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BACTERIAL ANALYSIS OF SOLID WASTE FROM REFUSE DUMPSITE IN IBI METROPOLIS, TARABA STATE NIGERIA

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ABSTRACT

Solid waste disposal remains a major environmental and public health challenge in many developing urban centers. Improper waste management practices promote the proliferation of pathogenic microorganisms that pose serious health risks to exposed populations. Culture media was prepared following the manufacturer's instructions and sterilized in an autoclave at a temperature of 121^{0}c and a pressure of 15psi. Biochemical test including catalase test, Coagulase test and IMViC Reactions were carried out according to clinical laboratory standard guidelines to further confirm the isolates from the solid waste dumpsites soil samples.The following biochemical test was carried out;Bacteriological analysis revealed the presence of five major bacterial isolates: *Bacillus subtilis* (24%), *Escherichia coli* (22%), *Klebsiella* spp. (20%), *Pseudomonas aeruginosa* (18%), and *Staphylococcus aureus* (16%). Both Gram-positive bacteria (40%) and Gram-negative bacteria (60%) were recorded, indicating diverse microbial contamination across the dumpsites. Enteric bacteria such as *E. coli* and *Klebsiella* spp., which together accounted for 42% of total isolates, suggest significant fecal contamination of the waste materials. Total viable bacterial counts varied across locations, with higher counts (5.2×10^6 cfu/ml) observed in dumpsites containing a greater proportion of decomposable organic waste and lower (3.5×10^6 cfu/ml), The findings indicate a high prevalence of pathogenic and opportunistic bacteria in solid waste dumpsites within Ibi Metropolis, posing notable public health risks. Improved waste segregation, sanitary disposal systems, routine microbial surveillance, and provision of personal protective equipment for waste handlers are strongly recommended to reduce bacterial contamination and protect community health.

Keywords: Frequency of Occurrence, Pathogenic Bacteria, Health, Microscopic, Media

INTRODUCTION

Solid waste refers to discarded materials that are no longer considered useful, including household garbage, industrial refuse, medical waste, and agricultural residues (Hoornweg and Bhada-Tata, 2012). Microbial analysis of solid waste is the scientific study of microorganisms such as bacteria, fungi, viruses, and parasites found in waste streams. These microbes may either contribute positively to waste degradation or pose serious risks to human health by acting as disease-causing pathogens (Guerrero *et al*., 2013). Human health, in this context, relates to the physical and biological well-being of populations that may be directly or indirectly exposed to microbial hazards in waste. Defining these key concepts is essential because it provides a foundation for understanding how waste management practices can either safeguard or endanger human life,from the reviewed literature reveal that solid waste, particularly when improperly managed, serves as a reservoir for diverse bacterial species, including pathogenic and antibiotic-resistant strains. The presence of organisms such as *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Clostridium perfringens* in municipal dumpsites underscores the significant health risks associated with open dumping and poor waste segregation. These bacteria can be transmitted to humans through contaminated water, food, air, direct contact, and environmental vectors such as flies and rodents. As a result, communities living near dumpsites and waste handlers are highly vulnerable to gastrointestinal infections, respiratory illnesses, skin diseases, and other bacterial-related health conditions. The situation is further compounded by inadequate infrastructure, limited microbial surveillance, and weak enforcement of environmental health regulations in many developing urban centers like Ibi.

MATERIALS AND METHODS

Study Area

Ibi Local Government Area is one of the 16 Local Governments in Taraba State, with Ibi town as its headquarters. It is located at the southern part of Taraba State and lies within latitude 7° 00' - 8° 10' North of the Equator and longitude 11° 10' - 12° 10' East of the Greenwich Meridian Time (GMT) (Akoga, 2012). Ibi has a landmass of 2,728.872 km^2 with a population of 84, 407 based on 2006 National Population Census. The area shares boundary with Plateau State to the North and Nasarawa State to the West. The southern part bordered to Ibi Local Government Area, while Gassol and Karim-Lamido Local Government Areas had it boarder to the North-East axis.

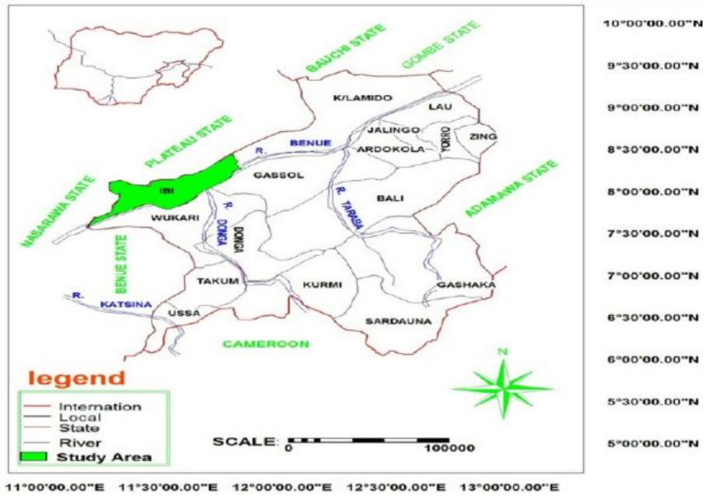

Figure 1: Map of Taraba State showing Ibi Local Government Area
Source: Geography Department, Kwararafa University, Wukari

Study Duration
The study lasted for one (1) week which involved collection of solid waste samples into sterile sample containers from selected solid waste dumpsites in Ibi metropolis. Isolation, identification and bacteria characterization was carried out in Biology Laboratory, Federal University Wukari.

Materials used Include
Petri dishes, sterile sample containers, inoculating needle**,** slide**,** bunsen burner**,** distilled water**,** cotton wool**,** alcohol**,** filter paper**,** hand gloves**,** test tubes, conical flask**,** measuring cylinder, beaker and spatula.

Media used Include
Nutrient agar,MacConkey agar,Simmons citrate agar,Glucose phosphate broth

Reagent used Include
Gram staining reagent (Crystal violet, lugol's iodine, acetone and safranin), Kovacs reagent, Hydrogen peroxide

Equipment used Include
Autoclave (Model Yx-280A), Analytical weighing balance (Model RS-232), Magnetic Stirrer (Model SB160), Incubator and Compound Binocular Microscope.

Methodology
Sample Collection
Solid waste samples were collected from differen tsolid waste disposal sites by using spatula, and placed into separate sterile sample containers, tagged appropriately and conveyed to Biology laboratory, Federal University Wukari for bacteriological isolation and analysis.

Media Preparation
Culture media was prepared following the manufacturer's instructions and sterilized in an autoclave at a temperature of 121^0C and a pressure of 15psi

Nutrient Agar Medium
Nutrient agar is a general-purpose medium used for the cultivation of bacteria and for the enumeration of bacteria in water, sewage, feces and other materials. Nutrient agar consists of peptone, beef extract and agar (Cheesbrough, 2009)

MacConkey Agar Medium
MacConkey agar is a selective and differential medium used for the detection of Enterobacteriaceae from clinical specimen and other materials (Cheesbrough, 2009)

Bacteria Isolation from Solid Waste Dumpsites in Ibi Metropolis
1g of the samples collected from the solid waste disposal sites was serially diluted in 9ml sterile distilled water before inoculation, 0.5ml of the dilution was aspirated by using pipette and poured into a sterile petri dish. The media that was used for bacteria isolation was Nutrient agar and MacConkey agar. The pour plate method was used for the primary isolation of bacteria from the serially diluted soil samples. Plates containing Nutrient agar and MacConkey agar was incubated for 24 hours at 37ºC. Bacteria isolates was purified by repeated sub-culturing onto freshly prepared sterile Nutrient agar using the streak plate method as described by Robert Koch.

Colony Morphology
The bacteria culture pure isolates were morphologically examined for size, color, shape and elevation and pigmentation. The isolates were morphologically identified based on cultural and microscopic characteristics as described by Valencia *et al*., 2019.

Microscopic Analysis
The bacteria culture (pure isolates) was picked using a sterile inoculating needle and placed on a sterile grease free microscope slide. The slide containing the bacteria culture was heat fixed under hot flame using the bunsen burner. The heat fixed slide was stained by using 3ml acetone,the gram staining reagent after which the stained slide was allowed to air dry. The slide was viewed under the microscope using x100 objective lens (oil immersion lens). This was done to check and determine the colony characteristics and gram reaction.

Gram Staining Techniques
Procedure
The smear of each isolated colony was prepared on a microscope slide and allowed to air dry. The air dried smear on the slide was heat fixed. The heat fixed smear was stained with 0.5ml of Crystal violet reagent for 60 seconds. The stained slides were in a slanting position and rapidly rinsed

with running water in a slanting position. The stained smear was then covered with Lugol's iodine for 60 seconds. The iodine was rapidly washed off with running water in a slanting position. The stained smear was decolorized with acetone for few seconds. Safranin was added to the stained smear for 30 seconds. The stained slide was rapidly washed off with running water. The smear preparation was subsequently air dried and microscopically examined using high resolution objective power (Cheesbrough, 2006).

Biochemical Test

Biochemical tests were done according to clinical laboratory standard guidelines to further confirm the isolates from the solid waste dumpsites soil samples.

The following biochemical tests were carried out;

Catalase Test

Using sterile glass rod, suspected colonies were immersed in tube containing 2 ml of 3% hydrogen peroxide. Presence of bubbles indicates a positive result (Cheesbrough, 2009; Collee *et al*., 2016).

Coagulase Test

1ml of human plasma were added to test tubes and a loopful of the bacteria isolates inoculated. The test tubes were incubated at 37^0C for 1 hour in a water bath to observe for presence of clouding and clotting.

Interpretation:

A positive coagulase test is represented by any degree of solid clotting.

IMViC Reactions

IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae (Aakanchha *et al*., 2020.

IMViC is used to study the physiological characteristics of bacteria from the family Enterobacteriaceae, especially *Escherichia* and *Enterobacter* (Aakanchha *et al*., 2020)

They are designed to differentiate Gram-negative intestinal bacilli of family Enterobacteriaceae which contains a large number of genera that are biochemically and genetically related to one another.

Indole Test: Isolated colonies were inoculated in peptone water, which contains amino acid tryptophan and incubated at 37^0C for 24 hours. After incubation, kovac's reagent was added and the test tube was shaken gently. The test tube was allowed to stand for 2 minutes.

Interpretation: Formation of a red or pink colored ring at the top of the test tube is a positive result. This indicate the presence of tryptophase enzymes

Methyl red (MR) Test: The isolated colonies were inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37^0C for 24 hours. The pH of the medium was tested by the addition of 5 drops of methyl red reagent.

Interpretation: Development of red color is a positive result. Gram Negative bacteria produce yellow color.

Voges-proskauer (VP) Test: The isolated colonies were inoculated into glucose phosphate broth and incubated at 37^0C for 24 hours. 0.6 ml of alpha-naphthol was added to the test broth and shaken. 0.2 ml of 40% KOH was added to the broth and shaken. The test tube was allowed to stand for 1 hour.

Interpretation: Appearance of red color indicate a positive result.This signifies a bacterium can produce acetoin. The test tube was allowed to stand for 1 hour since maximum color development occurs within 15 minutes after addition of reagents

Citrate Utilization Test: Isolated bacterial colonies were picked with a sterile inoculating loop and inoculated into slants of Simmon's citrate agar and incubated 24 to 48 hrs at 37^0C.

Interpretation: If the organism has the ability to utilize citrate, the medium changes its color from green to blue. This means the organism can utilize citrate as a sole carbon source and the result indicate that its help identify the organism.

Statistical Analysis

The result obtained from this research study were analyzed using simple statistical methods. The Frequency of occurrence was determined by dividing the number of isolates by the total number of isolates multiplied by one hundred.

Additionally, Chi-square statistical analysis was performed to assess the significance of the findings. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 24 software package.

RESULTS AND DISCUSSION

Table 1 shows the morphological characteristics and Gram reaction of bacteria isolated from solid waste disposal sites in Ibi town. *Bacillus subtilis* appeared as small, moist, white or slightly yellow colonies and was Gram-positive, consistent with its known aerobic, spore-forming rod morphology (Madigan *et al.,* 2018). *Escherichia coli* and *Klebsiella sp*. produced moist lactose-fermenting colonies, yellow for E. *coli* and pink for *Klebsiella,* both Gram-negative rods, confirming their classification within *Enterobacteriaceae* (Todar, 2020). *Pseudomonas aeruginosa* exhibited blue-green, non-lactose fermenting colonies and Gram-negative rod morphology, reflecting its typical pyocyanin pigment production (Murray *et al.,* 2023). *Staphylococcus aureus* formed small, yellow, lactose-fermenting colonies and Gram-positive cocci, which is characteristic of this pathogenic species (Forbes *et al.,* 2021).

Table 1: Morphological Characteristics of Isolated Bacteria on Nutrient Agar and MacConkey Agar and their Gram Reaction

Bacteria	Morphology	Gram Stain Reactions
Bacillus subtilis	Small, moist, white or slightly yellow colonies	Gram-positive rod
Escherichia coli	Moderate, moist yellow lactose fermenter colonies	Gram-negative rod
Klebsiella sp.	Moderate, moist pink lactose fermenter colonies	Gram-negative rod
Pseudomonas aeruginosa	Moderate, moist, blue-green non-lactose fermenter colonies	Gram-negative rod
Staphylococcus aureus	Small, moist, yellow lactose fermenter colonies	Gram-positive rod

Table 2 presents the biochemical test results for bacteria isolated from solid waste samples, confirming their identities. *Bacillus subtilis* was positive for catalase and citrate but negative for coagulase, indole, methyl red, and oxidase, consistent with its aerobic, non-pathogenic characteristics (Madigan *et al.,* 2018). *Escherichia coli* tested positive for catalase, indole, and methyl red but negative for citrate and oxidase, which is typical for this species (Todar, 2020). *Klebsiella sp.* was catalase and citrate positive but negative for other tests, validating its identity as a non-motile, lactose-fermenting Gram-negative rod. *Pseudomonas aeruginosa* exhibited oxidase, catalase, and citrate positivity while being negative for coagulase, indole, and methyl red, confirming its status as an opportunistic pathogen (Murray *et al.*, 2023). *Staphylococcus aureus* was catalase and coagulase positive, methyl red positive, and citrate positive, consistent with its pathogenic potential (Forbes *et al.*, 2021).

Table 2: Biochemical Reactions of Bacteria Isolated from Solid Waste Disposal Sites Samples from Different Locations in Ibi Town

Bacteria	Catalase Test	Coagulase Test	Indole Test	Methyl Red Test	Citrate Test	Oxidase Test	Gram Reaction
Bacillus subtilis	+	-	-	-	+	-	+ Rod
Escherichia coli	+	-	+	+	-	-	- Rod
Klebsiella sp	+	-	-	-	+	-	- Rod
Pseudomonas aeruginosa	+	-	-	-	+	+	- Rod
Staphylococcus aureus	+	+	-	+	+	-	+ Cocci

Table 3 shows the total viable counts of bacteria in cfu/ml across different soil depths in the selected solid waste disposal sites.

Table 3: Total Viable Counts of Bacteria in Colony Forming unit per Milligram (cfu/ml) of Solid Waste Disposal Samples from the Selected Solid Waste Disposal Sites in Ibi Town

Sample Location	Total Counts in cfu/ml
Nwonyo I (Top soil)	2.4×10^6
Nwonyo I (Middle soil)	3.5×10^6
Nwonyo I (Bottom soil)	1.7×10^6
Nwonyo II (Top soil)	2.0×10^6
Nwonyo II (Middlesoil)	2.4×10^6
Nwonyo II (Bottom soil)	1.2×10^6
Rimi Uku (Top soil)	1.6×10^6
Rimi Uku (Middle soil)	1.9×10^6
Rimi Uku (Bottom soil)	5.2×10^6

Table 4: Frequency of Occurrence of Bacteria Isolated from Solid Waste Samples from the three Selected Solid Waste Disposal Sites in Ibi Town

Bacteria	Number of Isolates	Total No. of Isolates	Percentage (%)
Bacillus subtilis	4	36	11.1%
Escherichia coli	4	36	11.1%
Klebsiella sp	4	36	11.1%
Pseudomonas aeruginosa	10	36	27.8%
Staphylococcus aureus	14	36	38.9%

X^2 = 4.128, Mean = 7.20 and P-value = 0.037

Table 4 displays the frequency and percentage occurrence of bacteria isolated from the three selected solid waste disposal sites.

Table 5 shows the site-specific distribution of bacterial isolates across Nwonyo I, Nwonyo II, and Rimi Uku.

Table 5: Total Frequency of Occurrence of Bacteria Isolated from Solid Waste Samples from the Three Selected Solid Waste Disposal Sites in Ibi Town

Bacteria	Nwonyo I	Nwonyo II	Rimi-Uku	Total
Baccillus subtilis	2	2	0	4
Escherichia coli	2	0	2	4
Klebsiella sp	0	2	2	4
Pseudomonas aeruginosa	4	4	2	10
Staphylococcus aureus	4	4	6	14
Total	12 (33.3%)	12 (33.3%)	12 (33.3%)	36

X^2 = 5.023, Mean = 12.00 and P-value = 0.044

Discussion

The differences in colony color, size, and texture indicate a diverse bacterial population in the solid waste environment. Morphological examination provided the initial step in identifying potential pathogens present in the sampled sites. These findings align with previous studies reporting similar bacterial isolates from Nigerian solid waste disposal sites (Okafor *et al.*, 2017.The biochemical reactions corroborated the morphological identifications and differentiated the bacterial species effectively. These results agree with prior studies that characterized bacterial populations in solid waste sites in Nigeria (Okafor *et al.*, 2017).

The highest bacterial load was recorded in Rimi Uku bottom soil (5.2×10^6 cfu/ml) and Nwonyo I middle soil (3.5×10^6 cfu/ml), suggesting that deeper or middle soil layers provide favorable conditions for microbial growth, possibly due to higher organic matter and moisture content (Atlas & Bartha, 2018). Top soil layers generally had lower counts, likely due to exposure to sunlight, desiccation, and human activity, which reduce bacterial survival. The variation in bacterial density across soil depths indicates that microbial populations are influenced by environmental conditions and nutrient availability. High microbial counts at all sites suggest that solid waste disposal areas are hotspots for bacterial proliferation. These observations are consistent with previous reports on Nigerian urban waste sites (Adeleke *et al.*, 2015). The cfu/ml data provide quantitative insight into bacterial prevalence and potential health risks associated with these sites. *Staphylococcus aureus* (38.9%) and *Pseudomonas aeruginosa* (27.8%) were the most frequently isolated species, indicating they dominate the microbial community in the waste samples. *Bacillus subtilis, Escherichia coli,* and *Klebsiella* sp. each accounted for 11.1% of isolates, reflecting lower prevalence. The χ^2 value of 4.128 with a P-value of 0.037 indicates a statistically significant difference in the distribution of bacterial species. The predominance of *S. aureus* and *P. aeruginos*a may be attributed to their ability to survive in harsh environmental conditions and utilize diverse substrates (Okafor *et al.*, 2017; Adeleke *et al.*, 2015). Less prevalent species, such as *B. subtilis*, may prefer specific microenvironments or face competition from more adaptable bacteria. The frequency data highlight the presence of pathogenic bacteria in solid waste environments, confirming public health concerns. These findings are consistent with other studies on microbial contamination of urban waste sites in Nigeria.

Each site contributed 12 isolates (33.3%), but species composition varied, with S. aureus most abundant in Rimi Uku (6 isolates) and Bacillus subtilis absent, suggesting environmental factors or waste composition influence bacterial presence. *Pseudomonas aeruginosa* and S. *aureus* were present in all three sites, demonstrating their resilience and adaptability. The χ^2 value of 5.023 with a P-value of 0.044 confirms statistically significant differences in bacterial distribution across locations. Differences in bacterial populations may result from variations in moisture, nutrient availability, and human activity at the sites (Adeleke *et al.*, 2015). This information highlights the importance of monitoring site-specific microbial risks in urban waste management.

CONCLUSION

This study revealed the presence of diverse bacteria species in solid waste refuse dumpsites within Ibi metropolis including *staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli.Klebsiella sp. And Bacillus subtilis*. The highest bacterial load was recorded at the bottom of the soil of Rimi Uku dumpsite,while the lowest count was observed in the bottom soil of Nwonyo II, indicating variation in microbial distribution across sampling location and soil depth. *Staphylococcus aureus* and Pseudomonas *aeruginosa* were the most frequently isolated bacteria, suggesting their strong adaptability to solid waste environments. The biochemical and morphological characteristics of the isolates confirmed significant microbial contamination of the dumpsites.This finding revealed the need for proper waste management and regular monitoring of refuse dumpsite to minimize environmental bacterial contamination.

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