Microbial Assessment and Antibiogram of Bacteria Isolated from Air samples around Dumpsites in Igando, Lagos, Nigeria

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Abstract: This study assessed the level of microbial contamination of air around three municipal dumpsites in Igando and determined the antibiogram of the bacterial isolates. Total aerobic bacteria count (TABC) and total enterobacterial count (TEC) of air around the dumpsites nearby amusement park and gas station were measured using the "sedimentation method. The microbial isolates were identified using phenotypic and biochemical characteristics and their antibiotic sensitivity was determined using Kirby Bauer disk diffusion. Total aerobic bacteria count (TABC) at the three dumpsites ranged between 1.9 x 10^7 and 2.6 x10^7cfu/m^3 while the amusement park and gas station recorded 2.2 x10^7 and 2.4 x 10^7cfu/m^3 respectively. The TECs of air at the dumpsites varied between 6.6 x 10^6 and 7.9 x 10^6 cfu/m^3, while the amusement park and gas station recorded 6.4 x 10^6 and 2.9 x 10^6 cfu/m^3 respectively. *Staphylococcus* sp. had the highest percentage occurrence of 47 followed by *Bacillus* 17. Among fungi, *Rhizopus* sp. had the highest percentage occurrence 26.67 followed by *Aspergillus* 26.47. For the Gram-positive bacteria, *Bacillus* spp. recorded the highest percentage resistance (HPR) of 40 against tetracycline. Twenty percent of the Gram positive bacterial isolates were resistant to tetracycline and erythromycin respectively. Among the Gram negative bacterial isolates, 33.33% were resistant to cefuroxime and 22.22% were resistant to gentamicin and ofloxacin respectively. The high level of microbial contamination of the air at and around the dumpsites constitute potential risk to health.
Key Words: Air quality, Antibiogram, Dumpsites, Igando, Microbial quality

INTRODUCTION

The increase in human population, industrial and technological revolutions has greatly contributed to the disposal of domestic, industrial and commercial waste in the world (Eni *et al.*, 2014). Solid waste management has remained an inevitable definite environmental issue in the developing countries of the world and it stands out among the arrays of global environmental hazards facing metropolitan cities (Geoffrey, 2005). Municipal solid wastes (MSW) are sources of environmental pollution through introduction of chemical substances above their threshold limit into the environment (Obasi *et al.*, 2012).

In Nigeria like most developing countries, unsegregated waste is disposed without the envisaged aim of reducing, reusing or recycling them. Due to poor handling of MSW and weak legislation to regulate, reduce, reuse and recycling waste (Angaye *et al.*, 2018), the magnitude of waste stream has acquired an alarming rapid dimension due to urbanization (Amuda *et al.*, 2014). These wastes are visually unpleasant, constitute eyesores, produce unpleasant odor especially when putrefying bacteria act upon their organic compositions (Guevart *et al.*, 2006). Two major methods of waste disposal remain in use in Nigeria cities, landfills and open dumping. Solid waste disposed in landfills is usually subjected to series of complex biochemical and physical processes, which lead to the production of both leachate and gaseous emissions (Ezenwa, 2014).

Municipal waste dumping sites as an alternative are designated places where wastes from various sources are deposited (Ekpo *et al.*, 2013). Most of the wastes generated in Nigeria especially Lagos State with a projected population of 12-18 million people are usually dump in open dumpsites most of which are not recognize by the government. Also, most of the designated sites are neither properly constructed nor designed, and consequently wastes dumped there over the year's biodegrade and generate leachates that ultimately become point source of pollution into soil and ground water (Agbor *et al*., 2013).

Dumpsites are breeding ground for vector insects and rodents capable of transmitting or causing diseases such as typhoid fever, bacillary dysentery, plaque, infantile diarrhea and cholera in humans. Most of these infections are also caused by bacteria suspended in air around the dumpsites which may later settle and cause contamination (Odeyemi, 2012). The quality of the air is one of the major factors that influence the surrounding environment. The atmospheric air contains microbiological contaminants in the form of bio aerosols. They are made by small liquid droplets or particles of solid matter that comprise bacteria, viruses and fungi as well as independently floating in a gaseous medium microorganism, pollens etc. (Despres *et al*., 2012). Infectious, saprophytic and mixed bio aerosols cause deterioration of the air hygienic conditions that lead to series of infectious diseases in humans, animals and plants, contamination of food products, medicinal products as well as bio-corrosion of building materials (Kalwansinska *et al*., 2014).

The negative environmental impacts of waste dumpsites have continued to generate public health concern. Pathogenic microorganisms transmitted in the air through dust or liquid droplets enter the body through the skin and mucous membranes or through the bite of hematophagous insects. The concentration of bacteria and fungi at dumpsites can be hundreds to thousands of times higher than what is obtained in homes and public buildings (Oyedele and Oyedele, 2017). World Health Organization estimates that about two million people die prematurely every year as a result of air pollution, while many more suffer from breathing ailments, heart disease, lung infections and even cancer (Madhukar and Srikantaswamy, 2013).

Igando dumpsites also known as Solous landfills comprise both closed and existing landfills and are one of the approved dumpsites for Lagos State Waste Management Authority (LAWMA). The landfill ranks the second largest after Olusosun dumpsite in Ojota area of Lagos State. Solous landfill is sub-divided into three (3) sections namely Solous I (closed), Solous II and III (existing). These sites began operation in the year 1996 with a projected lifespan of between 5 and 6 years (LAWMA, 2010). Soluos 1 covered about 3 hectares of land, Soluos II covered about 7.8 hectares of land with an average life span of 5 years while Soluos III is approximately 5 hectares of land with an average life span of 5 years and each site receives an average of about 2,250 m^3 of waste per day. Soluos dumpsites are surrounded by commercial, residential and industrial activities as a result of the high population of 309,347 recorded by Alimosho local government (NPC, 2006). The studied dumpsites were open and very close to residential and industrial areas. There was no attempt by the government to prevent people living or working around these dumpsites from the health implications that could arise as a result of their exposure. This research was therefore carried out to investigate the distribution and frequency of occurrence of bacteria and fungi isolates at Igando dumpsites in Lagos State as well as determine the antibiogram of the bacteria isolated.

MATERIALS AND METHODS

Description of the Study Areas

The study area is the Igando dumpsites along Iba-Egbeda Road in Alimosho Local Government Area of Lagos State, Nigeria. It lies approximately between longitude 3°13’30”E to 3°17’15”E and latitude 6’28°N to 6’42°N (Akoteyon *et al*., 2011). The other sample locations were the amusement park (ROSELLA) and gas station (LETO), both located along the same road, but few meters beside and opposite the dumpsites. The samples were collected from three different locations in and around the dump sites. A (150 m to the dumpsite and the closest point to neighborhoods), B (50 m away from the dumpsite) and C (a nearby stream 100 m away from the dumpsite).

Enumeration and isolation of Bacteria and Fungi from dumpsites air

Twenty milliliters (20 ml) of freshly prepared nutrient agar, MacConkey agar, and Potato dextrose agar medium was dispensed into sterile Petri-dishes and allowed to solidify. The plates were then sealed and labeled appropriately and taken to the dump site where each was exposed for ten minutes at the selected locations. The plates were afterwards covered and taken into the laboratory for incubation at 37^{o}C for 48 h for bacteria and 27^{o}C for 3-5 days for fungi. The colonies obtained after incubation were counted and the average counts for duplicate cultures were recorded.

Characterization and identification of isolated bacteria and fungi

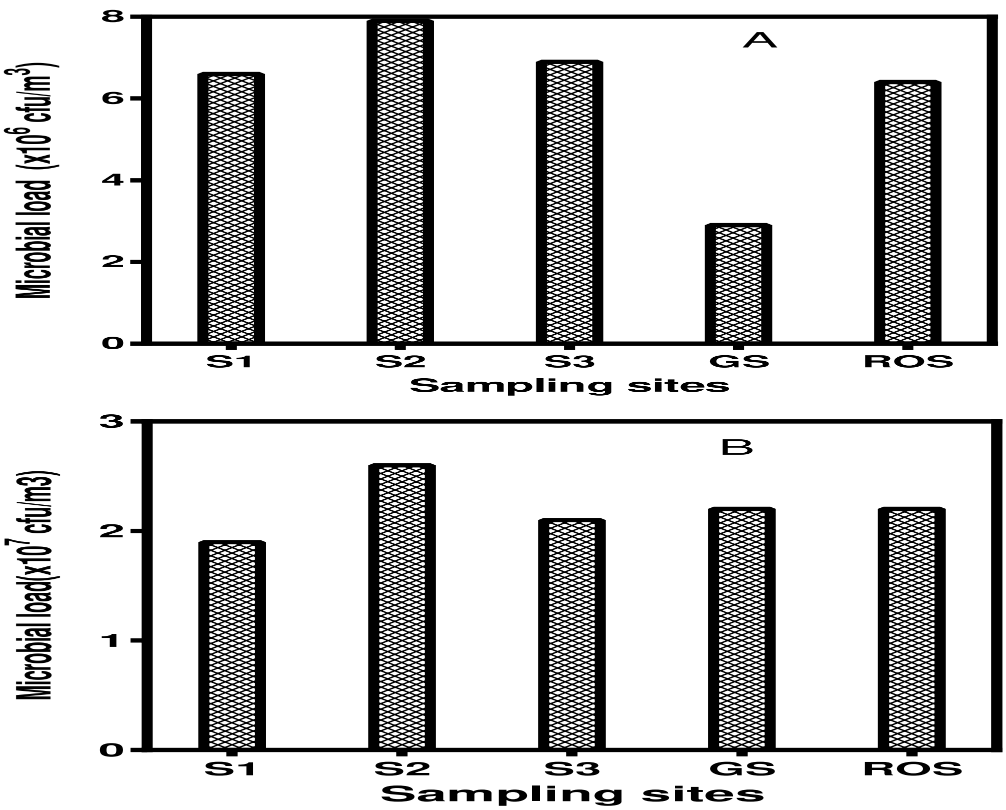
Pure cultures of bacteria and fungi were obtained by aseptically streaking representative colonies of different morphological types that appeared on the cultured plates onto freshly prepared Nutrient agar plates and MacConkey agar plates for bacteria and Potato Dextrose agar for fungi and incubated at 37^{o}C for 24 h for bacteria and 28± 2^{o}C for 5 days for fungi. The bacterial isolates were identified based on their colonial and cellular morphology and biochemical tests according to the scheme of Cowan and Steel's Manual (Barrow and Feltham, 1995). Purified selected fungal isolates were identified using their cultural and morphological characteristics as described by James and Natalie (2001).

Antibiotic susceptibility test

The antimicrobial susceptibility patterns of the isolates were determined by disk diffusion method as described by CLSI (2005). The inoculum was standardized by adjusting its density to equal the turbidity of 0.5 McFarland standards. A sterile cotton swab was dipped into the standardized suspension, drained and used for inoculating 20 ml of Mueller-Hinton agar in a 100 mm disposable plate (Sterilin, UK). The inoculated plates were allowed to dry and antibiotic discs (Oxoid, UK) were placed on the agar using flamed forceps and were gently pressed down to ensure contact. Plates were incubated at 28± 2^{o}C for 3-5 days for fungi and 37^{o}C for 18 h – 24 h for bacteria. Gram negative bacterial isolates were tested against eight antibiotics which comprised Cefotaxime (CAZ 30 µg), Cefuroxime (CRX 30 µg), Gentamycin (GEN 10 µg), Ofloxacin (OFL 5 µg), Augmentin (AUG 30 µg). Nitrofurantoin (NIT 300 µg), Ceftazidme (CTX 30 µg) and Amoxicillin (AMX 30 µg). Gram positive disc include Tetracycline (TET), Sulfamethoxazole (SMX), Erythromycin (ERY), Fusidic acid (FUS), Penicillin (PEN), Clindamycin (CLN), Trimethropin (TRM), Gentamicin (GEN), The diameter of zone of growth inhibition was measured to the nearest whole millimeter and interpreted on the basis of CLSI guideline (CLSI, 2005) and Cheesebrough (2006). All assays were carried out in triplicate.

RESULTS

The mean total of enteric and aerobic bacteria load of the sampling sites are presented in Figs. 1 A and B respectively. It was observed that the highest concentration of total aerobic bacterial population of 2.6 x10^7 was recorded at Soluos 2 while Soluos 1 recorded the least value of 1.9 x$10^{7.}$ Also, highest concentration of total enteric bacteria of 7.9 x10^6 was also recorded at Soluos 2 while the gas station recorded least value of 2.9 x10^6.



Key : S1 : Soluos 1, S2 : Soluos 2, S3 : Soluos 3, GS : Gas station, ROS : Rosella

Fig 1: Mean total of enteric (A) and aerobic bacteria (B) isolated from the studied sites

The sampling sites recorded various genera of bacteria and Fungi as shown in Figure 2A and B. *Staphylococcus* spp. was the most prevalent bacteria identified while *E. coli* was the most common enteric bacteria isolated from the air of the study sites. Among the fungi isolated, *Rhizopus* recorded the highest frequency of 26.67% followed by *Aspergillus* with 13.37%.

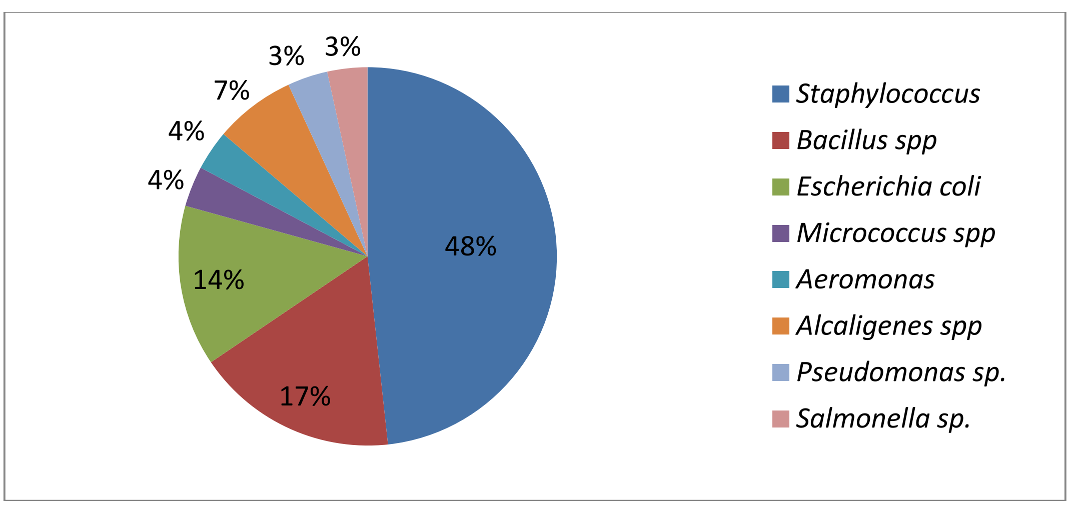


Figure 2A: Distribution of bacterial species isolated from the studied sites

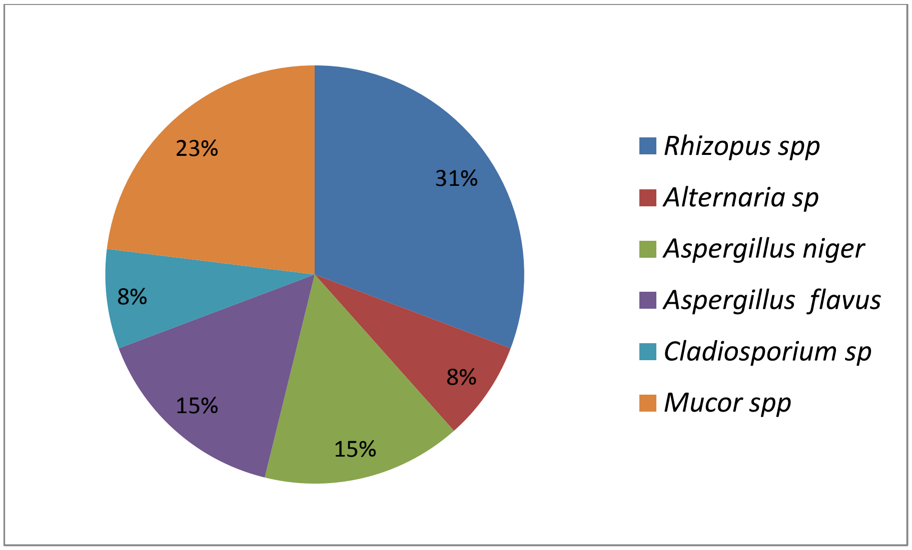


Figure 2B: Distribution of fungal species isolated from the sampling sites

The antibiotic sensitivity test showed that most of the bacterial isolates were sensitive to the test antibiotics. Low percentages of the bacterial isolates showed antibiotic resistance, such that for the Gram-positive bacteria the HPR was 40 recorded among *Bacillus* sp against tetracycline, followed by 23.1 among the *Staphylococcus* sp against erythromycin and 20 among *Bacillus* sp against penicillin. Lowest percentage resistance of 7.7 was recorded by fusidic acid (Table 1).

For the Gram-negative bacteria, the HPR was 50 recorded among *E. coli* against cefuroxime, gentamicin, cefotaxime, and among *Alcaligenes* sp against cefuroxime and ofloxacin. The Gram-negative bacteria isolates were sensitive to nitrofurantoin, amoxicillin, augmentin and ceftazidime. However, some of the isolates were resistant to cefuroxime, gentamicin, cefotaxime and ofloxacin as shown in Table 2.

Table 1: Percentage antibiotic sensitivity patterns of Gram-positive isolates from air around dumpsites.

	Staphylococcus sp (n = 13)			*Bacillus* sp (n = 5)			*Micrococcus* sp (n = 2)		
Antibiotic	S	I	R	S	I	R	S	I	R
Tetracycline	84.6	7.7	7.7	60.0	0.0	40.0	100	0.0	0.0
Sulfamethoxazole	84.6	7.7	7.7	80.0	20.0	0.0	100	0.0	0.0
Erythromycin	76.9	15.4	7.7	80.0	0.0	20.0	100	0.0	0.0
Fusidic acid	92.3	0.0	7.7	100	0.0	0.0	100	0.0	0.0
Gentamicin	84.6	7.7	7.7	80.0	20.0	0.0	100	0.0	0.0
Clindamycin	84.6	7.7	7.7	100	0.0	0.0	100	0.0	0.0
Penicillin	92.3	0.0	7.7	80.0	0.0	20.0	100	0.0	0.0
Trimethoprim	100	0.0	0.0	80.0	20.0	0.0	100	0.0	0.0

* n = Number of isolates; S= Sensitive, I = Intermediate, R = Resistant.

Table 2: Percentage antibiotic sensitivity patterns of Gram-negative isolates from air around dumpsites

Antibiotic	*Aeromonas* sp (n = 1)			*Escherichia coli* (n = 4)			*Salmonella* sp (n = 1)			*Alcaligenes* sp (n = 2)			*Pseudomonas* sp (n = 1)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ceftazidime	100	0.0	0.0	75.0	25.0	0.0	100	0.0	0.0	50.0	50.0	0.0	100	0.0	0.0
Cefuroxime	100	0.0	0.0	50.0	0.0	50.0	100	0.0	0.0	50.0	0.0	50.0	100	0.0	0.0
Gentamicin	100	0.0	0.0	50.0	0.0	50.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Cefotaxime	100	0.0	0.0	50.0	0.0	50.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Ofloxacin	100	0.0	0.0	75.0	0.0	25.0	100	0.0	0.0	50.0	0.0	50.0	100	0.0	0.0
Amoxicillin	100	0.0	0.0	75.0	25.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Augumentin	100	0.0	0.0	50.0	50.0	0.0	100	0.0	0.0	50.0	50.0	0.0	100	0.0	0.0
Nitrofurantoin	0.0	100	0.0	75.0	25.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0

* n = Number of isolates; S= Sensitive, I = Intermediate, R = Resistant

DISCUSSION

Waste deposition in dumpsites brings about a potential hazard for human and animal health, as they introduce notable amounts of gaseous pollutants, odorous and microbiological characters to the atmosphere (Marchand *et al*., 2012; Vilavert *et al.,* 2012). Airborne bacteria and fungi may lead to establishment of many diseases. These include cardiovascular diseases or allergic reactions (sinusitis or conjunctivitis), bronchitis pneumonia, tuberculosis, hay fever and asthma. The metabolites of these bacteria and fungi which are the endotoxins and mycotoxins, composing bioaerosol also play an important role in inflammatory reactions (Zucker and Muller, 2004; Lawniczek-Walcyk and Gorny, 2010).

The high total aerobic bacterial population recorded at the dump sites and its surroundings could pose threat for humans and the environment. Standard atmospheric concentration for bio-aerosol has been reported to be 3.0 log 10 cfu/ml (Panthi, and Shrestha, 2008) which is lower compared to the higher values recorded in this study. The result of this study is in contrast with the results of Huang *et al*. (2002); Heo *et al*. (2010); Williams and Hakam (2016), in which lower values were reported for total aerobic bacteria and this showed the magnitude of bacteria pollution of the waste dumpsites and its surroundings. The disparity could be as a result of the large amount of refuse dumped at these sites and the population of human, birds, and rodents occupying the dumpsites. The relatively high total aerobic bacteria population isolated around the amusement park may be due to the nearness of the amusement park to Soluos II and also human activities taking place there. Talking, sneezing and coughing by human's release bacteria into the air thereby accounting for the bacteria population.

The high population of total enteric bacteria (Figure 2A) recorded at most of the sampling sites is not surprising in view of the fact that, it is a common feature in developing countries such as Nigeria to defecate in dumpsites and also throw feces contained in disposable bags into dumpsites. Excreta from animals; mostly rodents are also passed out at these dumpsites. The enteric bacteria present in these wastes are blown into the air by wind. Furthermore, the high population, traffics and proximity of the waste dump sites to markets and schools may also be responsible for the high total enteric bacterial population isolated from the air of the dumpsites (Ugboma et al., 2018). The relatively low enteric bacterial population from the air of the gas station may be as a result of good management of the gas station with appropriate toilet facilities that prevent uncontrolled urination and defecation at the gas station. However, the presence of enteric bacteria in the gas station can be traced to its nearness to Soluos II dumpsites, as enteric bacteria in bio aerosols from the dumpsites could be blown via wind to the gas station.

The high percentage recorded for *Staphylococcus aureus* a normal human skin flora known to form aggregates in nature could be as a result of the possible breaking of the clusters (Ugboma *et al.,* 2018). This finding is in conformity with Obire *et al*. (2002); Mansour *et al.* (2012); Igborgbor and Ogu (2015), who also showed that Gram positive bacteria mainly *Staphylococci* predominate among bacteria isolated in bioaerosols in dump sites. This is essential, in as much as spreading infections by air cannot be excluded (Li *et al*., 2013). The genus *Staphylococcus* are known as sanitary indicators of atmospheric air pollution and signify a likelihood of the presence of pathogenic microorganisms (Małeckae-Adamowicz *et al*. 2007; Fraczek and Ropek, 2011). Although *Staphylococci* are non-spore formers, they show ability to survive in the air for a long time (Roodbari *et al*., 2013). *Stapylococcus aureus i*s one of the leading causes of bacteremia, skin and soft tissue infections, food poisoning, osteoarticular and toxic shock syndrome infections (Oliveira, 2018).

The presence and occurrence of *Bacillus* and *Micrococcus* sp. in the dumpsite could be due to the presence of damp organic materials, materials saturated with water, food and food products and spores of microorganisms propelled through the air. *Bacillus* sp. endospores have a usual resistance to chemical and physical agents. This makes them predominant in soils and explains their aerial distribution. Gołofit-Szymczak (2010), also reported the presence of these bacteria as air microflora. According to Douwes (2003), these bacteria can cause different forms of bacterial pneumonia, influenza and gastrointestinal diseases. *Bacillus* species such as *B.cereus* and *B.anthracis* are important pathogens that cause food poisoning and anthrax (Bhunia, 2018). *Micrococcus* spp. are also commensal organism that can become opportunistic pathogens especially in immune compromised hosts such as HIV patients. They have been implicated in other infections such as recurrent bacteremia and septic shock in immunosuppressed patients (Dada and Aruwa, 2014).

Unlike Gram-positive bacteria that recorded high percentage of occurrence, low percentage of occurrence was recorded for Gram negative bacteria which showed that they may be poorly adapted to living outside their natural environment. This result is in conformity with the result of Małeckae Adamowicz *et al.* (2007), who also reported higher percentage of Gram-positive bacteria as airborne microflora on the landfill site in Poland. This report does not agree with the result of Odeyemi (2012); Kazmierczuk and Bojanowicz-Bablok (2014), who reported higher percentage of Gram negative especially *E. coli* as compared to Gram positive bacteria. Although there are no threshold values for bacteria of the family Enterobacteriaceae indicating their permissible content in the air (Fraczek and Ropek 2011; Le Goff *et al.* 2012) it is important to note that they are potential source of endotoxins. Endotoxins inhaled with polluted air may lead to toxic pneumonitis and finally to adult respiratory distress syndrome (William *et al.*, 2017).

The fungi isolated from the air of these sampling sites were similar to those reported by Obire *et al.* (2002); Igborgbor and Odu, (2015) and Breza-Boluta, (2016). Fungal species present in the soil of dumpsites have been known to produce numerous spores in adverse conditions and modest nutritional and environmental demands. These spores develop into vegetative fungi when the environmental conditions and other factors become favorable. The spores form bio-aerosols in the dumpsites and are dispersed by wind into the surrounding air. This fact could be responsible for the fungal species isolated from the air of the sampling sites. The increase in human activity may also cause more disturbance and lead to increase in fungal pollutants in air.

The most common threat caused by fungi is spores and volatile toxic metabolites that are the main component of bioaerosol. Environmental pollution and threat connected with the presence of fungi in the air results from the fact that they can cause: allergies, asthma, broncho- and pulmonary mycoses and general infections (Kim *et al.*, 2013; Kalwasinska *et al.*, 2014). Exposure to molds has been associated with a variety of adverse health outcomes including respiratory, haematological, immunological and neurological symptom disorder and/or diseases (Yassin and Almouquatea, 2010). Toxinogenic species of the genera *Aspergillus, Penicillium* etc. may induce cytotoxic, neurotoxic, teratogenic and cancerogenic actions towards other organisms (O'Gorman and Fuller, 2008).

The antibiogram results revealed that most of the bacterial isolates in this study were susceptible to the test antibiotics. The prevalence of antibiotic resistant bacteria recorded in this study was lower compared to previous studies on bacteria isolated from dumpsites or air around dumpsites reported by Odeyemi (2012); Mwaikono *et al.* (2015);

Nyandjou (2017). The presence of the few resistant bacterial strains in this study remains still a source of concern, mainly because of the ease of resistance genes transfer among bacteria, especially among the enterobacteria, horizontally (Ugwu *et al*., 2015) and vertically (Mitra *et al*., 2019). Among the Gram-positive bacteria, the HPR was recorded against tetracycline by *Bacillus* sp., followed by erythromycin and penicillin by *Staphylococcus* and *Bacillus* species. These findings correlate with that of Cadena *et al.* (2018), who reported high levels of tetracycline resistant genes in soils from Nebraska organic farming operations. The prevalence of antibiotic resistance among the *Bacillus* sp. may be because they are spore formers, likely to possess resistance plasmids (Bhunia, 2018). The resistance to tetracycline might be due to the fact that tetracycline is a cheap, popular over - the counter drug in Nigeria and therefore prone to abuse.

Over 50% of the *E. coli* isolates in this present study were resistant to cefuroxime, gentamicin, cefotaxime, and half of the *Alcaligenes* sp showed resistance against cefuroxime and ofloxacin. This is in agreement with the findings of Mwaikono *et al.* (2015), who reported the presence of multidrug resistant *E. coli* in samples from a municipal dumpsite in Tanzania. The rate of resistance of *E. coli* to gentamicin in this study is comparable to that of Nwanze *et al.* (2007), who reported gentamicin resistance of 46% in *E. coli* but, it is higher than the 10.6% gentamicin resistance rate in *E. coli* reported by Mbata (2007). Also, Mwaikono *et al.* (2015), has reported high resistance rate in *E. coli* from dumpsites to the β-lactams. These differences in the prevalence of antibiotic resistant bacteria might be due to difference in geographical locations, strain difference, sampling size, and the prevalent antibiotic propriety practices. Human and animal feaces are common sights in many dumpsites and could therefore constitute veritable sources of resistant *E. coli* and other resistant enteric bacteria in dumpsites.

Many humans harbour resistant microorganisms due to selection pressure as a result of antibiotic abuse. The indiscriminate use of antibiotic in animal husbandry as prophylactics, therapeutics, and growth promoters has been reported to contribute mainly to the presence of antibiotic resistant microorganisms in farm animals, which often shed these organisms in their feaces. These animal feaces may somehow found their way into dumpsites by indiscriminate disposal by man or shedding by free - range animals (Odeyemi, 2012). There is also, a dynamic interaction of people working in dumpsites without protective clothing, people that openly defecate in dumpsites, domestic animals and rodents scavenging in dumpsites (and which may later visit peoples' homes). This interface, therefore, presents high risks of contacting resistant microorganisms from the dumpsites and spreading such in the public (Mwaikono *et al*., 2015). The presence of resistant bacteria in the air around dumpsites is of medical significance as these organisms can cause diseases difficult and expensive to treat.

CONCLUSION

This research affirms that dumpsites are potential hazards to the environment and its occupants, as they constitute sources of air pollution by fungi and bacteria, especially antibiotic resistant organisms whose infections in humans are difficult and expensive to treat.

Recommendations

In a bid to improve the quality of air, proper sanitary landfill waste disposal should replace open dump system to reduce the number of microbes that get into the atmosphere. Methods like incineration in a highly controlled environment should be encouraged. Waste management practices of waste reduction, wastes re-use and recycling should be emboldened. Legislative laws and regulations on use and effective waste disposal and management should be made to control the location of dumpsite which should be far removed from the community. Eminent health bodies and other public health organizations should create public awareness and educative campaigns to inform individuals on the effects of improper disposal of waste and open dump system of waste disposal.

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