observed to possess the potential of creating health hazards for neighboring populations when they are not properly sited (Gani et al., 2013). When waste is dumped on land, microorganisms proliferate, while utilizing the components of the wastes as nutrient sources and degradation of complex waste materials (Williams and Hakam, 2016). These dumpsites are usually accompanied by very offensive odors and serve as sources of infections to humans.

Waste dumpsites can serve as sources of pathogenic bacteria and fungi (Odeyemi, 2012). The use of dumpsites for defecation as well as animal sheds can promote the prevalence of antibioticresistant microorganisms. The improper disposal of unused and expired drugs contributes to the development of drug resistant microbial strains within the environment thereby posing more health risks (Borquaye *et al.*, 2019). The presence of pathogenic microorganisms in soil can bring about possible air pollution as dust particles are taken up by air currents into the atmosphere. It can also lead to contamination of ground water through leachates from the dumpsites. These are deemed possible because atmospheric transport has been identified as a key mode of microbial dispersal and transmission of diseases (Odeyemi, 2012). These pathogens can, therefore, find their way into the human body either through improper hygienic practices, injury sustained at dumpsites or through the process of inhalation. Also the high occurrence of rodents and other common disease vectors can promote the spread of these pathogens (Waturu *et al.*, 2017).

Multidrug resistance among microbial populations has become a major global problem (Mwaikono *et al.*, 2015). These microbes have developed mechanisms of evading the effects of various antibiotics currently in use. Several researches (Idahosa *et al.*, 2017; Mwaikono *et al.*, 2015) have pointed out that domestic dumpsites harbor high populations of multidrug resistant microbes. Oviasogie and Agbonlahor (2003), while studying the burden, antibiogram and pathogenicity of bacteria found in municipal waste dumpsites and on waste site workers in Benin City discovered that all isolates obtained showed multiple antibiotic resistance patterns. They also discovered that some of these isolates carried pathogenic traits. This high prevalence can be linked to the introduction of resistant strains via indiscriminate disposal of medical wastes as well as defecation by human carriers and livestock. Resistance genes may then be acquired by other susceptible strains in the environment via horizontal gene transfer. The fact that there is high diversity of antimicrobial resistant bacteria in dumpsites as well as frequent animal and human interactions on dumpsites increases the chances of infection with antibiotic resistant pathogens among human populations.

The most susceptible group of individuals to the public health hazards associated with domestic dumpsites are waste-pickers (scavengers) who live and work under unhygienic conditions. They are usually seen to visit dumpsites in search of recyclable materials (plastics, bottles, metallic objects, and clothes). This increases the risk of infections through inhalation of contaminated aerosols, cuts from sharp objects and infection of wounds by soil pathogens (Thirarattanasunthon et al., 2012). Another problem is the use of recycled packaging materials obtained from dumpsites for selling food items in markets. Also, most municipal dumpsites in Nigeria are located at distances not far away from residential houses. This makes these waste dumpsites an environmental interest as not just scavengers are prone to the health hazards associated with dumpsites but also residents of urban societies (Osazee et al., 2013). This study, therefore, focused on the prevalence of bacterial pathogens in waste dumpsites located in Abraka, Nigeria. It further provided information on their antibiotic susceptibility as more Multidrug Resistant (MDR) strains keep emerging.

## MATERIALS AND METHODS

### Study site description and sample collection

Soil samples were collected from four (4) major dumpsites in Abraka, Delta State, Nigeria. The study area (Abraka) is a growing university town, where Delta State University (DELSU) is located. The dumpsites assayed are situated close to residential buildings, hostels, trading centers and even small scale food industries. The first dumpsite (Dumpsite A) is situated off College road and in front of a private hostel; the second (Dumpsite B) is between Ethiope and Council Halls (residence of female students, Campus II, DELSU): the third (Dumpsite C) is located off Business centre road and a few meters from a bakery, while the fourth (Dumpsite D) is a major dumpsite along Winners Road. Soil samples were collected following the methods of Osazee et al. (2013). Top soil samples were collected at a depth of 2 -20cm from the surface with the use of an auger after removal of surface debris. Collection was done randomly at 5 points on each of the sites after which representative composite samples were obtained by mixing all samples for each dumpsite. A total of 8 composite samples were collected as each dumpsite was visited once in a fortnight (2 weeks). Samples were dispensed into new polythene bags previously soaked in 95% ethanol to sterilize, labeled appropriately and transported to the Microbiology laboratory (DELSU) for analysis. All samples were collected in the month

#### of February, 2020.

#### Isolation and Identification of bacteria from soil samples

Ten (10) grams of each sample was weighed and introduced into 90ml of sterile peptone water contained in cotton-plugged test tubes. The mixtures were homogenized by shaking. Six fold serial dilutions were made by withdrawing 1ml of the mixtures into subsequent test tubes containing 9ml of peptone water. These were then used to inoculate different sterile growth media (Nutrient, MacConkey, and Chocolate agars) using the pour plate method of inoculation. Inoculated plates were incubated at  $37^{\circ}$  C for 24-48 h in an incubator.

Distinct colonies were sub-cultured into fresh sterile medium using the streak plate method and further incubated for 24h to obtain pure cultures. Subcultures were observed for colony features such as color, shape, size, elevation, form, opacity and odor (Oviasogie and Agbonlahor, 2013). Isolates were identified based on their cultural, morphological and biochemical characteristics such as aerobic growth, anaerobic growth, catalase, oxidase, citrate, indole activities as well as glucose fermentation, lactose fermentation, acid and gas production (Cheesbrough, 2005).

### Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted for all isolates using the disk diffusion method outlined by the British Society for Antimicrobial Chemotherapy (BSAC, 2013). Each of the isolates was exposed to 10 antibiotics based on their gram reactions. Commercially prepared antibiotic disks (Maxicare medical laboratories, Lagos, Nigeria) were aseptically placed on the surface of agar culture plates with the use of sterile forceps. These were then incubated at 37° C for 24h. Antibiotic discs used against gram positive organisms contained ampicillin/cloxacillin (30µg), cefuroxime (20µg), amoxicillin (30µg), ceftriaxone (25µg), ciprofloxacin (10µg), streptomycin (30µg), cotrimoxazole (30µg), erythromycin (10µg), pefloxacin (10µg) and clindamycin (10µg), while those used for gram negative organisms contained cotrimoxazole (30µg), chloramphenicol (30µg), sparfloxaxin (10µg), amoxicillin (30µg), amoxicillin/clavulanic acid (30µg), gentamycin (10µg), ofloxacin (10µg), pefloxacin (10µg), streptomycin (30µg) and ciprofloxacin (10µg). Diameters of zones of inhibition were measured and interpreted according to interpretative charts (BSAC, 2013). MAR (Multiple Antibiotic Resistance) index was calculated as follows:

#### MAR = a/b

Where a = number of antibiotics to which the isolate was resistant; b = total number of antibiotics against which individual isolate was tested (Odjadjare *et al.*, 2012).

## **Statistical Analysis**

Statistical analysis was carried out using Microsoft Excel (version 2007). Mean distribution of isolates within the dumpsites and standard deviations were calculated based on the number of different bacterial species isolated.

# RESULTS

A total of 61 isolates were obtained from the various dumpsites which included bacterial species belonging to nine (9) genera. Among the gram- positive isolates were *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Micrococcus* spp. The gram-negative isolates obtained were *Escherichia* spp., *Klebsiella* spp., *Pseudomonas* spp., *Neisseria* spp. and *Proteus* spp.

The prevalence of isolates in the various dumpsites assayed is presented in Table 1. *Escherichia* spp. had the highest prevalence (26.23%) followed by *Bacillus* spp (24.59%).The least occurring isolate was *Neisseria* sp. with an occurrence frequency of 1.63%. Although there was an even distribution of gram-positive and gram-negative bacteria in the dumpsites, a total occurrence frequency of 50.82% was recorded for gram-positive isolates and 49.18% for gram negative isolates.

The antibiogram of the isolates are presented in Tables 2 and 3 respectively for gram positive and gram negative isolates. Susceptibility to the tested antibiotics among bacterial isolates was observed to be generally low. All the isolates were observed to present a resistant population of 50% and above to the antibiotics tested. However, pefloxacin was the least effective as 96% and 76.67% of gram-positive and gram-negative organisms respectively, were resistant to the drug. All the isolates obtained in this research were observed to have multidrug resistance traits. An MAR index of 0.8 was recorded for 31.15% of the isolates tested while an MAR index of 1.0 was recorded for one isolate (*Bacillus* spp.). Only 3.28% of isolates tested showed low MAR indexes of 0.3 and 0.4 (Table 4).

# DISCUSSION

The bacterial isolates obtained from the various dumpsites have been reported by several researchers to occur in waste dumps and the underlying soils. Williams and Hakam. (2015) reported the isolation of Bacillus spp., Escherichia coli, Klebsiella spp., Proteus spp., Pseudomonas spp., Staphylococcus aureus and Streptococcus spp. from dump sites in Port-Harcourt metropolis, Nigeria. Also, Oviasogie and Agbonlahor. (2003) reported the isolation of several bacteria, belonging to the same genera identified in this research, from dumpsites in Benin City, Nigeria. Similar isolates have also been obtained from dumpsites in the south-west regions of Nigeria (Achudume and Olawale, 2007; Adekanle et al., 2014; Odeyemi, 2012; Sulaimon et al., 2015). The bacterial isolates obtained are indicative of dumpsites harboring bacteria of public health importance. All isolates were obtained at the mesophilic temperature of 37° C, an optimum temperature for the proliferation of certain bacterial pathogens.

The high prevalence of *Escherichia* spp. and *Bacillus* spp. in the dumpsite soils is similar to the findings of Oviasogie and Agbonlahor. (2003) in Benin City, Nigeria. They reported that *Bacillus* spp. (18.20%) *Staphylococcus* sp (13.93%), *Escherichia coli* (12.72%) are among the predominant bacterial

species in dumpsite soils and leachate. Mwaikono *et al.* (2015) reported that *Bacillus* spp. was the second most abundant group in municipal dumpsites in Tanzania after *Escherichia* spp. The high frequency of occurrence recorded for *Escherichia coli* in this research is indicative of frequent human and animal interactions at the various dumpsites which was also suggested by Mwaikono *et al.* (2015). The disposal of human and animal wastes at the dumpsites can be a source of *Facillus* spp. calls for serious health concern as spores of *Bacillus* spp. are easily transported by air current and dust particles. These may cause food poisoning if they come in contact with food. These organisms are also important as they are the aetiological agents of common diseases (Egong *et al.*, 2016).

High tolerance of the isolates to the antibiotics is suggestive of a public health risk. Resistance to common antibiotics usually administered for the treatment of common bacterial infections makes dumpsites significant sites for the acquisition of antibiotic resistant strains and the emergence of new disease types. Sulaimon et al. (2015), reported the isolation of bacteria from a dumpsite of which all gram-positive isolates were resistant to cefuroxime and ampicillin/cloxacillin, while 75% were resistant to cotrimoxazole and streptomycin. Although Mwaikono et al. (2015) in their study of a municipal dumpsite in Tanzania reported that ciprofloxacin and gentamycin were effective against enteric bacterial isolates from dumpsites; they also stated that 56% (45/80) of the isolates obtained were resistant to at least two antibiotics. Some isolates were resistant to more than four of the tested antibiotics. This research presents a contrary opinion to that of Oviasogie and Agbonlahor. (2003) who stated that fluoroquinolones are effective against dumpsite isolates as high resistance to ciprofloxacin and pefloxacin were recorded.

Multidrug-resistance among dumpsite isolates has been commonly reported. All the bacteria isolated from the dumpsites in Abraka showed multiple drug resistance patterns. These finding are similar to those of Oviasogie and Agbonlahor. (2003) and Odjadjare et al. (2012). The high rate of multidrug resistance among the dumpsites isolates is suggestive of the introduction of resistant strains through the observed indiscriminate defecation and disposal of wastes at these dumpsites. The isolates must have originated from sources associated with high antibiotic use (Odjadjare et al., 2012). It is also indicative of the possible transfer of resistant genes via exchange of genetic materials (plasmids) among the microbial populations in dumpsite soils. A possible indication is the indiscriminate and high use of antibiotics among Abraka residents which corroborates reports of Nyandjou et al. (2019). The implication of these findings is a possible spread of persistent and "difficult to cure" bacterial infections among the resident population. Improper recycling of food packaging materials (bottles) picked up by dumpsite scavengers is a further means by which these isolates can spread to human hosts.

 Table 1: Prevalence of bacterial isolates at different waste dumpsites within Abraka, Delta State

S/N	Isolates		No. of bacteria at different dumpsites				Frequency of occurrence (%)	Mean ± SD
		Α	B	C	D		000ui i enec (70)	
1	Escherichia coli	5	3	4	4	16	26.23	1.3±1.6
2	Staphylococcus spp.	2	2	3	1	8	13.11	$0.66 \pm 0.8$
3	Streptococcus spp.	1	1	1	1	4	6.56	0.33±0.4
4	Proteus spp.	2	0	1	1	4	6.56	$0.33 \pm 0.4$
5	Pseudomonas spp.	1	1	0	1	3	4.92	0.25±0.3
6	Klebsiella spp.	1	1	0	0	2	3.28	0.16±0.1
7	Neisseria spp.	1	0	0	0	1	1.63	$0.08\pm0.1$
8	Micrococcus spp.	1	1	0	2	4	6.56	0.33±0.4
9	Bacillus spp.	0	2	6	3	11	18.03	$0.92 \pm 1.1$
10	Proteus spp.	0	1	1	0	2	3.28	0.16±0.1
11	Klebsiella spp.	0	1	0	1	2	3.28	0.16±0.1
12	Bacillus spp.	0	0	3	1	4	6.56	0.33±0.4
	Total					61	100	

Table 2: Antibiogram of gram-positive bacteria isolated from waste dumpsites within Abraka

Antibiotics (conc.)	Ν	umber of Isolates (%) (n=31)	
	Sensitive	Intermediate	Resistant
Ampicillin/Cloxacillin (30µg)	2(6.45)	5(16.13)	24(77.42)
Cefuroxime (20µg)	5(16.13)	3(9.68)	23(74.19)
Amoxicillin (30µg)	6(19.35)	6(19.35)	19(61.29)
Ceftriaxone (25µg)	4(12.90)	8(25.81)	19(61.29)
Ciprofloxacin (10µg)	1(3.23)	4(12.90)	26(83.87)
Streptomycin (30 µg)	2(6.45)	6(19.35)	23(74.19)
Cotrimoxazole (30µg)	7(22.58)	5(16.13)	19(61.29)
Erythromycin (10µg)	7(22.58)	6(19.35)	18(58.06)
Pefloxacin (10µg)	1(3.23)	0(0)	30(96.77)
Clindamycin (10µg)	2(6.45)	0(0)	29(93.55)

# Table 3: Antibiogram of gram-negative bacteria isolated from waste dumpsites within Abraka

Antibiotics (conc.)		Number of Isolates (%)	(n=30)
	Sensitive	Intermediate	Resistant
Cotrimoxazole (10µg)	11(36.67)	4(13.33)	15(50)
Chloramphenicol (30µg)	6(20)	8(26.67)	16(53.33)
Sparfloxacin (10µg)	7(23.33)	8(26.67)	15(50)
Amoxicillin (30µg)	9(30)	5(16.67)	16(53.33)
Amoxicillin/Clavulanic acid (10µg)	8(26.67)	7(23.33)	15(50)
Gentamycin (10µg)	3(10)	5(16.67)	22(73.33)
Pefloxacin (30µg)	4(13.33)	3(10)	23(76.67)
Ofloxacin (10µg)	7(23.33)	5(16.67)	18(60)
Streptomycin (30µg)	6(20)	5(16.67)	19(63.33)
Ciprofloxacin (10µg)	3(10)	3(10)	24(80)

No. of Antibiotics	No. of resisted antibiotics	MAR index (a/b)	Frequency of MAR Isolates (%)	Resistant gram positive organisms (No. of isolates)	Resistant gram negative organisms (No. of isolates)
10	3	0.3	2(3.28)	nil	Klebsiella spp.(2)
	4	0.4	2(3.28)	Bacillus spp. (1)	Pseudomonas spp. (1)
	5	0.5	7(11.47)	Bacillus spp. (1)	Pseudomonas spp. (1) Escherichia coli (3) Proteus spp. (1) Klebsiella spp. (1)
	6	0.6	12(19.67)	Bacillus spp. (1) Staphylococcus spp. (1) Micrococcus spp (1)	Neisseria spp. (1) Escherichia coli (3) Klebsiella spp. (1) Proteus spp. (3) Pseudomonas spp. (1)
	7	0.7	16(26.23)	Bacillus spp. (5) Staphylococcus spp. (2) Micrococcus spp. (1) Streptococcus spp. (2)	Escherichia coli (5) Proteus spp. (2)
	8	0.8	19(31.15)	Bacillus spp. (4) Staphylococcus spp. (5) Micrococcus spp. (2) Streptococcus spp. (2)	Escherichia coli (5)
	9	0.9	2(3.28)	Bacillus spp. (2)	nil
	10	1	1(1.64)	Bacillus spp. (1)	nil
Total			61(100)	31	30

Table 4: Multiple antibiotic resistance (MAR) among bacteria isolated from dumpsites within Abraka, Delta State

## CONCLUSION

It can be concluded from this study that multidrug resistance (MDR) is a common characteristic of bacteria present in dumpsite soils. The indiscriminate dumping of wastes (especially of medical origin) replete with MDR strains pose one of the greatest risks to public health. This study, therefore, serves as an exposé to the hazards inherent in using recycled materials (paper, plastic and glass bottles) from dumpsites for food packaging. Also, proper location of dumpsites and disposal of wastes is necessary to reduce the health risks associated with dumpsites. Waste workers should also be educated about the risks and hazards inherent in their job so that they can take appropriate precautions like, wearing personal protective clothing, shoes, hand gloves and face mask. There is the need to prevent indiscriminate disposal of wastes of medical origin.

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FUDMA Journal of Sciences (FJS) Vol. 4 No. 2, June, 2020, pp 639 - 644