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CULTURE-BASED MICROBIOLOGICAL ASSESSMENT OF BACTERIAL COMMUNITY IN SOILS FROM WASTE DUMPSITES WITHIN GOMBE METROPOLIS, NIGERIA

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ABSTRACT

Waste dumpsites or landfills is an area of land where refuse or waste materials from differed processes, activities and sources are deposited. This study was aimed at applying a culture-based approach for assessing the culturable bacterial community in soils from waste dumpsites within Gombe metropolis for environmental relevance. Exactly thirty (30) soil samples were collected from 10 different dumpsites and were examined bacteriologically by serial dilution then pour plating on several culture media. Afterwards, bacteria isolated after 24 h incubation were macroscopically, microscopically and biochemically examined for genuine identification and confirmation. Also, the bacterial plate counts of the respective dumpsites were determined. The bacterial load from the mean plate count were $3.33x10^9$, $3.1x10^9$, $1.43x10^9$, $2.77x10^9$, $2.13x10^9$, $3.20x10^9$, $4.20x10^9$, $3.23x10^9$, $2.03x10^9$ and $1.33x10^9$ for Arawa, Bolari, Bagadaza, Hammadu Kafi, Cecenia, Riyal, Pantami, Malam Inna, State low-cost and Kagarawal respectively. Nine (9) bacteria genera/species isolated and identified were *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Bacillus cereus, Salmonella* sp, *Enterobacter* sp, *Micrococcus* sp and *Shigella* sp though with varied percentage of the occurrence. Findings from this study understandably portray low diversity of the bacterial population as only culturable bacterial community was reflected in commensuration with the approach applied. The detection of *Staphylococcus aureus, Salmonella* and *Shigella* in the refuse dumpsites soils confirmed microbial pathogen presence and depict an environmental and public health significance thus government and municipalities should provide annexe laws or revise existing policies regarding the locations, operation and management of dumpsites to safeguard the health and safety of the populace.

Keywords: Bacterial community, Plate count, Colony-forming unit, Soil, and Waste dumpsites.

INTRODUCTION

Indiscriminate and unmanaged waste disposal in both rural and urban settings has caused dumpsites otherwise known as landfills to abound in many communities today. Even though with policies and regulations in place by state and national environmental regulatory bodies for proper waste disposal and management, these policies often lack stringent enforcement and penalties to ensure compliance except in major cities of the country. Consequently, most community parade unpleasant sites of waste dumpsites to the

detriment of the populace; an important instant of such is the health and safety of the receptors of the hazards from the dumpsites aside from the huge environmental implications.

Refuse or waste dumpsite refers to an area of land or site where all variety of wastes from several sources and processes are dumped. Refuse dumps are both municipal solid and industrial wastes including liquid effluent containing heavy metals (Olanrewaju, 2002). This provides a rich source of microorganisms most of which are pathogenic and more also serves as the sight of attraction to many insects and rodents, thereby contributing to the microbial load as dumpsites serve as their shelter and food sources (Odeyemi *et al.,* 2011). Many microbes can remain viable even after extended period despite the constrain associated with thriving in the atmosphere, including extended ultraviolet exposure, low moisture levels and extremely oligotrophic conditions (Jones and Harrison, 2004).

The US Law-solid waste Act 2, (1999) termed "Solid waste" to include garbage, refuse or sludge from a waste treatment plant, water supply treatment plant or air pollution control facility and other discarded material, including solid, liquid, semi-solid or contained gaseous material resulting from industrial, commercial, mining and agricultural operations. However, solid waste can be classified into different types depending on their source; a household waste is generally classified as municipal waste, industrial waste as hazardous waste while biomedical waste or hospital waste as infectious waste (Salam, 2010). Notably, man-made anthropogenic activities including industrial activities, small and large-scale businesses and manufacturing, food processing, farming activities and house-hold processing are the glaring major sources of assorted waste compounded in community waste dumpsites and this has encompassed an aspect of environmental pollution.

The group at risk from the unmanaged and indiscriminate disposal of solid waste are the general population (especially children) in areas where there is no proper waste disposal method, waste workers and workers in facilities producing toxic and infectious waste materials. Other high-risk groups include the population living close to a waste dump and those whose water supply has become contaminated from the dumpsites as a result of leakage from landfill sites. The uncollected refuse dump also increases the risk of injury and infection (Odeyemi, *et al.,* 2011).

Dumpsites are known for their smelly and unsightly condition; these conditions are worse in the summer due to extreme temperatures which speed up the rate of bacterial action on biodegradable organic materials (Salam, 2010). Disposal of solid waste on the land without careful planning and management can cause grave danger to the environment, and health and safety of the general populace. The health implications could be more destructive if the waste dumpsites contain hazardous chemical substances and/or pathogenic microbial infectious agents. This environmentally relevant study, therefore, applied a culture-based method for the assessment of a bacterial community in soils collected from waste dumpsites within Gombe metropolis. Findings are expected to unravel the type of bacteria inhabiting the waste dumpsites and advise on the health, safety and environmental implications of

dumpsite operations based on the findings of this study.

MATERIALS AND METHODS

Study Area

The study was carried out in 10 different areas within Gombe metropolis, namely; Bolari, Jekadafari, State Low-cost, Kagarawal, Malam Inna, Bagadaza, HammaduKafi, Riyal, and Cecenia, Arawa.

Collection of Soil Samples

A total number of 30 soil samples were collected from 10 selected areas within Gombe Metropolis. Before sampling, the surface refuse of the dumpsite was initially removed then the soil was dug out up to 5 to 10 cm depth by the use of pre-disinfected hand shovel. Foreign materials like pebbles, polythene bags and stones were separated and removed from the soil before sampling. Approximately 10 g soil was collected in triplicates from different points in one site then transferred into a sterile soil pick bags already labelled A, B, and C. All the samples collected in sample bags were properly sealed and transported to the microbiology laboratory at 4 ºC in an ice bucket for analysis.

Microbiological Analysis of Soil Samples

Stock Preparation and Serial Dilution

One gram of soil sample was transferred into a sterile test tube containing 9 mL of distilled water then thoroughly vortexed to give a stock homogenate solution. Ten-fold serial dilution was achieved up to 10^{-5} dilutions by taking 1 mL from the prepared stock homogenate then dispensed into 9 mL distilled water for the first dilution (10^{-1}). This was continued for other dilutions according to the standard serial dilution protocol to obtain discrete colonies (Marshal *et al.,* 1995)

Plating on Culture Media

Pour plating method was employed for culturing on solid media where 1 mL was taken from 10^{-2} and 10^{-4} dilutions and dispense in sterile Petri dishes. 15 to 20 mL of prepared culture media cooled to ~45 °C were poured into the Petri-dishes containing the 1 mL of serially diluted inoculum then gently rocked on the bench to mix and give a uniform homogenate. The inoculated plates were allowed to set firmly for at least five minutes afterwards inverted in the incubator for incubation at 37 °C for 24 hr.

Identification and Confirmation of Bacterial Isolates

Bacterial isolates identification was tentatively done using experimental microbiology manual by Aneja, (2003). Bacteria isolates were identified by macroscopic examination for colony morphology and cultural characteristics which includes colour, shapes or forms, elevation, margin and pigment formation on the colonies, especially on selective and differential media.

For the microscopic identification, bacterial colonies from the culture plates were initially subjected to Gram staining reaction then microscopy according to standard microbiological protocols to primarily reveal their gram reaction and enable proper examination of the microscopic morphology of bacteria isolated.

Gram Staining techniques

This staining procedure was employed for the identification of Gram-positive and Gram-negative microorganisms. A smear of the bacterial isolate was made on a clean grease-free slide then slightly heat-fixed before staining with gram staining reagents according to the standard gram staining protocol as described by Cheesbrough, (2006). Isolate's Gram reaction and cell morphology were observed under the microscope at x100 objective lens.

Confirmatory Biochemical test

After the identification of bacterial isolates, further confirmation via biochemical tests that includes Catalase, Coagulase, Indole, Citrate Utilization, Motility and Urease tests were carried out for genuine confirmation of the various bacteria isolated from the soil samples. Protocol for biochemical analyses described by Cheesbourgh (2005) was applied and complimented with techniques described by Fawole and Oso (2004). Biochemical test results obtained for this study were checked in Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham, 1993) for the genuine confirmation of bacterial isolates.

RESULTS

The findings of this study reveal the heterotrophic bacterial plate counts for each of the 10 dumpsites studied. Specifically, the mean bacterial plate count obtained in this study were $3.33x10^9$, $3.10x10^9$, $1.43x10^9$, $2.77x10^9$, $2.13x10^9$, $3.20x10^9$, $4.20x10^9$, $3.23x10^9$, $2.03x10^9$ and $1.33x10^9$ for Arawa, Bolari, Bagadaza, Hammadu Kafi, Cecenia, Riyal, Pantami, Malam Inna, State low-cost and Kagarawal respectively. As shown in Figure 1, the bacterial plate counts portray the bacterial load or enumerated viable bacterial population in various soil samples collected from 10 different refuse dumpsites in Gombe metropolis.

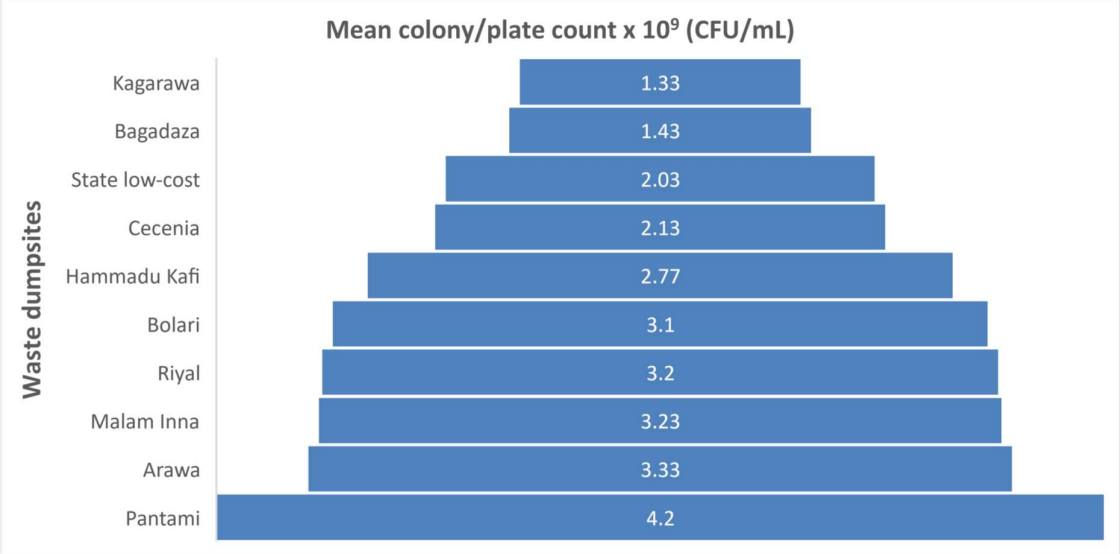

Figure 1: Funnel chart showing the mean bacterial plate count for the ten selected waste dumpsites within Gombe metropolis. Plate count established in colony-forming unit per mL of serially diluted inoculum. (CFU/mL).

Besides, it was deduced that 33.3% of the bacteria genera/species screened and identified were Gram-positive while the Gram-negative counterpart had a comparatively higher percentage of 66.6%. The results for biochemical tests, gram staining reaction, colonial and microscopic morphology of different bacteria isolated from the dumpsite soils is provided in Table 1 below. This table confirmed the identity of the varied bacterial colonies screened from all the culture plates representing the entire 10 waste dumpsites in this study. It must be acknowledged here that colonies were randomly screened from all the culture plate to give an overall picture of bacterial community proliferating in these waste dumpsites and this is without any focus to individual sites.

Table 1 shows that nine (9) different bacteria genera/species isolated from the waste dumpsites were identified and confirmed and these include *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Bacillus cereus, Salmonella* sp, *Enterobacter* sp, *Micrococcus* sp and *Shigella* sp. However, the frequency of occurrence of these bacterial isolates in the overall 10 waste dumpsite varies.

Table 1: Microscopic morphology and biochemical characteristics of identified and confirmed bacterial isolates.

Organisms	Biochemical tests										
	GR	Morp.	Ur	Cat	Co	Cit	Mot	Ind	MR	Ox	H_2S
E. coli	-	srs	-	+	-	-	+	+	+	-	-
S. aureus	+	cc	-	+	+	-	-	-	+	-	-
B. cereus	+	*lrc*	d+	+	nd	d+	+	-	-	d+	-
Proteus mirabilis	-	*rps*	+	+	-	-	+	-	+	-	+
Salmonella sp.	-	srs	-	+	-	+	+	-	+	-	+
Shigella sp.	-	srs	-	+	nd	-	-	d+	+	-	-
Enterobacter spp	-	rs	-	+	nd	+	+	-	-	-	-
Micrococcus	+	cpt	-	+	-	+	-	-	-	d+	-
Klebsiella pneumonia	-	*lrs*	+	+	-	+	-	-	+	-	-

Table showed the microscopic features and result of biochemical analyses applied to confirm all the bacteria isolated from the soils of waste dumpsites within Gombe metropolis. Key: Positive sign + signifies positive reaction, d+ signifies 16-84% strains are positive (mostly positive), Negative sign - signifies negative reaction, nd = Not determined, Ur = urease test, Cat = catalase, Co = coagulase test, Cit = citrate test, Mot = motility test, Ind = indole test, MR = Methyl red test, Ox = Oxidase test, Morp: microscopic morphology/shape represented as cc = cocci in cluster, rs = rods in singles, srs = short rods in singles, lrc = long rod in clusters, rps = rods in pairs and singles, cpt = cocci in pairs and tetrads and lrs = long rods in singles. Biochemical test results were checked with Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham,1993) for convincing confirmation of bacterial isolates.

Table 2 shows the frequency of occurrence of different bacterial isolates in the refuse dump soil samples, indicating *Klebsiella pneumonia* has the highest occurrence of 18 (17.8%), trailed closely by *E. coli* with 17 (16.83%), then *Salmonella* sp with 15 (14.85%), while *Micrococcus* had the lowest frequency of occurrence of 4 (3.96%) in the combined dumpsites studied.

Table 2: Frequency of occurrence of bacterial isolates from refuse dumpsite soil samples.

Organisms	Frequency of occurrence	Percentage (%)
Klebsiella pneumonia	18	17.82
Escherichia coli	17	16.83
Salmonella sp	15	14.85
Shigella sp	13	12.87
Proteus mirabilis	11	10.89
Staphylococcus aureus	9	8.91
Bacillus cereus	8	7.92
Enterobacter sp	6	5.95
Micrococcus sp	4	3.96
Total	**101**	**100**

DISCUSSION

In this study, the culturable bacterial community in 10 different waste dumpsites within Gombe metropolis was microbiologically assessed using the culture-based approach. It should be emphasized that this study is of environmental, health and safety relevance as it unravelled the bacterial community proliferating in the dumpsites thus prospecting microbial hazards looming in the community and predicting health and safety implications.

The findings from this study revealed a disparity in the heterotrophic bacterial plate counts (mean plate count) for the 10 different dumpsites within Gombe metropolis, where dumpsite soils from Pantami produced the highest mean plate count of $4.2x10^9$ CFU/mL while dumpsite sampled in Kagarawa established the lowest count of $1.33x10^9$ CFU/mL. A similar study by Ebe *et al.,* (2015) reported a heterotrophic bacterial count of $5.6x10^8$ CFU/mL from a domestic solid waste dump in Egbu, Owerri North Local Government, while Achudume and Olawale (2009) reported total heterotrophic bacteria count of $4.5x10^8$ CFU/mL. The variation in bacterial load or plant count from different dumpsites is reasonably expected as these dumpsites constitute differed quantities, types and degradation/decomposition status of waste in the study sites. Additionally, the age of the dumpsites could most likely contribute to the population of microbial communities as studies have revealed that microbial degradation and decomposition is more pronounced in aged polluted soils due to the long-term deposition of organic nutrients. On this note, higher bacterial count noticed in some dumpsites is attributed to the availability of rich organic substrates or nutrients required for microbial growth and metabolism. On the other hand, these aforementioned factors could be the determinants of heterotrophic bacterial load in waste dumpsites and may necessarily affect the microbial ecology and the roles of microorganisms in such an environment.

In this study, only nine different bacteria genera/species were identified and

confirmed from the entire colonies microbiologically and biochemically screened from all the culture plates representing the 10 waste dumpsites (Table 1), even though the frequency of occurrence of these bacterial isolates varies across the ten sites (Table 2) – this pointedly implies low diversity of the bacterial community in the ten dumpsites within Gombe metropolis. On second thought, this is only a fair perspective considering the culture-based approach applied for this study, accordingly, this does not portray a complete picture of the entire bacterial community in the waste dumpsites. To buttress this point, the soil has been termed the most diverse environment containing a vast abundance of diverse microorganisms (Kirk *et al.,* 2004). Specifically, Roesch *et al.,* (2007) stated that approximately 1g of soil possesses 10^9 - 10^{12} prokaryotic cells, and bacteria are the most predominant groups in soil (Gans *et al.,* 2005). However, crucial studies (e.g., Kirk *et al.,* 2004) have reported that only 1% or less of the soil microorganisms are culturable on common culture media under standard conditions (Torsvik and Ovreas, 2002) insinuating that traditional culturing methods limit analysis to those that only grow under laboratory conditions (Hugenholtz and Pace, 1996). Therefore, culturing techniques has failed to capture the full spectrum of microbial diversity (Ibrahim, 2020; Colwell and Grimes 2000), even though it possesses cherished historical unique values in the study of the phenotypic and biochemical characteristics of microbes in a petri dish. Fortuitously, Ibrahim, (2020) clarify that the advent of culture-independent approaches principally molecular biology and metagenomics augmented with sequencing technology has now made it possible to make a more comprehensive assessment of the scope of the microbial biodiversity including the uncultured species present in soil and other environmental samples.

The study established that majority (66.6 %) of bacteria genera/species isolated and identified from the ten selected different waste dumpsites are Gram-negative type while the Gram-positive bacteria constituted the remaining 33.3 % bacterial population. This is contrary to the study of Begum *et al.,* (2017), who reported 79 % Gram-positive and 26 % Gram-negative bacterial isolates from the 19 different single colonies isolated from food waste sites in Bangladesh. This further support the assertion that the microbial community in the waste dumpsites could largely depend on certain important factors previously mentioned.

The bacterial community in an environment is mostly predictive of the microbial activities and biological roles in such environment and conceivably denotes microbial relevance or significance in an environment. For instance, microorganisms in the diverse environment have been extremely useful for the production of antibiotics, biosurfactants and chemicals having biochemical, pharmacological and industrial importance. Although many useful chemicals have been discovered as microbial metabolites, and many more potential products yet to be discovered from soil microorganisms (Ibrahim *et al.,* 2016). This is aside from the crucial roles of microbes in essential elemental cycles (Nitrogen, Carbon, Phosphorus etc.), microbial decomposition, organic compound degradation and its application in the remediation of environmental pollutants. In this study, the profile of

bacterial community isolated and identified from the ten waste dumpsites includes *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Bacillus cereus, Salmonella* sp, *Enterobacter* sp, *Micrococcus* sp and *Shigella* sp. Firstly, it is justifiable that these bacterial isolates inhabit waste from different sources such as human, plant, animal and anthropogenic sources including industrial and commercial origin. The bacterial community identified in this study mostly conformed with the findings of previous works (e.g., Corker *et al.,* 2002) whose predominant isolates were *Klebsiella* sp, *Escherichia coli, Staphylococcus* sp, *Corynebacterium* sp and *Lactobacillus* sp. Also, Begum *et al.,* (2017) reported several microorganisms including *E. coli, S. aureus* among others in fermented fruits dump area from Dhaka City.

The presence of Coliform group (*Escherichia coli, Klebsiella pneumonia, Enterobacter* sp) among the bacteria identified from the dumpsites is an important pointer and mostly regarded indigenous bacterial population in faecal soil samples. Nevertheless, these coliform bacteria group are non-pathogenic microbes (except the rare strain *E. coli* O157: H7) but usually serves as an important indicator of pathogenic microorganisms mostly from the enteric origin. To corroborate this fact, some pathogenic or disease-causing bacteria such as *Salmonella* sp, *Shigella* sp, *Staphylococcus aureus* and *Proteus mirabilis* were isolated and identified from the waste dumpsite soils though few are opportunistic pathogens. Others are simply saprophytic bacteria mainly responsible for the decomposition of plant and animal materials or living as heterotrophs in the soil environment.

Importantly, since the presence of microbial pathogens and opportunistic pathogens have been established in the ten selected waste dumpsites within Gombe metropolis, this portrays a significant public health and safety hazard especially in addition to other chemical and physical hazards onsite. The population at risk of exposure to these hazards specifically microbial infections are humans (e.g., toddlers, children and dumpsite workers) and animals if dumpsites are not properly or entirely managed. Pathways such as water bodies and air could exacerbate transmission while insects are mostly vector serving as disease carriers. In some cases, affected buildings nearby are regarded as inanimate receptors. A crucial instance is a child who ingests 0.1g/day of contaminated soil, would potentially be consuming an average of 1.8×10^6 CFU/g per day, these ingested bacteria are a real risk for small children and immune-compromised persons who can develop pyrogenic reactions and peritonitis (Lewis *et al.,* 1994).

CONCLUSION

On a general note, findings from this study indicated high bacterial counts from the selected waste dumpsites studied and profiling of bacterial isolates indicated the presence of a variety of bacterial community ranging from indicator of pathogenic microbes to opportunistic pathogens and infectious bacterial agents. This critically suggests these microbial hazards could pose serious public health issues to receptors (people) residing in proximity to the dumpsites thus portray

public health, safety and environmental relevance. However, it is acknowledged that the low diversity of bacterial community unravelled from dumpsite soils is largely attributed to the culture-based approach applied in this study as this limit findings to only the culturable bacterial population present in the waste dumpsites. Molecular techniques and metagenomics have been highly appraised in several studies to capture the full spectrum of the bacterial community in any environmental sample consequently reflecting the genuine community including the uncultured microbial population.

Recommendation

The following recommendations are highlighted to ensure public health and safety especially in communities were waste dumpsites are operated.

I. For further study, culture-independent approaches such as molecular techniques and metagenomics could be applied to assess the microbial ecology of dumpsites in a bid to divulge the unculturable microbial population that may be involved in the degradation of biodegradable waste and compounds.
II. Dumpsites or landfills should be strategically situated in the outskirt of town and must be properly managed according to the recognised environmental regulations and policies.
III. Government and municipalities should revise laws regarding the locations of dumpsites within communities where applicable. These laws should include proper management of sites which should be fenced and situated away from human settlements or residential communities.
IV. Government establishment in charge of environmental issues should follow up on the functioning and management of the dumpsites to avoid environmental pollution and public health hazards.
V. There is need for continuous public sensitisation and awareness in the media and by environmental health workers in the rural and urban settlements on the need for proper waste disposal practices and the public health and safety implications of indiscriminate disposal of waste and environmental pollution.
VI. There is a need for regular enforcement of established environmental laws and policies, and stringent penalties enacted to ensure adherence to these laws.

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