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Occurrence of antibiotic resistant bacteria in waste site of Ede south west Nigeria

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Abstract: *Analysis of antibiotic resistant bacteria from composted waste site soil samples for two seasons were done, using prepared diagnostic test agar and direct sensitivity tests. All major isolated bacteria were subjected to various antibiotics. Concurrent positive and negative tests were simultaneously studied. Results show relatively high resistant and sensitive bacteria in both seasons. In wet seasons, the Bacillus cereus concentrations are considerably reduced than in the dry. In the dry seasons, Bacillus cereus was sensitive to Erythromycin (ERY) and Tetracycline (TET) and slightly resistant to Ampicillin (AMP), Chloramphenicol (CHL), Cloxacillin (CXC) and water (CNT). In the wet season, identified Pseudomonas aeruginosa was moderately resistant to Streptomycin (STR) and Tetracycline and remained resistant to Ampicillin, Cotrimoxazole (COT), Nitrofurantion (NIT) and water (CNT). The implications for human health are discussed.*

Key words: *Antibiotic, Resistance, Heterotrophic, Sensitivity, Bacteria*
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Introduction

The proportion of time spend in-doors is greater for children and healthy mothers (Simecox *et al.,* 1995; Whitmore *et al.,* 1994), but in Nigeria it is contrary because of self employment. People spread their wares on the common public sites. As a result children and adults get infected *via* air borne bacteria, food, dermal contact and oral ingestion.

The burden of improper disposal method has not been rigorously assessed in terms of mutagenicity, otherwise proper method of waste management might be altered if risks were adequately addressed and analyzed. Nigeria is a typical developing nation at a cross road because of growing population and consumption patterns which invariably compound the problem of waste collection and disposal. The magnitude of solid waste problem is worsening and the government at various levels failed to address and seek modern waste management study that will correctly analyze the waste composition characteristics and generation rate is lacking (Nwankwo, 1991).

Indiscriminate dumping of wastes causes traffic obstruction, intrusion of microbes into the immediate environment and a number of hazardous diseases. (Achudume and Olawale, 2007). Overall prevalence and severity of exposure to mutagens may represent a risk index for public health Atasoylu *et al.* (2007). The used antibiotics syringes from various origins (agricultural, hospitals/clinics and pharmaceutical industries) are dumped on waste sites and this has implication for public health *etc.* This has implication for public health specifically mothers and children who holds potential health risk (Achudume and Olawale, 2007). The health implication of bacterial sensitivity in waste sites is for sole purpose of hygienic assessment of the local environment from a socio-demographic perspective. Therefore the primary objective of the present study was to assess the occurrence of antibiotic resistance bacteria in waste site located in Ede, south west , Nigeria.

Materials and Methods

Study area: The study area Ede in south west Nigeria was chosen because of its relative by high population of about 145,000. The population figure has direct relationship with the volume of solid wastes generated (NEST, 1991) as a medium town with same characteristics as other medium cities in Nigeria. The geography and topography are described in Fig. 1. Composted municipal soil, samples were collected in dry (Nov. 2004 - Feb. 2005) and wet seasons (June-Sept. 2005) from the general common site located along "Awo Town junction" from four different locations. Three of the sites were chosen as the relatively high-use study site while the fourth was chosen as the low-use site.

Total heterotrophic bacteria (TAB) count: Multiple portions of soil samples were suspended in distilled deionized water. A 100 fold serial dilution of the mixture (1 ml) was plated in duplicate in a sterile nutrient agar medium. The culture plates were incubated aerobically at room temperature for 48 hr for isolation of aerobes and causative heterotrophic bacterial present in the samples (Kelly *et al.,* 1999).

Antibiotics sensitivity assay: The incubation assay was used to measure antibiotic sensitivity of the isolated organisms. In general, 100µl of the isolated bacterial suspension ($1x10^8$) cells was placed in glass reaction tube. Using prepared diagnostic test (DST) agar, the selected bacteria were subjected to various antibiotics, Ampicilin (AMP), Chloramphenicol (CHC), Cloxacillin (CXC), Erythromycin (ERY), Streptomycin (STR), Tetracycline (TET), Cotrimoxazole (COT) and Nitrofurantion (NIT) at recommended concentrations of 100 µl, 250 µl, 50 µl, 100 µl, 250 µl, 100 µl, 250 µl, 250 µl and 400 µl respectively (Stern *et al.,* 1989; Silkowski *et al.*, 1992). For direct sensitivity tests, the total volume of the reaction tube was brought up to 600 µl with potassium phosphate buffer. The required numbers of wells were filled up with appropriate previously prepared antibacterial

Table - 1: Mean and standard deviation of antibiotic patterns of isolated and type culture of heterotrophic bacteria (HTB) in dry season

Isolated HTB	Antibiotic pattern susceptibility								
	AMP	CHL	CXC	ERY	STR	TET	COT	NIT	CNT
Bacillus cereus	R	R	R	S	SR	S	-	-	-
Type culture NCIB 6349	R	R	R	S	SR	SR	-	-	-
Klebsiella pneumonia	S	-	-	-	SR	S	R	S	R
NCIB 418	SR	-	-	-	SR	S	R	S	R

All experiments were in triplicate. NCIB - National collection of industrial bacteria, R - Resistant, S - Sensitive, SR - Slightly resistant

Table - 2: Mean and standard deviation of antibiotic sensitivity patterns of isolated and type culture of heterotrophic bacteria (HTB) in wet season

Isolated HTB	Antibiotic pattern susceptibility								
	AMP	CHL	CXC	ERY	STR	TET	COT	NIT	CNT
Pseudomonas aeruginosa	R	-	-	-	SR	SR	R	R	R
NCIB 950	R	-	-	-	SR	SR	R	R	R
Micrococcus luteus	S	R	R	SR	R	S	-	-	R
NCIB 196	S	R	R	S	S	S	-	-	R

All experiments were in triplicate. NCIB - National collection of industrial bacteria, R - Resistant, S - Sensitive, SR - Slightly resistant

solution. Concurrent positive controls consisted of potassium phosphate buffer in nutrient agar medium. While concurrent negative test consisted of dimehyl sulfoxide (DMSO) as solvent or isolates mixed with the solvent in a range of microlitre amounts. All broth culture of each test organism was done in triplicate.

The culture media efficiency test was carried out with standard strains of *Pseudomonas aeruginosa* NCIB 950, *Bacillus cereus* NCIB 6349, and *Klebsiella pneumoniae* NCIB 418. These components were incubated at 37°C for 48 hr. The relative susceptibility of test organism was recorded. The means and their SD in the results were subjected to the test of significance by student's t-test.

Results and Discussion

Summary statistics for two groups of isolated heterotrophic bacteria for dry and wet seasons are given in Table 1 and 2 respectively. Soils from high-use and low-use dumping sites were analysed for antibiotic resistance bacteria using standard culture techniques. Analyses of the samples show relatively high number of resistant bacteria in both seasons. The concentrations of bacteria recovered in wet season samples were more than in dry season samples. The results indicate both antibiotic sensitive and resistant patterns of isolated and type culture of heterotrophic bacteria in varying seasons. Besides, the sensitivity ranking and significant interspecies were found in the type culture.

A chart consistent for screening sensitivity, resistance and monitoring phase of the study guides each assessment. In dry season *Bacillus cereus* was sensitive to Erythromycin (ERY) and Tetracyclin (TET), moderately resistance to Streptomycin (STR) and resistant to Ampicillin (AMP), Chloramphenicol (CHL), Cloxacillin (CXC) and water (CNT), while its typed culture NCIB 6349 was sensitive to Erythromycin (ERY), moderately resistant to Streptomycin (STR) and resistant to Ampicilin (AMP), Chloramphenicol (CHL), Cloxacillin (CXC) and water (CNT), while its typed culture NCIB 4349 was sensitive Erythromycin (ERY), moderately resistant to Streptomycin (STR) and Tetracyclin (TET) and showed resistance to Ampicillin (AMP), Choramphenicol (CHL), Cloxacillin (CXC) and water (CNT). *Klebsiella penumoniae* was sensitive to Ampicillin, Tetracyclin and Nitrofurantoin, moderately resistant to Streptomycin and resistant to Cotrimoxazole and water. The type culture NCIB 418 was moderately resistant to Ampicillin, Streptomycin, sensitive to Tetracyclin and Nitrofurantoin and resistant to Cotrimoxazole and water (Table1).

Composted waste soil contained large fraction of detectable level of antibiotic resistant bacteria particularly in wet season. Antibiotic products, and other toxic wastes are discharged in various amount in the environment as a result of the increasing and often indiscriminate use of antibiotics in homes, animals farming and clinics (Karlowsky *et al.,* 2003). The main receptacles for these pollutants are the waste dumps and the common sites.

Table 1 and 2 show different species of specific resistant bacteria. Generally these bacteria are normally found below infection levels in the environment, but when their concentration increases they can infect innocent persons working in nearby areas. These bacteria from garbage and trash causes several problems in human beings like of respiratory, cardiovascular and immune systems disorders and cancer in lungs liver and other vital organs of vulnerable persons (Kelly *et al.,* 1999).

Most investigations on antibiotic resistance in the ecological habitat are concerned with bacteria of fecal origin, because they are used as pollution indicators and may be associated with infectious

diseases. However, in many dumping sites, fecal bacteria are of little numerical significance. Thus, if the environmental pool of resistant bacteria is measured, bacteria other than those of fecal origin must also be considered. Many other studies are interested in whole bacteria population (Enterobacteriacaeae) because they are more commonly associated with hospital – acquired infections (Gresser *et al.,* 2003; Karlowsky *et al.,* 2003; Murray, 2005). In recent times, studies have immerged that dealt with global antibiotic resistance. The frequencies of these cells are able to grow on antibiotic-supplemented media (Besser, 2005).

In Ede common site the specificity of interaction may have been subjected to various protein sequences given rise to a very high ratio of mutations that completely disrupt the specific function of cells resulting in antibiotic resistance. In recent times, studies have immerged that dealt with global antibiotic resistant bacteria. In real life, this is exactly what happens. This has implication for human health, and spread of antibiotic resistant strains for mothers and children who for no fault of their own seek economic redress in and around the common site. Knowledge of the microbial diversity in waste sites should therefore provide a rational basis for development of an effective controlling waste sites programme.

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