Global Journal of the Built Environment Vol 5 No. 1 (2023)
print-ISSN: 2408-6223 | electronic-ISSN: 2992-5088

Global Journal of the Built Environment

(Published by the School of Environmental Studies, Federal Polytechnic Nasarawa)

Available Online at www.gjbeses.org

Isolation and Identification of Air Microflora Around Refuse Dumpsites in Nasarawa Town and their Sensitivity Pattern

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Abstract

Refuse dumpsites are found littered both within and on the outskirts of Nasarawa town due to poor and ineffective waste management, this posed health risk to the populace and add strain on diminishing land space as well as harboring pathogenic organisms. This study is aimed at assessing the air microflora around refuse dumpsites in Nasarawa town and their sensitive pattern to some common antibiotics. Nasarawa state were checked and the sensitivity of the isolates to some common antibiotics were evaluated. A total of sixteen plates of Nutrient and Sabouraud agar were prepared in duplicate and opened around the refuse dump sites for about 30 minutes at a height of about 130cm. Prepared plates were incubated appropriately for 24 and 42 hours for bacteria and fungi respectively. The average number of colonies of bacterial and fungi of the different dumpsites (Tammah, Oversea, Campus and Market) are; $8.5x10^1$, $7.55x10^1$, $6.55x10^1$, $9.4x10^1 cfu/m^3$ and $7.8x10^1$, $6.25x10^1$, $5.0x10^1$, $9.0x10^1 cfu/m^3$ respectively. Pure isolates were obtained by sub culturing into Mannitol salt agar, Salmonella shigella agar and MacConkey agar. The cultural and morphological of the isolates were revealed.The isolates were identified by biochemical test such as Catalase, Coagulase, Citrate, Sugar fermentation test. The bacteria isolated and some antibiotics they are sensitive to are as follow; Escherichia coli (Streptomycin, Oxofloxacine and Ciproflox), Staphylococcus epidermidis (Oxofloxacine, Streptomycin and Gentamicin), shigella spp (Ceporex, Oxofloxacine and Gentamicin,). Staphyloccus aureus (Streptomycin, Oxofloxacine and pefioxacine). The fungi identified are Rhizopus crazae, Mucor racemosus and Aspergilus niger. Some of the isolates are pathogenic which have the potential to cause infectious diseases, therefore indiscriminate dumping of refuse within and around the town should be avoided.

Keywords: Microflora, Refuse, Staphyloccus aureus, Catalase, Mucor, Nasarawa

Introduction

Refuse dumpsites are common sights in most developing countries towns and cities due urbanization and improper wastes management; these posed health risk to the populace. Refuse dumps are areas or land where material wastes from several sources and processes are deposited (Odeyemi, 2012). Solid waste disposal is crucial for the maintenance of both human and environmental health. Notwithstanding, in developing countries, waste dumps are

indiscriminately placed openly (Arigbede and Yusuf, 2010; Odeyemi, 2012). Waste management processes have been rigorously assessed with regards to potential dangers posse to the immediate environment and public health. Efficient wastes management is fundamental to the sustenance of physical and social infrastructure as well as the enhancement of the socio-economic well being of any community (Arigbede and Yusuf, 2010).

The isolation of pathogenic bacteria from waste dumps potent serious health risks to waste scavenger, waste handlers and people living in the communities around the vicinity of the dumps sites. So much risk is involved and is further worsen by the widely reported cases of waste dumps bacterial pathogens resistant to several antibiotics. For instance, in a study conducted on the antibiotics susceptibility pattern of bacterial isolates from air around dump sites, the isolated miroorganisms were observed to be resistant to most of the antibiotics on which they were tested (Odeyemi, 2012). *Escherichia coli, Salmonella species, Shigella species* and *Vibrio cholerae* are a few pathogenic bacteria thought to be associated with faecal contamination. In addition to these microorganisms causing human diseases, resistant to antibiotics have complicated the health problems (Glynn *et al.,*1998; Ajayi and Akonai, 2003).

Most microorganisms found in the waste can be transmitted by inhalation and contact (exposure to the dumpsite), with infection occurring in the respiratory tract, in skin lesion and mucus membrane (Awisan *et al.,*2011). faecal Coliform group of microorganisms are used as indicators of the sanitary quality of the environment especially the non spore forming Gram negative rods (Achudume and Olawale, 2007).

Therefore, microbial contamination of waste by human or animal excreta is hazardous and of public health significance. Due to inadequate and lack of government authorized waste disposal infrastructure in most urban and rural areas, almost all of the population dump wastes are in open spaces and drainage channels. These wastes are largely untreated and poorly managed and might serve as shelter and food source for rodents, flies and insect vectors. These are known to carryvarious pathogenic agents of diseases including typhoid fever, plague, cholera, bacillary and amoebic dysentery. A high percentage of these infections are caused by bacterial strains which can be found in these refuse dumps sites and could cause diseases (Arigbede *et al.,* 2010; Odeyemi *et al.,*2011; Ogunrinola and Adepegba, 2012).

In developing countries, towns and urban cities may be untidy, food chunks, cans, polythene bags, garbage. Upleasantly human and animal feces could be noticed accumulating on landfills in close proximity of up to 100m near surrounding houses. Dumps of this nature are usually worrisome and come irritating odour and smells especially when there is the decomposition organic matter occasioned by the presence of bacteria (Hassen, 2002). Waste dumps release bioaerosols into the air which are associated with pathogens known for causing fatal diseases such as diarrhea and cholera. The presence of bioaerosols of this nature in the environment is of much concern because this can be distributed over wide areas by various mechanisms into the indoor setting as well. Anaerobic microoganisms have the ability convert organic matter present in wastes into gases that could react with other compounds as they move through the air space to cause explosions, which are detrimental to human health (Molino,

2013).

This research is aimed at isolating and characterizing bacteria and fungi found in the air around refuse dump sites and to observe their sensitivity pathern to some common antibiotics.

Materials and methods

Study area

The study area is Nasarawa Local government, Nasarawa state located central of the Middle Belt region of Nigeria. Nasarawa Local Governmernt is located at western part of the state on longitude 7° 50'E and latitude 8° 50'N . It is covered a distance of about 40km to Keffi Local Governent Area and 90 km to Abuja, Federal capital Taritory (FCT) Akwa et al., 2007; Makut et al., 2014

Materials and Methods

Sample collection and microbial examination

In the month of February, 2022, the air samples were collected in duplicate at four different refuse dumpsites around Nasarawa town namely: Tammah, Oversea, Federal Polytechnic Nasarawa campus and Nasarawa market. Different culture media appropriate for the growth of the ogranisms were prepared according to the manufactural instructions. Nutrient agar, MacConkey Agar, Manitol salt Agar and Sabouraud Agar were prepared as outlined by methods described by Bailey and Scott (2007); MacFaddin (2000).

Following the methods decribed by Makut et al.(2014); Obiekezie et al.,2019), Sedimentation methods was used to collect the air samples onto the surfaces of the plates by opening of the petri dishes for about 20 munites at a height of about 130cm. The plates were covered and transported immediately to Microbiology Laborator, Federal Polytechnic Nasarawa for further analysis.

Enumeration and Identification of the Isolates

The plates where incubated for 24 hours at 37°C for bacterial isolates and 72 hours in a dark cupboard at room temperature for fungi isolates. The colonies were counted and recorded in colony forming units per cubic meter of air (Cfu/m^3) as described by Stryjakowska-Sekulska *et al*., 2007 and Makut *et al.,* 2014. Cultural and morphological characteristics of bacterial and fungi isolates were revealed, in addition Gram staining and biochemical test were conducted on the bacterial isolates adopting the methods described by Cheesbrough (2000).

Antibiotic susceptibility test

The antibiotics susceptibility tests for the isolates was determined by the disk diffusion method using Mueller-Hilton agar according to CLSI (2009). The Bacterial isolates were tested with seven ABTEK disc antibiotics which include Augmentin (AUG 30µg), Gentamycin (GEN 10µG), Ofloxacin (OFL 5µg), Cefotaxime (CAZ 30µg) and Cefuroxime (CRX 30µg). The Gram negative discs were fitted with additional constituent which include Amoxicillin (AMX 30 µg), Ceftazidme (CTX 30µg) and Nitrofurantoin (NIT 300µg). The Gram positive discs were incorporated with additional constituent such as Cloxacilin (COX 5µg), Oxacilin (OXA 10µg) and Lincomycin (LIN 2µg). The inoculum was standardized by to the 0.5 McFarland turbidity standard by adjusting inoculum density equal to that turbidity of Barium sulphate (BaSO4) and

incubated at 35°C for 18 hours. The inhibitions zones were measured and interpreted in accordance to the standards used by Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2009).

Results

Table 1 below shows the total bacterial and fungal counts of air samples around the refuse dumpsites of the four different locations within Nasarawa town; Tammah, Oversea, Campus and Market. From the table,the Market has the highest bacterial and fungal count, followed by Tammah and oversea while campus has the least count in both organisms.

Table 1: Bacterial and fungal count (cfu/m^3)

Sites	Bacterial	Fungal
Tammah	$8.5x10^1$	$8.8x10^1$
Oversea	$7.55x10^1$	$6.25x10^1$
Campus	$6.55x10^1$	$5.0x10^1$
Market	$9.4x10^1$	$9.0x10^1$

Table 2: The morphological characteristics and pigmentation of the colonies on various culture media plates revealed the following bacterial isolates; *Staphylococcus aureus, Escherichia. coli, Shigella spp, Staphylococcus epidemidis, Salmonella spp, Pseudomonas aeugenosa*

Table 2 Morphological Characteristics of bacteria Isolates

Sites	Media	Morphological Characteristics	Probable Isolates
Tam mah	Manitol salt agar	Yellow colonies with yellow zones	*Staphylococcus spp*
	MacConkey agar	Small, pink to red	*Escherichia coli*
	Salmonella shigella agar	Clear, colorless, transparent	*Shigella spp*
Over sea	Manitol salt agar	Yellow colonies with yellow zones	*Staphylococcus aureus*
	MacConkey agar	white, raised, cohesive colonies about 1-2mm in diameter	*Staphylococcus. epidemidis*
	SS agar	Clear, colourless, transparent appear as opaque/yellow, pink or red colonies with black centers.	*Shigella Salmonella*
Cam pus	MacConkey agar	Lactose fermenter, flat, red/pink non-mucoid colonies with surrounding darker pink area ppt bile salt	*Escherichia coli*
	Manitol salt agar	Gram positive bacteria, forms white, raised, cohesive colony about 1-2mm in diameter after over night incubation	*Staphylococcus epiidermidis*
	SS agar	Clear, colourless, transparent	*Shigella spp*
Mar ket	Manitol salt agar	Gram positive bacteria, forms white, raised, cohesive colony about 1-2 mm in diameter after overnight incubation	*Staphylococcus epidemidis*

MacConkey agar	Red/pink non-mucoid colonies with surrounding darker	*Escherichia coli*
	Pink area ppt tile salt, translucent-opaque	*Pseudomonas aeugenosa*
Salmonella shigella agar	Forms flat & smooth colonies that are between 2 and 3mm in diameter, colourless, gram negative rod-shape, clear, colourles, transparent	*Shigella spp*

Table 3:This table revealed the following fungal isolates based on their cultural, morphological characteristics and pigmentation on sabouroud agar; *Rhizopus cryzae*, *Mucor racemosus* and *Aspergillus niger*

Table 3: Cultural and Morphological characteristics of fungi isolates

Site	Probable organisms	Colony colour	Characteristics feature	Growth rate
Tammah	Rhizopus cryzae/mucor racemosus	White to grayish brown colonies about 1cm with tendency to collapse back	Colonies are raised, about 1-2cm high. Stolen hyaline sporangiophore arising directly from stolon.	Fast
	Mucor racemosus	White fluffy cotton	Coloured are raised in tact	
		Wool like.	Colonied heads non septate lyphe.	Fast
Oversea	Mucor racemosus	White fluffy cotton wool like	Colonied are raised infact conidia heads, non-septate hypae.	Fast
Campus	Aspergillus niger/mucor racemosus	Chocolate brown colonies	Intact conidia heads conidiophores arising from thick walled conidia conidiosphorehyelline to brown, mostly smooth walled conidia at maturity globose irregularly roughened with conspicuously ridges.	Fast
Market	Rhizopus cryzae/Aspergillus niger	White to grayish brown colonies about 1cm with tendency to collapse back	Colonies are arised, about 1-2cm high stolon hyaline sporongiophore arising directly from ston, craeriallyphae sporangia black	Fast

Table 4: Shows the biochemical, gram staining and morphological properties of the isolated microorganisms characterized into gram positive and gram negative microorganisms, the following bacterial strains were identified *Staphylococcus aureus* and *Staphylococcus*

epidermidis, Shigella and *Escherichia coli*. These findinge are similar with the work of Omusi et al. (2017).

Table 4:Biochemical Test, Gram staining and morphological characteristices of bacterial isolates

SHAPE	GR	CAT	COA	CIT	SUF	COLOUR	Organisms Identified
Cocci	+	+	+	-	Sucrose + G Lactose + G Maltose + G	Yellow	*Staphylococcus aureus*
Cocci	+	+	-	+	Sucrose + G Lactose + G Maltose + G	Milkfish	*Staphylococcus epidermidis*
Rod	+	+	-	+	Sucrose + G Lactose + G Maltose + G	colourless	*Shigiella spp*
Rod	-	+		-	+ Sucrose + G Lactose + G Maltose + G	Pink to red rose	*Escherichia coli*

Key: GR – gram staining reaction, CAT – Catalase test, COA – Coagulase test, CIT – Citrate test, SUF – Sugar fermentation test, COL – Colour, +=positive indicating presence of organism-=negative indicating absence of organism, G-Gas formation.

Table 5: This table shows the susceptibility pattern of the isolates to some common antibiotics.

Table 5: Sensitivity test after 24hours in (%)

Organisms	S	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
E.coli	24.0	12.37	0.00	21.90	0.00	16.17	18.07	16.58	21.34	11.80
S.aureus	5.78	0.00	0.00	5.09	0.00	3.67	0.22	0.00	0.00	0.00
S.epidemidis	18.02	13.98	0.00	22.13	15.33	17.5	17.5	0.93	6.21	0.00
Shigella	0.00	0.00	11.62	9.90	6.91	6.91	9.69	0.00	0.00	0.00

Key:S=Streptomycin, PN=Ampicillin, CEP=Ceporex, OFX= Oxofloxacine,NA=Nalidixic acid, PEF=pefioxacine, CN=Gentamicin, AU=Augmentin, CPX=Ciproflox, SXT=Septrin

Discussion

The microbial loads of the air samples collected around refuse dumpsites in the market area has the highest numbers of colonies of both bacterial and fungal isolates ($9.4x10^1$ and $9.0x10^1$cfu/m^3). This is due to lot of activites that goes on around the area especially on Fridays being the market day. Nasarawa is known of its rich agriculture and solid mineral exploration (Wang et al., 2011; Cetin, 2014). People comes from villages around the environs with their farm produce to sell to foriegners and the cities dwellers especially people

from Abuja (Federal Capital of Nigeria) which is just about 100km away. These activities led to littering of the areas which in turn result into heap of refuse dumpsites.

In this part of the world, toilet facilities and not adequately provided in the market areas, and also, the villagers are use to open air system of toilet therefore uses any available refuse dumpsites to unrinate of or even deficate when the need arises and these activites poses health risk to the populace (Longe and Williams, 2006; Agwu 2012). In addition, the wastes arising from the Market from rotten vegetable, fruits, tubers, meat, fish e.t.c are all dumped at the refuse sites there by polluting the air around the dumpsites.

Tammah is next to Market in bacterial and fungal load ($8.5x10^1$and $7.8x10^1$cfu/m^3), Tammah is known to be students residential areas, where off campus students are residing due to it proximity to the school. Huge refuse dumpsites are seen around the area due its high population and high number of activities ranging from, road side food vendors, sales of fruits and vegetables, restaurants bars and high number of commuters flying the road that connect the State with Benue and Eastern part of the country leading to accumulation of huge refuse dumpsites behind the residential areas. Furher more, children and and young adults within the area are also found urinating and defaecating around the dumpsites during the dawn and dusk period leading to the contamination of the air around the area. Oversea and campus have less colonies of both organisms ($7.55x10^1$, $6.25x10^1$ $6.55x10^1$, $5.0x10^1$ cfu/m^3), Oversea being purely a residential area is not highly populated and wastes generated in the campus are normally gathered within short period and burnt of in the incinerators within the campus.

The morphological characteristics of both bacterial and fungal isolates showed the presence of the following organisms; *Escherichia. coli, Staphylococcus aureus, Staphylococcus epidemidis, Shigella spp, Salmonella spp, Pseudomonas aeugenosa*, *Rhizopus cryzae*, *Mucor racemosus* and *Aspergillus niger*. Open system of toilet (latrine) is very common in our villages and small towns, the presence of *Escherichia coli*, *Salmonella spp* and *Shigella spp* which are enteric bacteria commonly found in the stool (feces) of people who are infected by these organisms in the air samples in these areas is not suprising as people found it easy to urinate and defecates in the dumpsites.

This finding is similar to the work reported by Obiekezie et al., 2019 in which some of the above microbial isolates were identified aound dumpsites in Keffi metropolis. Similary, Makut et al.,2014 also reported the presence of *Staphylococcus aureus*, *Escherichia coli*, *Shigella* spp, *Aspergillus niger mucor spp* in the outdoor air environment within the same Keffi Metropolis.

Omusi et al.,2017, reported the presence of *Staphylococcus aureus, Pseudomonas*, *Aspergillus, Mucor, Rhizopus spp* in the soil around refuse dumpsites in the southern part of the country which are similary to that where isolated in the work, although in the soil but most of the organisms found in the air around the refuse dumpsites comes for the soil environment during various activities such as scavenging (Aljaradin et al., 2015), digging and excavation at the dumpsites.

The sensitivity of the isolates to some common antibiotics tested, indicated some degree of susceptibility to most of the antibiotics. *Escherichia coli* was susceptible to Streptomycin, Oxofloxacine

and Ciproflox but resistance to Ceporex. *Staphylococcus epidermidis* was susceptible to Oxofloxacine, Streptomycin and Gentamicin but resistance to Ceporex and Septrin, *shigella spp* was susceptible to Ceporex, Oxofloxacine and Gentamicin resistance to Streptomycin, Ampicillin, Augmentin, Ciproflox and Septrin while *Staphyloccus aureus* was susceptible to Streptomycin, Oxofloxacine and pefioxacine but resistance to Ampicillin, Ceporex, Nalidixic, Augmentin, Ciproflox and Septrin. *Escherichia coli* revealed the highest level of susceptibility to the antibiotic tested followed by *Staphyloccus aureus*, Three out of the four bacterial isolates were reistance to Septrin, this antibiotic is over the counter drug which can be use without the prescription of the medical personals leading to it improper usage.

This is finding is similar to that reported by Obiekeze et al., 2019; Obiekeze et al., 2013.The resistance of some of these pathogenic bacteria to some common antibiotics is becoming more worrisome because they are mostly use in curing infectious diseases in both lower and higher animals.

Conclusion

The presence of bacterial and fungal strains found in the air flora around the refuse dumpsites around Nasarawa town is of public health significance which has received much attention in recent years. In this findings, four bacteria and three fungi genus were isolated of which some are pathogenic. Although, their count are not up to the threshold of 10^3 cfu/m^3 (1000cfu/m^3) recommended by National Institute of Occupational Safety and Health (NIOSH) as reported by Makut et al (2014) and Gorny and Dutkiewicz (2002) for both bacteria and fungi isolates

Recommendations

Dump sites are residence to many harmful microorganisms, good policies have to be adapted for waste handlers and populace living in the vicinity of the dumps to safeguard their lives. Health organizations and other relevant bodies should start up public awareness and enlightenment campaigns to educate individuals on the hazards involved in indiscriminate waste disposals and the open dump system of waste disposal. The public should be enlightened on the aspects for environment and health surveillance, town planners officials and public health should implement the critical (minimum) distance within which refuse dumps should not be sited. Subsquently open unrination and defaecation at waste dump sites and environs should be highly discourage by enforcing Laws and the penalty to defaulters. Government should provide adequate and functional public conveniences exspecially the markets areas and School environment.

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