FROM REFUSE OCCURRENCE OF PLASMID MEDIATED HEAVY METAL AND ANTIBIOTIC CO-RESISTANCE AMONGST BACTERIA ISOLATED DUMP SITES

BY

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CERTIFICATION

We certify that this research work titled: OCCURRENCE OF PLASMID MEDIATED METAL AND ANTIBIOTIC CO-RESISTANCE AMONGST BACTERIA ISOLATED FROM REFUSE DUMP SITES was carried out by MOKOGWU, Michael Chinedu of the Department of Microbiology of the Delta State University, Abraka.

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DECLARATION

I, MOKOGWU, Michael Chinedu declares that "OCCURRENCE OF PLASMID MEDIATED METAL AND ANTIBIOTIC CO-RESISTANCE AMONGST BACTERIA ISOLATED FROM REFUSE DUMP SITES, is my research work and all the literature used or quoted therein have been acknowledged by complete reference. This research work is an original work and has not been submitted, and will not be submitted to any university or institution other than Delta State University, Abraka, for the award of a Master degree.

_____

MOKOGWU, Michael Chinedu

DEDICATION

This Research work is dedicated to God almighty, my lovely wife Dr. (Mrs.) P.E. Mokogwu, my Son Michael C. Mokogwu (Jnr) and in memory of late Dr O.E Nwafor who left this sinful world on the 18th of July, 2016.

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ABSTRACT

The occurrence of plasmid mediated heavy metal and antibiotic co-resistance among bacteria isolated from refuse dumpsites was investigated. This was achieved by use of standard microbiological techniques for the isolation and identification of the bacterial isolates. The antibiotic and heavy metal susceptibility tolerance of bacteria was determined by the disc diffusion technique and minimum inhibitory concentration (MIC) respectively. The plasmid analysis was carried out by the conventional standard of Zymo research miniprep^{TM}-classic kit (Dolphin'Doc) according to the manufacturer instructions with minor alterations. A total of 61 bacterial isolates across 19 genera were identified. These genera were: *Proteus, Acinetobacter, Serratia, Klebsiella, Aeromonas, Erysipelothrix, Nocardia, Listeria, Corynebacterium, Cellulosimicrobium, Citrobacter, Bacillus, Pseudomonas, Arthrobacter, Enterococcus, Clostridium, Kurthia, Micrococcus,* and *Staphylococcus* sp. All the isolates were resistant to first line antibiotics Cefuroxime (100%), Ceftazidime (100%), Ceftriaxone (100%), Amoxicillin/clavulinic acid (100%), Erythromycin (100%), Cloxacillin (100%), Ampicillin (100%), and Ciprofloxacin (57%). However, the bacterial isolates were susceptible to Ofloxacin (84.21%), Gentamicin (94.73%) and Nitrofurantion (85.71%).The minimum inhibitory concentration (MIC) of cadmium (Cd^{2+}), copper (Cu^{2+}) and chromium (Cr^{6+}) against the bacterial isolates were 1000-1050, 250-300 and 200-250mg/l, respectively. Three isolates with the highest MIC values and antibiotic resistance were further identified to species level using specific primers and subjected to polymerase chain reaction. The isolates were confirmed as *Pseudomonas aeruginosa* (UGDS2), *Bacillus subtilis* (EDS2) and a species of *Klebsiella* sp (ODS2). Curing rendered *P. aeruginosa* sensitive to Cr^{6+} and Cu^{2+} but not to Cd^{2+}. However, curing did not affect the resistance of *B. subtilis* and *Klebsiella* sp to the three heavy metals. Plasmid profile analysis indicated that *P. aeruginosa* harbored plasmid DNA gene (100bp) while *B. subtilis* and *Klebsiella* sp did not. Plasmid mediated antibiotics and metals (Cr^{6+}, Cu^{2+} and Cd^{2+}) co-resistance were therefore indicated in *P. aeruginosa* since there was loss of resistance to the metal and antibiotics after curing. Although occurrence of plasmids associated with metal resistance is of potential benefit in bioremediation, co-resistance is of medical concern because of possible transfer of antibiotic resistance to pathogenic bacteria.

CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Refuse dump site is often subjective (because what is waste to one need not necessarily be waste to another) and sometimes objectively inaccurate (for example, to send scrap metals to a landfill is to inaccurately classify them as waste, because they are recyclable). Examples include municipal solid waste (household trash/refuse), hazardous waste, wastewater (such as sewage, which contains bodily wastes (feces and urine) and surface runoff), radioactive waste, and others, a wide ranging term encompassing most unwanted materials, defined by the Environmental Protection Act 1990. Waste includes any scrap material, effluent or unwanted surplus substance or article that requires disposal because it is broken, worn out, contaminated or otherwise spoiled. Wastes are 'those substances or objects which fall out of the commercial cycle or chain of utility' for

example glass bottles that are returned or reused in their original form are not waste, whilst glass bottles banked by the public and dispatched for remoulding are waste ‘until they have been recovered’(EPA, 1990).

The Department of the Environment identifies four broad categories of potential waste: Worn but functioning substances or objects that are still useable (albeit after repair) for the purpose they were made. Substances or objects that can be put to immediate use otherwise than by a specialised waste recovery establishment or undertaking for example ash from a power station used as a raw material in building blocks. Degenerated substances or objects that can be put to use only by establishments or undertakings specialised in waste recovery. These are always wasteseven if transferred for recovery for value for example contaminated solvents or scrap. Such substances only cease to be waste when they have been recovered. Substances or objects which the holder does not want and which he has to pay to have taken away. If substances or objects are consigned to the process of waste collection then they are waste but they may not be where they are fit for use in their present form by another identified person (Wilson *et al*., 2013).

Nigeria, rural and Urban landscapes like other developing countries are exposed to garbage, plastics, bottles, disposable cups, discarded tires and even human and live-stock faeces. These wastes are aesthetically unpleasant when their organic compositions are acted upon by putrefying bacteria, thereby constituting eyesores and producing unpleasant odours. Vector habitat are constituted from these refuse dumps and other nuisance organisms having the ability of transmitting or causing diseases such as infantile diarrhea, typhoid fever and cholera in humans and animals (Obire and Aguda, 2002).

Municipal solid wastes and industrial wastes including liquid effluents containing heavy metals are included in refuse dumps (Olanrewaju, 2002). Refuse dumps create a rich source of microorganisms which are mostly pathogenic in nature (Odeyemi *et al*., 2011). Refuse dumps serve as shelter and food source, and serves as attraction for rodents and vector insects (Maleicka-Adamowicz *et al.,* 2007). Although it is known that vector insects and rodents can transmit various causal agents of diseases and the co-resistance to heavy metal and antibiotics, a good percentage of this infections/resistance is as a result of bacteria found in these refuse dumps which may later settle and cause contamination. Activities involving the disposal of solid wastes may have adverse impact on the environment even if properly controlled with proper adoptive precautionary measures.

The presence of microorganisms in a refuse dump is due to their usage of such refuse dump as a food source. These microorganisms convert the organic material in the refuse dump to methane and carbon dioxide, under the anaerobic conditions especially in

most dumps. Other compounds are some evaporate, as the gas rises through the dump and escapes into the atmosphere. Explosions and fires in an uncontrolled environment may result in the presence of large amounts of methane. Additionally, this untreated gas may contain other compounds that pose a substantial health risk to nearby communities (Lewis and Gattie, 2002).

Antibiotics, also called antibacterials, are a type of antimicrobial (ASHP, 2015), drugs used in the treatment and prevention of bacterial infections (NHS, 2015; ECDPC, 2014). They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity (John, 2012; ECDPC, 2014). Antibiotics are not effective against viruses such as the common cold or influenza, and may be harmful when taken inappropriately (NHS, 2015). In 1928, Alexander Fleming identified penicillin, the first chemical compound with antibiotic properties. Fleming was working on a culture of disease-causing bacteria when he noticed the spores of a little green mold (*Penicillium chrysogenum*), in one of his culture plates. He observed that the presence of the mold killed or prevented the growth of the bacteria (*Brown,* 2015*).*

A substance, such as penicillin, that is capable of destroying or weakening certain microorganisms, especially bacteria or fungi that cause infections or infectious diseases. Antibiotics are usually produced by or synthesized from other microorganisms, such as molds. They inhibit pathogens by interfering with essential intracellular processes, including the synthesis of bacterial proteins. Antibiotics do not kill viruses and are not effective in treating viral infections (*Brown,* 2015*).*

A heavy metal is usually regarded as a metal with a relatively high density, atomic weight or atomic number, and is often assumed to be toxic. The criteria used, and whether metalloids or alloys are included, vary depending on the author and context. More specific definitions have been published, including those based on chemical behavior or periodic table position, but none of these have obtained widespread acceptance. Despite this lack of agreement, the term is widely used in science (Bánfalvi, 2011).

Some heavy metals, such as cadmium, copper, iron, chromium, mercury and lead, are notably toxic. The rest are relatively harmless but can be toxic in large amounts or certain forms. Potential causes of heavy metal poisoning include mining and industrial wastes, agricultural runoff, occupational exposure and contact with lead-based paints. Certain heavy metals are essential nutrients and these results in the human body containing, for example, several grams of iron (Bulkin, 2016).

Heavy metals are relatively scarce in the Earth's crust but pervade many aspects of economic activity. They are used in, for example, manufacturing and construction,

fertilisers, electronics, and jewellery; sport, mechanical engineering, military ordnance and nuclear science; and soap chemistry, glass making, pyrotechnics and medicine (Guney and Zagury, 2012).

A plasmid is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently. They are most commonly found in bacteria as small circular, double-stranded DNA molecules; however, plasmids are sometimes present in archaea and eukaryotic organisms. In nature, plasmids often carry genes that may benefit the survival of the organism, for example antibiotic resistance. While the chromosomes are big and contain all the essential genetic information for living under normal conditions, plasmids usually are very small and contain only additional genes that may be useful to the organism under certain situations or particular conditions. Artificial plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant DNA sequences within host organisms (Sinkovics *et al*., 1998).

Plasmids are considered replicons, a unit of DNA capable of replicating autonomously within a suitable host. However, plasmids, like viruses, are not generally classified as life (Sinkovics *et al*., 1998). Plasmids can be transmitted from one bacterium to another (even of another species) via three main mechanisms: transformation, transduction, and conjugation. This host-to-host transfer of genetic material is called horizontal gene transfer, and plasmids can be considered part of the mobilome. Unlike viruses (which encase their genetic material in a protective protein coat called a capsid), plasmids are "naked" DNA and do not encode genes necessary to encase the genetic material for transfer to a new host. However, some classes of plasmids encode the conjugative "sex" pilus necessary for their own transfer. The size of the plasmid varies from 1 to over 200 kbp (Thomas and Summer, 2008).

1.2 Statement of problem

In most developing countries such as Nigeria there is indiscriminate dumping of metals, which most times constitute both eyesores and environmental pollution with attendant toxicity to plants, animals and humans. These dump sites also encourage (by selection pressure) occurrence of microorganisms possessing resistance plasmids (R-plasmids) to heavy metals and equally having loci for antibiotic resistance. Such R-plasmids could be spread to humans by non-pathogenic organisms through contaminated food consumption or water, thus enabling opportunistic bacteria of the normal flora to acquire the plasmid. In addition the knowledge of co-resistance implies that resistant

antibiotic species can be used for bioremediation of effluents from metal processing industries.

The damage of the cell membranes can be cause by heavy metals, which result in enzymes specificity alteration, cellular functions disruption and damage of the DNA structure (Nies, 1999). Several bacteria which are resistant to the effects of high concentrations of heavy metals are concomitantly resistant to several antibiotics as shown in many Laboratory studies. The bacteria possess plasmid which is the source of different resistance factor to many heavy metals and antibiotics. Bacteria resistance to antibiotic is a serious threat to society today. Condition such as acute mild condition that hardly affect daily life to severe respiratory diseases that are chronic such as cancer, kidney/heart related diseases that required specialist's care. The reasons responsible for this problem is over usage of antibiotics in humans and high heavy metal concentration in the environment (Okeke *et al*., 2007).

Antibiotic/metal co-resistance possess a threat to every one, especially new neonate children and the immunocompromised person (HIV and elderly persons), who are more vulnerable to infectious disease.

For the general public as a whole, antibiotic/metal co-resistance limits number of effective drugs available leading to fewer treatment options for the sick.

1.3 Research questions

The following questions were used to guide the study:

- ✓ are there bacteria resistant to antibiotics and heavy metals in dumpsites?
- ✓ what is the antibiotic resistance profile of dump sites bacterial isolates?
- ✓ what is the test of heavy metal minimum inhibitory concentration?
- ✓ do plasmids play any role in the resistance of bacterial to antibiotics?
- ✓ are these bacterial resistance to heavy metal plasmid mediated?
- ✓ are the same plasmids involved in both antibiotic resistance and heavy metal?

1.4 Hypotheses

Antibiotic and heavy metal increasing concentrations of Cd^{2+}, Cu^{2+}, and Cr^{6+} has no co-resistance on bacteria isolates from refuse dumpsite.

- ✓ There is no relationship between heavy metal and antibiotic resistance in bacteria from the refuse dumpsites.
- ✓ Antibiotic resistance pattern in bacteria isolates before and after curing from the refuse dumpsite does not vary.

- ✓ Heavy metal resistance pattern in bacteria isolates before and after curing from the refuse dumpsite does not vary.
- ✓ The plasmid DNA profile pattern of the multi-drug resistant environmental bacteria before and after curing does not vary.

1.5: Objective

General objective of this study was to determine the occurrence of plasmid mediated metal and antibiotic co-resistance amongst bacteria occurring in refuse dump site.

The specific objectives are to;

1 isolate, characterize and identify bacteria from refuse dump sites and control site.

2 Determine antibiotic resistance pattern in bacteria isolates from both site.

3 determine minimum inhibitory concentration (MIC) of the antibiotic resistance against the test heavy metals like Chromium (Cr^{6+}), copper (Cu^{2+}) and cadmium (Cd^{2+}) isolates from both sites.

4 ascertain the involvement of plasmids in heavy metal and antibiotic co-resistance.

5 ascertain the relationship between antibiotics resistance plasmid and heavy metal test resistance.

1.6: Significance of the study is to;

1. Assist environmental authorities to develop strategies for reducing the discharge of heavy metals and other chemical pollutants into the environment in order to improve the quality of the environmental resources.
2. Sensitize the stake holders in the Ugborikoko, Effurun and Obiaruku environment and the municipal council authorities on the impact of indiscriminate discharge and raw effluent waste into the environment.
3. Create awareness among the Ugborikoko, Effurun and Obiaruku communities on the significance of environmental management and conservation in view of promoting public health.
4. Solve the increasing global problem on antimicrobial resistance by establishing the drug resistance trends in environmental bacteria as to provide the basis for making policy decisions on indiscriminate use of antibiotics and discharge of heavy metals and industrial waste.

1.7 Scope and limitations of the study

i. Due to the vast nature of the refuse dumpsites, this study was targeted to cover three main refuse dumpsites within Delta State. These included; Ugborikoko, Effurun and Obiaruku dumpsites. The dumpsites targeted were composed of domestic, industrial wastes and were approved public waste dumpsites. These dumpsites were suspected to be hot spots of pollution due to human and industrial activities.

ii. The study was aimed at establishing the occurrence of plasmid mediated heavy metal and antibiotic co-resistance bacterial isolates from refuse dumpsite status hence the analysis did not include water sources.

iii. Chromosomal and other gene factor were not included as part of the analysis of the co-resistance of antibiotic and heavy metals.

iv. The study did not take into account seasonal variations to account for the differences in plasmid mediated bacteria isolates from refuse dumpsite in the concentrations of the heavy metals and antibiotics co-resistance as well as the differences in the bacterial numbers as determined by plate counts.

CHAPTER TWO
LITERATURE REVIEW

2.1 Plasmid

An American molecular biologist Lederberg Joshua in 1952 was the first to introduce the term plasmid, which he used originally to describe any existing bacterial genetic material in an extra chromosomal condition for at least part of its cycle of replication. Later, the definition was narrowed to existing genetic elements that is predominantly or exclusively outside of the chromosome and can replicate autonomously, which was further used to distinguish it from viruses (Finbarr, 2003; Sheua *et al*., 2007).

Plasmid is a small molecular DNA that can be physically separated within a cell from a chromosome DNA and can as well replicate independently. In bacteria they are most commonly found as circular, small, double-stranded DNA molecules. However, plasmids are sometimes found within archaea and eukaryotic organisms. Plasmids many a times carry genes that may benefit the survival of the organism in nature; Plasmids hold genes that govern their replication, segregation and copy number with additional phenotypic functions such as resistance to antibiotic, metal resistance, synthesis of bacteriocins and ability to mediate cell to cell conjugation among others (Robicsek *et al*., 2006). While the plasmids are very small and contain only additional information, chromosomes are usually very big and contain all the necessary information for living. Artificial plasmids are widely used as vectors in molecular cloning, which served as driving in the replication of DNA recombination sequences within the organism's host (Hughes *et al*., 2011).

Plasmids are also known as replicons which are referred to as DNA unit capable of replicating autonomously within a host that are suitable. Plasmids are not generally classified as living just like viruses (Stephan *et al.*, 2013). Plasmids from a bacterium can be transmitted to another and even of another species by three main mechanisms: transformation, transduction, and conjugation. Transfer of genetic material from host-to-host is referred to as horizontal gene transfer, and plasmids can also be considered as part of the mobilome. Plasmids are "naked" molecules of DNA that do not encode their genes which are essential to encase the genetic material for new host transfer, unlike viruses that encase their genetic material in a capsid called protective protein coat. Except in some cases like the conjugative "sex" pilus, which encoding plasmid is necessary for their own transfer. Plasmid varies in size from 1 to over 200 kilo base pair (Schuurmans *et al*., 2014), and identical plasmids present in a single cell can range from one to thousands in some occurrence.

The microbes and plasmid DNA does not have a relationship that is neither parasitic nor mutualistic, because each involved the presence of species that is not dependent and is living in a detrimental or commensurable state with the host organism, instead, plasmids create a mechanism for horizontal gene transfer within population of microbes and typically create a selective beneficial environment under a given condition. Plasmids may carry genes that create resistance to antibiotics naturally occurring in a competitive habitat, or the proteins created may act as toxins under similar condition which allowed the organism to use a particular organic compound that would be beneficial when nutrients are scarce (Wolfgang, 2008).

Plasmids must possess a stretch of DNA that can act as an origin of replication in order for it to replicate independently within a cell. A replicon usually refers to a self-replicating unit, as in the case of plasmid. The gene for plasmid-specific replication initiation protein (Rep) is a typical bacterial replicon which consists of a number of elements; a unit that is repeatedly called iterons, an adjacent AT-rich region and DNAA boxes (Finbarr, 2003). Larger plasmids may carry genes specific for the replication of plasmids, but in the case of smaller plasmids, they make use of the host replicative enzymes to produced copies of more plasmids. The host chromosome usually has a few types of plasmids which are capable of inserting themselves, and the episomes in prokaryotes are sometimes referred to as plasmids integrative (Brown, 2010).

At least one gene is almost always carried by Plasmids. The host cells are usually beneficial to many of the genes carried by the plasmids. Such as enabling the host cell to survive in an environment that would have being referred to be lethal or restrictive for their growth. Some of these genes encode traits for resistance to antibiotics or heavy metals, while others may display virulence traits that enable a bacterium to colonize the host and overcome its defenses, or have specific metabolic functions that give room for bacterium to utilize a particular nutrient, including the ability or inability to reduced toxic organic or recalcitrant compounds (Finbarr, 2003). Bacteria can develop the ability to fix nitrogen through the means of plasmids. Cryptic plasmids are phenotype of host cell which has no observable effect by plasmids or the benefits of host cells which are yet to be discovered (Cook and Dunny, 2013).

The physical properties of naturaly occurring plasmids vary greatly. The size of plasmid can range from very small mini-plasmid that are less than a 1 kilobase pairs (Kbp), to very large mega plasmids with several megabase pairs (Mbp). There is usually little that can differentiate between a megaplasmid and a minichromosome at the upper end.

Plasmids are generally circular, such as linear plasmids which are also known to require specialized mechanisms for their ends replication (Finbarrs, 2003).

A plasmid may be present in an individual cell in varying number, between one to several hundreds of ranges. The copy number, which is determined by how the replication initiation is regulated and the size of the molecule, are usually the normal number of copies of plasmid that may be found in a single cell. Larger plasmids tend to occurr in lower copy number (Brown, 2010). Upon cell division, low copy number plasmids that occurred only as one or few copies in each bacterium, are in danger of being lost during bacteria segregation. Plasmids with a single-copy have systems that try to actively distribute a copy to both daughter cells. These systems, which include the parABS system and parMRC system, are often known as the partition system or partition function of a plasmid.

2.2 Bacteria resistance to antibiotics

Resistance to antibiotic is the ability of a bacterium to resist the effects of an antibiotic to which it should normally be susceptible. It evolves by natural selection, plasmid transfer and mutation. Antibiotics usage gives a selective advantage to bacteria resistant and over time the composition of the entire resistant strains population. Treatment of these resistant strains with antibiotics becomes ineffective (Laxminarayan and Brown, 2001). Resistance to antibiotic genes is normally carried on chromosomes, plasmids or conjugative transposons. These genes resistance are acquired through conjugation from antibiotic producers (Sarma *et al*., 2011).

Antibiotic resistant bacteria can transfer resistance to indigenous bacteria through plasmids or conjugative transposons. Plasmids transfer occurs within or between species (Ong *et al.*, 2009). Conjugative transposons are located in the chromosome of bacteria and can also transfer themselves from the chromosome of the bacteria donor to the chromosome of the bacteria recipient. Plasmids conjugation also integrates within plasmids. Conjugative plasmids can transfer antibiotic resistance genes among species within bacteria that is either Gram positive or Gram negative. Unlike plasmids, conjugative plasmids are not detected easily. Plasmid transfer is believed to be liable for the geographical spread of resistance to antibiotics (Laxminarayan and Brown, 2001).

Sensitive microorganisms resist antibiotics by modification of the target reaction so that is no longer sensitive to the antibiotic or cause a change that prevents the antibiotic from reaching the target reaction. Some bacteria destroy the antibiotics activity through enzyme productivity. Resistance to β-lactams, aminoglycosides and tetracyclines is achieved by enzymes whose activity inactivates the antibiotic (Sarma *et al*., 2011; Suzuki

and Horinouchi, 2015). A modification in the membrane of plasma wall may reduce its permeability to an antibiotic (Suzuki and Horinouchi, 2015).

Some bacteria have developed the capacity to actively pump the antibiotic away from the cytoplasm. This is termed efflux mechanism, which was described first in bacteria that were having the capacity to pump tetracycline out of their cells (Salyers and Whitt, 2005). Resistance to antibiotic is also acquired when a structure that is normally attacked by an antibiotic is modified so that it will no longer recognized by the drug. Bacteria resistant to streptomycin produce modified ribosomes to which the antibiotic cannot bind (Suzuki and Horinouchi, 2015). Antibiotic resistance is also achieved through by-passing the metabolic step inhibited by the drug.

A microorganism which develops cross-resistance becomes insensitive to all related antibiotics (Suzuki and Horinouchi, 2015). The resistance to multiple antibiotic (*rma*) loci of *E. coli* and *Salmonella* are one of the most researched and understood chromosomal resistance systems to multiple drugs (Randall and Woodward, 2002). The *mar*-locus is involved in antibiotics resistance such as chloramphenicol, cephalosporins, nalidixic acid and flouroquinolones, penicillins, puromycin, rifampicin and tetracycline (Collard *et al*., 2005). Development of resistance to antibiotics is associated with the antimicrobial agent's usage in veterinary medicine, plant agriculture, human medicine, animal husbandry, aquaculture and environmental contamination by industrial effluents (CCAR, 2002).

The spread of drug resistance within bacterial populations are due to the increasing number of diseases resistant to treatment, which many a times pose a threat to successful treatment of microbial infections such as typhoid fever, malarial (Mourão *et al*., 2015; Mishra and Prasad, 2015). For example gonorrhea caused by *Neisseria gonorrheae* was first treated successfully with sulfonamides in 1936 but by 1942 most strains had developed resistance and penicillin was turned to by physicians. However, resistant strains of penicillin later emerged within 16 years in the Far East (Harley and Prescott, 2016).

The use of bactericides, disinfectants and antiseptics as been an increasing concern in health care facilities and community due to it potential to induce the expression of resistance genes to antimicrobial which encode multi-drug efflux pumps and their regulators (CCAR, 2002). This mechanism almost always causes wide variety of antibiotics resistance especially in *E. coli*, *Salmonella*, *Pseudomonas* species and species of other bacteria (Matyar *et al.*, 2010).

Rapid resistant genes spread to antibiotics in a bacterial population can occur in between one ecosystem and other (Witte, 2004). Particular resistance to antibiotic genes

was first described in specific bacteria of human were also found in animal specific species of bacteria and vice versa, suggesting that bacterial populations can share and exchange these genes (Sternberg, 1999; Arias *et al*., 2009).

2.2.1 Antibiotic resistance

The emergence of bacteria resistance to antibiotics is a similar phenomenon. Evolutionary processes often reflect an emergence of resistance that take place during therapy with antibiotics. The survival of high doses of antibiotics for bacterial strains with physiological or genetically enhanced capacity may be selected by antibiotic treatment. This may be as a result of preferential growth of resistant bacteria, under certain conditions, while the drug inhibited growth of susceptible bacteria (levy, 2004). Such as selection for strains with previous acquired antibacterial resistance genes as demonstrated in 1943 by the Luria–Delbrück experiment (Luria and Delbruck, 1943). Penicillin and erythromycin antibiotics, which used to have a high efficacy against many bacterial species and strains that are now less effective, due to increase in many strains of bacterial resistance (Pearson, 2007).

Resistance pattern may appear in a way of biodegradation of pharmaceutical product, such as soil bacteria degradation of sulfamethazine which is introduced by means of medicated pig feces (Topp *et al*., 2013). For any bacteria to survival is often due to an inheritable resistance (Witte, 2004), but resistance development to antibacterial also occurs through horizontal transfer of gene. This horizontal transfer is more likely to occur in locations of frequent usage of antibiotics (Dyer, 2003).

Resistance to antibacteria agent may impose a biological cost, thereby resulting in reduction of strains resistant fitness that may limit the spread of resistant bacteria to antibacterial such as absence in antibacterial compounds. However, addition of mutations may compensate the cost of fitness and can aid the bacteria survival (Anderson, 2006).

It was shown by paleontological data that both resistance to antibiotic and antibiotics are ancient mechanisms and compounds respectively (D'costa *et al*., 2011). Targets of useful antibiotic are those for which negative mutations impact bacterial reproduction or viability (Gladki *et al* 2013).

Existence of several molecular mechanisms of resistance to antibacterial is in place. Resistance to intrinsic antibacterial may be part of the makeup of genetic bacterial strains (Alekshun and Levy, 2007). For instance, a target to antibiotic may be absent from the genome of the bacteria. This result in resistance that is acquired from a bacterial chromosome mutation or extra-chromosomal DNA acquisition (Alekshun and Levy,

2007). Bacteria that produce antibacterial have evolved resistance mechanisms that have been described to be similar to antibacterial-resistant strains and may also have been transferred to antibacterial-resistant strains (Nikaido, 2009; Forsbeg *et al*., 2012). The resistance to antibacterial spread often appears through vertical mutations transmission during growth and by DNA genetic recombination in horizontal genetic exchange (Witte, 2004). For instance, resistance genes to antibacterial can be distribution between different bacterial strains or via species that carry these resistance genes within the plasmids (Witte, 2004; Barker-Austin *et al.*, 2006). Many of the different resistance genes that carry plasmids can confer resistance to multiple antibacterial (Barker-Austin *et al.*, 2006). Too many of the antibacterial cross-resistance may also occur when there is encoding resistance mechanism by a single gene conveying resistance to several antibacterial compounds (Barker-Austin *et al.*, 2006).

Resistant strains to antibacterial and species, which are referred to as "superbugs" in some cases, has been contributed to the emergence of diseases that were well-controlled in a while. For instance, bacterial strains that cause tuberculosis (TB) emergent with previous resistant effect to antibacterial treatments have posed many therapeutic challenges. Yearly, nearly half a million new cases of tuberculosis multidrug-resistance (TB-MDR) are worldwide estimated to occur (WHO, 2015). For instance, NDM-1 is a new enzyme that is identified in conveying bacterial resistance to a broad range of antibacterial beta-lactam (Boseley, 2010).The resistance to all standard intravenous antibiotic treatment of severe infections are mostly cause by isolates with NDM-1 enzyme was stated by United Kingdom's Health Protection Agency (Health protection report, 2010).

2.2.2 Misuse of Antibiotics

The first principle of antibiotics Per The *ICU Book* is try not to use them, and the second principle is try not to use too many of antibiotics (Marino, 2007).

One of contributions to the emergence of bacteria resistance to antibiotic is inappropriate treatment and overuse of antibiotics. Such as self prescription of antibiotics which lead to misuse (Larson, 2007). The frequent prescription of many antibiotics to treat diseases or symptoms that do not respond to antibiotics or may resolve without treatment. Also, prescription of incorrect or suboptimal antibiotics for certain bacterial infections (Slama *et al*., 2005; Larson, 2007). Penicillin and erythromycin antibiotics overuse, has been associated with emerging resistance to antibiotic since the 1950s (Pearson, 2007; Hawkey, 2008). Antibiotics widespread usage in hospitals has also been associated with

bacterial strains and species increment with the most common antibiotics which no longer respond to treatment (Hawkey, 2008).

Excessive use of prophylactic antibiotics in travelers and medical professional's failure to prescribe antibiotics correct dosage on the basis of the patient's weight and history of prior use are common forms of antibiotic misuse. Again, failure to take the entire prescribed course of the antibiotic, sufficient recovery rest, incorrect dosage and administration are other forms of misuse. Antibiotic treatment that is inappropriate, for example, is the viral infections treatment prescription such as the common cold. One study on the infections of respiratory tract discovered that physicians were more likely to prescribe antibiotics to patients who appeared to expect the infection (Ong *et al*., 2007). The aim at both physicians and patient's multifactorial interventions can help in the reduction of inappropriate prescription of antibiotics (Metlay *et al*., 2007).

Lobbying to eliminate the unnecessary use of antibiotics as been shown concern by several antimicrobial resistance organizations (Larson, 2007). The issue of antibiotics misuse and overuse has been taken care by the US Interagency Task Force formation on Antimicrobial Resistance. This task force aims is to actively address resistance to antimicrobial, and is coordinated by the US Centers for Prevention and Disease Control (PDC), the Drug and Food Administration (DFA), and the National Institutes of Health (NIH), as well as other US agencies (Centers for Diseases Control and prevention, 2009). Keep Antibiotics Working by Non Governmental Organisation (NGO) campaign group (Keep Antibiotics Working, 2010). Antibiotics are not automatic" in France, but the government campaign started in 2002 and led to a clearly noticeable reduction of unnecessary prescriptions to antibiotic, especially antibiotics used for children (Sabuncu *et al*., 2009).

Restrictions on antibiotics usage in the UK in 1970 were prompted by the emergence of resistance to antibiotics as report by Swann (1969) and in 2003 the EU restrict the use of antibiotics as growth-promotional agents (Regulation, 2003). Moreover, the request for restrictions on antibiotic use in food animal production and an end to all no therapeutic uses were initiated by several organizations such as The American Society for Microbiology (ASM), American Public Health Association (APHA) and the American Medical Association (AMA). However, there are common delays to limit the use of antibiotics by the actions of regulatory and legislative, which contribute to the delay by industries using or selling antibiotics attitude against such regulation, and to the time required to test causal links between their use and resistance to antibiotics by researcher. The federal bills are of Two kind such as S.742 GovTrack.US, 2005 and H.R. 2562

GovTrack.US, 2005, which aimed at proposing the phasing out of no therapeutic antibiotics usage in US food animals, but have not been able to be pass in to law (GovTrack.US.S, 2005; GovTrack.US,H.R, 2005). A group of organization such as public health and medical organizations, the American Holistic Nurses' Association, the American Medical Association, and the American Public Health Association (APHA) endorsed these bills (Allison *et al*., 2011).

In animal husbandry there has been extensive use of antibiotics. In the year 1977 in the United States, the question of emergence of bacterial strains resistant to antibiotics was due to livestock usage of antibiotics which was raised by the US Food and Drug Administration (FDA). In March 2012, an action brought by the Natural Resources Defense Council and others in the United States District Court for the Southern District of New York, made a ruling ordering the FDA to revoke approvals for the use of antibiotics in livestock, which violated FDA regulations (John, 2012).

2.3 Heavy metals

There is widespread of heavy metal contamination. Any group of metals whose atomic density is greater than $5g/cm^3$ can be defined as heavy metals. About 50 heavy metals of special concern have been discovered in nature because of human beings toxicological effect and other living organisms. Several of them have the nutritional characteristics as "trace elements" for example Zinc (Zn), Copper (Cu), Cobalt (Co), Nickel (Ni), Manganese (Mn), Chromium (Cr), Cadmium (Cd) and Iron (Fe) and are necessary for enzyme activities in living organisms but in high concentrations, they produce toxic effects (Bruins *et al.*, 2000). Due to heavy metals stability they cannot be degraded or destroyed and as such they exhibit persistence as environmental contaminants (Gochfeld, 2003). Excess untreated effluent of both industrial and municipal origin have long time been channeled into Lake Victoria river. This rich effluent of metals flows from the towns of Kisumu, Eldoret, Kakamega and Kericho with sewage plants that are malfunctional and coupled with the discharge of semi-treated effluents with high biological oxygen demand (BOD) to the rivers draining into the lake from Agro based industries such as paper, sugar, tea, coffee, dairy, tanning hide into leather and fish (Nzomo, 2005).

A number of studies have showed that bacteria are influenced by heavy metals through growth adverse effect, morphology and biochemical activities, resulting in biomass and diversity decrease (Michael *et al*., 2013). Microbes have developed tolerance mechanisms to metals either through complexation, efflux or reduction of metal ions or use them in anaerobic respiration as terminal electron acceptors (Naik and Dubey, 2013). An

increasing problem in different treatment of infections is bacterial antibiotic resistance and other antimicrobial agents. The widespread of heavy metals in industrial effluent and sewage has led to considerable speculation about possible association of genes between metals tolerance to bacteria and multiple resistances to antibiotics (Bhattacharya *et al*., 2013; Pandit *et al*., 2013; Raquel *et al*., 2013).

It has been suggested that encoding resistance genes to heavy metals and biocides can be located together with resistance genes to antibiotics on either the same genetic structure such as plasmid, or different genetic structures within the same bacterial strain as reported by Guardabassi and Dalsgaard (2002). McArthur and Tuckfield (2000) had suggested that resistance to metals and antibiotics among bacteria are very closely linked and the expression of resistance to antibiotics may be dependent on the metals exposure. Metal tolerance and resistance to drug among bacteria have also shown proportional increase along gradients of industrial contamination and use of metal based antimicrobial agents (Stepanauskas *et al*., 2005; Bhattacharya *et al*., 2013). There is need to be more conscious of the impact of the products we put into the environment such as sterilants, disinfectants, heavy metals and other antimicrobials that cause an environment selective pressure leading to the mutation of bacteria that allow them to survive creating superbugs multiplication, thereby posing a potential public health hazard.

2.3.1 Heavy metal toxicity and resistance

Not all heavy metals are equally toxic to bacteria. Some are important trace metals involved in various cell physiological functions. For instance Zinc (Zn), nickel (Ni), iron (Fe) chromium (Cr), copper (Cu), and cobalt (Co) are metals of moderate to high physiological importance. They are essential micronutrients necessary for several cellular functions and components of DNA- and RNA-polymerases such as zinc (Zn) in Urease, nickel (Ni) in cytochrome, chromium (Cr) and cytochrome—c—oxidase (Cu). Lead (Pb), Cadmium (Cd), Mercury (Hg), silver (Ag), and gold (Au) have reduced relevance as trace nutrients and they have limited physiological function. Cd and Hg have been known as strong cellular toxins due to their ability to form complexes that are harmful (Nies, 1999). In contrast, trace metals toxicity such as Zn, Ni, Cu, Co, and Cr are dependent strongly on the concentration. As reviewed by Nies (1999), with limited toxicity, the elements Fe, Mn and molybdenum (Mo) were physiologically important. Metals such as Zn, Ni, Cu, Co, Cr, vanadium (V), and tungsten (W) are elements that are toxic with metabolic relevance, while the elements Ag, Cd, Hg, Pb, antimony (Sb), and uranium (U) are strong toxins.

The toxicity of heavy metals in the environment depends strongly on the environmental conditions because it influences the valence of the metal ions and therefore their bioavailability. Environmental Cr, for example mainly occurs in two different forms: as Cr^{3+} ion or as the hexavalent Cr with oxygen associated as chromate (CrO^{2-}_4). The Cr^{3+} ions are less toxic to bacteria compared to chromate (Nies, 1999). Conditions to the environment such as the redox potential, the concentration of organic matter and the pH-value affect the bioavailability and the concentrations of heavy metals in soil, sediment, and water. For example, the redox potential is influenced by the oxygen level and thereby affecting some metals solubility. The decomposition of high concentrations of organic matter in some water bodies lead to a reduction of the oxygen level down to anaerobic conditions. The solubility of Cd and Zn is reduced under such conditions (Schulz-Zunkel and Krueger, 2009). It also increases the solubility of the metals Pb, Cd, and Zn in the case of low pH-values. Schulz-Zunkel and Krueger (2009) reported that Cr and Zn bind to high concentrations of organic matter in sediment.

Generally, the toxicity of heavy metals to bacteria is not only due to their affinity to chemical such as thiol groups and macrobiomolecules but also depends on the metal compound solubility under physiological conditions (Nies, 1999). Bacteria evolved mechanisms of metal tolerance to prevent cellular damage caused by metal ions. The general mechanisms involved in resistance to heavy metal are of three types: The first are the toxic metals complex formation or sequestration (Silver and Phung, 2009). The cytoplasm concentration of the free toxic ions is minimized. The cell membranes, cell walls and extracellular polymeric substance (EPS) of biofilms are used in knowing the biosorption of toxic metals (Harrison *et al*., 2007). For instance, it was reported that heavy metals can bind to the EPS matrix and the contained polysaccharides Thus, resulting in the enhancement of the biofilms that belong to the bacteria metal tolerance (Teitzel and Parsek, 2003). The second is the reduction of intracellular ions through detoxification (Nies, 1999). A well understood instance is the encoding of the mercury reductase by the gene of *merA*. The decrease of Hg^{2+} to a less toxic Hg^0 is due to MerA protein (Schiering *et al*., 1991). Due to the low evaporation point of Hg^0 as resulted in it diffuses from cell (Nies, 1999). The third is the efflux systems used in toxic ions extrusion (Nies and Silver, 1995), known for the resistance to the metal ions Cd^{2+}, Zn^{2+}, and Co^{2+} were mediated by *Alcaligenes eutrophus* which is an example of the cation/proton antiporter Czc. This is done by the extrusion of metals from the cytoplasm through the inner and outer membrane to the immediate environment (Silver and Phung, 2009). Persisted cells help in the increase of wide metal tolerance population (Harrison *et al*., 2007). Time dependent tolerance to

toxic metal ions is mediated by Persisted cells which are due to continuous regulation of genes that are of resistant and stress response (Harrison *et al.*, 2007).

Toxic metal resistance mechanisms are known to be in existance (Ganiyu *et al.*, 2010). Bacteria resistance in metal is controlled by resistance genes that are found on chromosomes, plasmids and transposons. Although resistance mediated plasmid is the most common, other systems of resistance have been described (Bruins *et al.*, 2000). For instance mercury resistance in *Bacillus* sp, cadmium efflux by p-type ATPase also in *Bacillus* sp and arsenic efflux in *E. coli* are chromosomal systems of metal resistance (Silver and Phung, 2009).

There is considerable evidence that microorganisms can rapidly adapt to metals toxicity by altering their chemistry and mobility (Salas *et al*., 2010; Sevgi *et al*., 2010). The resistance mechanisms include metal reduction or transformation to more volatile or less toxic forms. Some bacteria including *Pseudomonas* sp, *E. coli* and *Clostridium* sp enzymatically reduce Hg^{2+} to Hg^{0} which is highly volatile and diffuses away from the cell of the bacteria. Others have a system that is specific to the metal efflux, which are the most commonly found plasmid mediated metal resistance mechanism (Robicsek *et al*., 2006). Other microbes tolerate metals through binding by extracellular polysaccharides (precipitation and exclusion) mediated by production of molecular weight that has low proteins binding such as phytochelatins (Borrok and Fein, 2005). Chelating and formation of heavy metal that is complex of metal species with the media components and organism induced pH changes can also contribute to metal tolerance. Chromate tolerance is achieved through methylation, reduction and precipitation at the surface of the cell (Solanki and Dhankhar, 2011), blocking cellular uptake by altering the uptake pathway and removal from cytoplasm by efflux pumps. In many cases these responses seem to be plasmid mediated (Lukasz *et al.*, 2015).

Cadmium binds to the group's sulfhydryl on essential proteins thus interfering with important cellular functions (Nies, 1992). It also can cause single-stranded breakage of DNA in *E. coli* (Williams *et al.*, 2010). Two cadmium plasmid mediated resistance efflux systems, that is, *cad* in *Staphylococcus aureus* and *czc* in *Acinetobacter eutrophus* are well characterized and documented (Szuplewska *et al*., 2014; Nies, 1992). The system of *czc* also causes resistance to zinc and cobalt. Another system that confers resistance to zinc and cobalt that is chromosomally encoded is known to exist (Bruins *et al.*, 2000). Resistance genes to cadmium located on transposons have been reported on *Listeria monocytogenes* (Lebrum *et al.*, 1994).

Bacteria among Gram positive and Gram negative have shown wide distribution of resistance to mercury. Some bacteria have the Hg (II) (*mer*) operon resistance. The *mer* operon detoxifies Hg (II) transports, and self regulates mercury resistance (Ni-chadhain *et al*., 2006; Boyd and Barkay, 2012). Mercury resistance is based on its potential redox and its low vapor pressure. Resistant bacteria could reduce Hg^{2+} to metallic mercury (Hg^0), which may not remain in the cell but uses passive diffusion method to leave the cell (Silver and Phung, 2009).

Bacteria heavy metals tolerance has been reported among Gram positive and Gram negative bacteria (Diptendu and Goutam, 2013). Gram positive bacteria are generally believed to be less heavy metal tolerant stress than Gram-negative bacteria. Some bacterial species such as *Bacillus* sp may be resistant due to their ability to sporulate, *Corynebacterium* sp have remarkable membrane lipids that protect the cells from environmental stress (Sevgi *et al*., 2010).

Bacteria resistance to heavy metals is caused by the presence or absence of the metal concentration in the environment. The metals absence from such environment reduces resistance power that is noticeable as reported by Parisa *et al.*, 2011 and Amalesh *et al*., 2012, found that although exposure to metals enhances resistance, the fact that resistant bacteria are found in environments never exposed to high heavy metals concentrations indicates that species tolerant to heavy metal already exist in non- pollutant habitats.

Nevertheless, bacterial sensitivity can be quite complex, in some possible generalizations. There has been more description of sensitivity to toxic metals by Gram positive bacteria than gram negative bacteria (Sevgi *et al*., 2010). Moreover, there was report on toxicity rankings of two general microbial (Nies, 1999; Harrison *et al*., 2007). In these rankings bacterial susceptibility is described as the particular metal sulfide dissociation constants function (pK_{SP}) (Nies, 1999) and as standard reduction potentials function (ΔE_0) (Harrison *et al*., 2007). Nevertheless, sensitivities to toxic metals have been shown by different types of bacteria. Even the susceptibility to heavy metal of bacteria belonging to the same genera can differ dramatically. Different strain from a particular general can be inhibited or show resistance to the metal concentration from the same sampling site, for instance in the strain of *Aeromonas* were one can be inhibited by a concentration of 100 μg Zn ml^{-1} while another strain can be resistant at a concentration of 3200 μg Zn ml^{-1} (Matyar *et al*., 2010). The toxicity pattern reported by Nies (1999) and

Harrison *et al*. (2007) is not the same with the above listed example. The adaptability of the environmental bacteria to their ecological conditions may have resulted to their selection of mechanisms for certain metal tolerance.

2.4 Antibiotics and metal co-resistance.

It as been proven that there is correlation between heavy metals tolerance and resistance to antibiotics, which is presently a global problem affecting some treatment of plants, animals and human infections (Knapp *et al.,* 2011). Resistance genes may be closely located together on the same plasmid in bacteria of both antibiotics and heavy metals co-resistance and may be transferred together in an environment. Tolerance to metal and antibiotic resistance in bacteria along industrial contamination gradients have been shown to increase proportionally (Stepanauskas *et al*., 2005). It has been shown that resistant bacterial isolates to vanadium show increased resistance to quoinolones (Ciprofloxacin and Norfloxacin), which are crucial in the management of *salmonella* sp infections. The genes can be relocated to indigenous populations of microorganisms occurring frequently in the environments thereby enhancing the resistance spread to antibiotics (CCAR, 2002).

Bacterial antibiotic resistance and other antimicrobial agents is a problem that is on the increase in today's society (Forsberg *et al*., 2012). Our current antibiotics are becoming less useful but used more heavily against resistant pathogenic bacteria, which result in infectious diseases becoming more difficult and more exorbitant to treat. This is because creation of environmental selective pressure leads to mutations in bacteria that permit them to survive better and multiply (Baquero and Teresa, 2014).

According to Lawrence (2000) on the Selfish Operon Theory discussion of plasmid of genes clustering. It was discussed that genes clustered if useful to the organism in terms of survival of its species and can be transferred together in the event of conjugation. In the case of multiple stresses in the environment, for instance it will be favorable ecologically, for a bacterium to acquire resistance to the stresses of both antibiotics and heavy metals. Plasmid mediated resistance if present in bacteria with clustered resistance genes have the tendency to pass on those genes to other bacteria simultaneously thereby creating a greater chance of survival. In situation as such, in the case of resistance to antibiotic with metal tolerance, one may suggest a positive association (Lawrence, 2000).

Several workers have reported the association between antibiotics resistance and heavy metals. It was discovered that genes with encoding resistance to metals are located on transmissible plasmids (Berg *et al*., 2010; Pal *et al.,* 2014; Pandit *et al*., 2013). The traits for both antibiotic resistance and metal resistance are coded in genes that are often carried on the same mobile genetic elements or plasmids (Raquel *et al.,* 2013). It has been reported that *Salmonella abortus equi* have a transferable plasmids encoding resistance to various antibiotics and heavy metals (Ghosh *et al.,* 2000).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of study areas

The map of delta state showing the three study areas is shown in fig 3.1. The study was carried out in Obiaruku (Ukwiani Local Government Area), Ogborikoko and Effurun (both in Uvwie L.G.A) refuse dumpsites which is indicated with star sign within the map. These refuse dumpsites is located within Delta State, Nigeria.

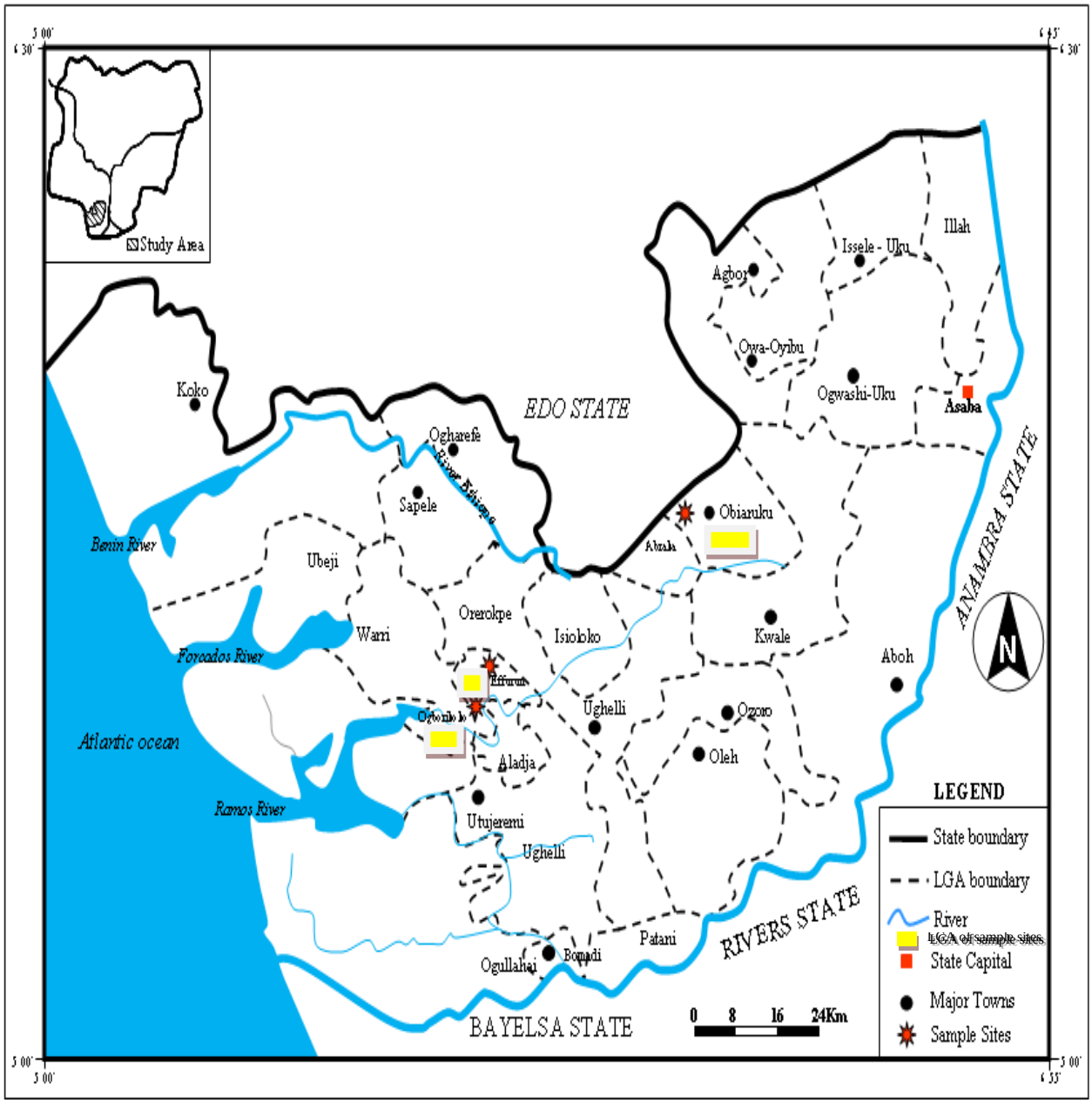

Fig 3.1: Map of Delta State showing the three Study Areas/Dump sites. (Source; Delta State ministry of land and survey)

3.2 Description of Obiaroko Refuse Dumpsite

The picture showing the type of waste in Obiaroko refuse dumpsite is shown in Fig 3.2. The study area is located at Obiaroko with a land use type of approximately $180m^2$ within latitude ($050\ 48.689^{I}$-707^{I}) N, longitude ($006^{0}\ 07.732^{I}$-747^{I}) E and with Elevation of between 23m-25m alongside glonass of 3m above the sea level. The tropic climate is marked with wet and dry seasons having a peak mostly in June to September and annual temperature of 22^{0}C to 35^{0}C and the soil sample was collected on March 2015. The soils in the study area are mostly white sandy loam at the top to black sandy loam sub soil well drain and form a plain level ground. The refuse dumpsite is compose of domestic waste, leaves waste, tires waste, electronic waste, human and live stock faeces, plastic and so many other waste.

Fig 3.2 Picture showing the type of waste in Obiaroko refuse dumpsite

3.3 Description of Ugborikoko Refuse Dumpsite

The picture showing the type of waste in Ugborikoko refuse dumpsite is shown in Fig 3.3. The study area is located at Ugborikoko with a land use type of approximately $180m^2$ within latitude (05^0 32.185^I -192^I) N, longitude (005^0 45.966^I-992^I) E and with Elevation of between 2m-3m alongside glonass of 3m above the sea level. The tropic climate is marked with wet and dry seasons having a peak mostly in June to September and annual temperature of 22^0C to 35^0C and the soil sample was collected on March 2015. The soils in the study area are mostly white sandy loam at the top to black sandy loam sub soil well drain and form a plain level ground. The refuse dumpsite is compose of domestic waste, leaves waste, tires waste, electronic waste, human and live stock faeces, plastic and so many other waste.

Fig 3.3 Picture showing the type of waste in Ugborikoko refuse dumpsite

3.4 Description of Effurun Refuse Dumpsite

The picture showing the type of waste in Ugborikoko refuse dumpsite is shown in Fig 3.4. The study area is located at Effurun with a land use type of approximately 360m^2 within latitude (05^0 34.356^I-375^I) N, longitude (005^0 44.744^I-776^I) E and with Elevation of between 10m-11m alongside glonass of 3m above the sea level. The tropic climate is marked with wet and dry seasons having a peak mostly in June to September and annual temperature of 22^0C to 35^0C and the soil sample was collected on March 2015. The soils in the study area are mostly white sandy loam at the top to black sandy loam sub soil well drain and form a plain level ground. The refuse dumpsite is compose of domestic waste, leaves waste, tires waste, electronic waste, human and live stock faeces, plastic and so many other waste.

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Fig 3.4 Picture showing the type of waste in Effurun refuse dumpsite

3.5: Collection and Transportation of Samples

With the aid of a sterile trowel, soil samples were collected from the entrance (Ogb 1, Ob 1 and Eff 1) and middle at 20m (Ogb 2, Ob 2 and Eff 2) of each dumpsite, from a depth of 15cm from the surface. A control soil sample was equally obtained from a location of 45m away from opposite the dumpsite. Each soil samples from each of the 3 dumpsite was collected in a sterile plastic bag and place in ice-packed coolers and immediately transported to the laboratory for microbial analyses.

3.6: Cultivation, isolation and enumeration of total aerobic bacteria and coliform bacteria.

One (1) gram of carefully weighed out soil sample was used for a tenfold serial dilution and then 0.1ml of 10^{-3} and 10^{-5} dilution was inoculated onto nutrient agar and MacConkey agar using spread plate technique. Developed colonies after incubation at room temperature at 37^0C for 24hrs, were sub-cultured on to fresh Nutrient agar media and incubation at room temperature at 37^0C for 24hrs for purification. The colonies count in the plate was multiplied by the dilution number ($3x10^3$) and divided by the measured milliliter ($\frac{3\ x\ 10^{-3}}{0.1}$) in getting the log cfu per gram and stored in a Macarthney bottle in refrigerators at 4˚C for future use (Cheesbrough, 2014).

3.7: Identification of microorganisms

Identification of the isolates was accomplished by the comparison of their morphological, cultural and biochemical characteristics with those of know Taxa by following Bergey's Mannual of Systematic Bacteriology (Whitman *et al*., 2012).

3.8: Antibiotics susceptibility test for standardize of bacterial isolate

The antibiotic susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hilton agar according to CLSI (2012). Bacterial isolates were tested against ABTEK multi disc eight antibiotics which comprised;

Ceftazidime (CAZ 30µg), Amoxycillin/clavulinate (AMC 30µg), Cefuroxime (CRX 30µg), Gentamycin (GEN 10µg) and Ofloxacin (OFL 5µg). Gram negative disc contains additional constituent such as Nitrofurantoin (NIT 300µg), Cefotaxime (CTX 30µg) and Ampicillin (AMP 30µg). Gram positive disc contains additional constituent such as Ceftriaxone (CTR 30µg), Erythromycin (ERY 30µg) and Cloxacilin (CXC 5µg).The inoculum was standardized by adjusting its density to equal the turbidity of a barium sulphate ($BaSO^4$) (0.5 McFarland turbidity standards). The isolates were inoculated

onto nutrient agar using spread plate techniques. The multi disc antibiotics were place on the inoculated nutrient agar and incubated at 35^{o}C for 24 hrs. The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted on the basis of clinical laboratory standard institute (CLSI) guideline (CLSI, 2012). The range between 0-14 is resistant, 12-21 intermediate and 15-16 and above as susceptible was used to interpreting the result.

3.9: Heavy metal susceptibility test

Stock solutions of cadmium chloride, potassium dichromate and copper, were prepared with deionized water and sterilized by autoclaving at 121°C and 0.15MPa pressure for 15 min. The minimum inhibitory concentration (MIC) of the antibiotic resistant isolates was determined against increasing concentrations of Cd^{2+}, Cu^{2+}, and Cr^{6+}, on nutrient agar at different concentration until no further growth was observed (Raja *et al.,* 2009). Starting with an initial concentration of 100mg/l, further MIC tests were carried out with concentrations of 150mg/l, 200mg/l, 250mg/l, 300mg/l, 350mg/l, 400mg/l, 500mg/l, 600mg/l, 700mg/l, 800mg/l, 900mg/l, 1000mg/ml and 1050mg/l. Cultures that showed growth at a particular concentration were repeated with higher concentration. The MIC tests were determined at 30°C for 24-48hours (Raja *et al.,* 2009).

3.10: Molecular identification of isolates

Three bacterial isolates *Pseudomonas* sp, *Bacillus* sp and *Klebsiella* sp were selected based on their 100% high resistance pattern to antibiotics and heavy metals among other bacterial isolates. Molecular identification was also carried out using specific premier DNA profile analysis that was grouped on the basis of visual similarities of the fragments analysed by electrophoresis in a 2% agarose gel stained with ethidium bromide according to the manufacturer's instructions (NewEnglandbiolab, England, United Kingdom) as follows;

3.10.1: Genomic DNA Extraction of Gram-Negative Bacteria for Ugb 2 (*Pseudomnas* sp) and Obia 2 (*Klebsiella* sp).

5'3' Frame 16S rDNA genes

Pseudomonas sp genus specific primers

PA-GS-F 5′-GACGGGTGAGTAATGCCTA-3′

PA-GS-R 5′-CACTGGTGTTCCTTCCTATA-3′

Primers specific for the capsule in *Pseudomonas aeruginosa*

Pa16S-F 5'-GGGGGATCTTCGGACCTCA -3'

Pa16S-R 5'-TCCTTAGAGTGCCCACCCG-3'

Klebsiella sp genus specific primers

FD1 -5'-AGA GTT TGA TCC TGG CTC AG-3′

RP2- 5′-AAG GAG GTG ATC CAG CC-3′

Primers specific for the capsule in *Klebsiella pneumoniae*

RmpA gene F 5' ACTGGGCTACCTCTGCTTCA-3'

R 5'- CTTGCATGAGCCATCTTTCA-3

$2x10^9$ bacterial cells were harvested in a 1.5ml microcentrifuge tube by centrifuging for 10mins at 5000 x g and the supernatant was discarding. Digested Solution of 180µl in a pellet was resuspended. 20µl of proteinase K solution was added and mix thoroughly by vortexing to obtain a uniform suspension. The sample was incubated at 56^0C and was vortex occasionally until the cells are completely lysed (~ 30 mins). 20µl of RNase A Solution was added, mix by vortexing and the mixture was incubated for 10mins at a room temperature. 200µl of the lysis Solution was added to the sample and mix thoroughly by vortexing for 15sec until a homogeneity mix was obtained. 400µl of 50% ethanol were added to the homogeneity mixture and mix by vortexing. The prepared lysate was transfer to a GeneJET Genomic DNA purification column that is inserted in a collection tube. The column was centrifuge for 1min at 600 x g. the collection tube containing the flow-through solution was discarding. The GeneJET Genomic DNA purification column was replaced in to a new 2ml collection tube. 500µl of wash Buffer I with addition of ethanol was added in to the new 2ml collection tube and centrifuge for 1min at 800 x g. the flow-through was discarded and the purification column was place back in to the collection tube. 500µl of wash Buffer II with addition of ethanol was added to the GeneJET Genomic DNA purification column and centrifuge for 3 min at a maximum speed of ≥ 12000 x g. the flow-through in the collection tube was discarded and the GeneJET Genomic DNA purification column was transfer to a 1.5ml sterile microcentrifuge tube. 200µl of Elution Buffer was added to the centre of the GeneJET Genomic DNA purification column membrane for the elution of genomic DNA. Was incubated for 2 min at room temperature and centrifuge for 1min at 8000 x g. The purification column was discarded and the purified DNA was stored (Lukasz *et al*., 2015).

3.10.2: Genomic DNA Extraction of Gram-Positive Bacteria for Eff 2 (*Bacillus* sp).

Bacillus sp genus specific primers

BacF-5' AGGGTCATTGGAAACTGGG-3'

BacR 5'-CGTGTTGTAGCCCAGGTCATA-3'

Primers specific for the capsule in *Bacillus subtilis*

EN1F (5'-CCAGTAGCCAAGAATGGCCAGC-3',

EN1R (5'-GGAATAATCGCCGCTTTGTGC-3')

Gram-Positive Bacteria lysis Buffer was prepared using 20mM Tris-Hydrochloride (HCL), pH 8.0, 2mM EDTA, and 1.2% Triton X-100, with addition of lysozyme to 2mg/ml immediately before starting the procedure. $2x10^9$ bacterial cells were harvested in a 1.5ml microcentrifuge tube by centrifuging for 10mins at 5000 x g and the supernatant was discarding. 180µl of the Gram-Positive Bacteria lysis Buffer was resuspended in a pellet and incubated for 30mins at 37^0C. 200µl of Lysis Solution and 20µl of Proteinase K was added and mix thoroughly by vortexing in other to obtained uniform suspension. The sample was incubated at 56^0C while vortexing occasionally until the cells was complete lysed (~ 30min). 20µl of RNase A Solution was added, mix by vortexing and the mixture was incubated for 10mins at room temperature. The mixture was added with 400µl of 50% ethanol and mix by vortexing. The prepared lysate was transfer to a GeneJET Genomic DNA purification column which was inserted in a collection tube and the column was centrifuge for 1min at 6000 x g. the collection tube containing the flow-through was discarded. The GeneJET Genomic DNA purification column was replaced into a new 2ml collection tube. 500µl of wash Buffer I with addition of ethanol was added in to the new 2ml collection tube and centrifuge for 1min at 800 x g. the flow-through was discarded and the purification column was place back in to the collection tube. 500µl of wash Buffer II with addition of ethanol was added to the GeneJET Genomic DNA purification column and centrifuge for 3 min at a maximum speed of ≥ 12000 x g. the flow-through in the collection tube was discarded and the GeneJET Genomic DNA purification column was transfer to a 1.5ml sterile microcentrifuge tube. 200µl of Elution Buffer was added to the centre of the GeneJET Genomic DNA purification column membrane for the elution of genomic DNA. Was incubated for 2 min at room temperature and centrifuge for 1min at 8000 x g. The purification column was discarded and the purified DNA was stored (Katoh and Standley, 2013).

3.10.3: Amplification of 16S rDNA Gene's polymerase Chain reaction (PCR).

Three of the most highly resistant isolates which are *Pseudomonas* sp, *Bacillus* sp and *Kliebsilla* sp PCR were carryout using the Genomic Guided Sequence method as followed:

The polymerase chain reaction (PCR) master mix (2x) after thawing was gently vortex and briefly centrifuge. A thin wall PCR tube was place on ice and the following components for each 50µl reaction were added.

Quick Load One Taq One Step PCR Master mix(2x)	25µl
Forward primer (2x)	(2.5µl)
Reverse primer (10µM)	(2.5µl)
Template DNA	10µl(1ng-1ug)
Nuclease free water	10µl
Total volume	50µl

The sample and spin down was gently vortex. The PCR was performed using the recommended thermal cycling condition as followed;

Step	Temperature	Time	Number of cycles
Initial denaturation	94^0C	3mins	1
Denaturation	94^0C	30sec	
Annealing	Tm-5	30sec	25-40
Extension	72^0C	1min	
Final extension	72	7mins	
Hold	4^0C	ω	

5-10µl of the PCR product was analyze on a 1.5% agarose gel electrophoresis and was stained with ethidium bromide.

3.10.4: Preparation of gel electrophoresis

1.5g (for PCR) agarose into 100ml. The agarose was Melt in 0.5X Tris-Acetate-EDTA (TBE) in the microwave at 20% power until all agarose is dissolved and there were no stringy pieces for about 45mins. The melted agarose gel liquid was cool under cold running water for 10-15 seconds. A very small amount stock solution of ethidium bromide (1ul per 20ml) was added and was swirling into the liquid agar. The gel was pour into the gel mold held in place by the clamp with leak-proof vessel with a comb 1 to 2 mm above the base place. Erlenmeyer was immediately rinse with distilled water and place on drying rack. the gel was allowed to dry for about 15 to 20 minutes. The comb was carefully

removed and the gel was transfer into gel mold to gel tank with TBE buffer and was ensure that the gel was completely submerged.

3.10.5: Polymerase Chain Reaction (PCR) DNA Detection

The amplified pcr DNA was purified by zymo research plasmid miniprep^{TM} - classic kit (NZYTech). Pcr DNA was visualized on 1.5% agarose electrophoreses gels in TBE (1X) buffer at constant voltage (90V) for 35 mins, stained with ethidium bromide and viewed under UV Transilluminator (Sayers *et al*., 2012).

3.11: Plasmid DNA extraction

Three of the most highly resistant isolates which are *Pseudomonas* sp, *Bacillus* sp and *Kliebsilla* sp plasmid Profile was carryout using Zymo Research DNA Extraction Kit as followed**;** 0.5-5ml[1, 2] of bacterial culture in a clear 1.5ml tube at full speed 15-20 seconds were centrifuge in a microcentrifuge and supernatant was discard.200µl of **P1 buffer** (Red) were added into tube and resuspend pellet completely (i.e. using vortexing or pipetting). 200µl of **P2 buffer** (Green) [3] were added and mix by inverting the tube 2-4 times. Cells were completely lysed due to the solution appearances which were clear, purple, and viscous and were proceed to the next step within 1-2 minutes. 200µl of **P3 buffer** (yellow) were added and mix gently by thoroughly, don't vortex. The samples were turned yellow at the completion of the neutralization. And the lysate were incubating at room temperature for 1-2 minutes and later centrifuge for 2 minutes. **Zymo^{TM}llИ**/column in a Collection Tube was place, and the supernatant from the centrifuge sample were transfer into the **Zymo^{TM}llИ** column and was centrifuge for 30 seconds. The flow through in the collection tube was discarded and the **Zymo^{TM}llИ** column was return into the collection tube. 200µl of **Endo-Wash buffer** were added to the column and centrifuge for 30 seconds. 400µl of **Plasmid-Wash buffer** were added to the column and centrifuge for 1 minute. The column was transfer into 1.5ml of microcentrifuge tube and 30 µl of **DNA** Elution Buffer were added to the column and centrifuge for 30 second to elute the plasmid DNA.

3.11.1: Preparation of Gel Electrophoresis for Plasmid

0.8g agarose into100ml, the agarose was Melt in 0.5X Tris-Acetate-EDTA (TBE) in the microwave at 20% power until all agarose is dissolved and there were no stringy pieces for about 45mins. The melted agarose gel liquid was cool under cold running water for 10-15 seconds. A very small amount stock solution of ethidium bromide (1ul per 20ml)

was added and was swirling into the liquid agar. The gel was pour into the gel mold held in place by the clamp with leak-proof vessel with a comb 1 to 2 mm above the base place. Erlenmeyer was immediately rinse with distilled water and place on drying rack. The gel was allowed to dry for about 15 to 20 minutes. The comb was carefully removed and the gel was transfer into gel mold to gel tank with TBE buffer and was ensure that the gel was completely submerged.

3.11.2: Plasmid DNA detection

The plasmid DNA was purified by zymo research plasmid miniprep^{TM} -classic kit (NZYTech). Plasmid DNA was visualized on 0.8% agarose electrophoreses gels in TBE (1X) buffer at constant voltage (90V) for 35 mins, stained with ethidium bromide and viewed under UV Transilluminator (Sayers *et al*., 2012).

3.12: Plasmid Curing Experiment

This was carried out using curing agent sodium dodecyl sulphate (SDS) with prepared graded concentration of 10µg/ml to 1000µg/ml. Tubes containing 10 ml peptone water supplemented with the curing agent, were inoculated with 0.1 ml of overnight broth culture containing cells and incubated at 37°C for 24h, an appropriate dilution 0.1ml of the culture was plated on nutrient agar to obtain descrite colonies isolates after 24h incubation at 37°C. Resulting colonies were tested for loss of plasmid on nutrient agar plates incorporated with the appropriate antibiotic/metal ion (Paul *et al*., 2008; Wang *et al*., 2011).

CHAPTER FOUR
RESULTS AND DISCUSSION

4.0 RESULT

4.1: Total aerobic bacteria count

Results of the total aerobic bacteria counts per gram of soil from the various dumpsites are presented in Figure 4.1. Centre of Effurun dumpsite had the highest count of 6.26cfu/g, closely followed by the centre of Ugborikoko dumpsite with 6.18cfu/g, and the edge of Obiaruku dumpsite with 5.70 cfu/g which was the least.

Comparing the various dumpsites and control sites, the lowest aerobic bacteria count in the dumpsite is higher than the highest in the control site, which indicates that the control sites had less aerobic bacteria counts than the dumpsite (Fig 4.1). In general observation between the aerobic and coliform counts, Ugborikoko and Effurun dumpsite had displayed consistent high counts and the lowest was centre of Obiaruku dumpsite of coliform counts.

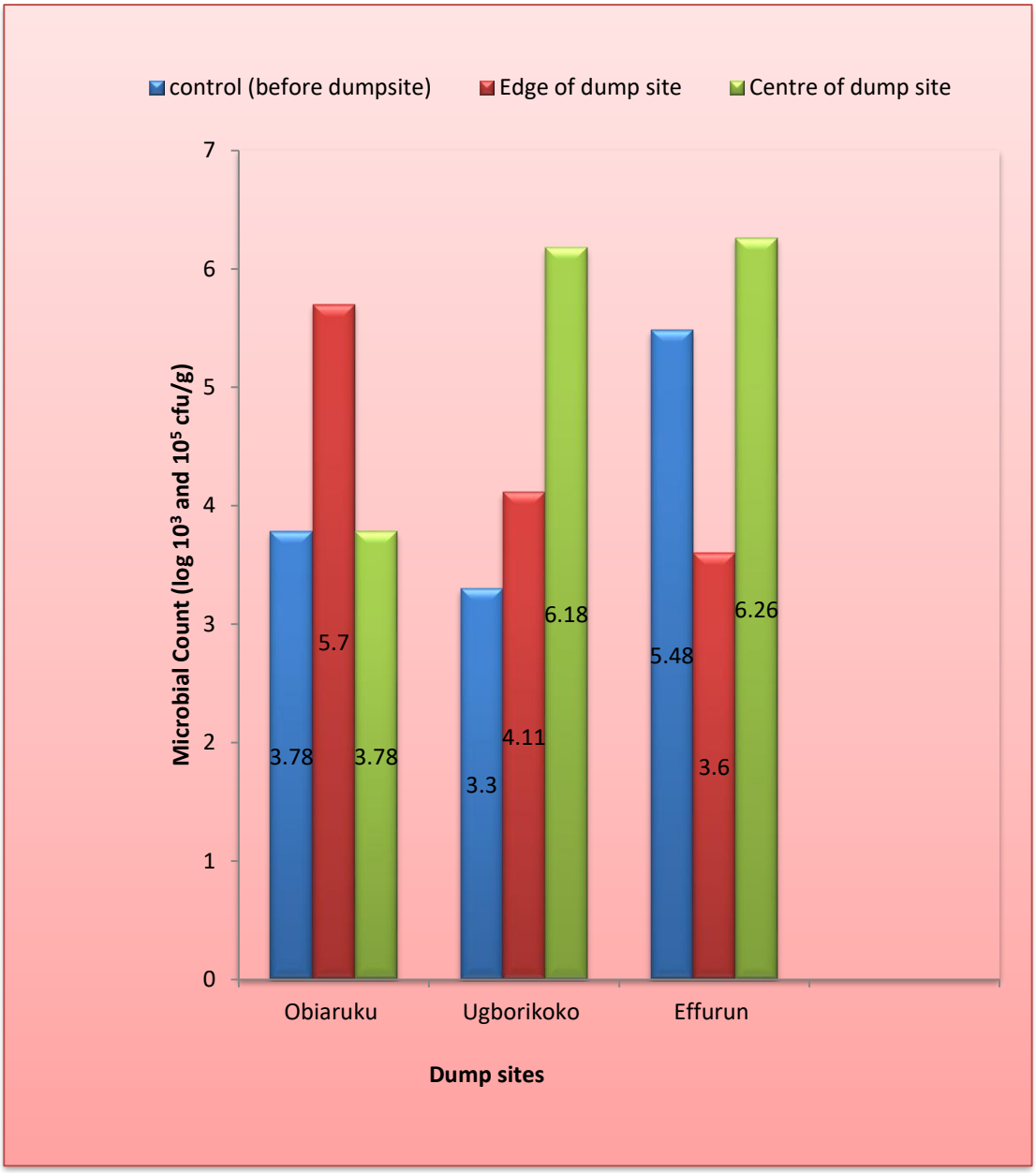


Fig 4.1: Total aerobic bacteria count from various dumpsites

4.2: Total coliform bacteria count

The total coliform bacteria counts per gram of soil from the various dumpsites, is presented in Figure 4.2. Centre of Ugborikoko dumpsite with 6.68 cfu/g had the highest count, followed by the centre of Effurun dumpsites with 5.04 cfu/g and the centre of Obiaruku dumpsite had the least with 0 cfu/g in that order. Generally, the Ugborikoko and Effurun dumpsites displayed higher counts than the Obiaruku dumpsite.

Comparing the various dumpsites and control sites, the lowest coliform bacteria count in the dumpsite is higher than the highest in the control site except in the case of centre of Obiaruku dumpsite with count of 0cfu/g, which indicates that the control sites had less coliform bacteria counts than the dumpsite (Fig 4.2). In general observation between the aerobic and coliform counts, Ugborikoko and Effurun dumpsite had displayed consistent high counts and the lowest was centre of Obiaruku dumpsite of coliform counts.

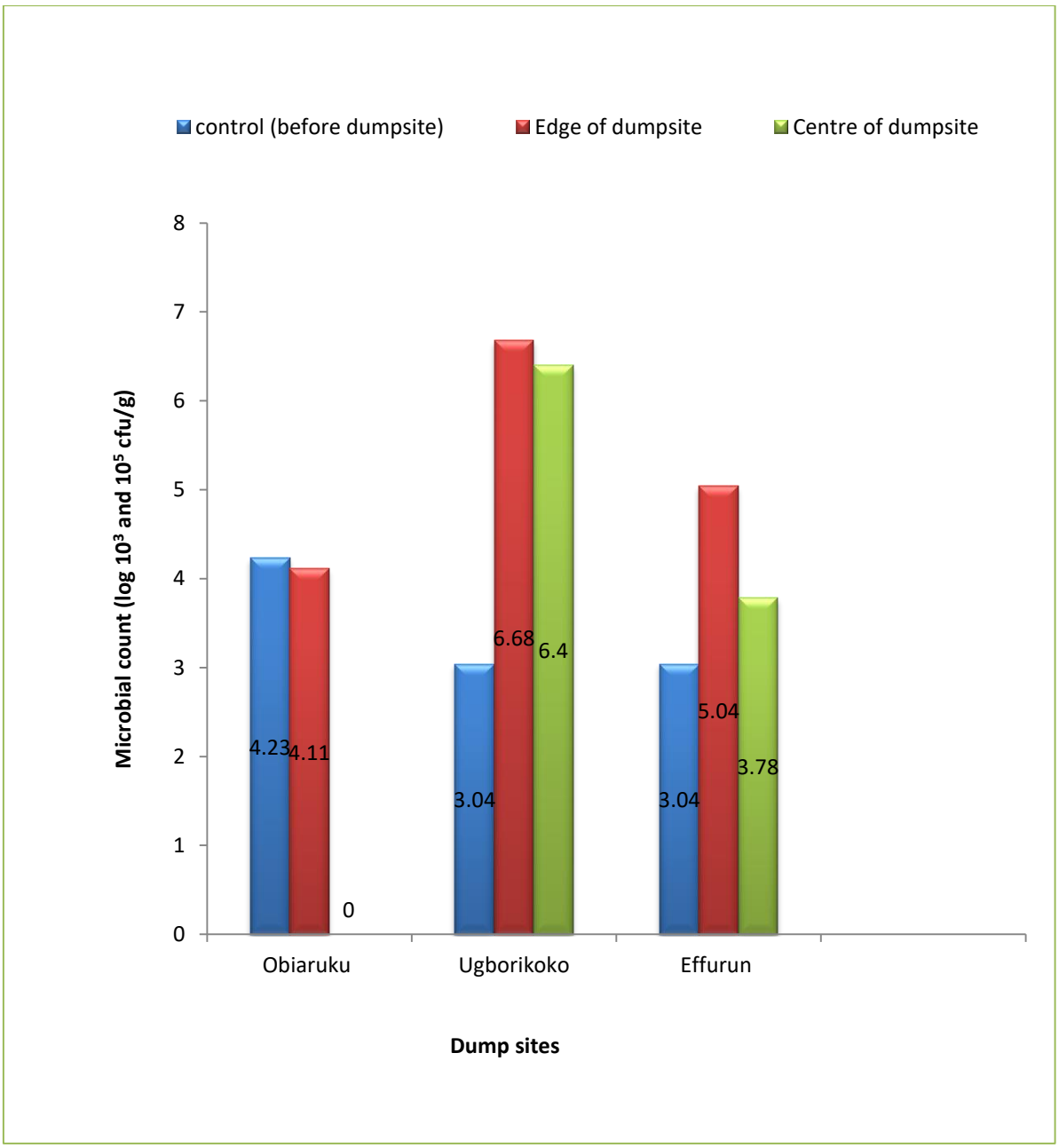


Fig 4.2: Coliform bacteria count from various dumpsites

4.3: Morphology and biochemical characteristics of bacterial isolates.

The morphology and biochemical characteristics of the bacterial isolates from the three dumpsites are shown in Table 4.1. A total of 61 bacterial isolates across 19 genera from the three dumpsites were identified. The bacteria isolated were identified to include twelve (12) Gram positive bacteria which are *Bacillus* sp, *Micrococcus* sp, *Staphylococcus* sp, *Kurthia* sp, *Arthrobacter* sp, *Listeria* sp, *Corynebacterium* sp, *Erysipelothrix* sp, *Nocardia sp, Cellulosimicrobium* sp, *Enterococcus* sp, and *Clostridium* sp as against seven (7) Gram negative bacteria *Pseudomonas* sp, *Aeromonas* sp, *Citrobacter* sp, *Proteus* sp, *Acinetobacter sp, Serratia* sp, and *Klebsiella* sp, on the basis of there difference in colony morphology and biochemical characteristics

Table 4.1: Morphology and biochemical characteristics of the bacterial isolates from the three dumpsites

Morphology test		Biological test										Identified isolates
Shape	**Gram reaction**	**Motility**	**Oxidase**	**Catalase**	**Citrate**	**Indose**	**Hydrogen Sulphur**	**Gas**	**Acid**	**Glucose**	**Lactose**	
Rod	-ve	+	+	+	-	-	+	+	+	+	-	*Pseudomonas* sp
Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
Cocci	+ve	-	-	+	+	-	+	-	-	+	+	*Staphylococcus* sp
Rod	+ve	+	-	+	+	-	-	-	-	+	-	*Kurthia* sp
Rod	-ve	+	+	+	+	+	+	-	-	+	-	*Aeromonas* sp
Rod	+ve	+	-	+	+	-	+	+	+	-	-	*Arthrobacter* sp
Rod	+ve	-	-	+	+	-	-	-	+	+	+	*Listeria sp*
Rod	+ve	-	-	+	-	-	+	-	+	+	-	*Corynebacterium* sp
Rod	+ve	-	-	-	+	-	+	-	+	+	+	*Erysipelothrix* sp
Rod	+ve	+	-	+	+	-	-	-	+	+	-	*Nocardia* sp
Rod	+ve	+	-	+	-	-	+	+	+	+	-	*Cellulosimicrobium* sp
Cocci	+ve	-	-	+	-	-	-	-	+	+	+	*Enterococcus* sp
Rod	+ve	+	-	+	+	+	+	+	+	+	+	*Clostridium* sp
Rod	-ve	+	-	+	+	-	+	+	+	+	+	*Citrobacter* sp
Rod	-ve	+	-	+	+	+	+	+	-	+	-	*Proteus* sp
Cocci	-ve	-	-	+	+	+	-	-	+	+	+	*Acinetobacter* sp
Rod	-ve	+	+	+	+	-	-	-	-	+	-	*Serratia* sp
Rod	-ve	-	+	+	+	-	-	+	+	+	+	*Klebsiella* sp

+ve = Positive

-ve = Negative

4.4: Prevalence of bacterial isolates.

The prevalence (%) of bacterial in soil samples from the various dumpsites is presented in Table 4.2. *Bacillus* sp had the highest prevalence (27.3%), which was closely followed by *Micrococcus* sp (18.2%) and the lowest prevalence of 4.5% was observed in five (5) of the bacterial isolates from *Listeria* sp, *Kurthia* sp, *Corynebacterium* sp, *Citrobacter* sp and *Cellusimicrobium* sp. The highest prevalence frequency of occurrence among the isolates from the various dumpsites was *Bacillus* sp at 27.3%, *Staphylococcus* sp at 13.6% and *Pseudomonas* sp at 9% from Ugborikoko dumpsite, while *Micrococcus* sp at 18.2%, *Bacillus* sp and *Staphylococcus* sp at 15.8% from Effurun dumpsite, and *Norcadia* sp, and *proteus* sp at 15% from Obiaruku dumpsite were observed respectively. Again it was also observed that the lowest prevalence isolates at 0.0% from the various dumpsite were *Proteus* sp, *Acinetobacter* sp, *Serratia* sp, *Clostridium* sp, *Enterococcus* sp, *Klebsiella* sp, *Aeromonas* sp, *Erysipelothrix* sp, and *Nocardia* sp from Ugborikoko dumpsite, while *Listeria* sp, *Corynebacterium* sp, *Nocardia* sp, *Cellulosimicrobium* sp, *Citrobacter* sp, *Proteus* sp, and *Serratia* sp and *Klebsiella* sp from Effurun dumpsite, and finally *Pseudomonas* sp, *Arthrobacter* sp, *Corynebacterium* sp, *Erysipelothrix* sp, *Cellulosemicrobium* sp, *Enterococcus* sp, and *Clostridium* sp from Obiaruku dumpsite were observed respectively.

Table 4.2: Prevalence (%) of bacterial in soil samples from the various dumpsites

Isolates	Number (%) Prevalence		
	Obiaruku dumpsite Isolates (N=20)	**Effurun dumpsites Isolates (N=19)**	**Ugborikoko dumpsites Isolates (N=22)**
Pseudomonas sp	0(0)	1(5.3)	2(9)
Bacillus sp	1(5)	3(15.8)	6(27.3)
Micrococcus sp	2(10)	3(15.8)	4(18.2)
Staphylococcus sp	1(5)	3(15.8)	3(13.6)
Kurthia sp	2(10)	1(5.3)	1(4.5)
Aeromonas sp	2(10)	2(10.5)	0(0)
Arthrobacter sp	0(0)	2(10.5)	2(9)
Listeria sp	2(10)	0(0)	1(4.5)
Corynebacterium sp	0(0)	0(0)	1(4.5)
Erysipelothrix sp	0(0)	1(5.3)	0(0)
Nocardia sp	3(15)	0(0)	0(0)
Cellusimicrobium sp	0(0)	0(0)	1(4.5)
Enterococcus sp	0(0)	1(5.3)	0(0)
Clostridium sp	0(0)	1(5.3)	0(0)
Citrobacter sp	1(5)	0(0)	1(4.5)
Proteus sp	3(15)	0(0)	0(0)
Acinetobacter sp	1(5)	1(5.3)	0(0)
Serratia sp	1(5)	0(0)	0(0)
Klebsiella sp	1(5)	0(0)	0(0)

4.5: Antibiotic resistance pattern of bacterial isolate before curing.

The antibiotic resistance pattern (%) of the bacterial isolates before plasmid curing for Gram positive isolates is shown in Table 4.3. Results revealed that most of the isolates were resistant to one or more antibiotics and a significant number of isolate from the various dumpsites showed multiple antibiotic resistance property. All bacterial isolates showed 50-100% resistance to Amoxicillin/clavulence acid, Ceftazidime, Cefuroxime, Ceftriaxone, Erythromycin, and Cloxillin, whereas, there was only one bacterial strain of the following bacterial Genus *Listeria* sp, *Micrococcus* sp, and *Bacillus* sp, were observed resistance and others are either intermediate or susceptible (0%) in Gentamicin, but in the case of ofloxacin only one isolate from *Bacillus* sp showed resistance and other bacterial isolates were either intermediate or susceptible (0%) on the Gram positive isolates from the 3 dumpsites.

Table 4.3: Antibiotics resistance pattern (%) of Gram positive bacteria Isolates before curing

	Number (%) resistance							
Bacterial isolates	**Amoxicillin/ clavulinic**	**Ceftazidime**	**Cefuroxime**	**Gentamicin**	**Ofloxacin**	**Ceftriaxone**	**Erythromycin**	**Cloxillin**
Bacillus sp *(N=10)*	10(100)	10(100)	10(100)	1(10)	1(10)	10(100)	9(90)	10(100)
Micrococcus sp (N=9)	9(100)	9(100)	9(100)	1(11.1)	0(0)	9(100)	7(77.8)	9(100)
Staphylococcus sp *(N=7)*	7(100)	7(100)	7(100)	0(0)	0(0)	7(100)	6(85.7)	7(100)
Kurthia sp *(N=4)*	3(75)	3(75)	4(100)	0(0)	0(0)	3(75)	4(100)	4(100)
Arthrobacter sp *(N=4)*	4(100)	4(100)	4(100)	0(0)	0(0)	4(100)	4(100)	4(100)
Listeria sp *(N=3)*	2(66.7)	3(100)	2(66.7)	1(33.3)	0(0)	1(33.3)	2(66.7)	3(100)
Corynobacterium sp *(N=1)*	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)
Erysipelothrix sp *(N=1)*	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)
Nocardia sp *(N=3)*	3(100)	3(100)	3(100)	0(0)	0(0)	3(100)	3(100)	3(100)
Cellulosemicrobium sp *(N=1)*	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)
Enterobacter sp *(N=1)*	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)
Clostridium sp *(N=1)*	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)

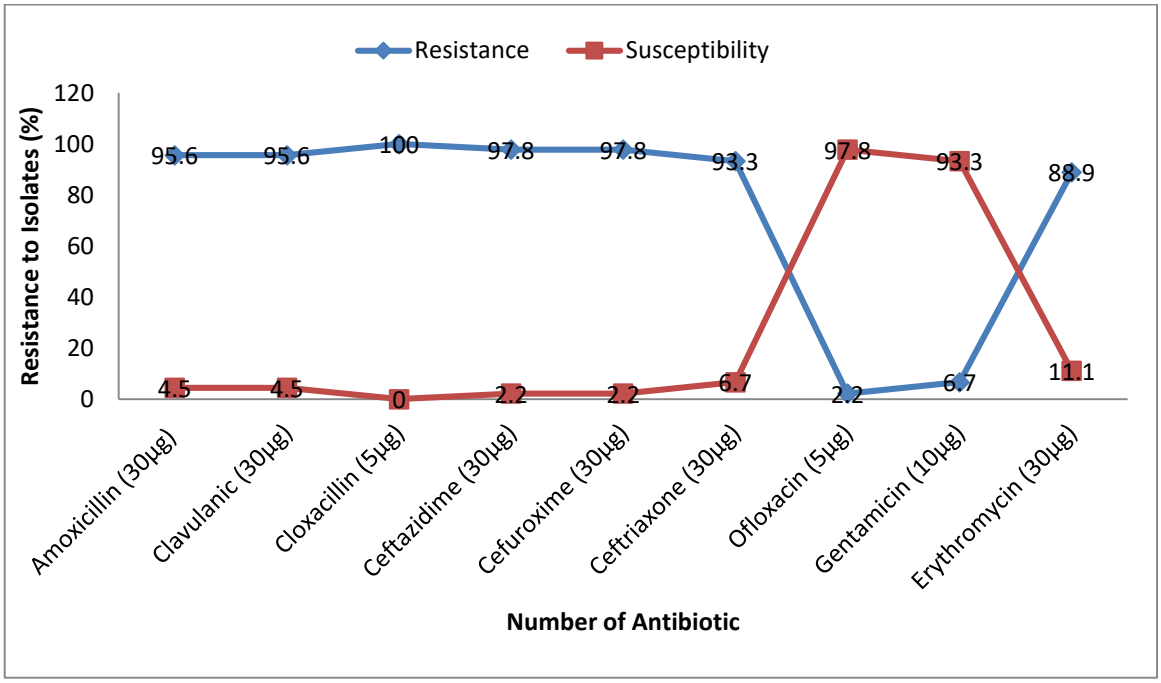
4.6: Antibiotic multi resistance pattern of gram positive bacterial isolates from refuse dump site before plasmid curing

The antibiotic resistance profiles of the 45 bacterial isolates from the three refuse dumpsites to the different antibiotics used and the antibiotic content of the disc and the resistance breakpoint used is shown in Table 4.4, while the graph illustration of Antibiotic Multi Resistance Pattern of Gram Positive Bacterial Isolates from Refuse Dump Site before Plasmid Curing is shown in Graph 4.1. The antibiotic multy resistance profile of bacterial isolates to 8 Gram positive different antibiotics was determined. A greater resistance was observed against cloxacillin (45,100%), Ceftazidime (44, 97.8%), Cefuroxime (44, 97.8%) and Amoxicillin/Clavulanic (43, 95.6%), while antibiotics such as Ceftriaxone (42, 93.3%) and erythromycin (40, 88.9%), had moderate resistance. The antibiotics Gentamicin (3, 6.7%) and Ofloxacin (1, 2.2%) were the most active.

Tabe 4.4: Antibiotic Multi Resistance Pattern (%) of Gram Positive Bacterial Isolates from Refuse Dump Sites before Plasmid Curing.

Group	Antibiotic (COD)	Resistance (%)	Susceptibity	RBP(mm)
Penicillin	Amoxicillin/Clavulanic (30µg)	43(95.6)	2(4.5)	<13
Penicillin	Cloxacillin (5µg)	45(100)	0(0)	< 10
Cephalosporin	Ceftazidime (30µg)	44 (97.8)	1(2.2)	< 17
Cephalosporin	Cefuroxime (30µg)	44 (97.8)	1(2.2)	< 14
Cephalosporin	Ceftriaxone (30µg)	42 (93.3)	3 (6.7)	< 19
Fluoroquinolone	Ofloxacin (5µg)	1 (2.2)	44 (97.8)	< 12
Aminoglycosides	Gentamicin (10µg)	3 (6.7)	42 (93.3)	< 12
Macrolides	Erythromycin (30µg)	40 (88.9)	5 (11.1)	< 14

COD = Antibiotic content of the Disc. RBP = Resistance Breakpoint.



Graph 4.1: Doughnut Graph Illustration of Antibiotic Multi Resistance Pattern of Gram Positive Bacterial Isolates from Refuse Dump Site before Plasmid Curing

4.7: Antibiotic resistance pattern of bacterial isolate before curing.

The antibiotic resistance pattern (%) of the bacterial isolates before plasmid curing for Gram negative isolates is shown in Table 4.5. Results revealed that most of the isolates were resistant to one or more antibiotics and a significant number of isolate from the various dumpsites showed multiple antibiotic resistance property. All bacterial isolates were observed resistance to Amoxicillin/clavulence acid, Ceftazidime, Cefuroxime, Ampicillin, and Gentamicin except *Serratia* sp and *aeromonas* sp where observed susceptible to Gentamicin, but in case of Ofloxacin and Ciprofloxacin all the bacterial isolates were observed susceptible except two isolates from *Klebsiella* sp and *Pseudomonas* sp where resistance to Ofloxacin and Ciprofloxacin alongside *Acinetobacter* sp where observed resistance to Ciprofloxacin and was susceptible to Ofloxacin.

Table 4.5: Antibiotics Resistances pattern (%) of Gram Negative Bacteria Isolates before curing

Bacterial isolates	Number (%) resistance							
	Amoxicillin /clavulinic	**Ceftazidime**	**Cefuroxime**	**Gentamicin**	**Ofloxacin**	**Ampicillin**	**Ciprofloxacin**	**Nitrofurantoin**
Citrobacter sp *(N=2)*	1(50)	2(100)	1(50)	2(100)	0(0)	2(100)	0(0)	0(0)
Proteus sp *(N=3)*	3(100)	2(66.7)	3(100)	1(33.3)	0(0)	3(100)	0(0)	2(66.7)
Acinetobacter sp *(N=2)*	2(100)	2(100)	1(50)	1(50)	0(0)	2(100)	1(50)	0(0)
Serretia sp *(N=1)*	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	0(0)
Klebsiella sp *(N=1)*	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)
Pseudomonas sp *(N=3)*	3(100)	3(100)	3(100)	1(33.3)	3(100)	3(100)	1(33.3)	1(33.3)
Aeromonas sp *(N=4)*	4(100)	4(100)	4(100)	0(0)	0(0)	4(100)	4(100)	0(0)

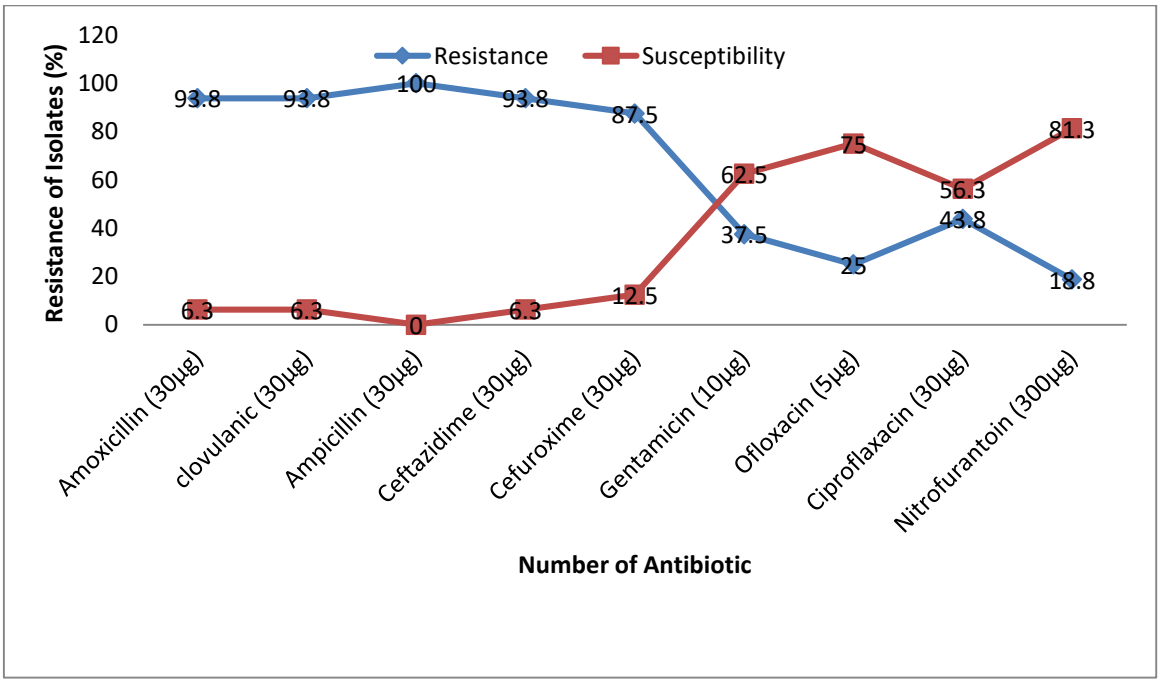
4.8: Antibiotic multi-resistance pattern of gram negative bacterial isolates from refuse dump site before plasmid curing.

The antibiotic resistance profiles of the 16 bacterial isolates from the three refuse dumpsites to the different antibiotics used and the antibiotic content of the disc and the resistance breakpoint used is shown in Table 4.6, while the graph illustration of Antibiotic Multi Resistance Pattern of Gram nigative Bacterial Isolates from Refuse Dump Site before Plasmid Curing is shown in Graph 4.2. The antibiotic multi resistance profile of bacterial isolates to 8 Gram negative different antibiotics was determined. A greater resistance was observed against Ampicillin (16, 100%), Ceftazidime (15, 93.8%), Amoxicillin/Clavulanic (15, 93.8%), and Cefuroxime (14, 87.5%) while antibiotics such as Ciprofloxacin (7, 43.8%) and Gentamicin (6, 37.5%), had moderate resistance. The antibiotics Ofloxacin (4, 25%) and Nitrofurantoin (3, 18.8%) were the most active.

Table 4.6: Antibiotic Multi Resistance Pattern (%) of Gram Negative Bacterial Isolates from Refuse Dump Sites before Plasmid Curing

Group	Antibiotic (COD)	Resistance (%)	Susceptibity	RBP(mm)
Penicillin	Amoxicillin/Clavulanic (30µg)	15(93.8)	1(6.3)	<13
Penicillin	Ampicillin (30µg)	16(100)	0(0)	< 13
Cephalosporin	Ceftazidime (30µg)	15 (93.8)	1(6.3)	< 14
Cephalosporin	Cefuroxime (30µg)	14 (87.5)	2(12.5)	< 14
Fluoroquinolone	Ofloxacin (5µg)	4 (25)	12 (75)	< 12
Fluoroquinolone	Ciprofloxacin (30µg)	7 (43.8)	9 (56.3)	< 15
Aminoglycosides	Gentamicin (10µg)	6 (37.5)	10 (62.5)	< 12
Azolidines	Nitrofurantoin (300µg)	3 (18.8)	13 (81.3)	< 14

COD = Antibiotic content of the Disc. RBP = Resistance Breakpoint.



Graph 4.2: Doughnut Graph Illustration of Antibiotic Multi Resistance Pattern of Gram Negative Bacterial Isolates from Refuse Dump Site before Plasmid Curing

4.9: Chromium (Cr^{6+}) susceptibility test before curing

The heavy metal susceptibility test of the antibiotic resistance bacterial isolates before curing is shown in Table 4.7. It was observed that the bacterial isolates from the various dumpsites were found to have multiple heavy metal and antibiotics co-resistance property. The sixty one (61) bacterial isolates from the dumpsites showed a wide range of minimum inhibitory concentration (MIC) values for the chromium (Cr^{6+}) tested heavy metals. 55 out of 61 bacteria isolates has MIC of 200mg/l while 6 have MIC of 250 mg/l respectively. Although some of the bacteria isolate show high resistance pattern than other such as *Pseudomonas* sp in Ugb2, *Bacillus* sp eff2, and *Klebsiella* sp in Obia2 were observed to have the highest resistance pattern of the 3 tested heavy metal at 1050mg/l for Cd^{2+}, 300mg/l for Cu^{2+} and 250mg/l for Cr^{6+}.

Table 4.7: Minimum Inhibitory Concentration (mg/l) of chromium on Bacterial Isolates before Curing

Isolates	Concentration			
	100mg/l	150mg/l	200mg/l	250mg/l
❖ 1	+	+	+	_
2	+	+	_	_
3	+	+	+	_
❖ 4	+	+	+	_
5	+	+	+	_
6	+	+	+	_
7	+	+	_	_
8	+	+	_	_
9	+	+	_	_
10	+	+	_	_
11	+	+	_	_
12	+	+	_	_
13	+	+	_	_
14	+	+	_	_
15	+	+	_	_
16	+	+	_	_
17	+	+	_	_
18	+	+	_	_
19	+	+	_	_
20	+	+	_	_
21	+	+	_	_
22	+	+	_	_
23	+	+	_	_
24	+	+	_	_
25	+	+	_	_
26	+	+	_	_
27	+	+	_	_
28	+	+	_	_
29	+	+	_	_
30	+	+	_	_
31	+	+	_	_
32	+	+	_	_
33	+	+	_	_
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38	+	+	_	_
39	+	+	_	_
40	+	+	_	_
41	+	+	_	_

42	+	+	_	_
43	+	+	_	_
44	+	+	_	_
45	+	+	_	_
46	+	+	_	_
47	+	+	_	_
48	+	+	_	_
49	+	+	_	_
50	+	+	_	_
51	+	+	_	_
52	+	+	_	_
53	+	+	_	_
54	+	+	_	_
55	+	+	_	_
56	+	+	_	_
57	+	+	_	_
58	+	+	_	_
59	+	+	_	_
60	+	+	_	_
❖ 61	+	+	+	_

Key:
+ = Growth
- = No Growth
❖ = selected isolate for further test

Isolate 1 to 3 = *Pseudomonas* sp, Isolate 4 to 13 = *Bacillus* sp, Isolate 14 to 22 = *Micrococcus* sp, Isolate 23 to 29 = *Staphylococcus* sp, Isolate 30 to 33 = *Kurthia* sp, Isolate 34 to 37 = *Aeromonas* sp, Isolate 38 to 41 = *Arthrobacter* sp, Isolate 42 to 44 = *Listeria* sp, Isolate 45 = *Corynebacterium* sp, Isolate 46 = *Erydipelothrix* sp, Isolate 47 to 49 = *Nocardia* sp, Isolate 50 = C*ellulosimicrobium* sp, Isolate 51 = *Enterobacter* sp, and Isolate 52 = *Clostridium* sp. 55 to 57 = *proteus* sp, Isolate 58 to 59 = *Acinetobacter* sp, Isolate 60 = *Serratia* sp, Isolate 61 = *Klebsiella* sp

4.10: Copper (Cu^{2+}) susceptibility test before curing

The heavy metal susceptibility test of the antibiotic resistance bacterial isolates before curing is shown in Table 4.8. It was observed that the bacterial isolates from the various dumpsites were found to have multiple heavy metal and antibiotics co-resistance property. The sixty one (61) bacterial isolates from the dumpsites showed a wide range of minimum inhibitory concentration (MIC) values for the copper (Cu^{2+}) tested heavy metals. 56 out of 61 bacteria isolates has MIC of 250mg/l while 5 have MIC of 300 mg/l respectively. Although some of the bacteria isolate show high resistance pattern than other such as *Pseudomonas* sp in Ugb2, *Bacillus* sp eff2, and *Klebsiella* sp in Obia2 were observed to have the highest resistance pattern of the 3 tested heavy metal at 1050mg/l for Cd^{2+}, 300mg/l for Cu^{2+} and 250mg/l for Cr^{6+}.

Table 4.8: Minimum Inhibitory Concentration (mg/l) of copper on Bacterial Isolates before Curing.

Isolates	Concentration				
	100 mg/l	150mg/l	200mg/l	250mg/l	300mg/l
❖ 1	+	+	+	+	-
2	+	+	+	-	-
3	+	+	+	-	-
❖ 4	+	+	+	+	-
5	+	+	+	+	-
6	+	+	+	-	-
7	+	+	+	-	-
8	+	+	+	-	-
9	+	+	+	-	-
10	+	+	+	+	-
11	+	+	+	-	-
12	+	+	+	-	-
13	+	+	+	-	-
14	+	+	+	-	-
15	+	+	+	-	-
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17	+	+	+	-	-
18	+	+	+	-	-
19	+	+	+	-	-
20	+	+	+	-	-
21	+	+	+	-	-
22	+	+	+	-	-
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26	+	+	+	-	-
27	+	+	+	-	-
28	+	+	+	-	-
29	+	+	+	-	-
30	+	+	+	-	-
31	+	+	+	-	-
32	+	+	+	-	-
33	+	+	+	-	-
34	+	+	+	-	-
35	+	+	+	-	-
36	+	+	+	-	-
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38	+	+	+	-	-
39	+	+	+	-	-
40	+	+	+	-	-
41	+	+	+	-	-
42	+	+	+	-	-
43	+	+	+	-	-
44	+	+	+	-	-
45	+	+	+	-	-
46	+	+	+	-	-
47	+	+	+	-	-

48	+	+	+	-	-
49	+	+	+	-	-
50	+	+	+	-	-
51	+	+	+	-	-
52	+	+	+	-	-
53	+	+	+	-	-
54	+	+	+	-	-
55	+	+	+	-	-
56	+	+	+	-	-
57	+	+	+	-	-
58	+	+	+	-	-
59	+	+	+	-	-
60	+	+	+	-	-
❖ 61	+	+	+	+	-

Key:
+ = Growth
- = No Growth
❖ = selected isolate for further test

Isolate 1 to 3 = *Pseudomonas* sp, Isolate 4 to 13 = *Bacillus* sp, Isolate 14 to 22 = *Micrococcus* sp, Isolate 23 to 29 = *Staphylococcus* sp, Isolate 30 to 33 = *Kurthia* sp, Isolate 34 to 37 = *Aeromonas* sp, Isolate 38 to 41 = *Arthrobacter* sp, Isolate 42 to 44 = *Listeria* sp, Isolate 45 = *Corynebacterium* sp, Isolate 46 = *Erydipelothrix* sp, Isolate 47 to 49 = *Nocardia* sp, Isolate 50 = C*ellulosimicrobium* sp, Isolate 51 = *Enterobacter* sp, and Isolate 52 = *Clostridium* sp. 55 to 57 = *proteus* sp, Isolate 58 to 59 = *Acinetobacter* sp, Isolate 60 = *Serratia* sp, Isolate 61 = *Klebsiella* sp

4.11: Cadmium (Cd^{2+}) susceptibility test before curing

The heavy metal susceptibility test of the antibiotic resistance bacterial isolates before curing is shown in Table 4.9. It was observed that the bacterial isolates from the various dumpsites were found to have multiple heavy metal and antibiotics co-resistance property. The sixty one (61) bacterial isolates from the dumpsites showed a wide range of minimum inhibitory concentration (MIC) values for the cadmium (Cd^{2+}) tested heavy metals. 56 out of 61 bacteria isolates has MIC of 1000mg/l while 5 have MIC of 1050 mg/l respectively. Although some of the bacteria isolate show high resistance pattern than other such as *Pseudomonas* sp in Ugb2, *Bacillus* sp eff2, and *Klebsiella* sp in Obia2 were observed to have the highest resistance pattern of the 3 tested heavy metal at 1050mg/l for Cd^{2+}, 300mg/l for Cu^{2+} and 250mg/l for Cr^{6+}.

Table 4.9: Minimum Inhibitory Concentration (mg/l) of Cadmium on Bacterial Isolates before Curing.

Isolates	Concentration mg/l				
	100	**200**	**300**	**400**	**500**
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	+
10	+	+	+	+	+
11	+	+	+	+	+
12	+	+	+	+	+
13	+	+	+	+	+
14	+	+	+	+	+
15	+	+	+	+	+
16	+	+	+	+	+
17	+	+	+	+	+
18	+	+	+	+	+
19	+	+	+	+	+
20	+	+	+	+	+
21	+	+	+	+	+
22	+	+	+	+	+
23	+	+	+	+	+
24	+	+	+	+	+
25	+	+	+	+	+
26	+	+	+	+	+
27	+	+	+	+	+
28	+	+	+	+	+
29	+	+	+	+	+
30	+	+	+	+	+
31	+	+	+	+	+
32	+	+	+	+	+
33	+	+	+	+	+
34	+	+	+	+	+
35	+	+	+	+	+
36	+	+	+	+	+
37	+	+	+	+	+
38	+	+	+	+	+
39	+	+	+	+	+
40	+	+	+	+	+
41	+	+	+	+	+
42	+	+	+	+	+
43	+	+	+	+	+
44	+	+	+	+	+
45	+	+	+	+	+
46	+	+	+	+	+
47	+	+	+	+	+

48	+	+	+	+	+
49	+	+	+	+	+
50	+	+	+	+	+
51	+	+	+	+	+
52	+	+	+	+	+
53	+	+	+	+	+
54	+	+	+	+	+
55	+	+	+	+	+
56	+	+	+	+	+
57	+	+	+	+	+
58	+	+	+	+	+
59	+	+	+	+	+
60	+	+	+	+	+
61	+	+	+	+	+

	600	**700**	**800**	**900**	**1000**	**1050**
1)	+	+	+	+	+	❖ -
2)	+	+	+	+	-	-
3)	+	+	+	+	+	-
4)	+	+	+	+	+	❖ -
5)	+	+	+	+	-	-
6)	+	+	+	+	-	-
7)	+	+	+	+	+	-
8)	+	+	+	+	-	-
9)	+	+	+	+	-	-
10)	+	+	+	+	-	-
11)	+	+	+	+	-	-
12)	+	+	+	+	-	-
13)	+	+	+	+	-	-
14)	+	+	+	+	-	-
15)	+	+	+	+	-	-
16)	+	+	+	+	-	-
17)	+	+	+	+	-	-
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21)	+	+	+	+	-	-
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24)	+	+	+	+	-	-
25)	+	+	+	+	-	-
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33)	+	+	+	+	-	-
34)	+	+	+	+	-	-
35)	+	+	+	+	-	-
36)	+	+	+	+	-	-

37)	+	+	+	+	-	-
38)	+	+	+	+	-	-
39)	+	+	+	+	-	-
40)	+	+	+	+	-	-
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43)	+	+	+	+	-	-
44)	+	+	+	+	-	-
45)	+	+	+	+	-	-
46)	+	+	+	+	-	-
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48)	+	+	+	+	-	-
49)	+	+	+	+	-	-
50)	+	+	+	+	-	-
51)	+	+	+	+	-	-
52)	+	+	+	+	-	-
53)	+	+	+	+	-	-
54)	+	+	+	+	-	-
55)	+	+	+	+	-	-
56)	+	+	+	+	-	-
57)	+	+	+	+	-	-
58)	+	+	+	+	-	-
59)	+	+	+	+	-	-
60)	+	+	+	+	-	-
61)	+	+	+	+	+	❖ -

Key:
+ = Growth
- = No Growth
❖ = selected isolate for further test
Isolate 1 to 3 = *Pseudomonas* sp, Isolate 4 to 13 = *Bacillus* sp, Isolate 14 to 22 = *Micrococcus* sp, Isolate 23 to 29 = *Staphylococcus* sp, Isolate 30 to 33 = *Kurthia* sp, Isolate 34 to 37 = *Aeromonas* sp, Isolate 38 to 41 = *Arthrobacter* sp, Isolate 42 to 44 = *Listeria* sp, Isolate 45 = *Corynebacterium* sp, Isolate 46 = *Erydipelothrix* sp, Isolate 47 to 49 = *Nocardia* sp, Isolate 50 = C*ellulosimicrobium* sp, Isolate 51 = *Enterobacter* sp, and Isolate 52 = *Clostridium* sp. 55 to 57 = *proteus* sp, Isolate 58 to 59 = *Acinetobacter* sp, Isolate 60 = *Serratia* sp, Isolate 61 = *Klebsiella* sp

4.12: Molecular identification of *Pseudomonas* sp.

Agarose gel electrophoresis of the DNA of *Pseudomonas aeruginosa* isolated from refuse dump soils is shown in Plate 4.1. The 16srDNA gene sequence analysis and DNA profile band results for the *Pseudomonas* sp isolate were analyzed with 1.5% agarose gel electrophoresis. L is 100bp-1000bp DNA ladder (molecular marker) for genus with sequence PA-GS-F 5′-GACGGGTGAGTAATGCCTA-3′ and PA-GS-R 5′-CACTGGTGTTCCTTCCTATA-3′ why capsule with sequence Pa16S-F 5'-GGGGGATCTTCGGACCTCA -3' and Pa16S-R 5'-TCCTTAGAGTGCCCACCCG-3' in *Pseudomonas aeruginosa* (Ugds1) against *Pseudomonas* sp (Ugds2) from initial Ugborikoko dumpsite 2 had a closest relatedness with that of *Pseudomonas* sp at the generic level with bands at 500base pair, it also had a closest relatedness with that of *Pseudomonas aeruginosa* at the species level with bands at 400bp .

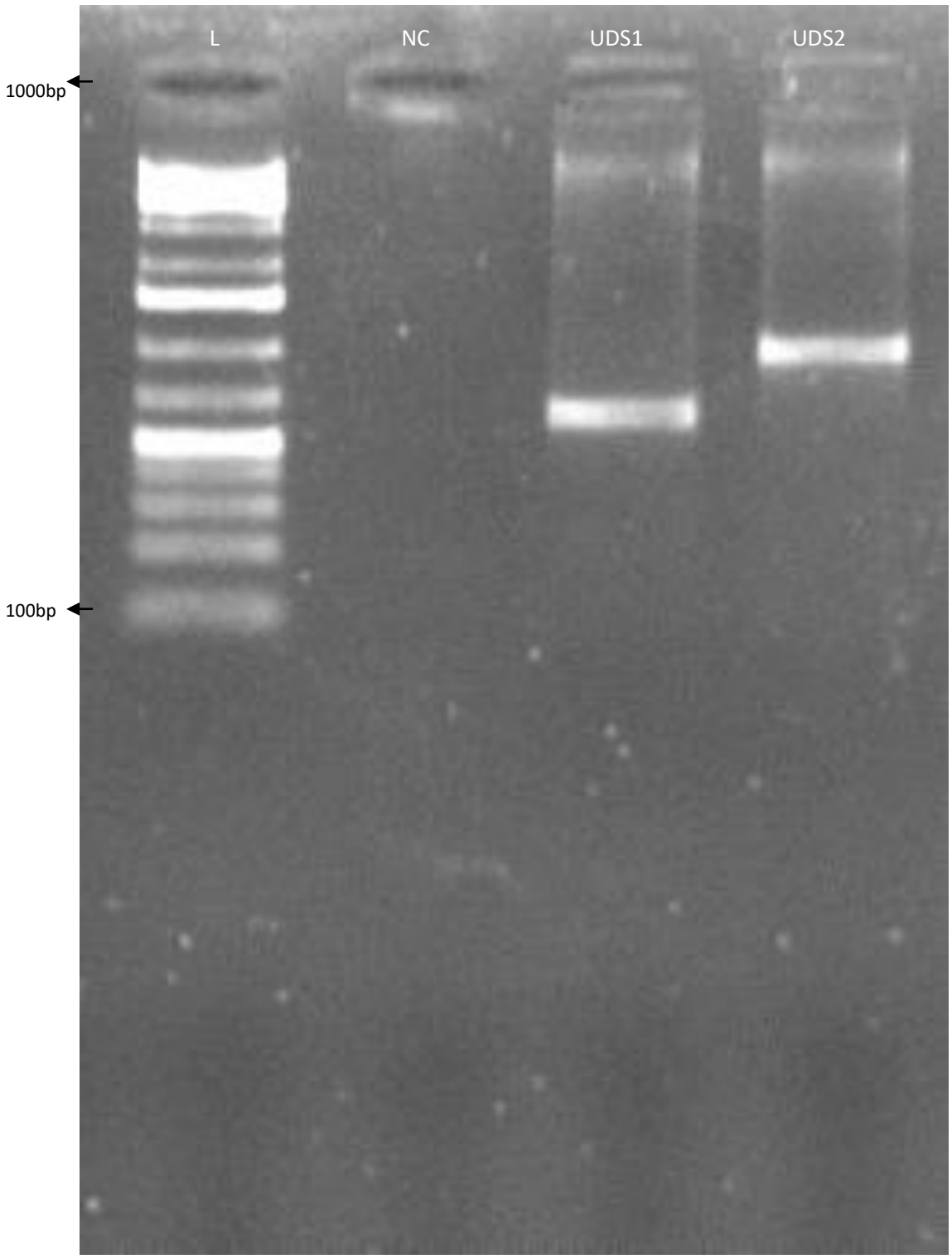


Plate 4.1: Agarose gel electrophoresis of the DNA of *Pseudomonas aeruginosa* isolated from refuse dump soils.

Key: UDS= Ugborikoko dumpsite, L = Ladder and NC= the no DNA template control.

4.13: Size and distance travel in gel of each DNA band of QuantDNA1000bp-100bp^{TM} DNA ladder/DNA marker at 5-10 ul loading

The exact distance travel in gel of each DNA band in base pairs is listed in the Table 4.10, while the generic and species level is listed at Table 4.11. Each DNA fragment contains 4 base single strand 5' overhangs at both ends. These overhangs allow convenient labeling of these DNA fragments and do not affect the migration properties of the DNA bands. The actual size of each DNA fragment runs at the apparent size on a agarose gel including a sequencing gel. Since each DNA fragment has 4-bases 5' overhangs at both ends, its migration property on a gel is indistinguishable from a blunt end DNA fragment of same molecular weight. This DNA ladder has 11 bands from smallest 100bp to largest 1000bp. The distance travel in gel of the DNA bands in the DNA ladder is 1-1.5mm. The highest distance DNA band is the 1000bp with 1.5mm distance at the 1st band which is closely followed by 6th and 11th band with distance travel in gel of 1mm at base pair of 550 and 100 but the rest was 0.5mm distance travel in gel from each other.

Table 4.10: DNA Size of Standard Molecular Marker

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
1st band	1.5	1000
2nd band	2	950
3rd band	2.5	850
4th band	3.5	750
5th band	4	650
6th band	5	550
7th band	5.5	500
8th band	6	450
9th band	6.5	400
10th band	7	300
11th band	8	100

Table 4.11: DNA Size in base pair of *pseudomonas aeruginosa* isolated from refuse dump soils

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
NC	Nil	Nil
UDS1	4	650
UDS2	5	550

4.14: Molecular identification of *Bacillus* sp.

Agarose gel electrophoresis of the DNA of *Bacillus subtilis* isolated from refuse dump soils is shown in Plate 4.2. The 16srDNA gene sequence analysis and DNA profile band results for the *Bacillus* sp eff 2 isolates were analyzed with 1.5% agarose gel electrophoresis. L is 100bp-1000bp DNA ladder (molecular marker) for genus with sequence BacF5^{I}-AGGGTCATTGGAAACTGGG-3^{I} and BacR5^{I}-CGTGTTGTAGCCC AGGTCATA-3^{I} why capsule with sequence EN1F-5^{I}-CCAGTAGCCAAGAAT GGCCAGC-3^{I} and EN1R-5^{I}-GGAATAATCGCCGCTTTGTGC-3^{I} in *Bacillus subtilis* (EDS1) against *Bacillus* sp EDS2 from initial Effurun dumpsite 2 had a closest relatedness with that of *Bacillus* sp at the generic level with bands at 350bp, it also had a closest relatedness with that of *Bacillus subtilis* at the species level with band at 100bp.

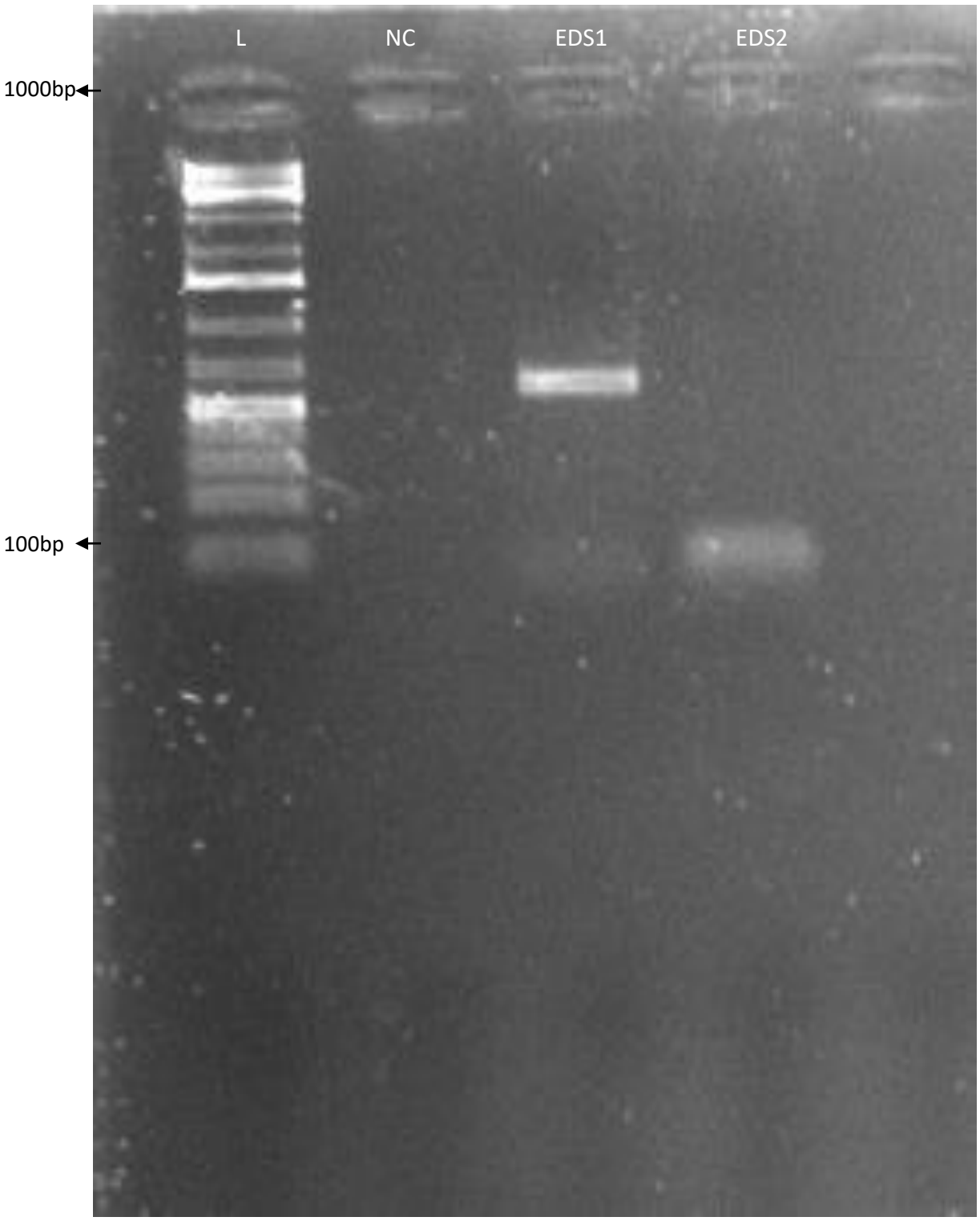


Plate 4.2: Agarose gel electrophoresis of the DNA of *Bacillus subtilis* isolated from refuse dump soils.

Key: EDS= Effurun dumpsite, L = Ladder and NC= the no DNA template control.

4.15: Size and distance travel in gel of each DNA band of QuantDNA1000bp-100bp^{TM} DNA ladder/DNA marker at 5-10 ul loading

The exact distance travel in gel of each DNA band in base pairs is listed in the Table 4.12, while the generic and species level is listed at Table 4.13. Each DNA fragment contains 4 base single strand 5' overhangs at both ends. These overhangs allow convenient labeling of these DNA fragments and do not affect the migration properties of the DNA bands. The actual size of each DNA fragment runs at the apparent size on an agarose gel including a sequencing gel. Since each DNA fragment has 4-bases 5' overhangs at both ends, its migration property on a gel is indistinguishable from a blunt end DNA fragment of same molecular weight. This DNA ladder has 11 bands from smallest 100bp to largest 1000bp. The distance travel in gel of the DNA bands in the DNA ladder is 1-1.5mm. The highest distance DNA band is the 1000bp with 1.5mm distance at the 1st band which is closely followed by 5th and 11th band with distance travel in gel of 1mm at base pair of 600 and 100 but the rest was 0.5mm distance travel in gel from each other.

Table 4.12: DNA Size of Standard Molecular Marker

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
1st band	1.5	1,000
2nd band	2	950
3rd band	2.5	800
4th band	3	700
5th band	4	600
6th band	4.5	500
7th band	5	400
8th band	5.5	350
9th band	6	300
10th band	6.5	250
11th band	7.5	100

Table 4.13: DNA Size in base pair of *Bacillus subtilis* isolated from refuse dump soils

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
NC	Nil	Nil
EDS1	4.5-5	500-400
EDS2	7.5	100

4.16: Molecular identification of *Klebsiella* sp.

Agarose gel electrophoresis of the DNA of *Klebsiella* sp isolated from refuse dump soils is shown in Plate 4.3. The 16srDNA gene sequence analysis and DNA profile band results for the *Klebsiella* sp isolate were analyzed with 1.5% agarose gel electrophoresis. L is 100bp-1000bp DNA ladder (molecular marker) for genus with sequence FD1- 5^{I}AGAGTTTGATCCTGGCTCAG3^{I} and RP2- 5^{I}AAG GAG GTG ATC CAG CC3^{I} why capsule with sequence RmpA gene F- 5^{I}ACTGGGCTACCTCTGCTTCA3^{I} and R 5^{I} CTTGCATGAGCCATCTTTCA3^{I} in *Klebsiella pneumonia* against *Klebsiella* sp obia 2 from Obiaruku dumpsite (ODS1) had a closest relatedness with that of *Klebsiella* sp at the generic level with bands at 200bp and while ODS2 didn't have a closest relatedness with that of *Klebsiella pneumonia* at the species level with bands at1000bp.

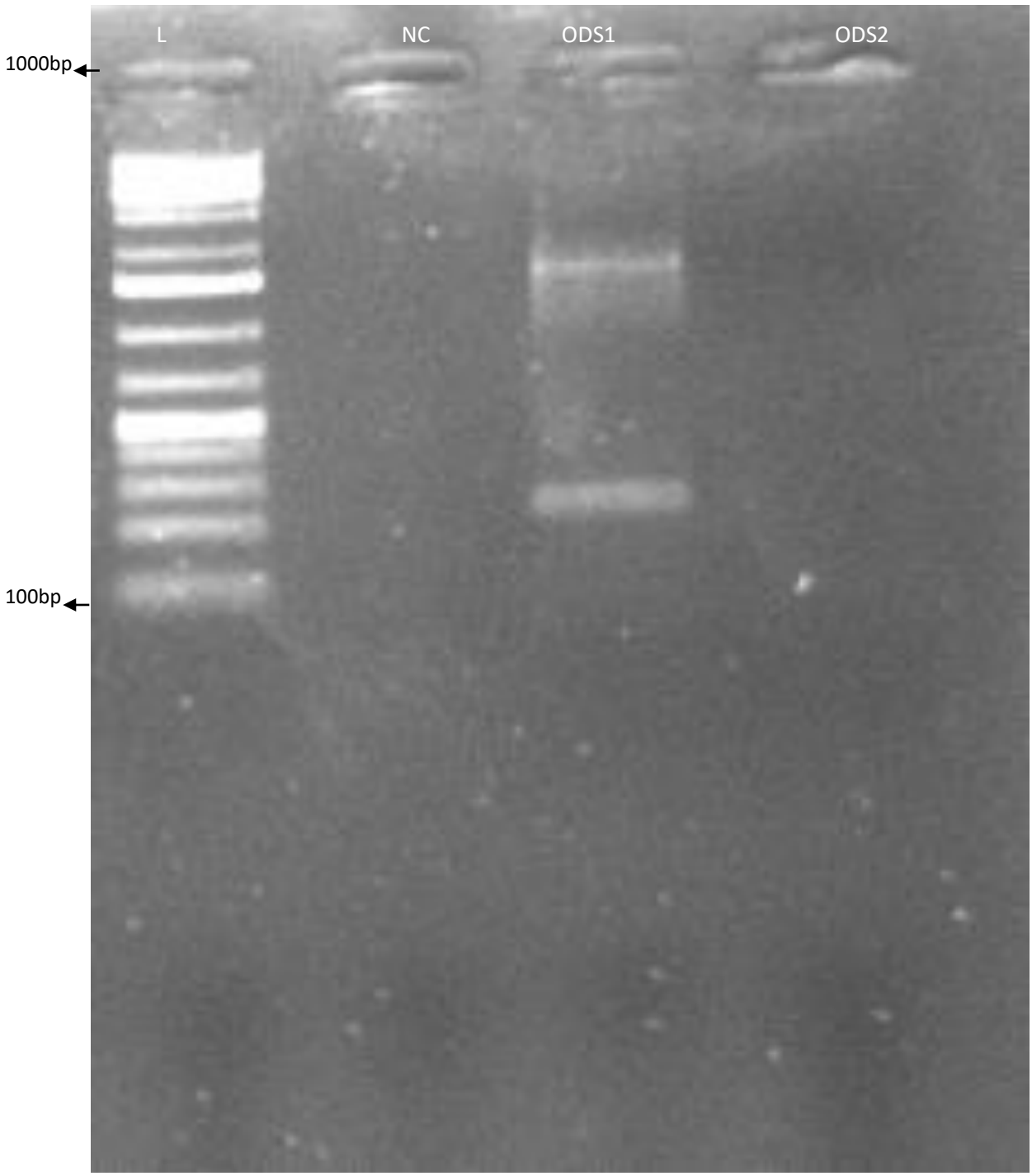


Plate 4.3: Agarose gel electrophoresis of the DNA of *Klebsiella species* isolated from refuse dump soils.

Key: ODS= Obiaroko dumpsite, L = Ladder and NC= the no DNA template control.

4.17: Size and distance travel in gel of each DNA band of QuantDNA1000bp-100bp^{TM} DNA ladder/DNA marker at 5-10 ul loading

The exact distance travel in gel of each DNA band in base pairs is listed in the Table 4.14, while the generic and species level is listed at Table 4.15. Each DNA fragment contains 4 base single strand 5' overhangs at both ends. These overhangs allow convenient labeling of these DNA fragments and do not affect the migration properties of the DNA bands. The actual size of each DNA fragment runs at the apparent size on a agarose gel including a sequencing gel. Since each DNA fragment has 4-bases 5' overhangs at both ends, its migration property on a gel is indistinguishable from a blunt end DNA fragment of same molecular weight. This DNA ladder has 11 bands from smallest 100bp to largest 1000bp. The distance travel in gel of the DNA bands in the DNA ladder is 1-1.5mm. The highest distance DNA band is the 1000bp with 1.5mm distance at the 1st band which is closely followed by 5th and 11th band with distance travel in gel of 1mm at base pair of 550 and 100 but the rest was 0.5mm distance travel in gel from each other.

Table 4.14: DNA Size of Standard Molecular Marker

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
1st band	1.5	1000
2nd band	2	950
3rd band	2.5	800
4th band	3	750
5th band	4	550
6th band	4.5	400
7th band	5.5	300
8th band	6	250
9th band	6.5	200
10th band	7	150
11th band	8	100

Table 4.15: DNA Size in base pair of *Klebsiella* sp isolated from refuse dump soils

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
NC	Nil	Nil
ODS1	2.5-6.5	800-250
ODS2	Nil	Nil

4.18: Plasmid DNA profile on *Pseudomonas aeruginosa*

Agarose gel electrophoresis of Plasmid DNA of multi drug resistant *Pseudomonas aeruginosa* isolated from refuse dump soils Plate 4.4. The result shows the plasmid profile of *Pseudomonas aeruginosa* which is among the 3 selected highly multiple drug resistant bacterial isolated from refuse dump soils at analysis of 0.8% agarose gel electrophoresis. L was 100bp-1000bp ladder (molecular marker) and it was observed that sample UDS appeared positive for plasmid genes at size of 330bp with travel distance of 13mm.

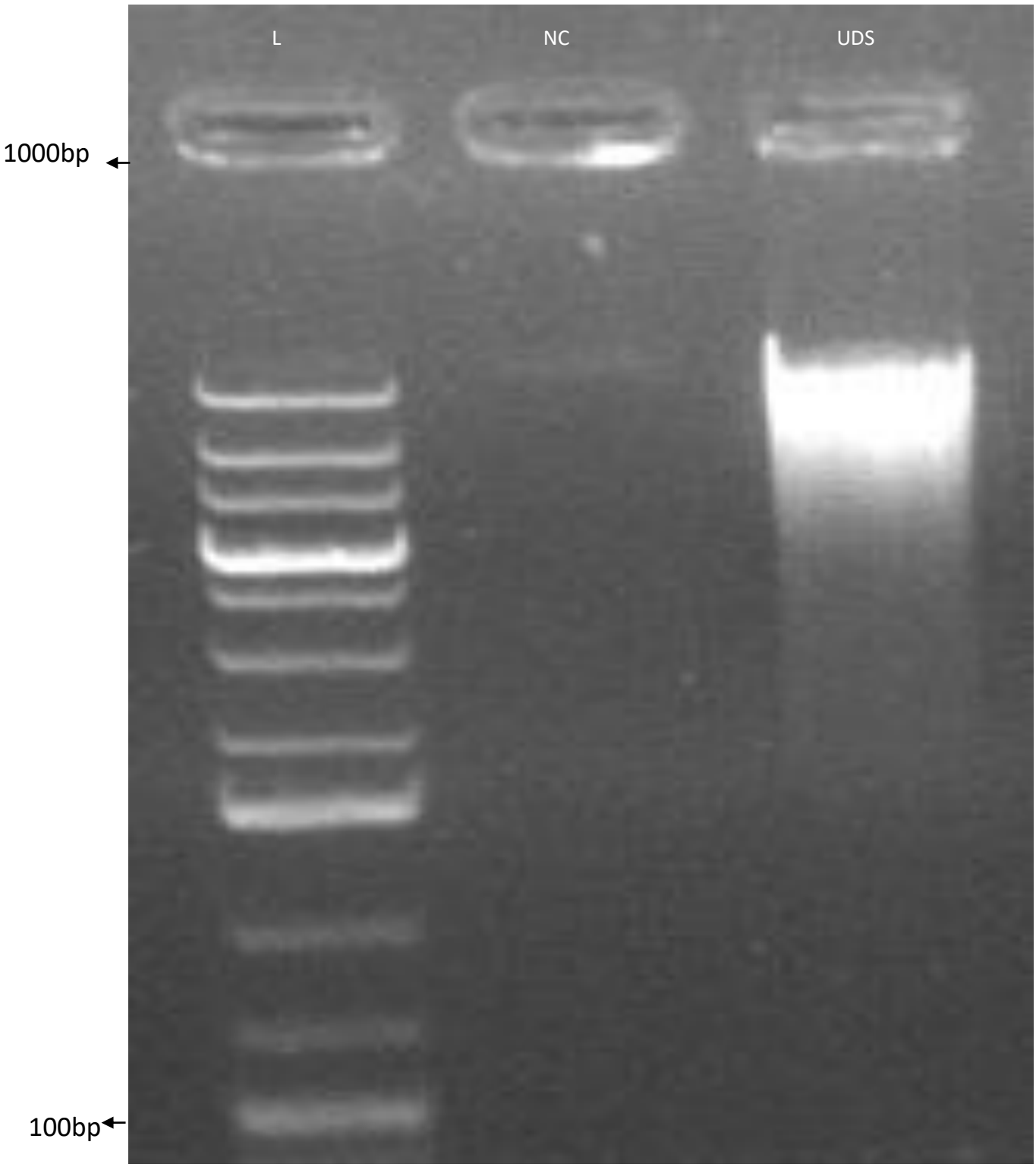


Plate 4.4: Agarose gel electrophoresis of Plasmid DNA of multi drug resistant *Pseudomonas aeruginosa* isolated from refuse dump soils.

Key: NC= the no template control, L = Ladder and ODS= Obiaroko dump site.

4.19: Size and distance travel in gel of each DNA band of QuantDNA1000bp-100bp^{TM} DNA ladder/DNA marker at 10 ul loading

The exact distance travel in gel of each DNA band in base pairs is listed in the Table 4.16, while the plasmid profile level is listed at Table 4.17. Each DNA fragment contains 4 base single strand 5' overhangs at both ends. These overhangs allow convenient labeling of these DNA fragments and do not affect the migration properties of the DNA bands. The actual size of each DNA fragment runs at the apparent size on a agarose gel including a sequencing gel. Since each DNA fragment has 4-bases 5' overhangs at both ends, its migration property on a gel is indistinguishable from a blunt end DNA fragment of same molecular weight. This DNA ladder has 11 bands from smallest 100bp to largest 1000bp. The distance travel in gel of the DNA bands in the DNA ladder is 1-1.5mm. The highest distance DNA band is the 1000bp with 4.5mm distance at the 1st band which is closely followed by 9th band with distance travel in gel of 2mm at base pair of 350 but the rest was 1.5-1mm distance travel in gel from each other.

Table 4.16: DNA Size of Standard Molecular Marker

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
1st band	4.5	1000
2nd band	5.5	950
3rd band	6.5	900
4th band	7.5	850
5th band	8	825
6th band	9	775
7th band	10.5	700
8th band	12	550
9th band	14	350
10th band	15.5	250
11th band	17	100

Table 4.17: DNA Size in base pair of Plasmid DNA of multi drug resistant *Pseudomonas aeruginosa* isolated from refuse dump soils

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
NC	**Nil**	**Nil**
UDS	**4.5-7.5**	**1000-700**

4.20: Plasmid DNA profile of *Bacillus subtilis*

Agarose gel electrophoresis of Plasmid DNA of multi drug resistant *Bacillus subtillis* isolated from refuse dump soils Plate 4.5. The result shows the plasmid profile of *Bacillus subtillis* which is among the 3 selected highly multiple drug resistant bacterial isolated from refuse dump soils at analysis of 0.8% agarose gel electrophoresis. L was 100bp-1000bp ladder (molecular marker) and it was observed that sample EDS appeared negative for plasmid genes at size of 330bp with travel distance of 13mm.

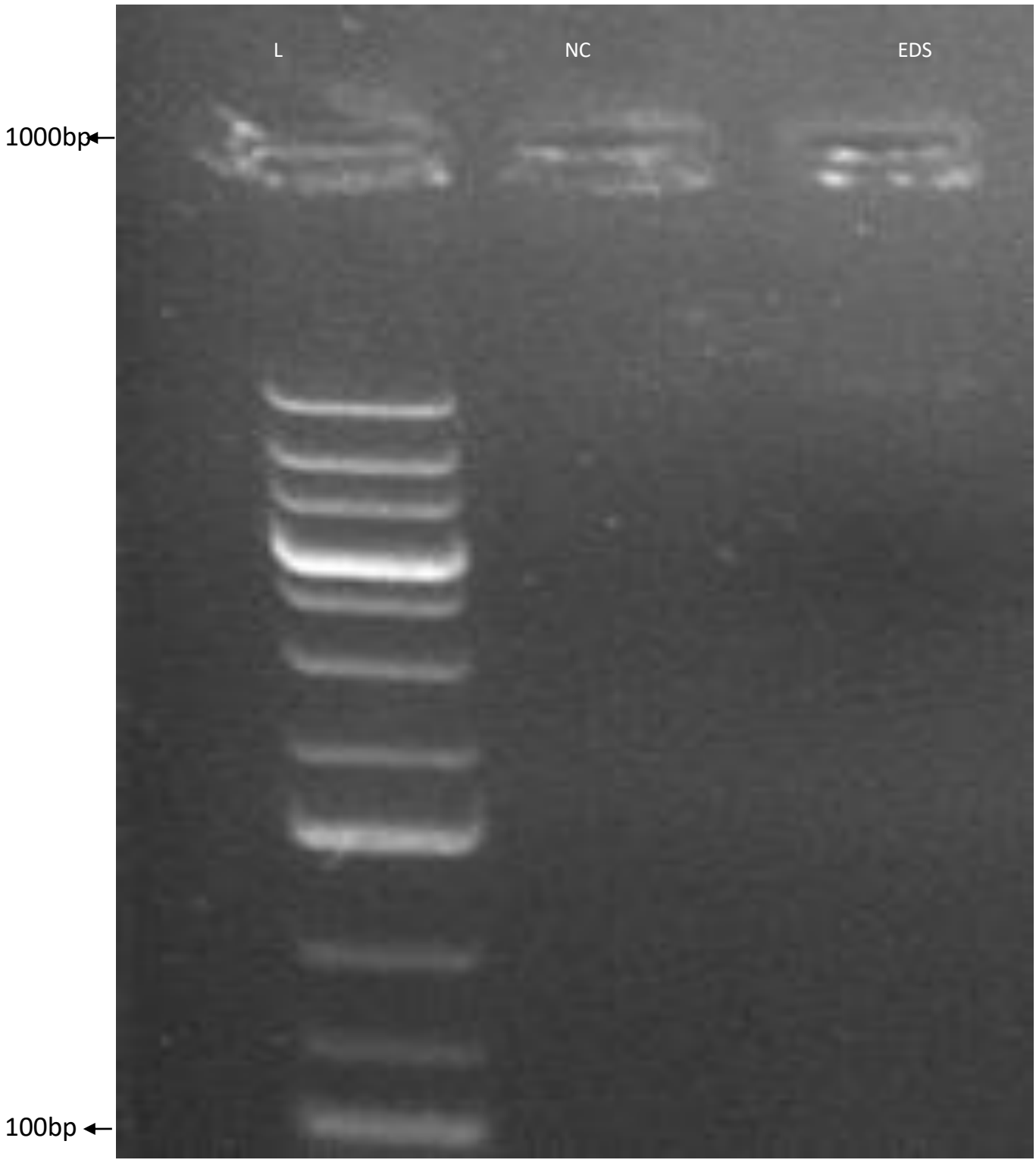


Plate 4.5: Agarose gel electrophoresis of Plasmid DNA of multi drug resistant *Bacillus subtilis* isolated from refuse dump soils.

Key: NC= the no template control, L = Ladder and EDS= Effurun dumpsite.

4.21: Size and distance travel in gel of each DNA band of QuantDNA1000bp-100bp^{TM} DNA ladder/DNA marker at 10 ul loading

The exact distance travel in gel of each DNA band in base pairs is listed in the Table 4.18, while the plasmid profile level is listed at Table 4.19. Each DNA fragment contains 4 base single strand 5' overhangs at both ends. These overhangs allow convenient labeling of these DNA fragments and do not affect the migration properties of the DNA bands. The actual size of each DNA fragment runs at the apparent size on a agarose gel including a sequencing gel. Since each DNA fragment has 4-bases 5' overhangs at both ends, its migration property on a gel is indistinguishable from a blunt end DNA fragment of same molecular weight. This DNA ladder has 11 bands from smallest 100bp to largest 1000bp. The distance travel in gel of the DNA bands in the DNA ladder is 1-1.5mm. The highest distance DNA band is the 1000bp with 4.5mm distance at the 1st band which is closely followed by 9th band with distance travel in gel of 2mm at base pair of 350 but the rest was 1.5-1mm distance travel in gel from each other.

Table 4.18: DNA Size of Standard Molecular Marker

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
1st band	4.5	1000
2nd band	5.5	950
3rd band	6	900
4th band	7	850
5th band	7.5	825
6th band	9	775
7th band	10.5	700
8th band	12	550
9th band	14	350
10th band	15.5	250
11th band	17	100

Table 4.19: DNA Size in base pair of Plasmid DNA of multi drug resistant *Bacillus subtilis* isolated from refuse dump soils

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
NC	Nil	Nil
EDS	Nil	Nil

4.22: Plasmid DNA profile of *Klebsiella* sp

Agarose gel electrophoresis of Plasmid DNA of multi drug resistant *Klebsiella* sp isolated from refuse dump soils Plate 4.6. The result shows the plasmid profile of *Klebsiella* sp which is among the 3 selected highly multiple drug resistant bacterial isolated from refuse dump soils at analysis of 0.8% agarose gel electrophoresis. L was 100bp-1000bp ladder (molecular marker) and it was observed that sample ODS appeared negative for plasmid genes at size of 330bp with travel distance of 13mm.

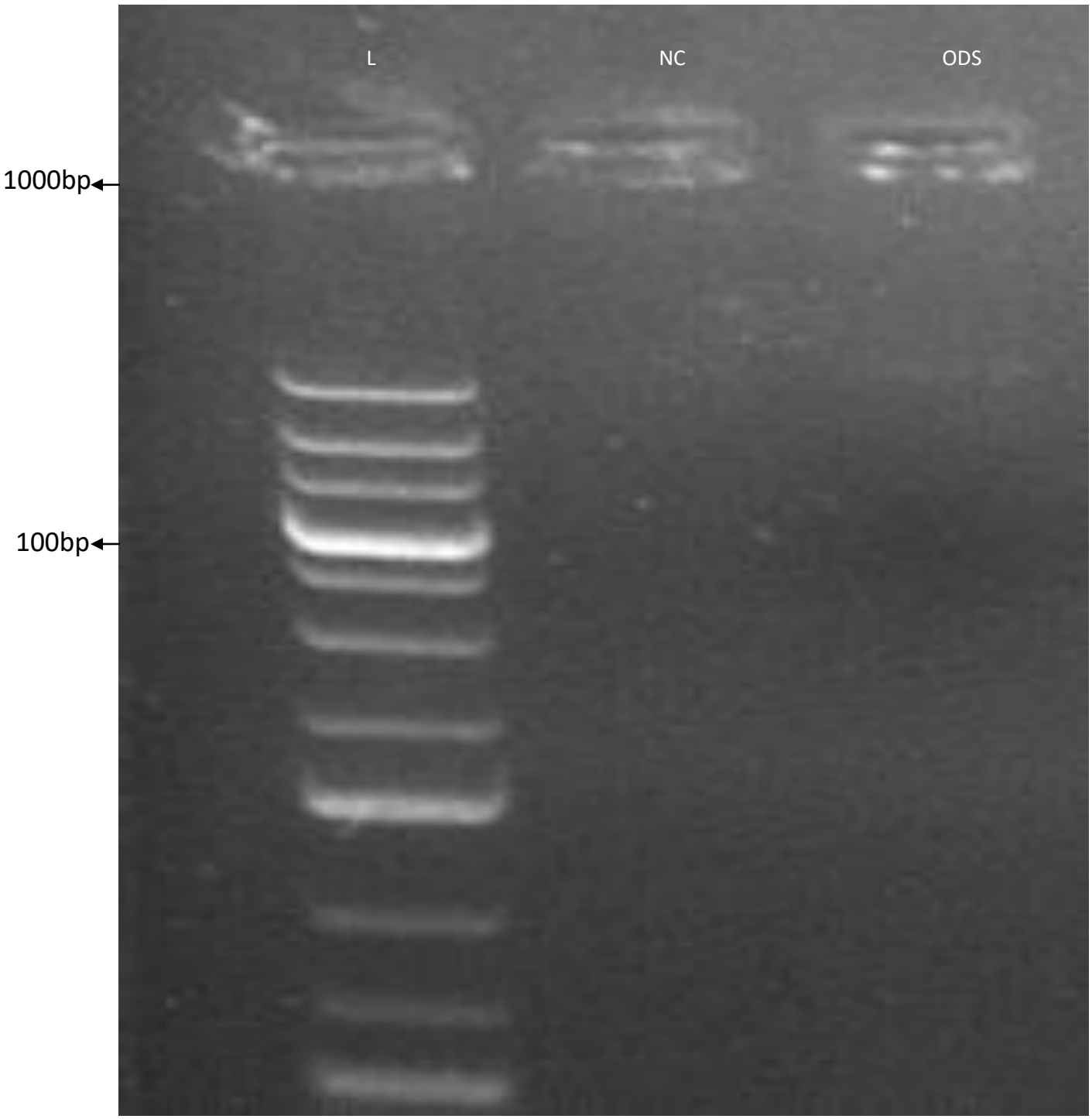


Plate 4.6: Agarose gel electrophoresis of Plasmid DNA of multi drug resistant *Klebsiella* sp isolated from refuse dump soils.

Key: NC= the no template control, L = Ladder and ODS = Obiaroko dumpsite

4.23: Size and distance travel in gel of each DNA band of QuantDNA1000bp-100bp^{TM} DNA ladder/DNA marker at 10 ul loading

The exact distance travel in gel of each DNA band in base pairs is listed in the table 4.20, while the plasmid profile level is listed at Table 4.21. Each DNA fragment contains 4 base single strand 5' overhangs at both ends. These overhangs allow convenient labeling of these DNA fragments and do not affect the migration properties of the DNA bands. The actual size of each DNA fragment runs at the apparent size on a agarose gel including a sequencing gel. Since each DNA fragment has 4-bases 5' overhangs at both ends, its migration property on a gel is indistinguishable from a blunt end DNA fragment of same molecular weight. This DNA ladder has 11 bands from smallest 100bp to largest 1000bp. The distance travel in gel of the DNA bands in the DNA ladder is 1-1.5mm. The highest distance DNA band is the 1000bp with 4mm distance at the 1st band which is closely followed by 9th band with distance travel in gel of 2mm at base pair of 175 but the rest was 1.5-0.5mm distance travel in gel from each other.

Table 4.20: DNA Size of Standard Molecular Marker

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
1st band	4	1000
2nd band	5	900
3rd band	5.5	850
4th band	6.5	750
5th band	7.5	650
6th band	8.5	550
7th band	9.5	450
8th band	11	325
9th band	13	175
10th band	14.5	125
11th band	15.5	100

Table 4.21: DNA Size in base pair of Plasmid DNA of multi drug resistant *Klebsiella* sp isolated from refuse dump soils

Marker Fragment	Distance travel in gel (mm)	DNA size in base pairs
NC	**Nil**	**Nil**
ODS	**Nil**	**Nil**

4.24: Antibiotics resistance pattern of *Bacillus subtilis* after curing.

The antibiotic resistance pattern (%) of *Bacillus subtilis* after curing is shown in Table 4.22. It was observed that *Bacillus subtilis* was having multi-drugs resistance to the entire Gram positive antibiotics except Gentamicin and Ofloxacin after curing.

Table 4.22: Antibiotic resistant pattern (%) of *Bacillus subtilis* after curing

Bacterial isolates	Number (%) resistance							
	Amoxicillin/ clavulinic	Ceftazidime	Cefuroxime	Gentamicin	Ofloxacin	Ceftriaxone	Erythromycin	Cloxacillin
Bacillus subtilis	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)

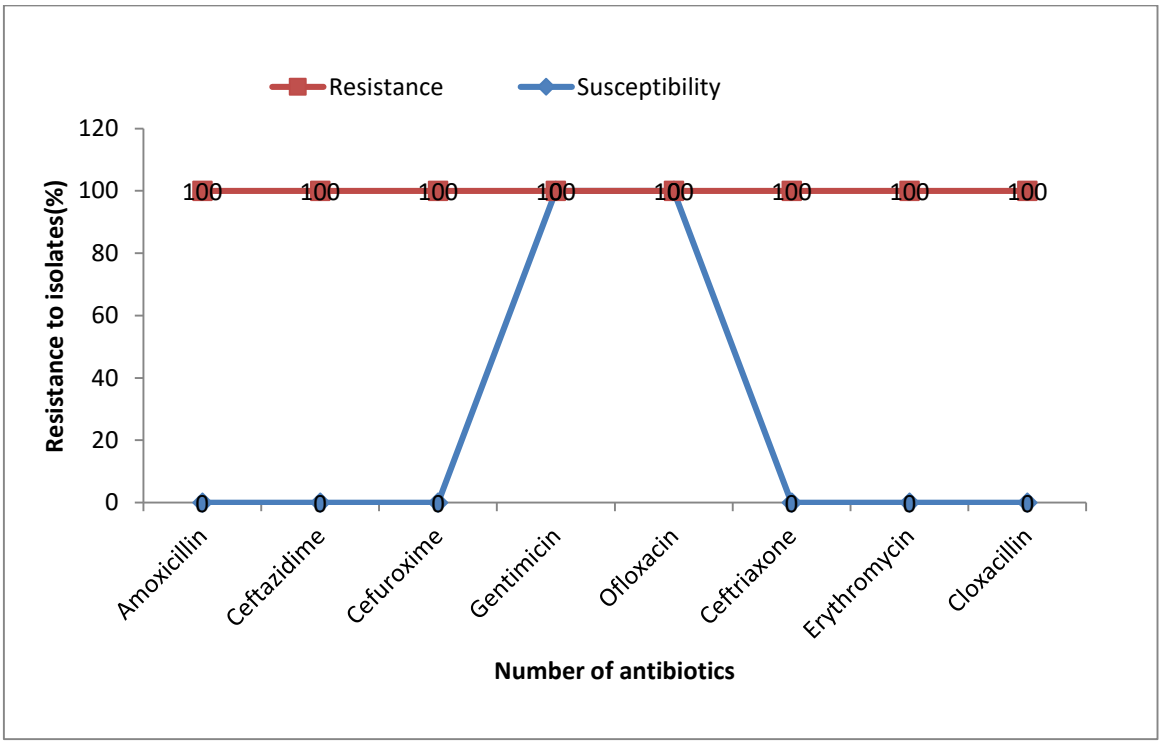
4.25: Antibiotic Multi Resistance Pattern of *Bacillus subtilis* from Refuse Dump Site after Plasmid Curing

The antibiotic resistance profiles of *Bacillus subtilis* from refuse dumpsites to the gram positive antibiotics used and the antibiotic content of the disc and the resistance breakpoint used is shown in Table 4.23, while the graph illustration of Antibiotic Multi Resistance Pattern of *Bacillus subtilis* from Refuse Dump Site after Plasmid Curing is shown in Graph 4.3. The antibiotic multy resistance profile of *Bacillus subtilis* to the 8 Gram positive multi-disk antibiotics was determined. A greater resistance was observed against Cloxacillin (1,100%), Ceftazidime (1,100%), Cefuroxime (1,100%), Amoxicillin/Clavulanic (1, 100%), Ceftriaxone (1, 100%) and Erythromycin (1, 100%). The antibiotics Gentamicin (0, 0%) and Ofloxacin (0, 0%) were the most active.

Tabe 4.23: Antibiotic Multi Resistance Pattern of *Bacillus subtilis* from Refuse Dump Sites after Plasmid Curing.

Group	Antibiotic (COD)	Resistance (%)	Susceptibity	RBP(mm)
Penicillin	Amoxicillin/Clavulanic (30µg)	1(100)	0(0)	<13
Penicillin	Cloxacillin (5µg)	1(100)	0(0)	< 10
Cephalosporin	Ceftazidime (30µg)	1(100)	0(0)	< 17
Cephalosporin	Cefuroxime (30µg)	1(100)	0(0)	< 14
Cephalosporin	Ceftriaxone (30µg)	1(100)	0(0)	< 19
Fluoroquinolone	Ofloxacin (5µg)	0 (0)	1 (100)	< 12
Aminoglycosides	Gentamicin (10µg)	0 (0)	1 (100)	< 12
Macrolides	Erythromycin (30µg)	1(100)	0(0)	< 14

COD = Antibiotic content of the Disc. RBP = Resistance Breakpoint



Graph 4.3: Doughnut Graph Illustration of Antibiotic Multi Resistance Pattern of *Bacillus subtilis* from Refuse Dump Site after Plasmid Curing

4.26: Antibiotics Resistance Pattern of *Pseudomonas aeruginosa* and *Klebsiella* sp after Curing

The antibiotic resistance pattern (%) of *Pseudomonas aeruginosa* and *Klebsiella* sp after curing is shown in Table 4.24. It was observed that *Pseudomonas aeruginosa* and *Klebsiella* sp was resistance to the entire gram negative multi drugs antibiotic except Nitrofurantoin which was susceptible to the isolates, but in the case of Ofloxacin was also observed susceptible to only *Pseudomonas aeruginosa*.

Table 4.24: Antibiotic resistant pattern (%) of *Klebsiella* sp and *Pseudomonas aeruginosa* after curing

Bacterial isolates	Number (%) resistance							
	Amoxicillin/ clavulinic	Ceftazidime	Cefuroxime	Gentamicin	Ofloxacin	Ampicillin	Cefotaxime	Nitrofurantoin
Klebsiella sp (N=1)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)
Pseudomonas aeruginosa (N=1)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	0(0)

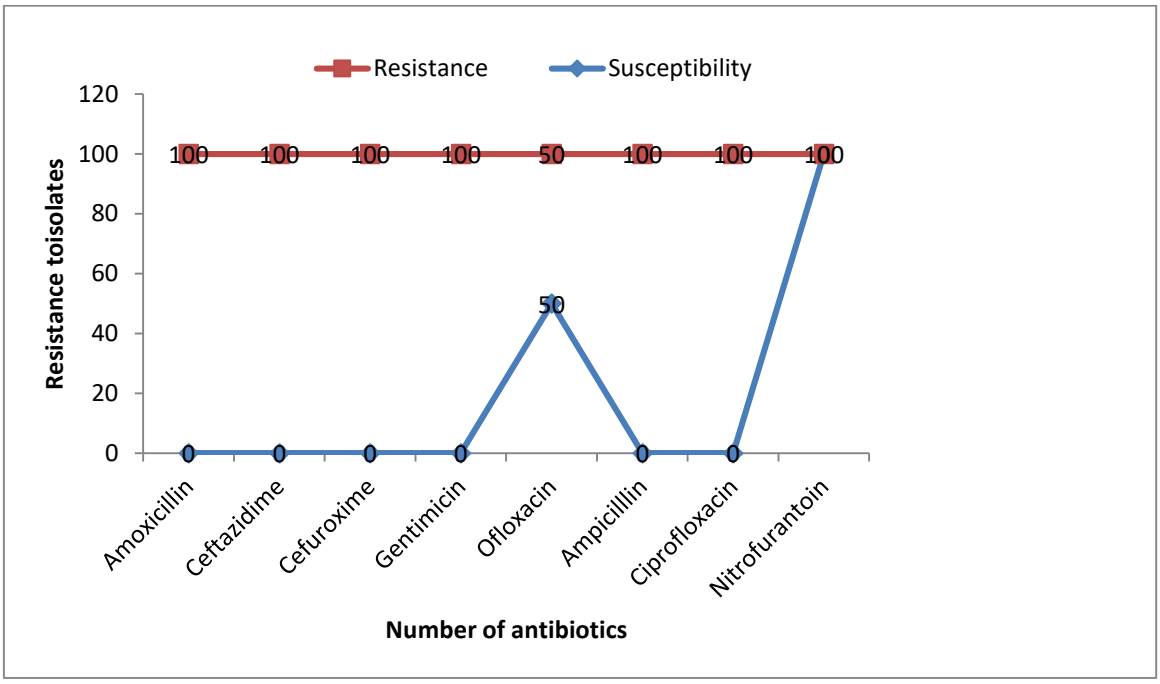
4.27: Antibiotic multi resistance pattern of *Klebsiella* sp and *Pseudomonas aeruginosa* from refuse dump site after plasmid curing.

The antibiotic resistance profiles of *Klebsiella* sp and *Pseudomonas aeruginosa* from refuse dumpsites to the gram nigative antibiotics used and the antibiotic content of the disc and the resistance breakpoint used is shown in Table 4.25, while the graph illustration of Antibiotic Multi Resistance Pattern of *Klebsiella* sp and *Pseudomonas aeruginosa* from Refuse Dump Site after Plasmid Curing is shown in Graph 4.4. The antibiotic multy resistance profile of *Klebsiella* sp and *Pseudomonas aeruginosa* to the 8 Gram negative multi-disk antibiotics was determined. A greater resistance was observed against Ciprofloxacin (2, 100%), Ceftazidime (2,100%), Cefuroxime (2,100%), Amoxicillin/Clavulanic (2, 100%), Ampicillin (2, 100%) and Gentamicin (2, 100%) while antibiotic such as Ofloxacin (1, 50%) had moderate resistance. The antibiotic Nitrofurantoin (0, 0%) were the most active.

Table 4.25: Antibiotic Multi Resistance Pattern of *Klebsiella* sp and *Pseudomonas aeruginosa* from Refuse Dump Sites after Plasmid Curing

Group	Antibiotic (COD)	Resistance (%)	Susceptibity	RBP(mm)
Penicillin	Amoxicillin/Clavulanic (30µg)	2(100)	0(0)	<13
Penicillin	Ampicillin (30µg)	2(100)	0(0)	< 13
Cephalosporin	Ceftazidime (30µg)	2(100)	0(0)	< 14
Cephalosporin	Cefuroxime (30µg)	2(100)	0(0)	< 14
Fluoroquinolone	Ofloxacin (5µg)	1 (50)	1 (50)	< 12
Fluoroquinolone	Ciprofloxacin (30µg)	2(100)	0(0)	< 15
Aminoglycosides	Gentamicin (10µg)	2(100)	0(0)	< 12
Azolidines	Nitrofurantoin (300µg)	0 (0)	2 (100)	< 14

COD = Antibiotic content of the Disc. RBP = Resistance Breakpoint.



Graph 4.4: Doughnut Graph Illustration of Antibiotic Multi Resistance Pattern of *Klebsiella* sp and *Pseudomonas aeruginosa* from Refuse Dump Site after Plasmid Curing

4.28: Heavy metal susceptibility test after curing

The heavy metal susceptibility test of the antibiotic resistance bacterial isolates after curing is shown in Table 4.26. The 3 selected highly resistant bacterial isolates *Pseudomonas aeruginosa, Klebsiella* sp and *Bacillus subtillis* where cured and re-introduced into the heavy metal concentration, it was observed that *Pseudomonas aeruginosa* were not able to show resistance for Chromium (Cr^{6+}) and copper (Cu^{2+}) except cadmium (Cd^{2+}), but *Klebsiella* sp and *Bacillus subtillis* were observed to showed resistance to chromium, copper and cadmium at their previous MIC.

Table 4.26: Minimum inhibitory concentration (mg/l) of heavy metala on *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella* sp after curing

Bacterial isolates	Minimum inhibitory concentration (mg/l)		
	Cadmium (Cd^{2+}),	Copper (Cu^{2+})	Chromium (Cr^{6+}),
Pseudomonas aeruginosa	1050	-	-
Bacillus subtilis	1050	300	250
Klebsiella sp	1050	300	250

4.27 Discussion

This present study has revealed the presence of some bacteria in waste dump soil from various parts of Delta State. A total of 61 bacterial isolates were obtain and they belong to 19 genera which were *Proteus, Acinetobacter, Serratia, Klebsiella, Aeromonas, Erysipelothrix, Nocardia, Listeria, Corynebacterium, Cellulosimicrobium, Citrobacter, Bacillus, Pseudomonas, Arthrobacter, Enterococcus, Clostridium, Kurthia, Micrococcus,* and *Staphylococcus*. *Bacillus* sp had the highest frequency of occurrence (27.3%). The high occurrence of *Bacillus* sp in this study may be attributed to the presence of waste from gastrointestinal tract of human and animals that provides the reservoir from which this bacterium can be introduced into the environment due to low hygiene and poor waste disposal method. The poor waste disposal and drainage in sample collection sites especially Effurun and Ugborikoko dumpsites could be responsible for the high number of *Bacillus* sp and also indicate the ability of *Bacillus* sp to withstand competition from indigenous microorganisms with higher growth rates. This result is in agreement with Rama *et al* (2005) who reported microbial virulence determinants and reactive oxygen species in urinary tract infection of human and animals.

The high viable counts of the aerobic and coliform bacterial recorded from the dumpsites suggest that these are hotspots of pollution from domestic, sewage and industrial wastes which increase the bacterial activities in the soils indicating presence of high microbial numbers. The higher the pollution in the soil, the higher the usage by aerobic microbes (Aspasia *et al.,* 2012). The low viable counts of aerobic and coliform bacterial in the control soils site could be due to dumping of relatively liquid waste, thereby lowering the bacterial activities in such soils and hence the low microbial populations. Presence of coliform pathogens is indicative of domestic sewage contaminated with fecal matter occasioned by breakdown of sanitary infrastructure such as seawage treatment plant or at the point of discharging into the municipalities. These are indications that the environment is hazardous and constitutes serious health risk and threat to both the waste workers and residents of the nearby municipalities. Similar to this present study, Viti *et al*., (2003); Chandra *et al*., (2011); Adeyemi, (2012) on the bacteriological analysis of soil and air collected from dumpsites revealed the presence of the diverse group of indicator bacteria as well as pathogenic bacteria like *Actinomycetes* sp, *Escherichia coli, Klebsiella* sp, *Serratia* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Enterococcus* sp and *Salmonella* sp, along with different *Bacillus* sp.

This study also revealed the antibiotic sensitivity profile of the bacteria that were isolated from the waste dumpsites. The isolates were highly resistant to first line drugs

such as Cefuroxime, Ceftazidime, Ceftriaxone, Amoxicillin/clavulinic acid, Erythromycin, Cloxacillin and Ampicillin (100%) and were 57% for Ciprofloxacin, but susceptible to second line drugs such as ofloxacin 84.21%, Gentamicin 94.73% and Nitrofurantoin 85.71%. The three areas of study have a high number of Patent Medicine Stores and hospitals that increases the level of antibiotics acquired without prescription with easy accessibility of these antibiotics from these patent medicine stores and hospitals hence the high resistance can partly be attributed to antibiotics misuse (Larson, 2007); But the widespread usage of antibiotics in hospitals around this area of study has been associated with increases in bacterial isolates being highly resistance to most common antibiotics, (Hawkey, 2008). Amoxicillin/clavulinic acid mode of action is the inhibition of cross links of peptidoglycan in the cell wall biosynthesis pathway, hence acts as a transition inhibitor resulting in bacterial cell death from modification of the cell wall to reduce permeability of heavy metals increases resistance since a reduction in permeability reduces antibiotic absorbance (Wolfgang, 2008).

The high sensitivityof the isolates to Nitrofurantoin, Gentamicin and ofloxacin may be due to the non-availability of these antibiotics easily limiting their abused and misuse (Marino, 2007). Multiple antibiotic resistances were exhibited with 12 genera demonstrating resistance to 6 antibiotics and 7 genera to 5 antibiotics. High sensitivity was exhibited to Gentamicin an aminoglycoside (94.73%) which acts by binding to the bacterial 30S ribosomal subunit inhibiting translocation or by binding to p10 in the 30S ribosome complex and the mRNA codon is misread and the wrong amino acids are incorporated into protein. Resistance to Gentamicin especially in *S. aureus* is mediated by a transposon carring gene found in large staphylococcal multi-resistance plasmids. In *P. aeruginosa* resistance is exhibited due to transport or membrane impermeability and it results in cross-resistance to all aminoglycosides with levels of resistance being seen as moderate (Hughes *et al*., 2011).

This study also revealed the tolerance of the isolates from refused dumpsites soil to some heavy metals. Metal tolerance varied according to species and the point of isolation of bacteria with isolates from the various dumpsites exhibiting high tolerance. Main source of metal pollution increase is the booming construction activities that utilize high volumes of metal components such as copper from iron complex, cadmium from steel, chromium from stainless steel, dyes, paints and tannery leather. Disposal of dyes, paints, tannery leather, complex iron and steel is a major source of concern in relation to chromium, copper and cadmium tolerance (Stepanauskas *et al*., 2005; Bhattacharya *et al*., 2013). The species minimum inhibitory concentration (MIC) was high among the isolates with

cadmium at 1000mg/l, copper at 250mg/l and chromium 200mg/l. In the case of the 3 selected highly resistant isolates *P. aeruginosa, B. subtilis* and *Klebsiella* sp for cadmium at 1050mg/l, copper at 300mg/l and chromium at 250mg/l. The high tolerance from this area of study can be attributed to continuous exposure to metals from vehicles, industries and indiscriminate disposal of heavy metal in such environment. The resistance to toxic metals in bacteria probably reflects the degree of environmental contamination with these metals and may directly relate to the bacterial cells with the toxic metals. However, the unpolluted environments may also harbor metal resistant organisms or organisms that readily adapted to high concentrations of toxic metals. The incident of this high metal resistant population is as result of increasing environmental pollution and plasmid bearing strains which are more in polluted sites than unpolluted sites. These reports are in agreement with the finds of Malik and Jaiswal, (2000) who studied Metal resistance in *Pseudomonas* strains isolated from soil treated with industrial wastewater.

Bacteria have devised specific mechanisms to tolerate different heavy metals. Ability of bacteria to tolerate chromium (Cr^{6+}) is dependent on the metal tolerance determinants, chromium resistance in gram-negative bacteria such as *P. aeruginosa* and *Klebsiella* sp. The periplasmic Cr^{6+}-binding protein (CRP) binds this cation as the first step of detoxification. Transports it into the cytoplasm where it is reduced by protein related to glutathione reductase and effluxes out of the cell. It is also based on unique peculiarities of chromium (Cr^{6+})-redox potential, vapor pressure, melting/boiling point of the metallic of this heavy metal, which is extraordinarily low for a metal with Melting point-1857.0^{o}C and boiling point-2672.0^{o}C. This enables living cells to reduce Cr to the metal and this metal does not remain inside the cell with the potential of being oxidized but it leaves the cell by passive diffusion, (Silver and Phung, 2009).

Two systems are used for copper detoxification in bacteria, P-type efflux ATPase's and Resistance-Nodulation-Cell Division (RND)-driven transporter. While P-type ATPases transport copper only across the cytoplasmic membrane, the RND-systems are hypothesized to efflux across the complete cell wall of gram-negative bacteria, which is energy dependent (Nies and Silver, 1995).

Resistance to cadmium is based on cadmium efflux and enzymatic activity of the bacteria. In gram-negative bacteria, cadmium is detoxified by Resistance-Nodulation-Cell division (RND) driven system (Nies, 1999). RND proteins mainly found in gram-negative bacteria play a role in export/efflux of Cr^{6+} and Cd^{2+}. RND-driven transporter protein families are involved in multi-drug resistance with ability to detoxify or breakdown betalactamase inhibitors.

In gram-positive bacteria an example of cadmium exporting P type ATPase is cadA pump found in *B. subtilis*. It is a single polypeptide chain that forms the trans-membrane channel of the transporter and the ATPbinding and hydrolysis site. *P. aeruginosa* and *Klebsiella* sp has four distinguished chromosomal genes of P type ATPase. This provides it with ability to tolerate metals and hydrolyze different antibiotics hence a higher degree of antibiotic resistance as exhibited in this study, i.e. the cadmium, copper, and chromium most tolerant isolates which are *P. aeruginosa, B. subtilis* and *Klebsiella* sp which was also resistant to about seven to eight tested antibiotics.

These metal specific mechanisms are complex with other non-specific mechanisms such as binding with bacterial cell envelope, metal reduction and metal efflux. These mechanisms are mainly encoded in plasmid genes facilitating their transfer from one cell to another (Naik and Dubey, 2013). The type of organic constituent and presence of negatively charged ions like chloride in the media also influence tolerance. This can explain low minimum inhibitory concentration to chromium (250mg/l) and copper (300mg/l) compared to cadmium (1050mg/l) among the 3 selected highly resistant isolates, which is due to its increased toxicity in media containing sodium chloride such as nutrient agar, caused by formation of soluble zinc-chloro complex which increases the availability of the cation to the cell (Bezverbnaya, *et al*., 2005). Positive co-resistance were observed between antibiotic and heavy metals at chromium = 200-250mg/l, cadmium = 1000-1050mg/l and copper = 250-300mg/l.

This study further selected 3 highly resistance bacteria isolates among the 61 bacterial isolated from the refuse dumpsite soils based on the high resisance pattern to the antibiotic and heavy metal tolerance. They were them subjected to curing techniques which later revealed the resistance to antibiotic and heavy metals tolerance after curing. It was observed that after curing, *Pseudomonas aeruginosa* which was the only isolate harboring the plasmid among the 3 selected highly resistant isolates, was found to exhibit multiple resistance to 6 out of the 8 antibiotics used in this research and with one of the heavy metal (cadmium), but did not exhibit resistance to the other heavy metals (copper and chromium). It clearly indicates that the antibiotics and heavy metals (cadmium, chromium, and copper) co-resistant properties observed among the 61 isolates, especially the 3 selected highly resistant isolates from *P. aeruginosa, B. subtilis* and *Klebsiella* sp were connected with the plasmid DNA and other gene factors. In spite of the wide range of plasmids present in bacterial isolates from the polluted environment, there was no constant relationship between plasmid profiles to antibiotics and heavy metal co-resistances. This is not unexpected since the same unrelated plasmid can be encoded in antimicrobial

resistance pattern, transposons, phages, and genes of chromosome (Karbasizaed *et al.,* 2003) and (Jain *et al.,* 2013). However, the co-resistance to antibiotics and heavy metal pattern and plasmid profile are sometimes inadequate to clarify the relationships between bacterial isolates of different kind from polluted environment such as this area of study, which lead to erroneous epidemiologic conclusion. Resistance properties have been well established to generally reside on DNA extra chromosomal molecule, i.e. plasmid, chromosome and other gene factors (Collard *et al*., 2005; Bharagava *et al*., 2014; Gullberg *et al*., 2014). Previous studies have demonstrated plasmids role in conferring both antibiotics and heavy metals resistance (Baker-Austin *et al.,* 2006). But these researches have emphasized that complicated sets of relationships exist between the host cell and the plasmid with respect to antibiotic and heavy metal co-resistance.

The exponential rise in antibiotic and heavy metal co-resistance has great implications on public health with the health risk further stressed by the occurrence of a high frequency of isolates that are typically resistant to several antibiotics. Although the heavy metal resistance is important to bioremediation and heavy metal detoxification at such level in the environment, which is of less clinical concern than the antibiotics resistance, this knowledge of heavy metal and antibiotic co-resistance mechanism has provided useful clues on plasmid and other genetic factors, physiology and ecology of bacteria present in this area of study.

Pollution to heavy metal has continually selected for tolerant organisms that has consequently resulted in increased levels of resistance to antibiotic. Bacteria species and heavy metal concentration influence the level of tolerance to both with bacteria being able to share these mechanisms. This relationship necessitates analysis and understanding adaptive stress contributing to drug resistance and mechanisms to combat them such as pollution management.

CHAPTER FIVE
SUMMARY, CONCLUSION AND RECOMMENDATION

5.1: Summary

1. In this study a total of 61 bacterial isolates across nineteen genera; *Proteus, Acinetobacter, Serretia, Klebsiella, Aeromonas, Erysipelothrix, Nocardia, Listeria, Corynebacterium, Cellulosimicrobium, Citrobacter, Bacillus, Serratia, Pseudomonas, Arthrobacter, Enterococcus, Clostridium, Kurthia, Micrococcus, and Staphylococcus* were isolated.
2. There were high viable counts recorded from the dumpsites, which suggest that these are hotspots of pollution from sewage, domestic and industrial wastes.
3. *Bacillus* sp has the highest frequency of occurrence across the 3 dumpsites with total of 27.3%.
4. There were multiple antibiotics resistances exhibited among bacteria isolates with 12 genera demonstrating resistant to 6 antibiotics and 7 genera to 5 antibiotics.
5. The bacterial isolates from the 3 dumpsites showed a wide range of minimum inhibitory concentration (MIC) values for the 3 tested heavy metals, 200-250mg/l for chromium (Cr^{6+}), 250-300mg/l for copper (Cu^{2+}) and 1000-1050mg/l for cadmium (Cd^{2+}).
6. It was observed that 3 bacterial isolate showed high level of co-resistances at 100% of the heavy metal MIC and antibiotics. These isolates were further identifying using molecular techniques and further used for the after curing experiment.
7. The molecular result came out positive for *Pseudomonas aeruginosa* and *Bacillus subtilis* in generic and species level, but was negative at species level and positive at generic level in the case of *Klebsiella pneumonia*, which therefore suggests that it was a different species of *Klebsiella*.
8. It was observed that Curing rendered *Pseudomonas aeruginosa* sensitive to Cr^{6+} and Cu^{2+} but not to Cd^{2+}. However curing did not affect the resistance of *Bacillus subtilis and Klebsiella* sp to the three heavy metals.
9. From the plasmid profile result, it was observed that only *Pseudomonas aeruginosa* were harboring plasmid, while other *Bacillus subtilis and Klebsiella* sp were not harboring plasmid.
10. It was observed that the bacteria isolate showed a linkage of heavy metal and antibiotics co-resistance.

5.2: Conclusion

From this study, it can be concluded that the occurrence of plasmids associated with metal resistance is of potential benefit in bioremediation, co-resistance is of medical interest due to possible transfer of antibiotic resistance to pathogenic bacteria. Open system of waste disposal dump is indeed a potential quality problem to the environment which takes the form of unsightliness, land and water pollution. It decreases the environment quality by the emission of foul odours and emission of different gases derived from the anaerobic and aerobic decomposition processes. These gases can result in hazards such as occasional burning. The bacterial isolates from these various refuse dumpsites soil are not only showing various resistance to the heavy metal due to harboring of plasmid, but was also due to other resistance gene factor within their cells, which also showed the linkage between co-resistance to heavy metals and antibiotics. It also serves as a possible source of pollution to the environment and contamination as it encourage dispersion of bacterial pathogens as either attached to particles or free entities into the environment. The presence of these pathogens in the environment is of great concern and become a source of immediate concern when they settle on surfaces as they cause varying kinds of infectious diseases, respiratory symptoms and lung function impairment which range from acute mild conditions that hardly affect daily life to severe respiratory diseases that are chronic such as cancer, acute diarrhea and so on, that require specialist's care.

Again there was a wide variation in the resistance of bacterial isolates to the heavy metal concentration from the different dumpsites soil. Sampling for heavy metals at one site might likely not provide a true reflection of the pollutants of heavy metal in the 3 dumpsites. The cadmium concentration resistance was the highest for bacterial isolates at the various dumpsites, perhaps due to the wide used of this heavy metal in the area as a result of the population of humans and cadmium related product usage in such locality. The high number of bacteria resistant to the heavy metal is a clear indication of the extent to which the 3 selected dumpsites within Delta state is polluted. This situation can be rectified by bioremediative and detoxificative methods. Bacterial isolates resistant to the heavy metal obtained in this study are autochthonous to the 3 dumpsites used. The uniqueness and characteristics of this bacterial resistance to heavy metals could be used as potential bioremediation and detoxification agents in environment with heavy metal pollution. Also, further research on heavy metal and antibiotic co-resistance genes of these isolates should be investigated because it may lead to the development of biosensors. The

information obtained in this study, will prove valuable in setting up bioremediation and detoxification projects of the three selected dumpsites within Delta state.

5.3: Contribution to Knowledge

- Refuse dumpsite was demonstrated as sources of bacterials that possess resistance to both antibiotics and heavy metals concurrently.
- The linkage between resistance to metals and antibiotics was identified
- Plasmids were identified as responsible for carrying genes associated with the co-resistance in some isolates.

5.4: Recommendation

In order to enhance environment quality and protect the lives of people, the following recommendations are suggested.

I. There should be legislative laws and regulations governing the disposal of waste from antibiotic and heavy metal industries far away from the community.

II. Harmful industries and hospitals wastes should be well treated or detoxified before disposal.

III. The disposal of waste on land fill should be preferred to open system so as to effectively control and prevent the release of harmful bacteria into the environment.

IV. Incineration under high heat in a controlled environment should be used in area with limited availability of waste disposal land.

V. Medium and small scale industries for the conversion of heavy metal wastes into useful products to the environment should be encouraged.

VI. The settlement patterns of individuals should be control by Government and communities to ensure that industrial area and residential area are far apart.

VII. Public health organizations with other relevant bodies should embark on public awareness and enlightenment campaigns to sensitize individuals on the hazards of indiscriminate disposal of heavy metal and antibiotic waste from homes, hospitals, patent medical stores and in some rare case industries into the environment.

5.5: Suggested Areas of Further Study

1. The occurrence of plasmid/ chromosomal mediated heavy metal and antibiotic co-resistance amongst bacteria isolated from refuse dump sites.
2. The occurrence of plasmid mediated heavy metal and antibiotic co-resistance amongst bacteria isolated from air of refuse dump site.
3. The heavy metal and antibiotic co-resistance effect amongst each bacterium isolated from refuse dump site.
4. The occurrence of plasmid/ chromosomal mediated heavy metal and antibiotic co-resistance and possible effect on bioremediation method amongst bacteria isolated from refuse dump site.

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APPENDICES

Appendix 1: Composition of Media

Formula per litre

Nutrient Agar

Peptic digest of animal tissue	5.0g
Beef extract	1.5g
Yeast extract	1.5g
Sodium chloride	5.0g
Agar	15.0g

Direction

28g of powder was dissolved in 1 liter of distilled water and heated for boiling for proper dissolution of the medium. After which it was sterilized by autoclaving at 121^0C for 15mins at 1.5PSI and then poured into Petri-dishes.

MacConkey

Peptone	20.0g
Lactose	10.0g
Bile Salt	5.0g
Sodium chloride	5.0g
Neutral red	0.0075g
Agar	12g

Direction

63g of powder was suspended in 1 liter of distilled water and heated for boiling for proper dissolution of the medium. After which it was sterilized by autoclaving at 121^0C for 15mins at 1.5PSI and then poured into Petri-dishes.

Appendix 2: Biochemical Test Preparation

1) Gram Staining:

Gram staining was donning to classify the bacterial isolates into either gram positive or gram negative. Smear of the bacteria isolates were aseptically prepared using sterile distilled water, and heat fixed on a clean slide. The smear was covered with crystal

violet for 30-60secs; the stained was washed off with tap water. Again, the smear was covered with lugol's iodine for 30secs; the stain was washed with 95% alcohol drop wise for about 5-10secs until the solvent flows colorlessly from the slide. The smear was washed with water, and consequently counterstained with safranin for 30secs. After which the smear was washed with water and then blotted dry. Following which the prepared slides were examined with oil immersion objective (X100) and observed. Gram-positive bacteria appeared as violet color, while Gram-negative bacteria appeared as red color. Identification of the isolates was done by carrying out various biochemical testing and using bacteriological analytical manual and Bergy's manual of determinative bacteriology as reference points. The following biochemical tests were carried out, oxidase test, indole test, catalase test, Glucose fermentation, lactose fermentation test, motility test, citrate test and H_2S production test.

2) Catalase test:

This test organism was aseptically picked from the slant with the aid of a sterile wire loop, and placed in drops of hydrogen peroxide (H_2O_2) on a clean grease free slide. The production of gas bubbles indicated catalase positive whiles the absence of bubbles catalase negative.

3) Indole test:

It is used to detect the ability of an organism to breakdown tryptophan to indole. Tryptic broth was inoculated with test organism and incubated at 35-37^{0}C 0vernight. A few drop of Kovac's reagent was added to the both, and observed for coloration. Red coloration on the upper layer of the broth indicated positive test, while light yellow coloration indicated negative test.

4) Oxidase test:

This test is used to determine if an organism possesses the cytochrome oxidase enzyme, and it is used to differentiate *Pseudomonas* from related species. The procedure is as follows: 2 drop of the reagent (1% oxidase reagent) was added to suspected colonies on an agar plate that are not flooded. Coloration was observed within 10secs. Development of a purple color indicated oxidase production positive, while no coloration is a negative result.

5) Lactose fermentation:

Lactose fermentation is used to differentiate bacteria that ferment lactose from that do not. It is simply tested by observing pinkish colony on MacConkey plates. Bacteria

colony with pink coloration is lactose fermenters, while those without pinkish colony are non-lactose fermenters.

6) Motility test:

This is done to differentiate between bacteria that are motile from those that are non-motile. A semi-solid nutrient agar medium was inoculated with a test organism by stabbing the medium with a straight wire containing the inoculums to about half the depth of the medium. Motile bacteria migrate from stab line and diffused through the medium causing turbidity and rendering it opaque while non-motile bacteria produced growth that are confined to the stab line.

7) Nitrate reduction Test

Drops of sulphanilic acid and N, N-Dimethyl-Napthylanine (1-2 drops each), reagent were added to the kit medium containing the isolates.

8) Citrate Utilization Test

This test was carried out using Simon's citrate agar. Slant of the medium were prepared in MacCartney bottles as prescribed by the manufacturer. Using a sterile straight wire loop, the slope was first streak with a saline suspension of the test organism and then stabbed and incubated for 18-24hrs. The color change from green to blue indicates positive result while negative is observed when it retains green color or change.

9) Hydrogen sulphide (H_2S) production test:

It involves stab inoculate of the bacteria into the slant of the TSI agar and also rubbing it at the surface to determine H_2S production at 37^0C for 8-24hrs in order to observe the following change within the medium, blackening which indicate positive for H_2S, pink at the bottom which indicate positive for glucose, yellow coloration at the top of the slant indicate positive for lactose, bubble in the slant indicate positive for gas and crack indicate positive for acid, respectively

Appendix 3: Bacterial Isolate Count Result

AEROBIC COUNT

Sample	Cfu	Cfu/per gram	Log Cfu per gram
Obiaruku Control	$\frac{3\ x\ 10^{-3}}{0.1}$	0.6 x 10^4	3.780
Dump 1	$\frac{5\ x\ 10^{-5}}{0.1}$	0.5 x 10^6	5.70
Dump 2	$\frac{6\ x\ 10^{-3}}{0.1}$	0.6 x 10^4	3.78
Ogborikoko Control	$\frac{2\ x\ 10^{-3}}{0.1}$	0.2 x 10^4	3.30
Dump 1	$\frac{13\ x\ 10^{-3}}{0.1}$	1.3 x 10^4	4.11
Dump 2	$\frac{15\ x\ 10^{-5}}{0.1}$	1.5 x 10^6	6.18
Effurun Control	$\frac{3\ x\ 10^{-5}}{0.1}$	0.3 x 10^6	5.48
Dump 1	$\frac{4\ x\ 10^{-3}}{0.1}$	0.4 x 10^4	3.60
Dump 2	$\frac{18\ x\ 10^{-5}}{0.1}$	1.8 x 10^6	6.26

COLIFORM COUNT (MACCONKEY)

Sample	Cfu	Cfu/per gram	Log Cfu per gram
Obiaruku Control	$\frac{17\ x\ 10^{-3}}{0.1}$	1.7 x 10^4	4.23
Dump 1	$\frac{13\ x\ 10^{-3}}{0.1}$	1.3 x 10^4	4.11
Dump 2	No growth		
Ogborikoko Control	$\frac{1\ x\ 10^{-3}}{0.1}$	0.1 x 10^4	3.04
Dump 1	$\frac{48\ x\ 10^{-5}}{0.1}$	4.8 x 10^6	6.68
Dump 2	$\frac{25\ x\ 10^{-5}}{0.1}$	2.5 x 10^6	6.40
Effurun Control	$\frac{1\ x\ 10^{-3}}{0.1}$	0.1 x 10^4	3.04
Dump 1	$\frac{1\ x\ 10^{-5}}{0.1}$	0.1 x 10^6	5.04
Dump 2	$\frac{6\ x\ 10^{-3}}{0.1}$	0.6 x 10^4	3.78

Appendix 4: Preparation of heavy metal

a. Chromium preparation

The molar mass of 294.2g/mol of potassium dichromate were divided with the atomic mass of 52amu of chromium to derive a pure chromium salt of 5.7g. 5.7g of potassium dichromate salt was dissolved into 1000ml of distilled water to get 1000mg/l of chromium concentration.

b. Copper preparation

1g of pure copper was dissolve with 5ml of Nitrate Acid (HNO_3). The 5ml of the pure copper solution was introduced in to 995ml of distilled water to get 1000mg/l of copper concentration.

c. Cadmium preparation

The molar mass of 231g/mol of Cadmium Acetate were divided with the atomic mass of 112.4amu of Cadmium to derive a pure Cadmium salt of 2.1g. 2.1g of Cadmium Acetate salt was dissolved into 1000ml of distilled water to get 1000mg/l of Cadmium concentration.

Appendix 5: Morphology and biochemical characteristics of the bacterial isolates from the three dumpsites

Refuse dumpsite code	Morphology test		Biological test										Identified isolates
	Shape	Gram reaction	Motility	Oxidase	Catalase	Citrate	Indose	Hydrogen Sulphur	Gas	Acid	Glucose	Lactose	
(eff2)	Rod	-ve	+	+	+	-	-	+	+	+	+	-	*Pseudomonas* sp
(ugb1)	Rod	-ve	+	+	+	-	-	+	+	+	+	-	*Pseudomonas* sp
(ugb2)	Rod	-ve	+	+	+	-	-	+	+	+	+	-	*Pseudomonas* sp
(eff2)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(ugb2)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(ugb2)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(eff1)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(obia1)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(eff2)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(ugb1)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(ugb2)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(ugb2)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(ugb2)	Rod	+ve	+	+	+	+	-	-	+	+	+	+	*Bacillus* sp
(obia1)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(obia2)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(eff2)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(eff2)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(ugb1)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(ugb2)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(ugb2)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(ugb2)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(eff1)	Cocci	+ve	-	+	+	+	-	-	-	-	+	+	*Micrococcus* sp
(eff2)	Cocci	+ve	-	-	+	+	-	+	-	-	+	+	*Staphylococcus* sp
(eff1)	Cocci	+ve	-	-	+	+	-	+	-	-	+	+	*Staphylococcus* sp
(obia2)	Cocci	+ve	-	-	+	+	-	+	-	-	+	+	*Staphylococcus* sp
(ugb1)	Cocci	+ve	-	-	+	+	-	+	-	-	+	+	*Staphylococcus* sp
(ugb2)	Cocci	+ve	-	-	+	+	-	+	-	-	+	+	*Staphylococcus* sp
(ugb1)	Cocci	+ve	-	-	+	+	-	+	-	-	+	+	*Staphylococcus* sp
(eff2)	Cocci	+ve	-	-	+	+	-	+	-	-	+	+	*Staphylococcus* sp
(ugb1)	Rod	+ve	+	-	+	+	-	-	-	-	+	-	*Kurthia* sp
(obia2)	Rod	+ve	+	-	+	+	-	-	-	-	+	-	*Kurthia* sp
(eff2)	Rod	+ve	+	-	+	+	-	-	-	-	+	-	*Kurthia* sp
(obia1)	Rod	+ve	+	-	+	+	-	-	-	-	+	-	*Kurthia* sp
(obia2)	Rod	-ve	+	+	+	+	+	+	-	-	+	-	*Aeromonas* sp
(obia2)	Rod	-ve	+	+	+	+	+	+	-	-	+	-	*Aeromonas* sp
(eff1)	Rod	-ve	+	+	+	+	+	+	-	-	+	-	*Aeromonas* sp

(eff2)	Rod	-ve	+	+	+	+	+	+	-	-	+	-	*Aeromonas* sp
(ugb2)	Rod	+ve	+	-	+	+	-	+	+	+	-	-	*Arthrobacter* sp
(ugb2)	Rod	+ve	+	-	+	+	-	+	+	+	-	-	*Arthrobacter* sp
(eff1)	Rod	+ve	+	-	+	+	-	+	+	+	-	-	*Arthrobacter* sp
(eff2)	Rod	+ve	+	-	+	+	-	+	+	+	-	-	*Arthrobacter* sp
(obia2)	Rod	+ve	-	-	+	+	-	-	-	+	+	+	*Listeria sp*
(obia2)	Rod	+ve	-	-	+	+	-	-	-	+	+	+	*Listeria sp*
(ugb2)	Rod	+ve	-	-	+	+	-	-	-	+	+	+	*Listeria sp*
(ugb2)	Rod	+ve	-	-	+	-	-	+	-	+	+	-	*Corynebacterium* sp
(eff2)	Rod	+ve	-	-	-	+	-	+	-	+	+	+	*Erysipelothrix* sp
(obia1)	Rod	+ve	+	-	+	+	-	-	-	+	+	-	*Nocardia* sp
(obia2)	Rod	+ve	+	-	+	+	-	-	-	+	+	-	*Nocardia* sp
(obia2)	Rod	+ve	+	-	+	+	-	-	-	+	+	-	*Nocardia* sp
(ugb2)	Rod	+ve	+	-	+	-	-	+	+	+	+	-	*Cellulosimicrobium* sp
(eff2)	Cocci	+ve	-	-	+	-	-	-	-	+	+	+	*Enterococcus* sp
(eff2)	Rod	+ve	+	-	+	+	+	+	+	+	+	+	*Clostridium* sp
(obia2)	Rod	-ve	+	-	+	+	-	+	+	+	+	+	*Citrobacter* sp
(ugb1)	Rod	-ve	+	-	+	+	-	+	+	+	+	+	*Citrobacter* sp
(obia1)	Rod	-ve	+	-	+	+	+	+	+	-	+	-	*Proteus* sp
(obia2)	Rod	-ve	+	-	+	+	+	+	+	-	+	-	*Proteus* sp
(obia2)	Rod	-ve	+	-	+	+	+	+	+	-	+	-	*Proteus* sp
(eff2)	Cocci	-ve	-	-	+	+	+	-	-	+	+	+	*Acinetobacter* sp
(obia2)	Cocci	-ve	-	-	+	+	+	-	-	+	+	+	*Acinetobacter* sp
(obia2)	Rod	-ve	+	+	+	+	-	-	-	-	+	-	*Serratia* sp
(obia2)	Rod	-ve	-	+	+	+	-	-	+	+	+	+	*Klebsiella* sp

Key:

+ve = Positive and -ve = Negative.

Obia 1 = Obiaroko enterance, Obia 2 = Obiaroko centre, Ugb 1 = Ughorikoko enterance, Ugb 2 = Ughorikoko centre, Eff 1 = Effurun enterance and Eff 2 = Effurun centr

Appendix 6: Antibiotics resistance pattern of the Gram positive isolates before curing in Measure zone diameters (mm)

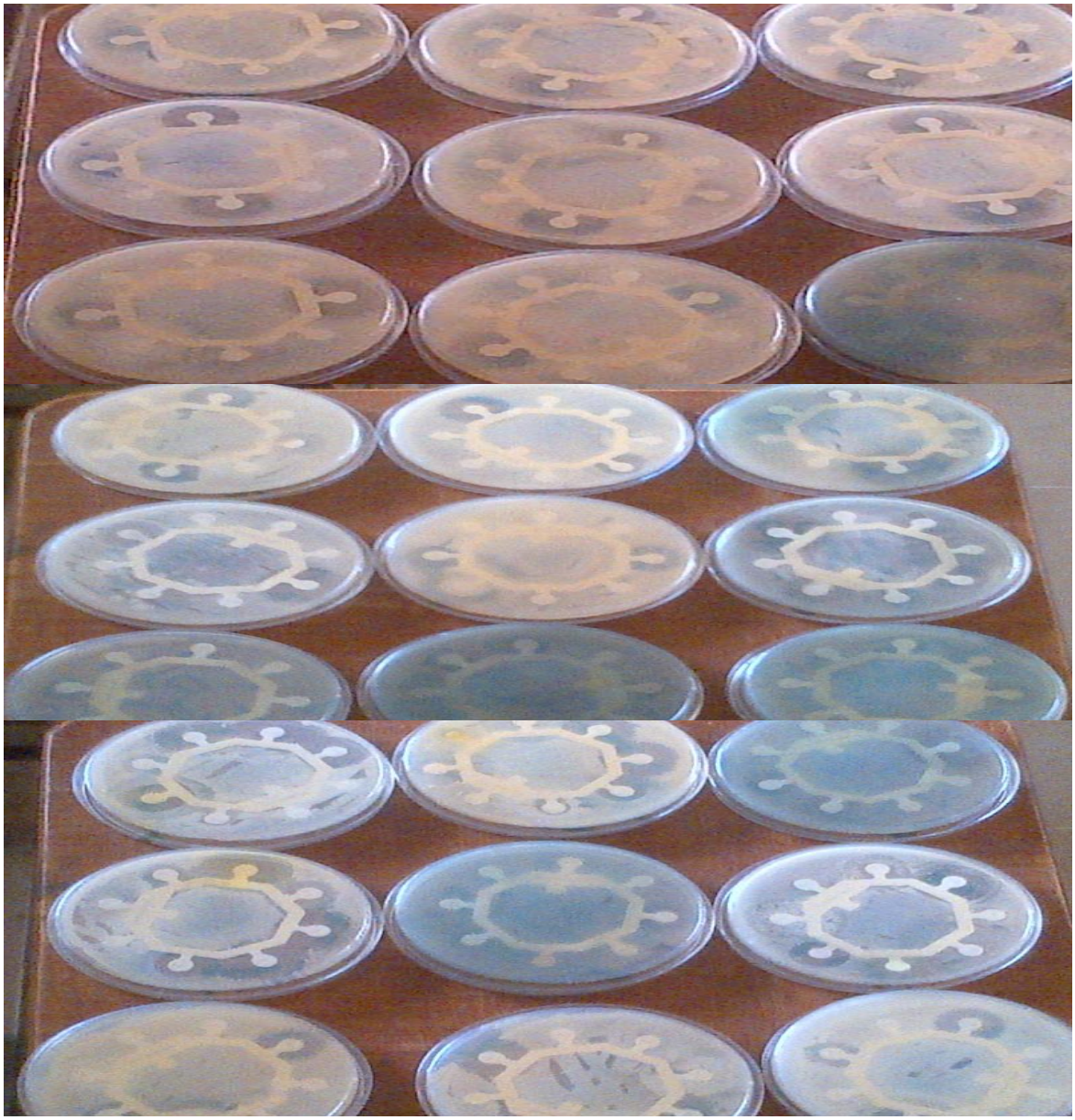
Isolates	Aug	Caz	Crx	Gen	Ctr	Ery	Cxc	Ofl
1)	R-0	R-0	R-0	I-14	R-0	R-0	R-0	S-17
2)	R-0	R-0	R-0	R-6	R-0	R-0	R-0	R-4
3)	R-0	R-0	R-0	I-12	R-0	R-0	R-0	S-22
4)	R-0	R-0	R-0	S-23	R-0	R-0	R-0	S-25
5)	R-0	R-0	R-0	S-22	R-0	S-23	R-0	S-21
6)	R-0	R-0	R-0	S-17	R-0	R-0	R-0	S-17
7)	R-0	R-0	R-0	I-12	R-0	R-0	R-0	S-19
8)	R-0	R-0	R-0	I-13	R-0	R-0	R-0	S-23
9)	R-0	R-0	R-0	S-19	R-0	R-0	R-0	S-18
10)	R-0	R-0	R-0	S-17	R-0	R-0	R-0	S-23
11)	R-0	R-0	R-0	R-0	R-0	R-0	R-0	S-17
12)	R-0	R-0	R-0	S-22	R-0	S-25	R-0	S-22
13)	R-0	R-0	R-0	S-18	R-0	R-0	R-0	S-19
14)	R-0	R-0	R-0	S-16	R-0	R-0	R-0	S-23
15)	R-0	R-0	R-0	S-18	R-0	R-0	R-0	S-18
16)	R-0	R-0	R-0	S-22	R-0	S -22	R-0	S-25
17)	R-0	R-0	R-0	S-19	R-0	R-0	R-0	S-17
18)	R-0	R-0	R-0	S-27	R-0	R-0	R-0	S-19
19)	R-0	R-0	R-0	S-26	R-0	R-0	R-0	S-24
20)	R-0	R-0	R-0	I-13	R-0	R-0	R-0	S-22
21)	R-0	R-0	R-0	S-18	R-0	R-0	R-0	S-19
22)	R-0	R-0	R-10	S-23	R-0	R-0	R-0	S-22
23)	R-0	R-0	R-0	S-19	R-0	R-0	R-0	S-27
24)	R-0	R-0	R-9	S-22	R-0	R-0	R-0	S-18
25)	R-0	R-0	R-0	S-26	R-0	S-19	R-0	S-22
26)	R-0	R-0	R-0	S-22	R-0	R-0	R-0	S-21
27)	R-0	I -12	R-0	S-23	S-22	R-0	R-0	S-24
28)	R-0	R-0	R-0	I-13	R-0	R-0	R-0	I-23
29)	I-12	R-0	R-5	S-26	R-0	R-0	R-0	S-25
30)	R-0	R-0	R-0	S-22	R-0	R-0	R-0	S-24
31)	R-0	R-0	R-0	S-25	R-0	R-0	R-0	S-22

32)	R-0	R-0	R-0	S-23	R-0	R-0	R-0	S-18
33)	R-0	R-0	R-0	I-12	R-0	R-0	R-0	S-16
34)	R-0	R-0	R-0	S-22	R-0	R-0	R-0	S-19
35)	S-23	R-0	S-19	S-19	S-19	S -22	R-0	S-21
36)	R-0	R-0	R-0	R-0	I -14	R-0	R-0	S-24
37)	R-0	R-0	R-0	I-13	R-0	R-0	R-0	S-21
38)	R-0	R-0	R-0	S-23	R-0	R-0	R-0	S-22
39)	R-0	R-0	R-0	S-19	R-0	R-0	R-0	S-27
40)	R-0	R-0	R-0	I-12	R-0	R-0	R-0	S-19
41)	R-0	R-0	R-0	I-14	R-0	R-0	R-0	S-17
42)	R-0	R-0	R-0	S-22	R-0	R-0	R-0	S-18
43)	R-0	R-0	R-0	I-12	R-0	R-0	R-0	S-23
44)	R -0	R-0	R-0	S-26	R-0	R-0	R-0	S-27
45)	R-0	R-0	R-0	S-19	R-0	R-0	R-0	S-19

Key:

Caz- Ceftazidime , Crx- Cefuroxime , Gen- Gentamicin , Ctr- Ceftriaxone, Ofl- Ofloxacin , Aug- Amoxicillin/clavulinic acid , Ery- Erythromycin , Cxc – Cloxillin, R = Resistant S = Sensitive I = Intermediate , Isolate 1 to 10 = *Bacillus subtilis*, Isolate 11 to 19 = *Micrococcus* spp , Isolate 20 to 26 = *Staphylococcus spp*, Isolate 27 to 30 = *Kurthia* sp, Isolate 31 to 34 = *Arthrobacter* sp, Isolate 35 to 37 = *Listeria* sp, Isolate 38 = *Corynobacterium* sp, Isolate 39 = *Erydipelothrix* sp, Isolate 40 to 42 = *Nocardia* sp, Isolate 43 = C*ellulosemicrobium* sp, Isolate 44 = *Enterobacter* sp, and Isolate 45 = *Clostridium* sp.

Appendix 7: Medium Plate Showing Antibiotic Multi Resistance Pattern of Gram Positive Bacterial Isolates from Refuse Dump Sites



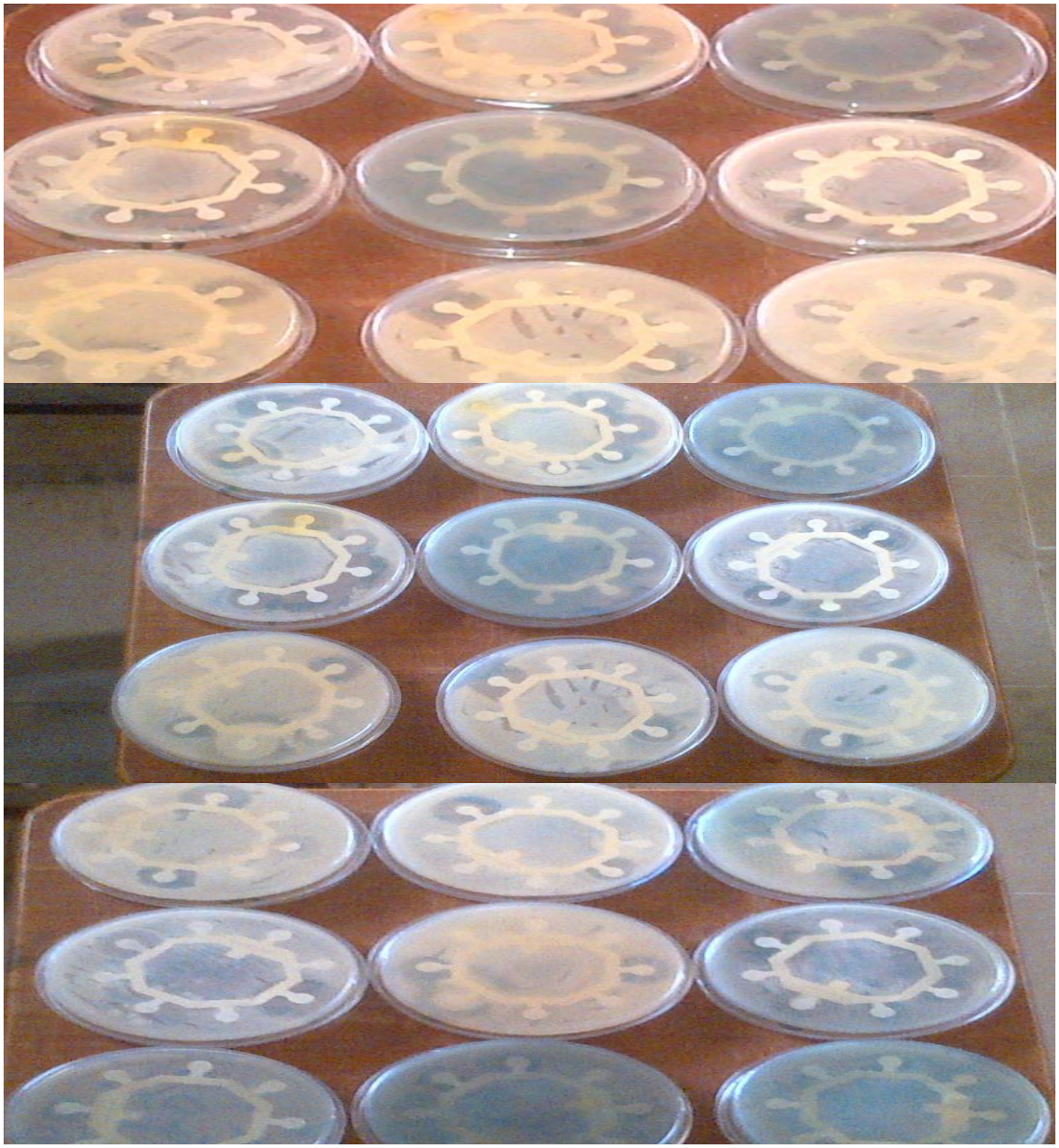
Appendix 8: Antibiotics resistance pattern of the Gram - negative isolates before curing in Measure zone diameters (mm)

Isolate	Amp	Caz	Crx	Gen	Cpr	Ofl	Aug	Nit
46)	R-0	R-0	R-0	R-0	I-12	I-12	R-0	S-17
47)	R-0	R-0	S-17	R-0	S-16	S-21	I-14	S-22
48)	R -0	S-22	R-0	I-13	I-12	I-13	R-0	R-0
49)	R-0	R-0	R-0	R-0	S-17	S-23	R-0	R-0
50)	R-0	R-0	R-0	I-12	S-23	S-21	R-0	S-25
51)	R-0	R-0	S-22	I-12	S-22	S-26	R-0	S-21
52)	R-0	R-0	R-0	R-0	R-0	I-12	R-0	S-24
53)	R-0	R-0	R-0	S-22	S-21	S-22	R-0	S-27
54)	R-0	R-0	R-0	R-0	R-0	R-0	R-0	R-6
55)	R-0	R-0	R-0	I-12	I-13	R-0	R-0	S-16
56)	R-0	R-0	R-0	S-17	I-12	R-0	R-0	S-21
57)	R-0	R-0	R-0	R-0	R-0	R-0	R-0	S-19
58)	R-0	R-0	R-0	I-12	R-0	S-23	R-0	S-17
59)	R-0	R-0	R-0	S-18	R-0	S-21	R-0	S-23
60)	R-0	R-0	R-0	I-12	R-0	I-14	R-0	S-21
61)	R-0	R-0	R-0	I-13	R-0	S-25	R-0	S-22

Key:

CAZ - Cefotaxime , CRX - Cefuroxime , GEN - Gentamycin , OFL – Ofloxacin, AUG – Amoxicillin/clavulinic acid, NIT - Nitrofurantoin Cpr - Ciprofloxacin , AMP- Ampicillin , Isolate 46 to 47 = *Citrobacter* sp, Isolate 48 to 50 = *proteus* sp, Isolate 51 to 52 = *Acinetobacter* sp, Isolate 53 = *Serretia* sp, Isolate 54 = *Klebsiella* sp, Isolate 55 to 57 = *Pseudomonas* sp, and Isolate 58 to 61 = *Aeromonas* sp.

Appendix 9: Medium Plate Showing Antibiotic Multi Resistance Pattern of Gram Negative Bacterial Isolates from Refuse Dump Sites



Appendix 10: Heavy metals susceptibility test before curing

	Minimum inhibitory concentration (mg/l)		
Bacterial isolates	**Cadmium (Cd^{2+})**	**Copper (Cu^{2+})**	**Chromium (Cr^{6+})**
❖ *Pseudomonas* sp (eff2)	1000	300	250
Pseudomonas sp (ugb1)	1050	250	200
Pseudomonas sp (ugb2)	1050	300	250
Bacillus sp (eff2)	1000	250	250
❖ *Bacillus* sp (ugb2)	1050	300	250
Bacillus sp (ugb2)	1000	250	250
Bacillus sp (eff1)	1050	250	200
Bacillus sp (obia1)	1000	250	200
Bacillus sp (eff2)	1000	250	200
Bacillus sp (ugb1)	1000	250	200
Bacillus sp (ugb2)	1000	250	200
Bacillus sp (ugb2)	1000	250	200
Bacillus sp (ugb2)	1000	250	200
Micrococcus sp (obia1)	1000	250	200
Micrococcus sp (obia2)	1000	250	200
Micrococcus sp (eff2)	1000	250	200
Micrococcus sp (eff2)	1000	250	200
Micrococcus sp (ugb1)	1000	250	200
Micrococcus sp (ugb2)	1000	250	200
Micrococcus sp (ugb2)	1000	250	200
Micrococcus sp (ugb2)	1000	250	200
Micrococcus sp (eff1)	1000	250	200
Staphylococcus sp (eff2)	1000	250	200
Staphylococcus sp (eff1)	1000	250	200
Staphylococcus sp (obia2)	1000	250	200
Staphylococcus sp (ugb1)	1000	250	200
Staphylococcus sp (ugb2)	1000	250	200
Staphylococcus sp (ugb1)	1000	250	200
Staphylococcus sp (eff2)	1000	250	200
Kurthia sp (ugb1)	1000	250	200
Kurthia sp (obia2)	1000	250	200
Kurthia sp (eff2)	1000	250	200
Kurthia sp (obia1)	1000	250	200
Aeromonas sp (obia2)	1000	250	200
Aeromonas sp (obia2)	1000	250	200
Aeromonas sp (eff1)	1000	250	200
Aeromonas sp (eff2)	1000	250	200
Arthrobacter sp (ugb2)	1000	250	200
Arthrobacter sp (ugb2)	1000	250	200
Arthrobacter sp (eff1)	1000	250	200
Arthrobacter sp (eff2)	1000	250	200
Listeria sp (obia2)	1000	250	200
Listeria sp (obia2)	1000	250	200
Listeria sp (ugb2)	1000	250	200
Corynobacterium sp (ugb2)	1000	250	200
Erydipelothris sp (eff2)	1000	250	200
Nocardia sp (obia1)	1000	250	200
Nocardia sp (obia2)	1000	250	200
Nocardia sp (obia2)	1000	250	200
Cellulosemicrobium sp (ugb2)	1000	250	200
Enterobacter sp (eff2)	1000	250	200
Clostridium sp (eff2)	1000	250	200
Citrobacter sp (obia2)	1000	250	200
Citrobacter sp (ugb1)	1000	250	200
Proteous sp (obia1)	1000	250	200
Proteous sp (obia2)	1000	250	200
Proteous sp (obia2)	1000	250	200
Acinetobacter sp (eff2)	1000	250	200
Acinetobacter sp (obia2)	1000	250	200
Serretia sp (obia2)	1000	250	200
❖ *Klebsiella* sp (obia2)	1050	300	250

Key; Ugb 1= Edge of Ugborikoko dumpsite and Ugb2= centre of dumpsite
Eff 1= Edge of Effurun dumpsite and Eff2= centre of dumpsite
Obia 1= Edge of Obiaruku dumpsite and Obia2= centre of dumpsite

Appendix 11: Procedure for curing of plasmid DNA

The curing of plasmid was done to determine whether a plasmid encode a trait or not.

a. Materials and Stock Preparations:

Sodium deodecyl sulphate (SDS) curing agent X 100ml

Sodium deodecyl sulphate (SDS) 10g (10%)

b. Nutrient Broth 100ml

- The mixture was autoclaved
- The pH adjusted to 7.6
- The final solution steamed for 1hour
- Autoclave

c. Methods

The nutrient agar slant was subculture into a Luria-Bartani (LB) culture medium containing antibiotics for 24hrs at 37^0C. 9ml of freshly prepared nutrient broth was inoculated with an aliquot from an overnight culture growth on LB medium and was incubated for 3-4hrs at 37^0C for minimal growth of the microorganism. 1ml of sodium deodecyl sulphate (SDS) curing agent was added to the mixture, which sufficiently brought the concentration 1% and was also incubated for 24-48hrs at 37^0C. 1ml of the cured culture was inoculated into a 9ml freshly prepared nutrient broth and was incubated for 24hrs at 37^0C. Poured plate method was carryout using the overnight broth culture to flood the nutrient agar plat and the multi antibiotics paper disc was place, after which was incubated for 24hrs at 37^0C. The susceptibility test was read.

Appendix 12: Antibiotics Resistance Pattern of *B. subtilis* after Curing in Measure Zone Diameters (mm)

Isolates	Aug	Caz	Crx	Gen	Ctr	Ery	Cxc	Ofl
B. subtilis	R-0	R-0	R-0	R-23	R-0	R-0	R-0	R-22

Appendix 13: Antibiotics Resistance Pattern of *P. aureginosa* and *Klebsiella* sp after Curing in Measure Zone Diameters (mm)

Isolate	Amp	Caz	Crx	Gen	Cpr	Ofl	Aug	Nit
P. aureginosa	R-0	R-0	R-0	R-0	R-0	S-22	R-0	S-21
Klebsiella sp	R-0	R-0	R-0	R-0	R-0	R-0	R-0	S-19

Appendix 14: *P. aeruginosa, B. subtilis* and *Klebsiella* sp Minimum Inhibitory Concentration Pattern 0f Chromium after Curing

Isolates	Concentration			
	100mg/l	150mg/l	200mg/l	250mg/l
P. aureginosa,	-	-	-	-
B. subtilis	+	+	+	-
Klebsiella sp	+	+	+	-

Appendix 15: *P. aeruginosa, B. subtilis* and *Klebsiella* sp Minimum Inhibitory Concentration Pattern of Copper after Curing.

Isolates	Concentration				
	100 mg/l	150mg/l	200mg/l	250mg/l	300mg/l
P. aureginosa,	-	-	-	-	-
B. subtilis	+	+	+	+	-
Klebsiella sp	+	+	+	+	-

Appendix 16: *P. aureginosa, B. subtilis* and *Klebsiella* sp Minimum Inhibitory Concentration Pattern of Cadmium after Curing.

ISOLATES	Concentration in mg/l						
	100	200	300	400	500	600	700
P. aureginosa	+	+	+	+	+	+	+
B. subtilis	+	+	+	+	+	+	+
Klebsiella sp	+	+	+	+	+	+	+
	800	900	1000	1050			
P. aureginosa	+	+	-	-			
B. subtilis	+	+	+	-			
Klebsiella sp	+	+	+	-			