

Assessment of Airborne Microbial Composition and Antibiotic Susceptibility Profiles in Dumpsite Environments within Rivers State University

ABSTRACT

The study was aimed at determining the microbial air quality and antimicrobial susceptibility of dumpsites within Rivers State University. The samples were collected from 5 different dumpsites and 10 meters away using the plate sedimentation method. Petri dishes containing culture media were used as sampling surfaces. The sampled media were incubated at 37°C and 30°C for bacteria and fungi respectively. The isolates from the culture were subcultured and identified by biochemical test methods. The multidrug resistance sensitivity of the isolates was determined by Kirby-Bauer disc diffusion method and multiple antibiotic resistance (MAR) index was determined. The total heterotrophic bacterial count of the dumpsites ranged from 1.43×10^3 CFU/m³ to 5.8×10^4 CFU/m³. The total heterotrophic fungal count of the dumpsites ranged from 1.06×10^3 CFU/m³ to 2.3×10^3 CFU/m³. The different bacteria identified were *Bacillus* sp, *Micrococcus* sp, *Pseudomonas* sp, *Serratia* sp, *Staphylococcus* sp. The fungi, *Aspergillus niger*, *Saccharomyces* sp, *Fusarium* sp, *Aspergillus flavus*, *Candida albicans*, *Penicillium* sp and *Mucor* sp, *Rhodotorula* sp, *A. fumigatus* were identified. The antibiotic sensitivity study showed that 100% of the Gram-positive isolates resistant to augmentin, cefuroxime, 66.6% were resistant to cefotaxime, 50% were resistant to ceftriaxone, sulbactam, 33.3% were resistant to cefexime and erythromycin while 16.66 of the Gram-positive bacteria were resistant to, levofloxacin, gentamycin, ofloxacin, imipenem and cefotaxime. For the Gram-negative bacteria, 83.3 were resistant to ampiclox, cefuroxime, levofloxacin, 66.6% were resistant to cefexime, 50% were resistant to augmentin, cefotaxime, 33.3% were resistant to imipenem, gentamycin, nitrofurantoin, 16% were resistant to ceftriaxime and all (100%) the bacterial isolates were sensitive to nalidixic acid. The study revealed that the dumpsite had impact on both the microbial load and quality of such environment. Also, the microbial load of air in dumpsites decreases with increase in distance from the dump sites. Multidrug resistance can be said to be associated with bacteria within the air of dumpsites hence its exposure could be of public health concerns.

Keywords: Dumpsites, microbes, Resistance, MAR index

Introduction

Waste generation by man started since the beginning of civilization because of human activities, involving the production of goods and services and the consumption of natural resources. Dumpsite is a piece of land where waste materials are disposed. Waste materials could be garbage, rubbish, yard waste, toxic waste, and domestic refuse (Uzoigwe & Agwa, 2012). Municipal solid waste dump areas commonly referred to as waste dumpsites constitute environmental and public health hazards all over the world (Bassey *et al.*, 2015). More so, human activities such as sewage treatment, plants and animal rendering, fermentation processes and agricultural activities do emit microorganisms into the air (Recer *et al.*, 2019; Adhikari *et al.*, 2020; Gillum & Leventin, 2018; Makutet *et al.*, 2014).

Air serves as a mode of transport for the dispersal of bioaerosols (particles of biological origin e.g., bacteria, fungi, pollen, viruses that are important constituents of the atmosphere and could possess the potential to cause a variety of diseases in humans and animals). The composition and concentration of the microorganisms comprising the bioaerosols vary with source and their dispersal in air (Fowoyo & Igbokwe, 2014). In the indoor air environment for instance, most of the air pollution comes from sources inside the building itself, hair spray, perfume, room deodorizer, paints, thinners, home appliances, photo copiers, printers, computers, and air purifiers (Rylander, 2016). The use of disinfectants (linear alkyl benzene sulfonates) and fatty acid salts (soap) in cleaning agents (rug shampoo) can cause enhanced eye and airway irritation (Guo, 2011; Khan & Kamppayal, 2012).

The survival of microorganisms in air varies, though generally, fungal spores, enteric viruses and amoebic cysts are somewhat resistant to environmental stresses encountered during transport through air. Bacteria and algae are more susceptible, although bacterial endospores for example spores of *Bacillus* spp. are quite resistant. In recent years, outdoor air quality has become an important issue, because of industrialization (Medrela-Kuder, 2017). Microorganisms present in the air originate from soil, plants, water and dispersed by dust. However, spore-forming bacteria and fungi can survive in bioaerosols and stay viable for a long time in the air (Fowoyo & Igbokwe, 2014).

Several studies have been conducted to examine the health and environmental effects arising from waste dumps. Such studies showed that a link exists between the two (Nwanta&Ezenduka, 2010). The conclusion from this and other studies has led to an increasing interest of researchers in the study relating to environmental pollution as well as its effects on plants and animals. Few of these studies examined the environmental and health implications of solid waste disposal to people living in proximity of wastes dumpsites (Nebegu, 2020; Sankoh *et al.*, 2013).

In Nigeria, as well as in most developing countries, the urban landscapes are littered with garbage, plastics, bottles, disposable cups, discarded tires, and even human and live-stock faeces. These wastes are aesthetically unpleasant, constitute eyesores, produce unpleasant odour especially when their organic compositions are acted upon by putrefying bacteria. These refuse dumps thus constitute a habitat for vector and other nuisance organisms capable of trans-mitting or causing diseases such as typhoid, infantile diarrhoea and cholera in humans and animals (Obire& Aguda, 2018).

Refuse dumps refer to areas or land sites where material wastes from several sources and processes are deposited. Refuse dumps include both municipal solid wastes and industrial wastes including liquid effluents containing heavy metals (Olanrewaju, 2016). Refuse dumps provide a rich source of microorganisms most of which are pathogenic (Odeyemi *et al.*, 2017). This is usually because of the attraction of rodents and vector insects for which the dump serves as shelter and food source (Womiloju *et al.*, 2020). Although it is known that vector insects and rodents can transmit various pathogenic agents of diseases such as amoebic and bacillary dysentery, typhoid fever, salmonellosis, cholera, plague and so on. A good percentage of these infections are caused by bacteria which are suspended in air around these refuse dumps which may later settle and cause contamination. Activities involving the disposal of solid wastes even if properly controlled with proper precautionary measures adopted may have adverse impact on the environment especially air since most of the dumps are open.

Microorganisms present in the refuse use the refuse as a food source. Under the anaerobic conditions typical in most dumps, these microorganisms convert the organic material in the refuse to methane and carbon dioxide. As the gas rises through the dump and escapes into the atmosphere, it some-times picks up other compounds. The presence of large amounts of methane in this uncontrolled environment may result in explosions and fires. Additionally, this untreated gas may contain other compounds that pose a substantial health risk to nearby communities (Onyido *et al.*, 2019). Many microbes can remain viable even after extended periods of time aloft despite the challenges associated with surviving in the atmosphere, including extended UV expo-sure, low moisture levels and extremely oligotrophic conditions. Atmospheric transport is a key mode of microbial dispersal (Stetzenbach *et al.*, 2018) and the transmission of airborne plant and animal pathogens can have significant impacts on ecosystems, human health, and agricultural productivity. This study is therefore aimed at assessing microbes and their antibiotic pattern, around dumb sites in Rivers State University.

MATERIALS AND METHODS

Description of the Study Area

The study was carried out with the Rivers state University dumpsites. Rivers state university is located in Diobu (mile 3) area of Port Harcourt, Rivers state, Nigeria. The University community has a number of obvious dumpsites within the vicinity. Five (5) locations (dumpsites) within the University environment in which the samples were collected were Mechanical workshop (location A), NDDC hostel (location B), Deeper life (location C), Hostel H (location D) and Hostel B (location E). The samples collected at the locations and 10 meters away from the dumpsites for each of the locations.

Sample collection

The samples were collected using the plate sedimentation methods as described by Makute *et al.* (2014). Petri dishes containing culture media suitable for bacteria and fungi growth were used as sampling surfaces. Nutrient agar (NA) was used for the determination of the total number of bacteria, while Potatoes dextrose agar (PDA) supplemented with 0.1% lactic acid (which inhibits the growth of bacteria) was used for the determination of the total heterotrophic fungi. The plates were set up at a height representative of the normal human breathing zone, five feet, 150 cm, 180 cm above ground level. Thereafter, plates in duplicates for each type of culture medium were exposed to air in each of the five waste dumpsites for at least 20 minutes to allow air microorganisms to settle gravitationally directly on the media surfaces of the plate and transported immediately to the laboratory for incubation analyses. The nutrient agar (NA) was incubated at 37°C for 24 hours while the PDA was incubated for 48-72 hours.

Microbial Enumeration

The colonies were enumerated, and pure culture of the isolates were obtained. The total number of colony forming units were enumerated and expressed as colony forming units per cubic meter of air (CFU/m³) (Ohagimet *et al.*, 2017) for the bacterial and fungal count.

Purification of Isolates

After incubation, pure isolates were obtained by picking (with sterile inoculating loop) distinct culturally and morphologically different colonies from the various plates. These were subjected to streaking on sterile nutrient agar plates to obtain pure distinct colonies of the isolates.

Identification of Bacterial Isolates by Cultural Methods

Pure bacterial isolates were subjected to Biochemical tests which include oxidase test, Catalase test, Indole test, methyl red test, Voges Proskauer test, Starch hydrolysis test, Urease test, Citrate test, Sugars fermentation test and Triple sugar iron agar test. Bacterial isolates were identified with reference to the Bergey's Manual of Determinative Bacteriology.

Microscopic Identification of Fungi

To appreciate the microscopic feature of the fungi isolated, lactophenol cotton blue was dropped on a clean glass slide, little growth of the fungus was removed with a sterile inoculating needle, and the preparation was covered with a clean coverslip and examined under the microscope with x10 magnification.

Antibiotics Susceptibility Testing

The antibiotic susceptibility patterns of the isolates to common antibiotics were evaluated using the Kirby Bauer disc diffusion technique and 0.5 McFarland's (1.5×10^8 cfu/ml) was employed in inoculum suspensions preparation according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) and Clinical and Laboratory Standards Institute. Mueller-Hinton agar medium recommended by CLSI for sensitivity analysis was used, 0.1% peptone water diluents was prepared. Five discrete colonies of the different identified isolates were inoculated into 5 ml of the broths and incubated at 35°C for 4 – 6 h. The inoculum for primary sensitivity testing was prepared from a broth that has been incubated for 4 – 6 h. The density of the suspension was adjusted by adding the bacterial suspension to a sterile saline tube to match the density of the desired 0.5 McFarland standard. Each of the isolates was uniformly and aseptically inoculated into a different Mueller-Hinton agar plates by spread plate method. The antibiotic sensitivity test was performed by disc diffusion technique using commercially available discs on Mueller Hinton agar plates. The appropriate antibiotic discs were aseptically placed on the agar using sterile forceps. The plates were then incubated at 37°C for 24h. Interpretation of results was done using the zones of inhibition sizes as recommended by CLSI.

Multiple Antibiotic Resistance (MAR) Indexing of the Isolates

The multiple Antibiotic resistance (MAR) indexing of the isolates was determined. The MAR index when applied to a single isolate is defined as a/b where 'a' represents the number of antibiotics to which the isolate

was resistant, and ‘b’ represents the total number of antibiotics to which the isolate was exposed. Isolates with a MAR index value higher than 0.2 was considered to have originated from high-risk source of resistance (Krishna *et al.*, 2012).

RESULTS&DISCUSSION :

Total Heterotrophic Bacterial Count of the Sampled Locations

The count of total heterotrophic bacteria of the sampled locations is shown in Table1. Dumpsites sites recorded higher count of bacteria and lower count was recorded 10metres away the dumpsites. The total heterotrophic bacterial count of the dumpsites ranged from $1.43\times10^3\text{CFU/m}^3$ to $5.8\times10^4\text{CFU/m}^3$ with the sample E (Hostel B dumpsite) having the highest count and the lowest count was observed in sample B (NDDC hostel dumpsite). The count of heterotrophic bacteria of location 10 meters away from the dumpsites was recorded with the range from $6.1\times10^2\text{CFU/m}^3$ to $1.12\times10^4\text{CFU/m}^3$ with the sample location E recording the highest heterotrophic bacterial count and the lowest count was observed in the sample, B.

Table 1: Total Heterotrophic Bacterial Count of the Sample locations

Sample location	(Dumpsites) CFU/m ³	10 meters away from the dumpsites (CFU/m ³)
A	5.7×10^4	1.66×10^3
B	1.43×10^3	6.1×10^2
C	2.7×10^4	1.3×10^3
D	4.0×10^4	7.7×10^3
E	5.8×10^4	1.12×10^4

Key;

A=Mechanical Workshop Dumpsites, B=NDDC Hostel Dumpsite, C= Deeper life Dumpsite, D= Hostel H Dumpsites, E=Hostel B Dumpsite

Total Heterotrophic Fungal Count of the Sampled Locations

The count of total heterotrophic fungi of the sampled locations is shown in Table 2. Dumpsites sites recorded higher count of fungi and lower count was recorded 10metres away the dumpsites. The total heterotrophic fungal count of the dumpsites ranged from $1.06\times10^3\text{CFU/m}^3$ to $2.3\times10^3\text{CFU/m}^3$ with the sample E (Hostel B dumpsite) having the highest count and the lowest count was observed in sample B (NDDC hostel dumpsite). The count of heterotrophic fungi of the locations 10 meters away from the dumpsites was recorded with the range from $3.0\times10^2\text{CFU/m}^3$ to $9.9\times10^2\text{CFU/m}^3$ with the sample location E recording the highest heterotrophic fungal count and the lowest count was observed in the sample, B.

Table 2: Total Heterotrophic Fungal Count of the sampled locations

Sample location	(Dumpsites) CFU/m ³	10 meters away from the dumpsites (CFU/m ³)
A	1.39×10^3	4.7×10^2
B	1.06×10^3	3.01×10^2
C	2.21×10^3	6.6×10^2
D	1.34×10^3	5.6×10^2
E	2.3×10^3	9.9×10^2

Key;A=Mechanical Workshop Dumpsites, B=NDDC Hostel Dumpsite, C= Deeper life Dumpsite, D= Hostel H Dumpsites, E=Hostel B Dumpsite

Macroscopic, Microscopic and Biochemical Test Result of the Isolates

The different bacteria identified were *Bacillus*sp, *Micrococcus*sp, *Pseudomonas*sp, *Serratia*sp, *Staphylococcus* sp. shows the macroscopic and microscopic identification of the fungi isolated from the locations. The fungi, *Aspergillus niger*, *Saccharomyces* sp, *Fusarium* sp, *Aspergillus flavus*, *Candida albican*, *Penicillium* spand *Mucor* sp, *Rhodotorulas*sp, *A. fumigatus* was identified.

Distribution, Prevalence and Frequency of the isolated microorganisms in sampled locations

The distribution and prevalence of the isolates in the different sampled locations is shown in Table 3. The microorganism, *A. niger*, *Staphylococcus*sp, *Bacillus*sp, were observed to have 100% distribution in all the sampled locations (including 10meters away). The organisms, *E. coli*, *Candida* sp, *Saccharomyces* sp, were observed to be present in the air of 80% of the sampled location. The genera, *Microococcus*, *Pseudomonas* were recorded in 70% of the sampled locations, *Aspergillus flavus*, *Fusarium*sp, *Penicillium*sp were also observed to be present in 60% of the sampled locations, *Serratia*sp was isolated from 50% of the sampled locations while *Rhodotorulas*sp, *A. fumigatus*, *Mucors*sp and *Enterobacters*sp were observed to be present in 30% of the sampled locations.

The frequency of occurrence of the bacteria and fungi is shown in Figure 1 and Figure 2 respectively. The bacteria with highest frequency of occurrence of 19% were *Staphylococcus*sp, *Bacillus*sp, followed by *E. coli*,

with the frequency of 17%, followed by *Micrococcus*sp with the frequency of 15% followed by *Serratiasp* and *Proteussp* with the frequency of 10% and, *Enterobacter* sp had the least frequency of 6%.For the fungi, the highest frequency of occurrence of 19% was *A. niger* followed by *Saccharomycessp* and *Candidasp* with the frequency of 15%, followed by *Fusarium*sp and *Penicillium* with the frequency of 11% while *A. fumigatus*, *Rhodotorullasp*, and *Mucorsp* had the least frequency of 6%.

Table 3: Distribution of the microbial isolates in the different sampled locations

Bacteria isolates	Dumpsites					10m away from the Dumpsites					Percentage occurrence (%)
	A	B	C	D	E	A	B	C	D	E	
<i>Micrococcus</i> sp	+	+	+	+	+	+	-	-	+	-	70
<i>Proteus</i> sp	+	+	+	-	+	-	+	-	-	+	50
<i>Pseudomonas</i> sp	+	+	+	+	+	+	-	-	+	-	70
<i>E. coli</i>	+	+	+	+	+	-	+	+	-	+	80
<i>Bacillus</i> sp	+	+	+	+	+	+	+	+	+	+	100
<i>Serratia</i> sp	+	-	+	-	+	+	-	+	-	-	50
<i>Staphylococcus</i> sp	+	+	+	+	+	+	+	+	+	+	100
<i>Enterobacter</i> sp	+	+	+	-	-	-	-	-	-	-	30
<i>Aspergillus flavus</i>	-	+	+	+	+	-	+	-	+	-	60
<i>Rhodotorulasp</i>	+	-	-	+	+	-	-	-	-	-	30
<i>Fusarium</i> sp	+	+	+	+	-	+	-	-	+	-	60
<i>Mucor</i> sp	+	+	-	-	+	-	-	-	-	-	30
<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+	+	+	100
<i>Penicillium</i> sp	+	+	+	+	-	+	+	-	+	-	60
<i>A. Fumigatus</i>	+	+	-	-	+	-	-	-	-	-	30
<i>Candida</i> sp	+	+	+	+	+	+	-	+	+	-	80
<i>Saccharomyces</i> sp	+	+	+	+	+	+	+	-	-	+	80

Key;

A=Mechanical Workshop Dumpsites, B=NDDC Hostel Dumpsite, C= Deeper life Dumpsite, D= Hostel H Dumpsites, E=Hostel B Dumpsite

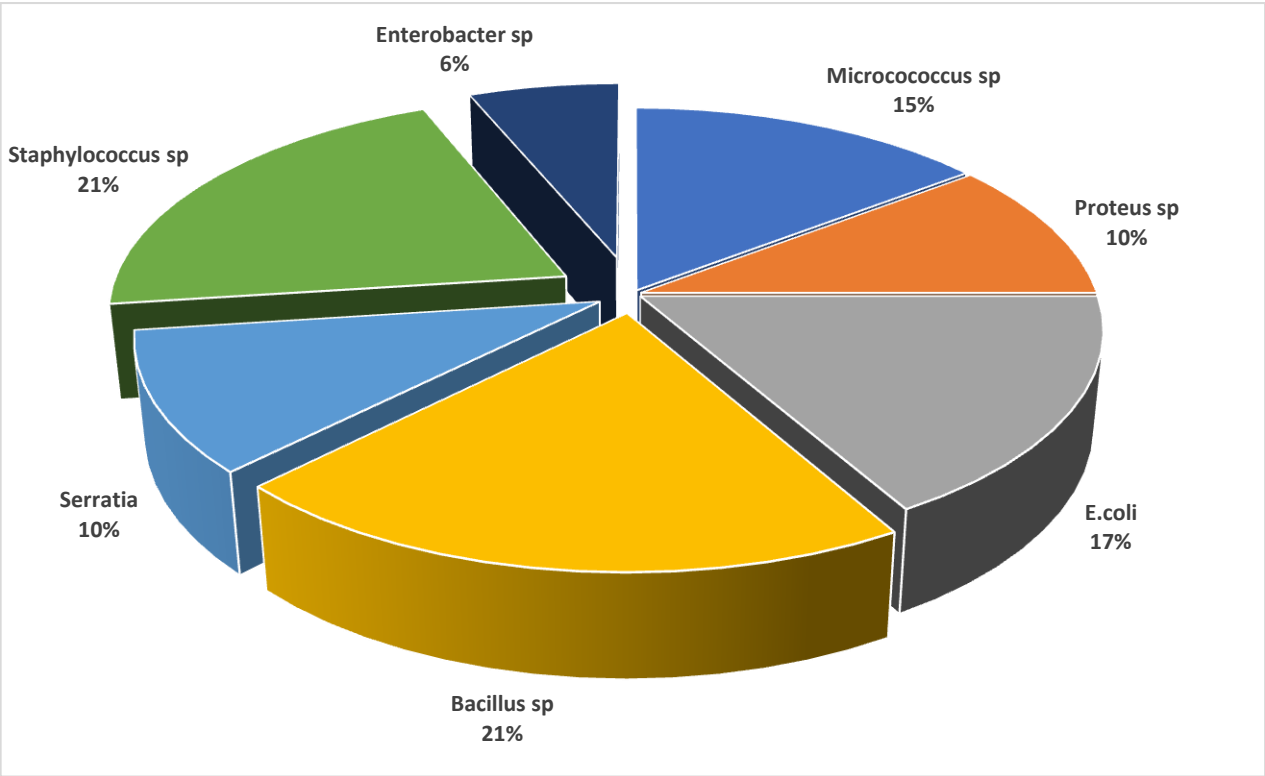


Figure 1: The frequency of occurrence of the isolated bacteria in the sampled locations

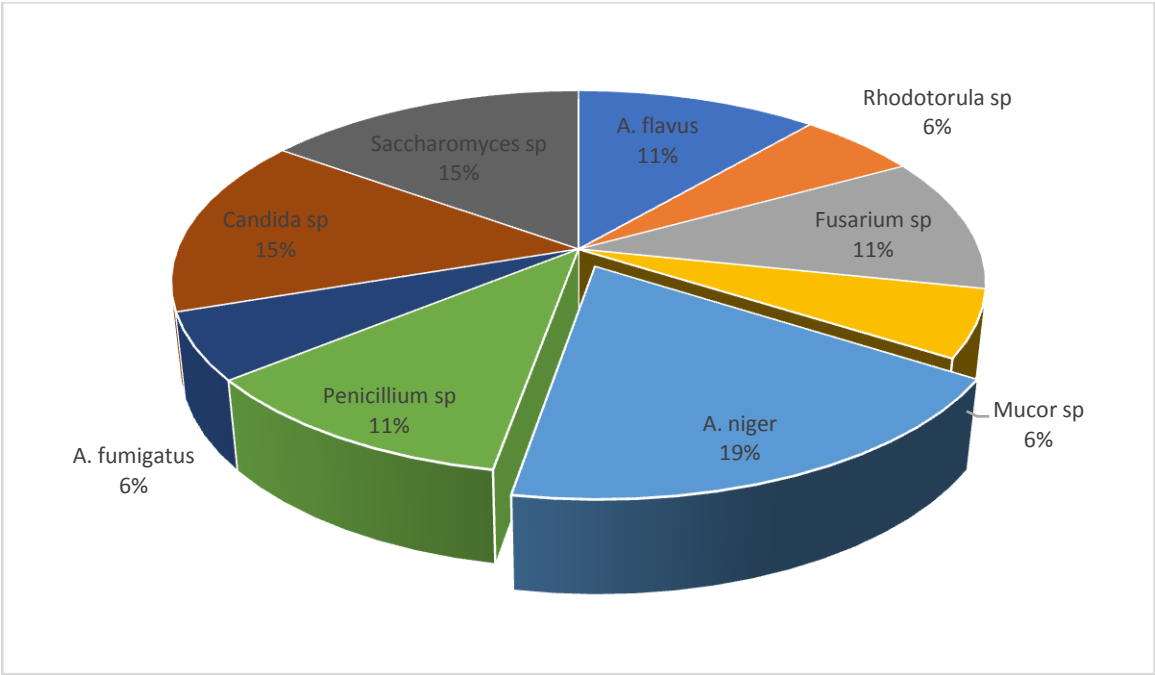


Figure 2: Frequency of occurrence of the fungal isolates

Antimicrobial Sensitivity of the Bacterial Isolates

Table 4 and Table 5 show the different sensitivity of the isolates to the antibiotics used for both Gram negative and Gram-positive isolates respectively.

As shown in Table 4 83.3% of the Gram-negative isolates were resistant to ampiclox, cefuroxime, levofloxacin, 66.6% were resistant to cefexime, 50% were resistant to Augmentin, cefotaxime, 33.3% were resistant to imipenem, gentamycin, nitrofurantoin, 16% were resistant to ceftraxime and all the bacterial isolates were sensitive to nalidixic acid.

As shown in Table 5, the Gram-positive isolates, 100% of the isolates resistant to Augmentin, cefuroxime, 66.6% of the Gram-positive isolates showed resistant to cefotaxime, 50% of the isolates were resistant to ceftriaxone, sulbatam, 33.3% of the Gram-positive bacteria were recorded to be resistant to cefexime and erythromycin while 16.66 of the Gram-positive bacteria were resistant to, levofloxacin, gentamycin, ofloxacin, imipenem and cefotaxime.

Multiple Antibiotic Resistant Index

Multi-drug resistance in this study was taken as resistance to more than one of the antimicrobial drugs tested. Multidrug-resistant (MDR) status of the isolates was tested against 12 different antimicrobials. Accordingly, the overall rate of MDR was observed in 9 (75%) out of the 12 different bacterial isolates identified as they recorded value more than 0.2 MAR index. The MAR index calculated for all the isolates ranged from 0.083 to 0.91 as shown in Table 4 and 5.

Table 4.: Antibigram of Gram-negative Bacterial Isolates

ISOLATE		AUG	CTX	IMP	OFX	GN	NA	NF	CXM	CRO	ACX	ZEM	LBC	MAR index
Iso2	<i>Proteus</i> sp	S	S	S	S	I	S	R	R	S	R	I	S	0.25
Iso3	<i>Pseudomonassp</i>	S	S	S	S	S	S	S	S	S	R	I	S	0.083
Iso4	<i>E. coli</i>	S	I	S	S	S	S	S	R	S	I	R	S	0.16
Iso7	<i>Pseudomonassp</i>	R	R	S	S	R	S	S	R	S	R	R	S	0.5
Iso8	<i>Serratiasp</i>	R	R	R	S	S	S	R	R	S	R	R	S	0.58
Iso9	<i>Enterobactersp</i>	R	R	R	R	R	S	R	R	R	R	R	R	0.91
Percentage of resistance isolates		50	50	33.3	16.6	33.3	0	33.3	83.33	16	83.33	66.6	83.33	

Key;AUG= Amoxicilin Clavulanate, NF= Nitrofurantoin, CTX= Cefotaxime CXM= Cefuroxime, IMP= Imipenem / Cilastatin, CRO= Ceftriaxone Sulbactam, OFX= Ofloxacin, ACX= Ampiclox, GN= Gentamycin ZEM= Cefexime, NA= Nalidixic Acid, LBC= Levofloxacin

Table 5: Antibigram of the Gram-positive Bacterial Isolates

ISOLA TE		AU G	CT X	CR O	ZE M	LBC	CI P	IM P	CX M	OF X	ERY	GN	AZ N	MA R inde x
Iso1	<i>Staphyloco ccussp</i>	R	R	R	R	S	S	S	R	R	R	R	R	0.75
Iso6	<i>Micrococcu ssp</i>	R	R	S	I	R	S	R	R	S	R	S	I	0.5
Iso5	<i>Bacillussp</i>	R	R	S	S	S	S	S	R	S	S	S	S	0.25
Iso10	<i>Bacillus sp</i>	R	S	R	S	S	S	S	R	S	S	S	S	0.25
Iso11	<i>Bacillus sp</i>	R	S	I	S	S	S	S	S	S	S	S	S	0.08 3
Iso12	<i>Staphyloco ccus sp</i>	R	R	R	R	S	S	S	R	S	S	S	S	0.41
Percentage resistance (%)	of isolates	100	66.6	50	33. 33	16.6	0	16. 66	100	16.6 6	33.3 3	16. 6	16. 6	

Key;AUG= Amoxicilin Clavulanate, NF= Nitrofurantoin, CTX= Cefotaxime CXM= Cefuroxime, IMP= Imipenem / Cilastatin CRO= Ceftriaxone Sulbactam, OFX= Ofloxacin, ACX= Ampiclox, GN= Gentamycin ZEM= Cefexime, NA= Nalidixic Acid LBC=Levofloxacin

Discussion

From the result of the study, the bacterial and fungal population as shown in the microbial count were recorded to be higher at the dumpsites compared to distance (10meters) from the dumpsites. This finding is consistent with the report of Odonkors and Mahami (2020) which recorded higher count of fungi and bacterial in samples collected in dumpsites compared to 2km away from the sites. Dumpsites have a considerable level of microbial contamination because whenever waste is dumped on land, soil microbes especially anaerobic fungi and bacteria inhabit the waste and extract nutrients by carrying out decomposition and dumpsites are points of convergence of different wastes which serves as nutrients for microbes (Ventorinoet al., 2018). Microbial population tends to multiply at the dumpsites/landfill sites and these dumpsites act as a source of microbiological agents (bioaerosols) in the atmosphere (Akpeimehet al., 2019). The soil is not the only source of microbes in dumpsites. Wastes like feces from both animals and humans contain loads of bacteria and fungi cells already. Thus, in the study, samples from the dumpsites had higher microbial counts as opposed to their respective air condition away from the dumpsites irrespective of the location. Nevertheless, the dumpsites contributed significantly to the bioaerosol population in the nearby vicinities. This is because the air samples collected from neighboring air that were 10metres away from the landfills across the locations had significantly lesser microbial counts (Table 1 and Table 2) than those from dumpsites. According to Burkowskaet al. (2011), dumpsites could have a negative impact on the atmosphere in surroundings from few hundreds of meters up to one kilometer apart even if they are well protected. In a

similar study by Odeyemi *et al.* (2011), the author found out that microbial loads in the air decreased further away from the dumpsites and this occurrence was attributed to the antimicrobial action of UV rays from sunlight that reduces atmospheric nutrients available for microbial use. The same reasons could explain similar observation in this study. WHO's guideline for indoor air quality in 2009 revealed that indoor microbial pollutants usually originate from the outdoor environment which includes dumpsites (WHO, 2018, Odonkor and Mahami, 2020).

The American Conference of Governmental Industrial Hygienists (ACGIH) standard microbial levels for bacteria in the atmosphere is 100 CFU/m³. In this study, the counts of total bacteria load recorded in the sampled locations exceeded the limit irrespective of the locations, which have serious health implications. According to reports, terrorist attacks using biological agents and the flu pandemonium in 2009 are important highlights calling for the need to carry out more research studies regarding the population of bioaerosols in the atmosphere especially in the indoor environment (Adams and Kanaroglou, 2016). Furthermore, the authors revealed that numerous diseases have been linked with poor air quality caused by bioaerosols with tuberculosis and severe acute respiratory syndrome (SARS) wreaking the greatest havoc socioeconomically. For fungi, WHO estimates a limit of 500 CFU/m³ which is higher than that for bacteria since most infectious diseases are not associated with them. The fungi count from this study in all the locations exceeded the limit. This observation could be as a result of higher concentration of the fungi, as well as their favorable environmental conditions which might have led to the propagation of the fungi in the air as most fungi are known to possess spores which helps them against adverse conditions (Gutaroska *et al.*, 2014).

The different bacteria, *Bacillus* sp, *Micrococcus* sp, *Pseudomonas* sp, *Serratia* sp, *Staphylococcus* sp and the fungi, *Aspergillus niger*, *Saccharomyces* sp, *Fusarium* sp, *Aspergillus flavus*, *Candida albicans*, *Penicillium* sp and *Mucor* sp, *Rhodotorula* sp, *A. fumigatus* sp identified in this study are in line with those isolated in the study of Obumneme *et al.* (2019) which isolated similar microorganisms in the study of microbial air assessment of environment around waste dumpsites in northern Nigeria. Several studies have reported similar microorganisms isolated from the outdoor air (Makur *et al.*, 2014; Udi-Ibiam *et al.*, 2016).

The bacteria, *Bacillus* sp, *Staphylococcus*, *E. coli*, *Pseudomonas*, *Micrococcus* were the most occurring and fungi *A. niger*, *Candida*, *Saccharomyces*, *Fusarium* sp, *A. flavus* and *Penicillium* sp were the most occurring microorganisms identified during the study in both the dumpsites and meters away from the site of refuse disposal. The predominance of these microorganisms agrees with the findings of the studies of Makur *et al.* 2014 and Obumneme *et al.* (2019) in which similar biotic agents were reported as the most encountered airborne pathogens.

Members of the Enterobacteriaceae, *E. coli* and *Proteus* sp encountered in this study could have emanated from wastewater and faecal matter of both human and animal source deposited around these waste environments which may contaminate the air and even foods sold around these environments. This is in consistent with some work on the distribution and public health implication of enteric pathogens (Fowoyo and Igbokwe, 2014; Obiekezie *et al.*, 2013). Bacteria such as *Staphylococcus*, *Candida* and microorganisms are known for being commensal of the human origin and their presence within the dumpsites can reflect the fact that most of the dirt within the dumpsites come from the household and human activities. According to the literature, the most common sources of *Bacillus* include soil, dust, plants, (Kayser *et al.*, 2005). According to Gutaroska *et al.* (2014), *Bacillus* mostly affect occupational groups such as farmers engaged in mixed (animal and plant) production, warehouse workers dealing with raw materials and products of plant and animal origin, and workers in the grain industry, and learning environment thus, these bacteria could be indicators for hazardous biological agent. The presence of *Staphylococcus* and *Micrococcus* might emanate from the mucous, and skin through respiratory exhalation of the people who live in the environment as they are commensal of the skin and mucous part of the body ((Makur *et al.*, 2014). Obumneme *et al.* (2019)

Ekhaiese *et al.* (2008) reported *Aspergillus* spp, *Mucor* sp. and *Penicillium* spp. as the predominant contaminating fungi genera of the air environment in their study. Remarkably, these fungi contaminants particularly the *Aspergillus* spp. and *Mucor* spp. are pathogenic and had been implicated as the etiological agents of several mycotic infections (Odonkor and Mahami, 2020). *Aspergillus* species are ubiquitous opportunistic fungi that cause pulmonary infections in immunocompromised patients. Fungi that were

previously thought to be of uncertain pathogenicity are emerging as causes of infections in immunosuppressed host (Stanley *et al.*, 2019). *Penicillium* genus constitute a work hazard that may cause exposed persons to suffer from alveolitis allergica, bronchial asthma, allergic rhinitis, and allergy-related conditions. These molds are known to produce citrinin, citreoviridin, cyclopiazonic acid, secalonic acid D, patulin, rubratoxin A and B, and viridicatin (Gutarowska *et al.*, 2015).

The antibiotic susceptibility pattern of the bacterial isolates against conventional antibiotics displayed varying degree of susceptibility. Higher percentage of the bacterial isolates were observed to be resistant to antibiotics, ampiclox, cefuroxime, levofloxacin, augmentin and cefotaxime which is of public health concerns. Most of the wastes at the dumpsites could be traced to human or house origin hence, a route of antibiotic resistance to the environment (Chen *et al.*, 2019).

The MAR index is an important risk assessment tool in determining the susceptibility ratio of microorganism to drugs, and the value of the MAR index (nominally 0.2) has been applied to differentiate low- and high-risk regions where antibiotics or growth promoters are overused (Riaz *et al.*, 2011). The analysis of MAR index indicates the number of bacteria showing antibiotic resistance in the risk zone of the susceptibility study (Amoako *et al.*, 2019). Multidrug-resistant (MDR) status of the bacterial isolates tested against 12 different antimicrobials in this study showed that 75% of the bacteria isolates were multidrug resistant. This suggest that the isolates showed resistance to most of the antibiotics tested and this could be attributed to possession of multiple resistance genes in the bacterial genome that enable them to resist all the antibiotics. Similar high-resistance patterns have been observed against these antibiotics in other studies elsewhere (Asante *et al.*, 2021).

Conclusion

The study revealed that the dumpsite had impact on both the microbial load and quality of such environment. Also, the microbial load of air in dumpsites decreases with increase in distance from the dump sites. The bacteria, *Bacillus* sp, *Staphylococcus*, *E.coli*, *Pseudomonas*, *Micrococcus* were and the fungi *A. niger*, *Candida*, *Saccharomyces*, *Fusarium*sp, *A. flavus* and *Penicillium*sp were the most occurring microorganisms identified during the study in both the dumpsites and meters away from the site of refuse disposal. Multidrug resistance can be said to be associated with bacteria within the air of dumpsites hence its exposure could be of public health concerns.

References

- Adams, M. D. and Kanaroglou, P. S. (2016). Mapping real-time air pollution health risk for environmental management: combining mobile and stationary air pollution monitoring with neural network models. *Journal of Environmental Management*, 168, 133–141.
- Adhikari, A., Reponen, T., Lee, S. & Grinshpun, S. (2020). Assessment of human exposure to airborne sampling. *Annals of Agricultural and Environmental Medicine*, 11, 269-277.
- Akpeimeh, G. F., Fletcher, L. A. and Evans, E. B. (2019). Exposure to bioaerosols at open dumpsites: a case study of bioaerosols exposure from activities at Olusosun open dumpsite, Lagos Nigeria. *Waste Management*, 89,37–47.
- Amoako, D. G., Somboro, A. M., Abia, A. L. K., Molechan, C., Perrett, K., Bester, L. A. and Essack, S. Y. (2019). Antibiotic Resistance in *Staphylococcus aureus* from Poultry and Poultry Product in uMgungundlovu District South African Using Farm to Fork Approach. *Microbial Drugs Resistance*, 2,1-11.
- Asante, J., Hetsa, B. A., Amoako, D. G., Abia, A. L. K., Bester, L. A. and Essack, S. Y. (2021). Multidrug-Resistant Coagulase-Negative *Staphylococci* Isolated from Bloodstream in the uMgungundlovu District of KwaZulu-Natal Province in South Africa: Emerging Pathogens. *Antibiotics*, 10,198-208.
- Bassey, I. U., Brooks, A. A., Asikong, B. E. & Andy, I. E. (2015). Environmental and Public Health Aspects of Solid Waste Management at the Lemna Dumpsite in Calabar, Cross River State, Nigeria. *International Journal of Tropical Disease and Health*, 10 (3), 1-13.

- Burkowska, A., Swiontek-Brzezinska, M. and Kalwasińska, A. (2011). Impact of the municipal landfill site on microbiological contamination of air. *Contemporary Problems of Management and Environmental Protection. Some Aspects of Environmental Impact of Waste Dumps*, 9, 71–87.
- Chen Q., Cui H., Su, J. Q., Penueluss J., Zhu, Y. (2019). Antibiotics Resistome in Plant Microbiome. *Trend Plant Science*, 24:530-541
- Fowoyo, P. T. & Igbokwe, O. E. (2014). Impact of Air Pollution on the Microbiological Quality of Ready to Eat Hawked Foods Sold around a Cement Factory in Lokoja, Nigeria. *American Journal of Research Communication*, 2 (11), 138-157.
- Fowoyo, P. T. and Igbokwe, O. E. (2014). Impact of Air Pollution on the Microbiological Quality of Ready to Eat Hawked Foods Sold around a Cement Factory in Lokoja, Nigeria. *American Journal of Research Communication*, 2 (11), 138-157
- Gillum, S. &Leventin, E. (2018). The air spore close to a compost facility in Northeast Oklahoma, part 1: spore trap sampling. *Aerobiologia*. 24, 3-12.
- Guo, H. (2011). Source apportionment of Volatile organic compounds in Hong Kong homes. *Building and Environment*, 46 (11), 2280–2286.
- Gutarowska, B., Skora, J., Sepien, L., Szponar, B., Otlewska, A., Pielech-Przybylska, K. (2015). Assessment of microbial contamination within working environments of different types of composting plants. *Journal of the Air and waste Management Association*, 65(4),466-478
- Khan, A. A. H. &Karuppaiyl, S. M. (2012). Fungal pollution of indoor environments and its management. *Saudi Journal of Biological Sciences*, 19, 405–426.
- Makut, M. D., Nyam, M. A., Shehu, L. &Anzaku, S. J. (2014). A survey of the microflora of the outdoor air environment of Keffi metropolis, Nasarawa State, Nigeria. *African Journal of Microbiology Research*, 8 (27), 2650-2655.
- Medrela–Kuder, E. (2017). Seasonal variations in the occurrence of culturable airborne fungi in outdoor air in Cracow. *International Biodeterioration and Biodegradation*, 52, 203-205.
- Nabegu, A. B. (2020). An Analysis of Municipal Solid Waste in Kano Metropolis. *Journal of Human Ecology*, 31 (2), 111-119.
- Nwanta, J. A. &Ezenduka, E. (2010). Analysis of Nsukka Metropolitan Abattoir Solid Waste in South Eastern Nigeria: Public Health Implications. *Archives of Environmental and Occupational Health*, 65(1), 21-26.
- Obiekezie, S. O., Ndimele, E. C. and Otti, T. A. (2013). Antibiotic Resistance Pattern of Bacteria Species Isolated From Wastewater in Bingham University Clinic, Karu, Nasarawa State, Nigeria. *International Journal of Biotechnology and Allied Science*, 8 (1), 1070-1076.
- Obire, O. & Aguda, M. (2018). Bacterial community of leachate from a waste dump and adjacent stream. *Journal of applied sciences and environmental management*, 6 (2), 71-75.
- Odeyemi, A. T., Faweya, E. B., Agunbiade, O. R. & Ayeni, S. K. (2017). Bacteriological, mineral and radioactive con-tents of leachate samples from dumpsite of Ekiti State Government Destitute Centre in Ado-Ekiti. *Archives of Applied Science Research*, 3 (4), 92-108.
- Olanrewaju, A. O. (2016). Dangers of indiscriminate refuse dumps in metropolis countries. *Environment opinions*, 35, 567-571.
- Onyido, A. E., Okolo, P. O., Obiukwu, M. O., & Amadi, E. S. (2019). A survey of vectors of public health diseases in un-disposed refuse dumps in Akwa town, Anambra state, south eastern Nigeria. *Research Journal of Parasitology*, 4, 22-27.
- Recer, G., Browne, M., Horn, E., Hill, K. & Boehler, W. (2019). Ambient air levels of *Aspergillus funigatus* and *Thermophilic actinomycetes* in a residential neighborhood near a yard-waste composting facility. *Aerobiologia*, 17, 99-106.

- Riaz, S., Faisal, M. and Hasnain, S. (2011). Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum-lactamase (ESBL) producing *Escherichiacoli* and *Klebsiella* species in Pakistan. *African Journal of Biotechnology*, 10,6325–6331
- Rylander, R. (2016). Microbial cell wall agents and sick building syndrome. *Advances in Applied Microbiology*, 55, 139–154.
- Sankoh, F. P., Yan, X. & Tran, Q. (2013). Environmental and Health Impact of Solid Waste Disposal in Developing Cities: A Case Study of Granville Brook Dumpsite, Freetown, Sierra Leone. *Journal of Environmental Protection*, 4, 665-670.
- Stanley H. O., Anele, B. C., Okerentugba, P. O., Immanuel, M. O. and Ugboma C. J. (2019). Isolation and Molecular Identification of Biodeteriogens isolated from Painted Classroom Wall surfaces in University of Port Harcourt, Nigeria. *South Asian Journal in Microbiology*, 9 5(3),1-6
- Stetzenbach, L., Buttner, M. & Cruz, P. (2018). Detection and enumeration of airborne bio contaminants. *Current Opinions on Biotechnology*, 15, 170-174.
- Udu-Ibiam, O. E., Maduka, A. V., Samuel, C. O., Oluwatoyin, O. O., Orji, J. O. and Elsie Ekeghalu, C. (2016). Microbiological Analysis of Outdoor Air Quality of Male and Female Hostels in Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. *Journal of Pharmacy and Biological Sciences*,11 (3),68-73.
- Uzoigwe, C. I. & Agwa, O. K. (2012). Microbiological quality of water collected from boreholes sited near refuse dumpsites in Port Harcourt, Nigeria. *African Journal of Biotechnology*, 11 (13), 3135-3139.
- Ventorino, V., Romano, I., Pagliano, G., Robertiello, A. and Pepe, O. (2018). Pre-treatment and inoculum affect the microbial community structure and enhance the biogas reactor performance in a pilot-scale biodigestion of municipal solid waste. *Waste Management*, 73,69–77.
- Womiloju, T. O., Miller, J. D., Mayer, P. M. & Brook, J. R. (2020). Methods to determine the biological composition of particulate matter collected from outdoor air. *Atmospheric Environment*, 37, 4335 - 4344.
- World Health Organization, Air Pollution, World Health Organization, Geneva, Switzerland, 2018, <https://www.who.int/airpollution/e>

UNDER PEER REVIEW