

Public Health Implications of Waste Dump to Inhabitants in the Environment

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Abstract: Dump sites are naturally associated with foul odour which obviously is product of microbial activities. The bacterial agents responsible for this decomposition may be injurious to humans. In order to determine the genera of bacteria responsible, Petri dishes containing three different types of media, nutrient, MacConkey and blood agars were exposed for fifteen minutes, and incubated at 37°C for 24 hours. Organisms that grew were analyzed to determine if they are pathogens. Five plates of each media were exposed, and the mean of the colony counts were expressed as colony forming units per millilitre (cfu/ml). Three different dump sites were studied, namely school waste dump (SWD), Village waste dump (VWD) and Market waste dump (MWD). Results of Petri dish exposure for 15 mins showed mean cfu/ml of 300, 250 and 300 on nutrient agar for SWD, VWD and MWD respectively. 100, 120 and 150 on MacConkey agar for SWD, VWD and MWD respectively then 20, 15 and 25 on blood agar for SWD, VWD and MWD respectively. Cultural characteristics of the isolates revealed diverse shapes, margins, colours and sizes. Phenotypic characterization showed the Grain reaction, *catalase*, *coagulase*, *motility*, *indole*, *oxidase tests*, *glucose fermentation*, *capsule and spore tests results of the isolates*. Isolates were tentatively identified to be *Listeria spp*, *Streptococcus spp*, *Escherichia spp*, *Staphylococcus spp*, *Bacillus spp*, *Shigella spp* and *Micrococcus spp*, by *Bergey's manual of determinative bacteriology*. Virulence test by haemolysis assay showed both *Streptococcus spp* and *Listeria spp* to be α -haemolysis negative and β -haemolysis positive. Their zones of inhibition were 2-2.5 mm and 3.0-3.5 mm for *Streptococcus spp*, while *Listeria spp* was 1.5 mm. Capsule stain was positive for *Streptococcus spp* and *Escherichia spp*. Spore stain was positive only for *Bacillus spp*. These finds show that pathogenic bacteria are associated with waste dumps and therefore can be injurious to public health.

Keywords: Waste Dumps, Public Health Implication, Pathogens

1. Introduction

Waste dump sites are associated with a myriad of microorganisms, many of which undoubtedly are pathogenic to man, plants and animals. On the occasion of dry seasons, some of these organisms change to forms like spores, capsules etc. and are deposited on buildings and other objects where they stay and de-face such buildings when water is made available. Is it not possible that some of these organisms are responsible for diseases and sickness of inhabitants in the environment? Ebe, *et al*, [8] in their work on bacteria associated with domestic solid waste dump in Egbu, reported of isolating five genera of bacteria from the waste and that *Klebsiella spp*. was most common organism present. Other bacteria they isolated were *Staphylococcus*

ureus, *Escherichia coli* and *Corynebacteria*. Similarly, Obire, *et al*, [14] isolated these bacteria *Arthrobacter spp*, *Micrococcus spp*, *Proteus spp*, *Serratia spp*, *Streptococcus spp*, *Staphylococcus aureus*, *Pseudomonas spp*, *Bacillus*, *Klebsiella* and *Escherichia coli*. These isolates are causative agents of diseases, some could be detrimental. They also isolated fungi among which are *Aspergillus*, *Fusarium*, and so on. Other researchers like Williams and Hakam, [19] and Odoyemi, [15] have reported of the presence of organisms identified to be pathogens of man, animals and plants from such waste dumps.

It is of crucial importance to address this issue of waste dumping as the health problems arising from the act are unquantifiable. The possible organisms associated with the waste dumps need to be isolated, characterized and

studied in order to determine their role to public health. The investigation should determine the presence of virulent factors e.g. presence of capsule, Acharya [1], formation of spores, Cogliati, *et al*, [6] and virulence, Granato *et al* [10], toxins, enzymes (Casadevall & Profsk [4]). These investigations will help to educate the public on issues of waste collection and control. Therefore the aim of this paper is to investigate the virulence properties of bacterial isolates from waste dump in a college neighborhood.

2. Materials and Methods

- i. *Sample Collection:* Five plates for each of the three different media, nutrient agar, MacConkey agar and blood agar, used were exposed for fifteen minutes according to (Menezes *et al.*, [12]) on three waste dumps, (i) school waste dump (SWD) (ii) Village waste dump (VWDs) and (iii) Market waste dump (MWD). These are all in Uli, Ihiala Local Government Area, Anambra State, Nigeria. At end of the 15 minutes exposure, the Petri dishes were covered, sent to the laboratory and incubated at 37°C for 24hrs. The media were prepared according to Chessborough [5].
- ii. *Isolation of Pure bacteria from the exposed Petri dishes.* The pure cultures of the bacterial isolates were obtained by aseptically streaking representative colonies of different morphological types which appeared on the exposed plates onto freshly prepared nutrient agar plates, according to (Jahir and Syed, [11]) and were incubated at 37°C for 24hrs. Discrete colonies which developed were sub-cultured on nutrient agar slopes and incubated at 37°C for 24hrs. These served as pure stock cultures for subsequent characterization and analytical tests.
- iii. *Characterization and identification of isolations:* The isolates were characterized phenotypically by means of Gram stain, motility, catalase, coagulase, indole, oxidase, and glucose fermentation tests. They were also identified on the basis of their cultural and morphological characteristics in accordance to Cruickshank *et al*, [7].

2.1. Analytical Tests

Spore staining. Smears of the various bacterial isolates which have incubated for 48 – 72 hrs. as pure cultures were made by collecting the culture with a sterile wire loop placed on the center of a clean slide and dropped sterile water on the culture. It was made molten by gentling swelling the slide to mix the culture and water. It was air dried and heat fixed by passing it through Bunsen flame three times. Then it was flooded with Malachite green and heated on Bunsen burner for 3 minutes. After heating, the slide was rinsed with water and air dried before dropping the counter stain Safranin which lasted for 2 minutes and was rinsed off with gentle

running water. After air drying, the slides were viewed under oil immersion using 100 x objective. The spores appeared green on a pink background. This is according to Chessborough, [5]

Capsule Staining: This was done according to the method of Barrow *et al.*, [3]. A loopful of the respective isolates was collected with sterile wire loops from the pure cultures which had incubated for 48 – 72 hrs. and placed on slides. This was followed by a drop of Indian ink placed next to the cultures on the same slide. Carefully cover slips were placed over the two drops so that they mixed together. After this, the slides with their contents were examined under the microscope and the observation recorded.

2.2. Virulence Test

A modified method of Noble and Vosti [13] was used to do this test. Blood agar plates were exposed to the waste dump for 15 minutes and incubated for 48hr at 37°C. After the incubation, the plates were observed for the development of zones of hemolysis. The developed zones of hemolysis were measured; the colour of the organisms noted and the nature of the hemolysis whether they are alpha (α -) or beta (β -) hemolysis.

2.3. Statistical Analysis

Results of enumeration of isolates were analyzed using the mean

3. Results

Table 1. Number of bacterial colonies on the different media after 15mins exposure to waste dumps and 37°C incubation.

Media	SWD	VWD	MWD
Nutrient agar	300 ^a cfu	250 ^a cfu	300 ^a cfu
MacConkey agar	100 ^a cfu	120 ^a cfu	150 ^a cfu
Blood agar	20 ^a cfu	15 ^a cfu	25 ^a cfu

Sample Sites

a is the mean of five plates.

Table 2. Cultural Characteristics of Isolates on the Three Different Media in School Waste Dump (SWD).

Type of Media	Shape	Margin	Colour	Size
Nutrient agar				
Isolate 1	Round	Entire	Dark Yellow	1-2mm.5
Isolate 2	Irregular	Obate	Creamy	3-3.5mm
Isolate 3	Irregular	Filamentous	Creamy	0.5-1mm
Isolate 4	Convex	Lobate	Light yellow	1.5-2mm
MacConkey agar				
Isolate 1	Convex	Entire	Pink	3.5-5mm
Isolate 2	Irregular	Lobate	Pink	2-3mm
Isolate 3	Convex	Entire	Colourless	2.3mm
Blood agar				
Isolate 1	Irregular	Entire	Light Yellow	1-2mm
Isolate 2	Round	Entire	Cream	2.5-3.0mm

Table 3. Cultural Characteristics of Isolates on the Three Different Media in Village Waste Dump (VWD).

Type of Media	Shape	Margin	Colour	Size
Nutrient agar				
Isolate 1	Circular	Entire	Light Yellow	2-3mm
Isolate 2	Irregular	Filamentous	Creamy	3.5-5mm
Isolate 3	Circular	Entire	Creamy	0.5-1mm
Isolate 4	Circular	Erode	Creamy	0.5-1mm
MacConkey agar				
Isolate 1	Circular	Entire	Pink	0.5-1mm
Isolate 2	Round	Entire	Colourless	1-1.5mm
Isolate 3	Convex	Erode	Pink	3-4mm
Blood agar				
Isolate 1	Circular	Entire	Creamy	2-2.5mm
Isolate 2	Round	Entire	Light Yellow	1.5-2.0mm

Table 4. Cultural Characteristics of bacterial Isolates emanating from VWD, on the Three Different Media.

Media type	Shape	Margin	Colour	Size
Nutrient agar				
Isolate 1	Filamentous	Filiform	Creamy	23.5-5mm
Isolate 2	Irregular	Lobate	Light yellow	0.5-1mm
Isolate 3	Circular	Entire	Creamy	1-1.5mm
Isolate 4	Irregular	Undulate	Colourless	3-4mm
MacConkey agar				
Isolate 1	Circular	Entire	Pink	3-4mm
Isolate 2	Irregular	Undulate	Colourless	1.5-2mm
Isolate 3	Filamentous	Filiform	Creamy	3.5-5mm
Blood agar				
Isolate 1	Irregular	Lobate	Colourless	2-2.5mm
Isolate 2	Circular	Entire	Light Yellow	2-3mm

Table 5. Virulence status of the isolates by haemolysis assay.

Medium	Organism	α -Haemolysis	β -Haemolysis	Zone of Inhibition
Blood agar	<i>Streptococcus spp</i>	Negative	Positive	2-2.5mm
	<i>Streptococcus spp</i>	Negative	Positive	3.0-3.5mm
	<i>Listeria spp</i>	Negative	Positive	1.5-2mm

Table 6. Characterization and Identification of Isolates on nutrient, MacConkey and Blood agars.

Isolate	Gram reaction	Shape	Catalase	Coagulase	Motility	Indole	Oxidase	Spore test	Capsule test	Glucose fermentation	Identification
1	+	Rod	+	-	+	-	-	-	-	A/G	<i>Listeria spp.</i>
2	+	Cocci in chains	-	-	-	-	-	-	+	A/G	<i>Streptococcus spp.</i>
3	-	Rod	-	-	+	+	-	-	+	A/G	<i>Escherichia spp.</i>
4	+	Cocci in chains	+	+	-	-	-	-	-	A	<i>Staphylococcus spp.</i>
5	+	Rod	+	-	-	-	-	+	-	A	<i>Bacillus spp.</i>
6	-	Rod	+	-	-	-	-	-	-	A	<i>Shigella spp.</i>
7	+	Cocci in chains	+	+	-	-	-	-	-	-	<i>Micrococcus spp.</i>

Key – Negative A-acid.

+ Positive G-gas.

4. Discussion

The characterization and identification of the isolates revealed that the following bacteria are resident in the waste dumps. *Listeria spp.*, *Bacillus spp.*, *Streptococcus spp.*, *Staphylococcus spp.*, *Escherichia spp.*, *Shigella spp.* and *Micrococcus spp.* (Table 6). This is in agreement with Obire *et al.*, [14]; Odeyemi, [15] and Ezechi *et al.*, [9]. All of these bacterial isolates are pathogenic and are causative agents of

some noted human diseases. *Listeria spp.* have been reported to cause diseases like sepsis, meningitis and listeriosis in neonates and immune compromised patients (Al –Ghazali and Al-Azawi [2]). *Bacillus spp.* have also been reported to cause diseases like anthrax, eye infections and food poisoning (Shoeni and Wong, [16]). *Streptococcus spp.* have been implicated in bacterial endocarditis, urinary tract infections, ear infections, mouth infections, pharyngitis, scarlet fever, wound infections, skin infections and rheumatic fever (Seng *et al.*, [17]). *Staphylococcus spp.* are noted for

causing diseases such as wound infections, food poisoning, abscesses, toxic shock and urinary tract infections. *Escherichia* spp. cause diseases that include gastroenteritis, meningitis, urinary tract infections, wound and intra-abdominal infections.

Lastly, *Shigella* spp. have been shown to be responsible for these diseases, bacillary dysentery and gastroenteritis. The only isolated non-pathogenic bacteria was *Micrococcus* spp. The activities of these organisms result in economic consequences as the lead to reduction in food security, loss of drinking water supplies and loss of economic opportunity since they contaminate food and drinking water supplies as well. The result of the virulence assay showed positive β -haemolysis which implies complete lysis of the erythrocytes. This suggests the extent of danger such waste dumps pose to public health. The colony forming units (cfu/ml) of the organisms were 300 cfu/ml and Nutrient agar (Table 1), 100 cfu/ml, 120 cfu/ml and 150 cfu/ml on MacConkey agar and 20 cfu/ml 15 cfu/ml and 30 cfu/ml on blood agar. This shows that the organisms in waste dumps have very high population densities so that its only by removing the waste dumps from neighborhood that their health problems can be reduced to a minimum. The result of the capsule and spore stains were positive for *Escherichia* spp and *Bacillus* spp (Table 5). This is a factor that makes it very possible for air to carry these organisms, a means to perpetuating their virulence actions.

5. Conclusion

Waste dumps are associated with bacteria, many of which are pathogenic to humans. A good number of them possess β -haemolysis and other virulent qualities like capsule and spore formation.

Recommendation

Waste dumps should not be in residential neighborhood, because they provide every conditions necessary for the living of these organisms.

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