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Bacterial community of some waste dumpsites in Lagos, Nigeria

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ABSTRACT

Bacteriological analysis of 120 soil samples from dumpsites in Ojo, Lagos, with standard microbiological techniques revealed the total viable aerobic bacterial count as 2.19 x10^7 cfu/mg soil on eosin methylene blue (EMB) agar and 5.12 x 10^8 cfu/mg soil on MacConkey (MCC) agar, indicating that the bacterial count is higher on MCC than on EMB. The bacterial isolates and their frequency of isolation from dumpsites were Escherichia coli (33.3%), Klebsiella spp. (16.7%) ,Pseudomonas spp. (13.3%), Proteus spp.(10%), Streptococcus spp. (8.3%), Salmonella spp. (6.7%), Staphylococcus aureus (6.7%) and Enterobacter spp. (5.0%). The high presence of pathogenic bacteria from dumpsites is of serious public health concern and highly significant. Antibiotic susceptibility testing using the disc diffusion technique showed that most of the bacterial species isolated were susceptible to ciproval, traflox and norfloxacin, but were highly resistant to cephalaxin and chloramphenicol.

Keywords: Bacteria, dumpsites, Bacterial counts, pathogens, antibiotic resistance.

INTRODUCTION

Waste disposal poses a threat to man, animals and soil, particularly in less developed nations of the world where such wastes are disposed untreated (Akano et al, 2013; Anyim et al, 2014; Yoada et al., 2014). A biological waste is said to be infectious when it contains viable microorganisms or their toxins which are known or suspected to be pathogenic to man and animals (Atuanya et al, 2012). Microbial pathogens, like chemical hazards, are dispersed in the environment through water and wind (Obire et al, 2002, Ogunnusi and Dahunsi, 2014).

When waste is dumped on land, soil microorganisms readily colonize the waste as the soil is generally favourable to the proliferation of microorganisms, with micro- colonies developing on soil particles (Atlas and Bartha, 1996). The number of microorganisms found in soil habitats is normally much higher than those found in fresh water or marine habitats which include bacteria, fungi, algae, protozoa and even particles like viruses. The incidence and distribution of many

pathogenic bacteria and intestinal worms from refuse which infects both man and animals have been reported (Lee and Ventillo,1996; Chikh et al,1997).

The most commonly found bacterial agents from waste dumpsites include Gram negative bacteria such as *E. coli, Klebsiela* spp*., Proteus* spp., *Pseudomonas* spp*, Shigella* spp. and Gram positive bacteria such as *Bacillus* spp, *Staphylococcus* spp*., Streptococcus* spp. and *Closridium* spp. (Obire et al, 1997). Some of these bacteria which are either pathogens or opportunistic pathogens such as *E. coli, P. aeruginosa, Klebsiella pneumoniae* and *Proteus* spp. have been implicated in cases of urinary tracts infections (UTI) (William et al., 2001). Furthermore, these bacterial pathogens have been associated with several other infectious diseases of man such as nosocomial infections, food poisoning, gastroenteritis, bacillary dysentery, typhoid fever, diarrhoea, anthrax etc. Antibiotic treatment failure has equally characterized treatment of these infections due to the high level of multiple drug resistance often shown by these bacteria.

This study was therefore carried out to isolate, identify and characterize bacteria from some dumpsites in Ojo Local Government Area of Lagos State, Nigeria. Estimation of microbial load at dumpsites and determination of antibiotic susceptibility pattern of the bacterial isolates were also carried out.

MATERIAL AND METHODS

Collection of soil samples

Soil samples were collected from various dumpsites in Ojo Local Government Area of Lagos State. These randomly chosen sites included Village, Iyana School, Iba, Barracks and Agboroko. At each site, four samples were collected and labeled 1,2,3,4. The same procedure was also repeated in the collection of the remaining 4 sites, thereby making a total of 20 samples. All samples were collected aseptically with the aid of sterile spatulas into sterile screw capped bottles at each point of collection. The sterile spatula in each case was used to till the soil to about 10 centimeters into the soil and a little sample size of the soil was taken into the sterile bottle. The bottle was quickly screwed immediately to avoid contamination from the environment. Samples were taken to the Microbiology laboratory for bacteriological analyses.

Bacteriological studies of soil samples

Each soil sample collected from each site was inoculated into sterile buffered peptone water (BPW) in bijou bottles with the aid of sterile spatula. The mouth of each bottle was cleaned before opening with cotton wool soaked in 70% ethanol and the bottle closed immediately after inoculation. The inoculated BPW were incubated at 37^0C for 18-24 h and were used for cultural, morphological and biochemical analysis.

Cultural analysis was carried out on eosin methylene blue (EMB), MacConkey agar and blood agar. The samples from BPW was inoculated into the three different agar plates using the streak plate technique and all plates were incubated at 37^0C for 18-24 h .These plates were observed culturally and morphologically after which biochemical characterization was carried out.

Serial Dilution

Serial dilution was carried out on the soil samples collected. Ten grams of each soil sample was weighed and dissolved in 90 milliliters of sterile deionized water in a beaker and was shaken for 5 minutes.

For each sample, six test tubes were set up in a test tube rack with each test tube containing 9 ml of

distilled water labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} respectively. One milliliter of the sample mixture in the first beaker of each sample was pipetted into the first test tube labeled 10^{-1} and 1 ml was in turn pipetted from the first test tube to the second test tube and this was repeated up to the test tube labeled 10^{-6} .

From the fourth and fifth test tubes, concentrations of 10^{-4} and 10^{-5}, 0.1 ml was pipetted into the Petri dishes containing EMB and MacConkey agars. With a sterile hockey stick, each sample in the Petri dishes was spread and at each turn, the hockey stick was dipped in ethanol and flame sterilized before and after each use. The plates were incubated for 24 h at 37^0C after which the bacterial colonies were counted.

For the second stage of the analysis, soil sample collected from the same sites with the aid of sterile spatula were inserted into sterile buffered peptone water (BPW) in bijou bottles. A total of 20 spatula and bijou bottles containing BPW were used. The mouth of each bottle was cleaned before opening with cotton wool soaked in 70% ethanol and the bottle closed immediately after inoculation.

The samples were taken to the microbiological laboratory and incubated at 37^oC FOR 18-24 hrs. Each of the samples was sub-cultured onto sterile EMB agar, MCC agar and blood agar. These three agar plates were inoculated using the streak plate technique and all plates were incubated at 37^oC overnight (18-24hrs.) and were observed culturally and morphologically, after which biochemical characterization was carried out.

Antibiotic susceptibility test

Antibiotic susceptibility test was performed using the disc diffusion method. Mueller-Hinton agar was used. Commercially prepared antibiotics (Poly-Test Laboratories, Enugu, Nigeria) were employed. The concentration of the antibiotics discs tested were as follows in micrograms: Nitrofurantoin (N) 10, traflox (TFX) 5,norxloxacin(NB) 10, contrimoxazole (CO) 50, gentamicin (GN) 10, tetracycline (TE) 50, ciproval (CVI) 5, nalidixic acid (NA) 30, chloramphenicol(C) 10, ampicillin(AM) 25, cephalexin(CX) 30, cloxacillin (CL) 5, erythromycin(E) 10, doxycycline (DX) 30 and clindamycin (CD) 10.

The bacterial inoculum was standardized by inoculating 5 ml of peptone water with 3 colonies of 18 h culture of *E. coli*. The broth was incubated until its turbidity matched that of 10% BaSO4 solution. Standardized inoculum was swab-inoculated into Mueller-Hinton agar and the excess fluid was allowed to adsorb for 5 mins. The antibiotic disc were aseptically placed on the agar and incubated at 37^0C for 24 h. The zones of inhibition were measured and recorded.

RESULTS AND DISCUSSION

The results obtained after overnight incubation at 37^0C are presented in Table 1.The spread method was used for total bacterial counts of the various soil samples. It was observed that the lowest bacterial load of 6.0 x 10^6 cfu/mg and the highest count of 4.32 x10^7 cfu/mg on EMB agar. Iba 4 had the highest while Iyana School 4 had the lowest bacterial count respectively. On MacConkey agar, the highest bacterial load was 1.60 x 10^8 cfu/mg while the lowest was 6.0 x 10^6 cfu/mg and both were from village 1 and village 4 respectively. The mean bacterial load on both EMB and MacConkey based on samples sources showed that village 1 had the highest load of 9.25 x 10^7 cfu/mg, followed by Iyana School 3 with bacterial count of 3.55 x 10^7 cfu/mg and Iba 3 with mean bacterial load of 9.25 x 10^6 cfu/mg (Table 1). MacConkey agar had mean bacterial growth count of 5.12 x 10^8 cfu/mg while EMB agar had mean total count of 2.19 x 10^7, thus showing that MacConkey agar supported growth better than EMB agar (Table1).

Table 1: Total bacterial counts of soil samples on MacConkey agar and EMB agar.

Sample source	EMB(cfu/mg) (cfu/mg)	MacConkey	Mean count.
Village 1	3.90×10^7	1.6×10^8	9.95×10^7
Village 2	1.74×10^7	4.00×10^7	2.87×10^7
Village 3	1.43×10^7	1.45×10^7	1.44×10^7
Village 4	1.90×10^7	6.0×10^6	1.25×10^7
Iyana Schl.1	7.75×10^6	3.1×10^7	1.94×10^7
Iyana Schl 2	1.40×10^7	1.95×10^7	1.68×10^7
Iyana Schl 3	2.55×10^7	4.55×10^7	3.55×10^7
Iyana Schl 4	6.00×10^6	9.0×10^6	7.50×10^6
Barracks 1	3.14×10^7	1.18×10^7	2.16×10^7
Barracks 2	2.32×10^7	1.75×10^7	2.04×10^7
Barracks 3	9.65×10^6	2.4×10^7	1.68×10^7
Barracks 4	2.47×10^7	1.23×10^7	$1.85\ 10^7$
Iba 1	4.00×10^7	1.55×10^7	2.87×10^7
Iba 2	2.54×10^7	1.56×10^7	2.87×10^7
Iba 3	6.1×10^7	1.24×10^7	9.25×10^6
Iba 4	4.32×10^7	8.85×10^6	2.60×10^7
Agboroko 1	1.92×10^7	8.0×10^6	1.36×10^7
Agboroko 2	1.38×10^7	1.63×10^7	1.51×10^7
Agboroko 3	2.85×10^7	3.10×10^7	2.98×10^7
Agboroko 4	3.27×10^7	1.35×10^7	2.30×10^7
Mean	2.19×10^7	5.12×10^8	

The bacterial isolates identified on the basis of standard microbiological techniques are shown in Table 2.

Table 2: Distribution of bacterial isolates from the dumpsites.

Organism	Frequency	Percentage (%)
Escherichia coli	120	33.3
Klebsiella spp.	60	16.7
Pseudomonas spp.	48	13.3
Proteus spp.	36	10.0
Streptococcus spp.	30	8.3
Salmonella spp.	24	6.7

Staphylococcus aureus	24	6.7
Enterobacter spp.	18	5.0

TABLE 3: Antibiotic resistance pattern of bacteria from various dumpsite: Number of resistant isolates (% Resistance).

Organism	Number tested	N	NB	TFX	CO	GN	TE	CVL	NA	C	AM
Escherichia coli	60	60(100)	60 (100)	0(0)	60 (100)	60 (100)	60 (100)	0(0)	60 (100)	24(40)	60 (100)
Klebsiella spp.	30	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	15(50)	30 (100)	30 (100)
Pseudomonas spp.	24	12(50)	0(0)	0(0)	0(0)	0(0)	12(50)	0(0)	24 (100)	24 (100)	24 (1000
Proteus spp	18	12 (66.7)	0(0)	0(0)	18 (100)	12 (66.7)	18 (100)	0(0)	18 (100)	18 (100)	18 (100)
Staphylococcus spp.	12	12(100)	0(0)	0(0)	12 (100)	12 (100)	12 (100)	0(0)	0(0)	12 (100)	6(50)
Enterobacter spp.	9	0(0)	0(0)	0(0)	0(0)	9(100)	0(0)	0(0)	0(0)	0(0)	9(100)

KEY:

N - Nitrofurantoin NB - Norfloxacin TEX - Traflux

CO - Cotrimoxazol GN – Gentamicin TE -Tetracycline

CVL - Ciproval NA - Nalidixic acid

C - Chloramphenicol AM - Ampicillin

Discussion

This study showed that there was high incidence of *Escherichia coli* in the various dumpsites. This constitutes a health hazard since the organisms could contaminate flowing streams and broken pipes during rainfall. A high load of *E. coli* in foods is indicative of the possibility of faecal contamination and possibility of the presence of other enteropathogens such as *Salmonella* (Jay, 1996). The high incidence of *E. coli* shows that there is probably a high level of human faeces at the sample sites, and this agrees with others studies (Guerrant et al., 1980: Jay, 1996). There was a wide range of microorganisms from the refuse dumpsites which included *Pseudomonas spp. Klebsiella spp. Proteus spp. Streptococcus spp., Staphylococcus spp., Salmonella spp. and Enterobbacter spp.* This finding is similar to a report by Obire et al, 2002.

E. coli had the highest occurrence of 33.3% compared to other bacterial isolates such as *Staphylococcus aureus* (6.7%), *Klebsiella spp*. (16.7) *Pseudomonas spp.* (13.3%), *Proteus spp*. (10.0%) and *Enterobacter spp*. (5.0%) from the dumpsites.

The bacterial count varied from site to site and from one culture medium to the other. This may be due to environmental factors that affect the growth of the organisms.

Two sample sites, Village I and Iyana-School 3 which were both highly polluted with faeces and

urine as at the time of sample collection had the highest mean total viable counts of 9.95 x 10^7 and 3.55 x 10^7 cfu/mg respectively while Iba 3 and Iyana – School 4 sample sites which have more dry soil and thus less moistened as at the time of sample collection, had means total viable count of 9.25 x 10^6 cfu/mg and 7.50 x 10^6cfu/mg respectively. This revealed that most of the organisms isolated from the dumpsites grow more in areas contaminated with faeces and urine than in dry soil.

Furthermore, bacteria were recovered from MacConkey agar than EMB with mean total viable counts of 5.12 x 10^8 cfu/mg and 2.19 x 10^7 respectively, indicating that MacConkey agar supports the growth of the bacteria more than EMB. E .coli is a pathogenic bacterium that can contaminate food and water through houseflies that carry them from the refuse dumps (Jay, 1996; Adeyeba and Okpala, 2000). *E. coli* strains involved in food –bone illness can be placed into five groups: Enteropathogenic (EPEC), Enterotoxigenic (ETEC). Enteroinvassive (EIEC), Enterohaemorrhagic (EHEC) and Facultatively Enteropathogenic (FEEC). The last strains (FEEC) are associated with sporadic diarrhea outbreak (Jay, 1996).

Apart from food borne illness, *E. coli* has also been implicated in urinary tract infection, neonatal meningitis and nosocomial infections which include bacteremia and pneumonia (William et al., 2001).

Schardinger was the first to suggest the use of *E. coli* as an index of facecal pollution because it could be isolated and identified more readily than individual water borne pathogens (Reinbold, 1983). *E .coli* was the first faecal indicator and one of most attractive properties of E. coli as a faecal indicator for water pollution is its period of survival (Jay, 1996).

Most of the bacteria isolated from the refuse dumpsites belong to the family Enterobacteriaceae with the exception of *Streptococcus sp.* and *Staphylococcus aureus* (William et al., 2001). These organisms have been associated with a wide array of infectious diseases, thus their high occurrence and presence in the dumpsites constitute a source of health hazard to human being within the immediate environment of the dumpsites (Bryan, 1997).

In order to reduce infection due to *E. coli* and other bacterial pathogens, refuse dumpsites should be sited away from human habitation and marker place so as to reduce the contamination of food and water by leaching and houseflies . Regular monitoring of market places by health officials should be carried out.

Indiscriminate dumping of refuse are fertile breeding grounds for mosquitoes and other insects. Some of which could carry life threatening diseases such as dengue fever, yellow fever and encephalitis.

The release of methane by decomposing garbage, as a by-product of anaerobic respiration of bacteria in land fills, often seeps into soil surrounding the waste and eventually migrate into basements and home, thereby constituting a risk of fire explosion (Atlas and Bartha, 1998).

The simplest and most effective way to reduce the health risk of waste is to recycle useable waste. Studies on the prevalence of infectious diseases in and around the dumpsite environment would help in the reduction of the morbidity and mortality rates of such infections.

Regular information on the awareness of hygienic disposal of refuse and improved personal hygiene practices must be emphasized by Local Government authorities.

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