ISOLATION, ANITIBIOTIC AND HEAVY METAL SUSCEPTIBILITY PATTERNS OF SOME PATHOGENS FROM DOMESTIC DUMPSITES AND WASTE WATER

BY

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M.Sc/Sci/44534/04-05

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY) DEPARTMENT OF MICROBIOLOGY AHMADU BELLO UNIVERSITY, ZARIA NIGERIA

NOVEMBER, 2007

DECLARATION

I declare that the work in the thesis entitled "Isolation, antibiotic and heavy metal susceptibility patterns of some pathogens from domestic dumpsites and waste water" has been performed by me in the department of microbiology under the supervision of Dr. S.O Olonitola and Prof. (Mrs.) V.J. Umoh.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

_______________	_____________	______________
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CERTIFICATION

This thesis entitled "Isolation, antibiotic and heavy metal susceptibility patterns of some pathogens from domestic dumpsites and waste water" by Mzungu Ignatius meets the regulations governing the award of the degree of Master of science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my mother, Agnes Mzungu.

ACKNOWLEDGEMENT

My gratitude goes first to Almighty God for the success of this work. Special thanks to my supervisory team; Dr. S.O. Olonitola, Professor (Mrs) V.J. Umoh and Dr. (Mrs.) H.I. Inabo who provided a great deal of insight and encouragement during the cause of this work and supervised each aspect of the work.

I also like to acknowledge Mr. Joseph Nashakyaa, Barrister J. Okoduwa, Mr. Lawrence Kuma and Mr. Y.S. Makama who provided financial assistances towards the success of my laboratory work.

My gratitude also goes to the following colleagues who in a special way assisted me in the course of my laboratory work, materially and otherwise; Grace Abakpa, Edia Uregu, Habiba Attah, Tafena Amapu and Hawa Ujo. Others are Micheal Dashen, Aliyu Mohammed, Al-Muktar Yahuza, Rimanshong Atiribum, Grace Oshagbemi and Nike Odede.

Special thanks to Mr. David A'hemba Mkovough who provided the facilities for typing and printing of this work and to Miss Ngumimi

Makpam, who typed the draft copy of the manuscript. Thanks to Dr. Hambesha Terkende Paul, for his tremendous support during the cause of my study.

Also not left out are the staffs of Material Science section of centre for Energy Research and Training (CERT). A.B.U., Zaria, for technical assistance in sample analysis of the heavy metals, the staff of Pharmaceutical Microbiology laboratory for providing the control strains used and to Mr. Ella, E. whose advice contributed immensely to the success of this work. Lastly, thanks to my siblings, Mr. Nicodemus Mzungu, Augustine Mzungu, Eunice Mzungu, Elizabeth Mzungu and Christiana Mzungu for all their support and prayers during the course of my studies.

ABSTRACT

The impact of domestic wastes on the antibiotic susceptibility patterns of environmental pathogens was evaluated by isolating some pathogens from dumpsite soil and wastewater. All the isolates were screened for susceptibility to tetracycline (30µg), ofloxacin (30µg), gentamicin (10µg), contrimoxazole (25 µg), nitrofurantoin (300 µg), augmentin (30 µg), amoxicillin (25 µg) and nalidixic acid (30 µg), using the CILS disk diffusioin technique. Ten samples each of dumpsite and wastewater samples were also analyzed for the presence of heavy metal ions using the energy dispersive X-Ray Fluorescence (EDXRF). All isolates were also tested for heavy metal tolerance on Mueller-Hinton Agar supplemented with heavy metals, using the serial dilution technique. One hundred and eighty bacteria isolates obtained were characterized. These comprised *Staphylococcus aureus* (56), *Escherichia coli* (55), and *Pseudomonas aeruginosa* (69). At least 16(29%) of *Staphylococcus aureus,* 35(64%) of *Escherichia coli* and 59(86%) of *Pseudomonas aeruginosa* were resistant to at least two of the antibiotics tested. None of the *Staphylococcus aureus* was tolerant to any of the heavy metals tested at the highest concentration(s) used, but 17(31%) of *Escherichia coli* were tolerant

to copper and 25(45%) were tolerant to cobalt, while 47(68%) of *Pseudomonas aeruginosa* were tolerant to at least two heavy metals. Correlation between metal tolerance and antibiotic resistance using χ^2 test of association (P=0.05) showed (χ^2 = 11.5). This suggests that, the presence of heavy metals in the environment may have constituted a selective pressure for multiple antibiotic resistance.

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Chapter One INTRODUCTION

In the early 1970s, physicians were finally forced to abandon the belief that, given the vast array of effective antimicrobial agents, virtually all-bacterial infections were treatable. This optimism was shaken by the emergence of resistance to multiple antibiotics among such pathogens as *Staphylococcus aureus, Pseudomonas aeruginosa* and a host of others (Lowy, 2003).

Multidrug resistance is now the norm among these pathogens. S.*aureus* is perhaps the pathogen of greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life-threatening infections, and its capacity to adapt to different environmental conditions (Lowy,2003). *Pseudomonas aeruginosa* is a highly relevant opportunistic pathogen. One of the most worrisome characteristics of *Ps.aeruginosa* is its low antibiotic susceptibility.

Escherichia coli is responsible for more than 80% of urinary tract infections (UTI) (Wikipedia, 2007), and is noted for producing an enzyme (β- lactamase) which degrades penicillin during clinical trials, and currently resists a vast number of other antibiotics (Sherman, 2006).

Antibiotic resistance is the ability of a microorganism to resist the effect of an antibiotic; these organisms thwart antibiotics by interfering with their mechanism of action.

Resistance to antibiotics has spread fast to a number of commonly used antibiotics such that “in the 1990s, we have come to a point for certain infections that we do not have agents available”. (Lewis, 1995).

Antimicrobial drug resistance in bacterial pathogens is of National and International concern (WHO, 2001; CDC, 2001). Although the misuse of antimicrobial agents is accepted as a major driving force behind the spread of resistance, the nature of this relationship is complex. Resistance to antibiotics is sometimes acquired by a change in the genetic make-up of a bacterium, which can occur by either a genetic mutation or by transfer of antibiotic resistance genes between bacteria in the environment (Williams, 2002).

Infectious diseases are becoming more difficult and more expensive to treat, because our current antibiotics are becoming less useful against antibiotic resistant pathogenic bacteria. When antibiotics fail to work,

the consequences include extra consultation with the doctor, hospitalization or extended hospital stays, a need for more expensive antibiotics to replace the older ineffective ones, loss of workdays, and sometimes, death (APUA, 1999).The increased used of antibiotics in health care, as well as in agriculture and animal husbandry, is in turn contributing to the growing problem of antibiotic resistant bacteria. The antibiotic does not technically cause resistance, but allows a situation where an already existing variant can flourish (APUA, 1999). Products such as disinfectants, sterilants, and heavy metals used in industries and in household products along with antibiotics, are creating selective pressure in the environment that lead to mutations in microorganisms (Baquero *et al.,* 1998). In environment with multiple stresses, such as antibiotics and heavy metals, it would be ecologically favorable, in terms of survival, for a bacterium to acquire resistance to both stresses. If the resistance is carried on plasmid, those bacteria with clusters of resistance genes on plasmids are likely to simultaneously pass on those genes to other bacteria, with a better chance of survival (Lawrence, 2000). In such a situation, one may suggest an association between antibiotic resistance and metal tolerance. Calomiris *et al.* (1984) found that a high percent of bacteria

isolated from drinking water that were tolerant to metals were also antibiotic resistant.

Although some heavy metals are essential trace elements, most are toxic at high concentrations to all organisms by forming complex compounds within the cell (Alm, 2002). Microbes have evolved several mechanisms to tolerate the pressure of heavy metals by either efflux, complexation, or reduction of metal ions or using them as terminal electron acceptors in anaerobic respiration (Alm, 2002). This is because heavy metals are increasingly found in microbial habitats due to natural and industrial processes (Nies and Silver, 1995).

Another implication of heavy metal tolerance in the environment is that it may contribute to the maintenance of antibiotic resistance genes by increasing the selective pressure in the environment (Calomiris *et al.*, 1984). The most likely mechanism underlying this indirect selection is microbial co-resistance; when genes encoding for metal and antibiotic resistance are located on the same plasmid or transposons, and across-resistance; when the same gene is responsible for a broad range of resistances to antibiotics and heavy metals

(Summers, 2002). Many researchers have speculated and shown that a correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both antibiotics and heavy metals are located closely on the same plasmid and thus more likely to be transferred together in the environment (Vajiheh *et al.,* 2003).

Growth in human populations has generally been matched by a greater formation of a wider range of waste products, many of which cause serious environmental pollution if they are allowed to accumulate in the ecosystem. Microorganisms, biodegradable organic compounds, nitrates, salts, nutrients, organic micro-pollutants and heavy metals are known pollutants of domestic waste (Ivanildo and Richard, 1995). According to Ivanildo and Richard, (1995), there are basically five sources of heavy metals contributing to environmental pollution: geological weathering, which provide the background level; industrial processing of ores and metals; the use of metals and metal compounds, such as chromium salts in tanneries, copper compounds in agriculture, and tetraethyl lead as an anti-knock agent in gasoline; leaching of heavy metals from domestic wastes and solid waste dumps; and heavy metals in human and animal excretions, particularly

zinc.

Efficient waste collection and specific treatment processes are generally lacking in developing countries and Nigeria in particular, dumpsites are located close to residential homes, along watercourses and near water sources. The accumulation of pollutants within the environment has numerous public health implications, among which is the spread of infectious diseases (Ivanildo and Richard, 1995). There are also speculations that, the presence of pollutants such as heavy metals could provide selective pressure for antibiotic resistance among the micro flora of the environment (Alm, 2002).

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1.1 Statement of Problem

Antibiotic resistance is a global problem currently threatening the treatment of infections in plants, animals and humans. Bacteria with elevated antibiotic resistance levels present a potential public health risk because infections with these organisms do not respond to one or more of the drugs commonly used to treat them. According to CDC, (2000), drug resistant pathogens are a growing menace to all people, regardless of age, gender, or socioeconomic background.

There is also, the risk of transferring antibiotic resistance to bacterial populations in the environment because antimicrobial resistance genes and their genetic vectors, once evolved in bacteria of any kind anywhere, can spread indirectly through the world's interconnecting commensal, environmental, and pathogenic bacteria anywhere else (O'Brien, 2002).

1.2 Justification

The proliferation of dumpsites and open drainages within residential areas, and the lack of efficient waste collection and treatment processes, is of public health concern because microorganisms and heavy metals among others are the major pollutants of dumpsites and wastewater.

Some studies (Calomiris *et al.*, 1984; Vajiheh *et al.,* 2003). have shown that, a correlation exists between antibiotic resistance and heavy metal tolerance in bacteria isolated from heavy metal contaminated sites. Therefore, there is need to study the occurrence of heavy metals in domestic wastes, as well as their effects on the microbial community of these environments.

In view of the increasing resistance among various pathogens to

common antibiotics, including broad-spectrum antibiotics, there is need to research into preventable causes of antibiotic resistance in order to lower the incidence of drug resistance.

Antimicrobial resistance is driving up health care cost, increasing the severity of disease, and increasing the death rates from certain infections (NIAID, 2006). A number of clinically important microbes have developed resistance to available antimicrobials including *Staphylococcus aureus* and *Pseudomonas aeruginosa* which cause skin, bone, lung and bloodstream infections and *Escherichia coli* which causes urinary tract infections (Wikipedia, 2007).

1.3 Objectives

The objectives of this work are therefore:

1. To isolate selected bacteria pathogens from domestic wastewater
2. To determine antibiotic resistance of the Isolates.
3. To determine metal tolerance of the isolates.
4. To determine the level of heavy metals in domestic wastewater effluents and waste dumpsites.

5. To determine the correlation between antibiotic resistance and heavy metal tolerance of isolates.

Chapter Two LITERATURE REVIEW

2.1 Antibiotic Resistance

When penicillin became widely available during the Second World War, it was a medical miracle, rapidly vanquishing the biggest wartime killer infected wounds. Discovered initially by a French medical student, Ernest Duchesne, in 1896, and rediscovered by Scottish physician Alexander Fleming in 1928, the product of the soil mold (-*Penicillium*) crippled many types of disease-causing bacteria. But just four years after Drug companies began mass-producing penicillin in 1943, resistant microbes emerged (Lewis,1995).

Resistance is a description of the relative insusceptibility of a microorganism to a particular treatment under a particular set of conditions. (Kummerer, 2004). For antibacterial, resistance is usually quantified as the minimum concentration required to assert a definable effect (e.g. growth inhibition) on a population of cells.Wherever there is a change in susceptibility that renders an agent ineffective against a certain organism, then the organism is referred to as resistant.

2.1.1 Emergence of Resistance

Low-level antibiotic resistance in bacteria can be found in pristine habitats, suggesting that antibiotic resistance is of minimal importance under natural conditions (Leff *et al.,* 1993). In the early 1940s, when sulphonamides and penicillin first came into widespread clinical usage, the common bacterial infections presenting in hospitals were due to the *Pneumococcus,* the β-haemolytic *Streptococcus* and penicillin-susceptible strains of *S aureus.* Very few antibiotic-resistant strains of these organisms were encountered. However, the explosion in antibiotic usage worldwide was followed by the appearance of clinical infections with organisms resistant to the agents in common use (Finland *et al*., 1959). Therefore, with respect to the causes of resistance, the focus is on the use of antimicrobials in hospitals by medical practitioners and in animal husbandry (Kummerer, 2004). The occurrence of antibiotic resistant bacteria in aquatic (Andersen and Sandaa, 1994; Miranda and Castillo, 1998; Boon and Cattanach, 1999; Huys *et al*., 2000).this has been the consequence of uncontrolled discharge of urban wastewaters and animal wastes in the aquatic environment (Guardabassi *et al*., 1998; Gonni Urriza *et al*., 2000). In addition, most the compounds used in

medicines are only partially metabolized by patients and then discharged into the hospital sewage system or directly into municipal wastewater if used at home. These flow along with excreta and municipal wastewater to the sewage treatment plant (Kummerer, 2004). For this reason, many investigations have recognized that, wastewater treatment plants are the principal recipients of enteric bacteria with multiple antibiotic resistance (Iverson *et al.,* 2002; Selvaratnan and Kurnbergar, 2004) and an important site for horizontal gene transfer, by containing nutrients and high concentration of microorganisms (Goni-Urriza *et al.,* 2000; Barberio *et al.,* 2001; Vilanova *et al.,* 2004).

Similarly, it has been noted that, environmental release and agricultural use of treated sewage effluent containing bacteria having elevated antibiotic resistance levels presents a potential public health risk, as well as the risk of transferring the antibiotic resistance to bacterial populations in the environment. (House of Lords, 1998; Silva *et al.,* 2006). Data also indicate that the strongest prediction of antibiotic resistance is the heavy metal concentration in sediments of polluted water such as mercury (McArthur and Tuckfield, 2000).

In general, the emergence of antibiotic resistance is a highly complex process, which is not yet fully understood with respect to the significance of the interaction of bacterial populations and antibiotics (Khachatourians, 1998; Witte *et al.,* 1999; Aarestrup *et al.,* 2001), even in an environment contaminated with antibiotic compounds (Bjorkman *et al.,* 2000; Martinez, 2000).

While the use of antibiotics in medicine and agriculture clearly stimulates the proliferation of antibiotic resistance (Neu, 1992), there is evidence for metal selection for antibiotic resistance in diverse ecosystems, including coastal oceans (Sabry *et al.,* 1997, Rasmussen and Sorensen, 1998), freshwater streams (McArthur and Tuckfield, 2000), digestive tracts of primates with mercury containing dental amalgam, coal - fired power plants as well as freshwater microcosms amended with cadmium and Nickel. The most likely mechanism underlying this indirect selection are microbial co-resistances (multiple resistance genes located on the same genetic element) and cross-resistance - same gene responsible for a broad range of resistance to antibiotics and metals (Summers, 2002).

2.2 Problems of Antibiotic Resistance

Antibiotic resistance has been called one of the world's most pressing public health problems. Over the decade, almost every type of bacteria has become stronger and less responsive to antibiotic treatment when it is really needed. These antibiotic resistant bacteria can quickly spread within the community thus, the threat of new strains of infectious aetiological agents which are more difficult and expensive to treat.

NIAID Fact Sheet,(2006) observed that, people infected with antibiotic resistant organisms are more likely to have longer hospital stays and require treatment with second-or third-choice medicines that may be less effective, more toxic, and more expensive.

Antibiotic resistance is also an ecological problem in the sense that, when antibiotics are used in humans or animals, approximately 80-90% of the ingested antibiotics is not broken down, but passes through the body intact and enters the environment as waste (APUA, 1996). Thus, they retain their ability to affect bacteria and promote antibiotic resistance even after they enter the soil or water as waste product.

Economically, antibiotic resistance is found to be a very serious

problem all over the world. The 1995 US Office of Technology Assessment Report attributes a cost of 1.3 billion dollars per year for antibiotic resistant infections due to six species of bacteria in US hospitals. While the real magnitude of the problem is unknown, the financial burden of treating antibiotic resistant infections worldwide is estimated to be many billions of dollars per year.

Some experts predict that, as resistance to antibiotics is increasing at a faster pace than it can be controlled, the future will resemble the pre-antibiotic era. Others are more optimistic that research and careful drug management can reverse the trend, if global efforts are focused on recognizing and controlling it (APUA, 1996).

2.3 Genetic Basis of Antibiotic Resistance

2.3.1 Inherent resistance

Resistance of bacteria to antibiotic agents may be inherent or acquired. Inherent (innate) resistance to some antibiotics is the natural resistance possessed by most strains of bacterial species and is part of their genetic makeup, encoded on the chromosome. Inherent multiple resistance is characteristic of free-living organisms, which may have evolved because of exposure to natural antibiotics in the environment.

The organisms have low virulence, but their multiple resistance allow them to produce opportunistic infections in hospital environment. An example of a free-living opportunistic pathogen with a high degree of inherent resistance is *Ps. aeruginosa.* (French and Phillips, 1997).

2.3.2 Mutational resistance

Acquired resistance may be due either to mutations affecting genes on the bacterial chromosome or to acquisition of genes conferring resistance upon a previously sensitive microorganism. Mutations usually involve deletion, substitution, or addition of one or a few base pairs, causing substitution of one or a few amino acids in a crucial peptide. This usually results in a gene product with reduced or absence of the ability to bind the antibiotic. Examples include, broad-spectrum aminoglycoside resistance in *Ps. aeruginosa* due to altered cell membrane permeability; and fluoroquinolone resistance in *E.coli* resulting from alteration in DNA gyrase (French and Phillips, 1997).

2.4 Mechanism of Resistance Transfer

2.4.1 Horizontal transfer of antibiotic resistance genes

Horizontal gene transfer is distinguished from vertical transfer, which

occurs between a parent and its offspring. Bacterial genetic material spreads vertically by inheritance, and diverse horizontal transfer mechanisms, often involving phylogenetically distant cells (Lorenz and Wackernagel, 1994; Davison, 1999; Sobecky, 1999). For example, identical *aphA-3* genes encoding Kanamycin resistance have been found in various species of *Campylobacter* as well as in *Enterococcus faecalis* (Papadopoulou and Courvalin, 1988). Most likely aphA-3 was transferred from Gram-positive enterococcal bacteria to the gram-negative *Campylobacter coli* and later disseminated to other *campylobacter* via the conjugative transposon Tn1545 (Papadopoulou and Courvalin, 1988). Similarly diverse Gram-negative and Gram-positive bacteria share identical tetracycline resistance genes *tetM and tetQ* (Davison, 1999).

Conjugation

Conjugation involves cell to cell contact through pilli and the transfer of plasmids and genetic materials from one bacterial cell to another. Conjugation is probably the major route of horizontal transmission of resistance genetic elements. Exchange of plasmids conferring antibiotic and metal resistance by conjugation occurs in a large range

of Gram-positive and Gram-negative bacterial genera inhabiting various environments such as marine sediments, water column and human digestive tract (Salyers *et al.,* 1995; Davison, 1999; Liebert *et al*., 1999; Sobecky, 1999).

Transposition

Transposons are segment of DNA which have the ability to move to other sites as discrete units. They are movable genetic elements that contain insertion sequence and additional genes and such genes can carry specific characteristics like antibiotic resistance. Chromosomal resistance factors can move to plasmid by means of transposition and become mobilizable to other bacteria. Tn21. (Liebert et al., 1999) and Tn916 (Rice, 1998) are among the best-studied transposons that carry resistance to antibiotics and metals. According to Rice, (1998),Tn916 and Tn21 are conjugative transpons which are mobile elements that possess genetic machinery to facilitate their own transfer between bacterial cells.

There are several mechanisms by which conjugative transposons can have significant impact on the dissemination of antimicrobial resistance determinants among pathogenic bacteria. The first and most obvious of these is the direct transfer of resistance determinants that

are encoded by the genes within the transposons themselves. The most obvious impact of conjugative transposons has been on the spread of resistance to tetracycline and minocyclines (Liebert et al., 1999). A second mechanism by which conjugative transposon can disseminate antimicrobial resistance determinants is by mobilizing plasmids or other transposons that encode antimicrobial resistance (Rice, 1998).

Tn916 - Like elements also contribute to the dissemination of multidrug resistance by integrating into larger conjugative transposons that encode additional antimicrobial resistance determinants. A final mechanism by which Tn916 - like elements influence the transfer of multiple antimicrobial resistance is by allowing homologous recombination between donor and recipient chromosomes during mating events.

Transformation

Transformation is a process where foreign or naked DNA is adsorbed unto the surface of recipient cell and then transferred across the cell membrane into the cytoplasm and incorporated by recombination into recipient cell's chromosome. Many bacteria are competent for the uptake and incorporation of exogenous DNA via natural

transformation (Lorenz and Wackernagel, 1994). Acquisition of antibiotic resistance via transformation among bacteria has been demonstrated in coastal waters and sediments (Paul *et al.,* 1991; Frischer *et al*., 1994) and in soils (Nielson *et al*., 1997) particularly in *Pseudomonas* and *Acinetobacter spp*. Environmental DNA is relatively abundant and can either be actively secreted from viable bacteria or released non-specifically following bacteria cell death (Liebert *et al.,* 1997). Free DNA can be quite stable and can survive in the environment for months adsorbed to sand or clay particles (Liebert *et al.,* 1999).

Transduction

This is a mode of genetic exchange between bacteria cells that is mediated by viruses especially bacteriophages. It is a third possible means of transmitting genetic material horizontally among bacteria. The virus comes in contact with the bacterium through their lysogenic cycle and injects its genetic materials, which acts as extra-chromosomal genetic materials or plasmid. Phages are extremely abundant in nature and outnumber bacteria in aquatic system (Wommack and Colwell, 2000), Transduction of antibiotic reistance

determinants from *Pseudomonas aeruginosa* (Blahova *et al.,* 2001), *Staphylococcus aureus* (Fouace, 1981), and *Streptococcus pyogenes* Hyder and streifeld, 1998) to other bacteria is documented and transduction is recognized as a major factor in the spread of bacterial virulence factor (Cheetham and Katz, 1995).

Horizontal transfer may result in new strains that are co-resistant or cross-resistant to metals and antibiotics providing new material for indirect selection of strains of bacteria. In the presence of metal, such strains would have selective advantages over non-resistant strains.

Metal exposure may also promote horizontal gene transfer by induction of conjugation (Smit *et al.,* 1998), transformation (Baur *et al*., 1996) and transposition (Beaber *et al.,* 2004).

2.5 Specific Mechanisms of Resistance

2.5.1 *Staphylococcus aureus*

Staphylococcus aureus is one of the major resistant pathogens. It is found on the mucous membranes and the skin of around a third of the population, it is extremely adaptable to antibiotic pressure. It was the first bacterium in which penicillin resistance was found in 1947, just four years after the drug started being mass-produced.. Methicillin

was then the antibiotic of choice, but has since been replaced by oxacillin due to significant kidney toxicity.

Most strains (approximately 90%) of *S. aureus* whether isolated in hospital or in the community now produce ß-lactamases and are resistant to penicillin and ampicillin. This type of resistance is plasmid mediated, resulting from bacteriophage transduction and is often associated with resistance to erythromycin and other agents (French and Phillips, 1997). *S. aureus* strains resistant to penicillin, tetracycline, erythromycin, chloramphenicol and other drugs have been reported, but after the introduction of the penicillinase-stable methicillin, the incidence of hospital infection with multiple resistant *S.aureus* declined during the 1960s and 1970s (Shanon, 1981). However, in the late 1970s, mehicillin resistant *S.aureus* (MRSA) emerged as a major pathogen of hospital infection throughout the world (Haley *et al.*, 1982;Keane and Catterky, 1984; Cookson and Phillips, 1988). MRSA are resistant to methicillin and other penicillinase-stable ß-lactams by the production of an altered penicillin-binding protein (PBP2a). the chromosomal region encoding the mecA gene contains a number of insertion sites that permit the accumulation of multiple chromosomal resistances to other classes of

antibiotics. In addition, MRSA may acquire other resistances encoded on plasmids.

Aminoglycoside resistance in *S.aureus* is mediated by a bifunctional protein with both aminoglycoside acetyltransferase and phosphotransferase activities and is encoded by a transposon (French and Phillips, 1997).

Following the rapid emergence of resistance to quinolones, many strains of MRSA remain sensitive only to glycopeptides, vancomycin and teicoplanin. (Schaefler, 1998; MacDoughall *et al.,* 2005).

2.5.2 *Escherichia coli*

E. coli is naturally sensitive to ampicillin and amoxicillin. Acquired resistance conferred by a plasmid-encoded TEM-1 ß-lactamase was first described in 1965 (French and Phillips, 1997), and this has spread so extensively throughout the world that 40-60% of both hospital and community strains are now resistant. Other plasmid-mediated ß-lactamases are sometimes seen in *E. coli* (French and Phillips, 1997). The plasmid, IncP (ß) which confers gentamycin resistance was identified in enterobacteria isolated from sewage (Heuer *et al*., 2002).

In another study, pathogenic *E. coli* isolated from hospital patients were found to be resistant to ampicillin, trimethoprim-sulphamethoxazole, tetracycline, chloramphenicol, kanamy cin, gentamycin, ciprofloxacin, nalidixic acid and cefalothin (Vajiheh *et al.,* 2003).

2.5.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a hghly relevant opportunistic pathogen. One of the most worrisome characteristics of *Ps. aeruginosa* is its low antibiotic susceptibility (Wikipedia, 2007). This low susceptibility is attributable to the concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes (Wikipedia, 2007). Besides intrinsic resistance, *Ps. aeruginosa* easily develop acquired resistance either by mutation in chromosomally-encoded genes, or by the horizontal gene transfer of antibiotic resistance determinants. Development of multidrug resistance by *Ps. aeruginosa* isolates requires several different genetic events that include acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes. Hypermutation favours the selection of mutation-driven antibiotic resistance in *Ps. aeruginosa* strains producing chronic

infections, whereas the clustering of several different antibiotic resistance determinants. Some recent studies have shown that phenotypic resistance associated to biofilm formation or to the emergence of small-colony-variants may be important in the response of *Ps*. *aeruginosa* populations to antibiotic treatment.

Ps. aeruginosa is inherently resistant to most ß-lactam antibiotics, tetracycline, chloramphenicol, sulphonamides and nalixic acid, and acquired resistance to other antibiotics is common (French and Philips, 1997), it has been shown that fluoroquinolone resistance in *Ps. aeruginosa* rose from 33.6% to 40.5% in a study involving some hospitals in USA (MacDougall *et al.,* 2005). This resistance is believed to arise largely from the selection of organisms with point mutations in the topoisomerase enzymes that are targets for the fluoroquinolone (Hopper, 2001). Two types of aminoglycoside resistances are seen: high-level plasmid-mediated resistance to one or two aminoglycosides and a low level plasmid-mediated resistance. The former type of resistance, which is due to the production of aminoglycoside-modifying enzymes, is often observed during nosocomial outbreaks (Philips and Shanon, 1997). Resistance to antipseudomonal cephalosporins by mutation to constitutive

production of chromosomal ß-lactamase may also occur (Wise *et al.*, 1996).

2.6 Microbial Heavy Metal Tolerance

The production of heavy metals has increased rapidly since industrial revolution (Ayres, 1992). Toxic metals are mobilized from industrial activities and fossil fuel consumption and eventually are accumulated through the food chain, leading to serious ecological and health problems (Nriagu and Pacyna, 1988). Since the natural mineralization of metal is slow process, pollution by heavy metal constitutes one of the most important environmental problems of industrial societies (Ayres, 1992, Nriagu and Pacyna, 1988).

Antibiotic resistant and heavy metal tolerant microorganisms have been isolated from nosocomial and burn wound infections, infections treated with metal-based antimicrobial agents, and various metal contaminated environments such as estuaries, soil, and sewage (Vajiheh *et al.,* 1997). It has been suggested that the combined expression of antibiotic resistance and metal tolerance may not be a fortuitous phenomenon but rather is caused by selection resulting from metals present in an environment (Calomiris *et al.,* 1984).

Microorganisms have evolved several mechanisms to tolerate heavy

metals or use them as terminal electron acceptors in anaerobic respiration because heavy metals are increasingly found in microbial habitats due to the natural and industrial processes (Alm, 2002).

2.7 Metal Tolerance Mechanisms

Heavy metal ions must enter the cells first and attain a high concentration to have toxic effects (Nies, 1999). However, some heavy metals are necessary in trace amounts for enzymatic functions and bacterial growth, hence; uptake mechanisms exist that allow the entrance of metal ions into the cell. There are two general uptake systems: the quick and unspecific system - driven by a chemiosmotic gradient across the cell membrane and thus requiring no ATP: and the slow and substrate specific system - driven by energy from ATP hydrolysis.

While the first mechanism is more energy efficient, it results in an influx of a wider variety of heavy metals and when these metals are present in high concentrations inside the cells, they are more likely to have toxic effects (Nies and Silver, 1995). To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These

mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state (Nies, 1999).

Biomass metal absorption has been demonstrated to be a powerful process for the mineralization and concentration of metals from toxic industrial residues (Gadd and White, 1993; Volesky and Holan, 1995; Unz and Shuttleworth, 1996; Hernandez *et al.,* 1998). Several bacteria and fungi that accumulate an ample range of metal species have been described (Fourest *et al.* 1994; Volesky, 1994; Volesky and Holan, 1995). Metal accumulation or biotransformation is an alternative mechanism for metal detoxification in bacteria (Gadd, 192). Bacteria have evolved resistance mechanisms to certain metal ions, which have been well studied and include;

2.7.1 Copper

Copper is used by cells in small quantities in cellular enzymes (e.g. Cytochrome c oxidase). However, copper is so widely used in mining industry, and agriculture that high levels may exist in some environments. As such, bacteria have evolved several types of mechanisms to resist toxicity due to high copper concentrations. With

respect to the prevalence of copper resistance in the environment, Lin and Olson (1995) studied bacteria isolated from a water distribution system experiencing copper corrosion and 62% of the bacteria were found to be copper resistant. Of these resistant bacteria, 49% had cop or cop-like gene systems, including both compartmentalization and efflux systems (Cooksey, 1993).

In the plant pathogen *Pseudomonas syringae*, resistance to copper via membrane is due to four proteins encoded on the plasmid-borne *cop* operon (Cooksey, 1994). The proteins are found in the periplasm (cop A and cop C), the outer membrane (cop B) and the inner membrane (cop D) and work together to compartmentalize copper away from sensitive cellular functions.

In *E. coli* resistance to copper is based on an efflux mechanism by which copper is removed from the cell. The efflux proteins are expressed by plasmid-bound pco genes (Cooksey, 1993). Two *cut* genes (*cut* C and *cut* F) were identified by Gupta *et al*. (1995) and were shown to encode a copper-binding protein and an outer membrane lipoprotein. Cooksey (1993) also stated that most bacteria species in the environment have acquired at least one of the

aforementioned management systems and that the evolution of copper resistance may be through the modification of copper uptake genes found on chromosomes.

2.7.2 Zinc

Zinc is another essential element**.** It is not biologically redox reactive and is thus not used in respiration. It is however, important in forming complexes (such as zinc fingers in DNA) and as a component in cellular enzymes. Bacterial cells accumulate zinc by a fast, unspecific uptake mechanism and it is normally found in higher concentrations (but is less toxic) than other heavy metals (Niles, 1999). Uptake of zinc ions is generally coupled to that of magnesium and the two ions may be transported by similar mechanisms in bacteria (Nies and Silver, 1995).

Two general efflux mechanisms are responsible for bacteria resistance to zinc. One is a P-type ATPase efflux that transport zinc ions across the cytoplasmic membrane by energy from ATP hydrolysis. A chromosomal gene*, znt* A, was isolated from E. coli K-12 and was found to be responsible for the ATPase that transport zinc and other cations across cell membranes (Beard *et al*., 1997). The other mechanism involved in zinc efflux is an RND-driven[2] transporter

system that transport zinc across the cell wall of Gram-negative bacteria and is powered by a proton gradient and not ATP (Nies, 1999).

2.7.3 Mercury

Mercury is one of the most toxic metals in the environment (Alm, 2002). Mercury pollution of areas can be caused both by geological processes and anthropogenic activities. Bacteria play an important role in the global mercury cycle, and they have evolved resistance mechanisms to detoxify several chemical forms of mercury (Smalla *et al.*, 2006). Molecular analysis of mercury-resistant bacterial isolates has revealed an enormous diversity of mercury resistance genes, which has been reviewed by Barkay *et al.* (2003). Mercury resistance genes are often located on mobile genetic elements (MGE), which can be located either chromosomally or on plasmid. Mercury resistance genes were reported to occur on a wide range of plasmid belonging to various incompatibility groups.

In general, it is proposed that MGE facilitate bacterial adaptability in response to environmental stresses and contributes considerably to their diversity. (Tieze *et al.,* 1989). Resistance for many mercury-

resistant strains, plasmids, and transposons have been studied at a molecular level in great details, however, studies making the link with their ecology and providing data on their abundance and diversity depending on environmental mercury pollution are relatively rare (Donen *et al.,* 1998).

In a study by Smalla *et al.,* (2006), further evidence was provided that as a result of mercury pollution, the abundance of bacteria carrying merRTAP target sequences in sediment samples was strongly increased compared to those in non-polluted sediment samples. It was also observed that the abundance of bacteria with IncP-1ß plasmids was increased in sediment samples taken from polluted sites. Considering their broad host range (Bathe *et al.,* 2004; Tauch *et al.,* 2003) and their transfer frequencies observed in soils and sediments (Osborn *et al.,* 1993). IncP~1 plasmids carrying mer operons could be an efficient means for microbial communities to rapidly adapt to mercury selective pressure. The spread of mercury resistance genes is similar to the worldwide spread of antibiotic resistance genes (Yurieva *et al.*, 1997) and has resulted in a worldwide population of mercury resistant species. Indeed, the presence of *mer* genes in bacteria collected from deep sediment cores indicates that *mer* is an

ancient system (Osborn *et al.,*1997*)*. Three major factors affecting the distribution of *mer* genes have been identified by Osborn *et al.,* (1997).

1. Long ancestry.

2. Coupling of localized selection pressures inform of mercury compounds.

3. Spread of *mer* sequences by a powerful array of broad-host-range plasmids and transposons.

2.8 Association of Metal Tolerance and Antibiotic Resistance

Bacteria have evolved a variety of plasmid and chromosomal-encoded systems to resist toxic agents including antibiotics and metals (Silver and Phung, 1996; Walsh, 2000). In some cases, single enzyme function as efflux pumps for multiple antimicrobials that is, cross-resistance (Perron *et al***.,** 2004). In other cases genes encoding for metal and antibiotic resistance may be located on the same plasmid and/or transposons, conferring co-resistance (Liebert *et al.,* 1999; Summers, 2002; Wireman *et al* ., 1997; Yurieva, *et al.,* 1997). In either case, selective pressure by toxic metals could co-select for

horizontally transferable antibiotic resistance factors. The co-occurrence of bacterial resistance to antibiotics and metals has been recognized since the discovery of resistance plasmids in the 1960s (Richmond and John, 1964). Metal tolerance and resistance of bacterial have been shown to increase proportionally along industrial contamination gradients (Osborn *et al.,* 1997; Roane and Kellogg, 1996).

Researchers have found relationships between the occurrence of antibiotic resistance and the occurrence of metal tolerance; examples includes lead and multiple antibiotic resistances in *Pseudomonas, Bacillus, corybacterium* and *Enterobacter (* Roane and Kellogg, 1996). Mercury and tetracycline resistance in unidentified marine bacteria (Rasmussen and Sorensen, 1998); lead, cobalt, nickel, copper and penicillin resistance in *Klebsiella pneumoniae* (Choudhury and Kumar, 1996); Vanadium and multiple antibiotic resistance in *Enterobacter cloacae* (Hernandez *et al.,* 1998) and cadmium and erythromycin resistance in *Stenotrophomonas maltophilia* (Alonso *et al.,* 2000). In another study, mercury-resistant strains of *Acinetobacter* were found to be resistant to ampicillin, chloramphenicol, kanamycin

and tetracycline (Dhakephalkar and Chopade, 1994).

Furthermore, antibiotic resistance under field conditions is made more complex by frequent genetic association with metal tolerance and resistance genes (Dhakephalkar and Chopade, 1994; Roane and Kellogg, 1996; Wireman *et al.*, 1997). Metal resistance and antibiotic resistance may be linked too closely to conclude that they are independent through incidental field sampling. Antibiotic resistance may not prove to be a singular event but may be a complex of events dependent on exposure to metals (McArthur and Tuckfield, 2000).

Multidrug resistance (MDR) has been described for several bacterial species and is associated with the expression of outer membrane profiles (OMPs) involved in the transport of antibiotics inside bacterial cells. Similar types of pumps are involved in heavy-metal resistance (Silver, 1996; Silver and Phung, 1996). Most MDR systems so far described for Gram-negative are three-component pumps (Nikaido, 1996; Paulson *et al.,* 1996; Saier *et al.,* 1998); the regulation of their expression, however is still not completely understood.

Most antibiotics are readily degraded in the environment (Kolpin, *et al.,* 2002), but metals are not and so can represent a long term selective pressure. Pathogenic and non-pathogenic microorganisms selected for antibiotic resistance in metal-contaminated environments may reach human hosts through bathing and other domestic uses of contaminated water due to run-off from dump sites and effluents from homes.

In a survey carried out by Sabry *et al.,* (1997), on aerobic heterotrophic and metal-resistant bacterial communities in marine water, the resistance patterns, expressed as MICs, for 81 bacterial isolates to eight heavy metals were surveyed by using the agar dilution method. A great proportion of the isolates were sensitive to cadmium (99%), mercury (91%), zinc (84%) and cobalt (83%). On the other hand, 94%, 40%, 35% and 22% were resistant to lead, nickel, arsenate and copper, respectively. The majority of the tested strains (95.06%) were multiple metal-resistant. The responses of the isolates to 11 antibiotics was tested and ranged from complete resistance to total sensitivity and multiple antibiotic resistance was exhibited by 70.38% of the total isolated population. The highest incidence of metal and antibiotic resistance existed between lead and

all antibiotics (100%), copper and penicillin (95%) and nickel and ampicillin (83.3%).

In a similar study, the antarctic waters from the Indian side were examined for the incidence of metal and antibiotic-resistant bacteria. The bacterial isolates from the waters showed varying degrees of resistance to antibiotics (Chloramphenicol, ampicillin, streptomycin, tetracycline and kanamycin) and metals (K_2CrO_4, $CdCl_2$, $ZnCl_2$ and $HgCl_2$) tested. Of the isolates screened, about 29% and 16% were resistant to 100 ppm of cadmium and chromium salt respectively. Tolerance to lower concentration (10ppm) of mercury (Hg) was observed in 68% of the isolates. Depending on the antibiotics the isolates showed different percentages of resistance. Multiple drug and metal-resistance were observed, and high incidence of resistance to both antibiotics and metals were common among the pigmented bacterial isolates. (De Souza *et al*., 2006).

In the above studies the agar dilution technique was employed to study resistance to heavy metals, consequently, this method is also applied in the heavy metal tolerance tests in this study.

2.9 Waste

Waste can be considered as any material or energy form that cannot be economically used, recovered or recycled at a given time and place (Goel, 1994). Growth in human populations has generally been matched by a greater formation of a wider range of waste products, many of which cause serious environmental pollution if they are allowed to accumulate in the ecosystem. In rural communities recycling of human, animal and vegetable waste has been practices by man for centuries, providing in many cases valuable fertilizers or fuels.

In urban communities where most of the deleterious wastes accumulate, efficient waste collection and specific treatment processes have been developed since it is impracticable to discharge high volumes of waste into natural land and waters. The development of these practices in the last centuries was one of the main reasons for the spectacular improvement in the health and well being of the community (Goel, 1994).

Unfortunately, developing centuries, on the whole, are lagging behind in getting control over their major pollution sources. As a

consequence, their environmental quality is gradually deteriorating. In the past, water pollution in the developing countries resulted mainly from the discharge of untreated wastewater. Today it is more complex as a result of the production of hazardous waste from industries and the rapidly increasing use of pesticides in agriculture. In fact, water pollution today in some developing countries at least in the newly industrializing ones, is worse than in industrialized countries (Goel, 1994).

2.10 Pollution

2.10.1 Types and Sources

There are large numbers of microbial agents, elements and compounds, which may cause water pollution. They are classified as: microorganisms, biodegradable organic compounds, suspended matter, nitrates, salts, heavy metals, nutrients and organic micro pollutants (Ivanildo and Richard, 1995).

2.10.2 Microorganisms

Microorganisms are common in freshwater polluted particularly by discharges of untreated domestic wastewater. These microbial agents

include pathogenic bacteria, viruses, helminthes, protozoa and several more complex multicellular organisms that cause gastro-intestinal illnesses. The other organisms are more opportunistic in nature, infecting susceptible individuals through body contact with contaminated water or by inhalation of poor quality water droplets in aerosols of various origins (Ivanildo and Richard, 1995). particulate matter is a major carrier of organic and inorganic pollutants. Most toxic heavy metals, organic pollutants, pathogens and nutrients, such as phosphorus, are found in suspended matter.

2.10.3 Heavy Metals

Heavy metals such as lead, cadmium and mercury are micro-pollutants and of special interest, as they have health and environmental significance due to their persistence, high toxicity and bio-accumulation characteristics. There are basically five sources of heavy metals, contributing to water pollution: Geological weathering, which provides the background level; industrial processing of ores and metals; the use of metal and their compounds such as chromium salts in tanneries, copper compounds in agriculture and tetraethyl leads as an anti-knock agent of gasoline; leaching of heavy metals

from domestic wastes and solid waste dumps; and heavy metals in human and animal excretions, particularly zinc, metals released into the air from automobiles, fuel burning and industrial process emissions may settle on land and ultimately run off to surface water (Ivanildo and Richard, 1995).

2.10.4 Urban Pollution

Owing to continuously expanding, aggressive and multi-faceted pollution scenario, the problem of maintaining the quality of water resources has become acute, particularly in the more urbanized areas of the developing world. Maintaining water quality is hampered by two factors: Failure to enforce pollution control at main sources, especially industrial and inadequacy of sanitation system and of garbage collection and disposal.

Urban effluents are known to contain antibiotics and antibiotic resistant bacteria belonging to the human and animal commensal flora, mainly enterobacteriacaeae (Jones *et al.,* 1986; Bhattacherjee *et al* ., 1988; Halling-sorensen *et al.,* 1998). On the other hand, vanadium and nickel are constituents of crude oil in the form of inorganic and metallo-organic compounds. Both metals are not only

present in residual waters from the oil industry but also can be present as particulate matter in inhaled air (Madany and Raveendran, 1992) and as environmental heavy metals emitted from automotive material (Falahin-Ardakani, 1984). The recovery of heavy metals from industrial residues is an important task for environmental and economic reasons (Ayres, 1992). Nickel and vanadium are toxic even at low concentrations (Kadiiska *et al.,* 1997).

2.10.5 Health Impacts of Microbial Pollution

Diseases arising from the ingestion of pathogens in contaminated water have the greatest impact worldwide. An estimated 80% of all diseases, and over one-third of deaths in developing countries are caused by the consumption of contaminated water, and on average as much as one-tenth of each person's production time is sacrificed to water-related diseases, (WHO, 2001).

Waste-borne diseases are the largest single category of communicable diseases contributing to infant mortality in developing countries and second only to tuberculosis in contributing to adult mortality, with one million deaths per year. The total annual number of cholera cases alone reported to the WHO by its member states has reached levels

unprecedented during the seventh pandemic, with a peak of 595.000 cases in 1991 (WHO, 2001).

2.10.6 Health Impacts of Chemical Pollution

The health problems associated with chemical substances dissolved in water arise primarily from their ability to cause adverse effects after prolonged periods of exposure; of particular concern are contaminants that have cumulative toxic properties such as heavy metals and some organic micro pollutants. Others include the substances that have carcinogenic, reproductive and developmental effects. Other dissolved substances in water are essential ingredients of dietary intake and yet others are natural with regards to human needs (Ivanildo and Richard, 1995). Chemicals in water may be classified into three categories for the purpose of health impacts;

Substances exerting an acute or chronic toxicity upon consumption; the severity of the health impairment increases with the increase of their concentration in drinking water. On the other hand, below a certain threshold concentration no health effects can be observed, that is, the human metabolism can handle this exposure without measurable long-term effects. Various metals, nitrates, cyanides and

so on fall within this category (Ivanildo and Richard, 1995).

Genotoxic substances; these cause health effects such as carcinogenicity, mutagenicity and birth-defects. According to present scientific thinking there is no threshold level which could be considered safe, since any amount of the substance ingested contributes to an increase in cancer and similar risks (Ivanildo and Richard, 1995). Complex mathematical extrapolation models are used to determine such risks, since very little epidemiological evidence exist. Synthetic organics, many chlorinated organic micropollutants, some pesticides and arsenic fall within this category.

For some element, such as fluoride, iodine and selenium, the contribution made by drinking water is crucial and, if deficient, causes more or less severe health effects as reported by Ivanildo and Richard, (1995). At high concentrations, however, these same substances cause equally severe health effects, but of a different nature.

2.10.7 Environment Impact

The impacts of environment pollution on freshwater quality are numerous and have existed for a long time. Industrial development, the advent of human populations and the production and use of tens of

thousand of synthetic chemicals are among the main causes of water quality deterioration at local, national and global levels (Ivanildo and Richard, 1995). The major issue of water pollution is the interference with actual or planned water uses. One of the most severe and ubiquitous causes of environment degradation is the discharge or organic waste into water courses.

The economic consequences of water pollution can be severe due to detrimental effects on human health or on the environment. The economics of disease burden can be expressed not only in cost of treatment, but also in quantifying the loss of productivity (Ivanildo and Richard, 1995).

Chapter Three MATERIALS AND METHODS

3.1 Study Sites

The study sites include Samaru, Sabon-gari and Tudun wada areas of Zaria metropolis. Common features of the dumpsites include deposits of agricultural waste such as vegetables, left over food materials, bottles, polythene materials, fabrics, potash, and metal cans. The proportions of these vary with the major activities within the area studied. A total of 52 dump-sites were used in this study.

3.2 Media Sterilization

Media employed in this study were sterilized at a temperature of 121-124 0C and standard pressure of 1.1 bar for 30min hold time in the autoclave (Astel). Glasswares were sterilized at 160-180 0C in the hot air oven (Memmert), before use (Mackie, 1989).

3.3 Sample Collection

Soil samples were obtained from 52 dump-sites randomly selected on the bases of their large size and location near residential homes, water source and watercourses. Wastewater samples from domestic effluents were also obtained from 52 source within the study area. These were

obtained from exposed drains from homes.

Representative samples of both soil and wastewater were collected separately from 3-5 different locations within a sampling site and mixed together to obtain a single sample. Soil samples were collected aseptically using sterile spatula into sterile wide mouth bottles, while wastewater sample were collected into 200 ml sterile bottles. Samples for microbiological analysis were brought to microbiology laboratory within 24 hrs for analysis.

Wastewater samples for heavy metal analysis were filled into plastic bottles previously soaked in 10% nitric acid and rinsed with deionised water (Erah *et al.,* 2002).

3.4 Microbiological Analysis

Three pathogens were selected for this study viz: *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* The selection of these organisms is based on the fact that they are common pathogens of public health importance and are widely known for resistance to various antimicrobial agents in common use. *S.aureus* is a major human pathogen of increasing importance as a result of the spread of antibiotic resistance. It causes a wide range of disease and

survives outside the host by virtue of its adaptability and resistance to environmental stress. *E.coli* and *Ps. aeruginosa* are common in the environment as opportunistic pathogens.

3.4.1 Isolation and Characterization of *S. aureus*

Ten grams of the soil sample was dissolved in 10 ml sterile distilled water and the supernatant serially diluted out ten folds into 10^{-1}, 10^{-2}, and 10^{-3}. The 10^{-3} dilution was used to inoculate Mannitol salt agar (Oxoid) by spread plate technique to obtain isolated colonies; plates were incubated at 37^{0}C for 18-24 hrs and only colonies which fermented Mannitol, giving a yellow colour in the medium were selected for further tests. All selected colonies were further purified by repeated culturing and stained for observation under the microscope. Gram-positive cocci isolates were then biochemically characterized using catalase, coagulase and Dnase tests. Wastewater samples were similarly diluted out and cultured as described above, and only isolates that produced clumping in citrated human plasma in case of coagulase test, bubbling and frothing in catalase test and a zone of clearing when the colonies on Dnase Agar (Oxoid) were flooded with 10% HCl solution were considered as pathogenic *S.aureus* (Cheesbrough, 2000). Isolates were designated SS-for soil

samples, and SW- for wastewater samples, and preserved on nutrient agar slants and stored bellow 8^0C in the refrigerator for further use.

3.4.2 Isolation and Characterization of *E. coli*

The dilution of the samples prepared was also used to inoculate Eosin methylene blue (EMB) agar (Oxoid) by spread plate and incubated at 37^0C for 18-24hrs colonies showing greenish metallic sheen on EMB were stained and viewed under the microscope. Only Gram-negative isolates were then tested for indole, methyl red, Voges Proskauer, citrate utilization (IMVIC) and Triple Sugar Iron (TSI) agar (Oxoid). Isolates that gave the characteristic IMVIC reaction that is ++-- and which gave (acid/acid) with gas production in TSI were then designated ES-for soil samples, and EW- for wastewater samples, and preserved on nutrient agar (Oxoid) slants and stored bellow 8^0C in the refrigerator for further use.

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3.4.3 Isolation and Characterization of *Ps. aeruginosa*

The dilution from both soil and wastewater samples were also inoculated on cetrimide agar (Oxoid) and incubated at 37^0C for 18-24 hrs for isolation of *ps.aeruginosa.* Colonies were sub-cultured on

nutrient agar (Oxoid) slants for further identification. After Gram staining and viewing smears of the isolated colonies under the microscope, presumptive *ps.aeruginosa* were confirmed using biochemical tests such as oxidase test, gelatin liquefaction and Triple sugar iron (TSI) test. Isolates which developed purple colour on filter paper soaked with oxidase reagent, liquefied nutrient gelatin (Oxoid) and produced (Alkaline/Acid) without gas production from TSI agar were confirmed isolates. Pigment production was an additional confirmation (Cheesebrough, 2000). Isolates were designated PS-for soil samples, and PW- for wastewater samples, then preserved on nutrient agar slants and stored bellow 8 0C in the refrigerator.

3.5 Antibiotic Susceptibility Testing

3.5.1 Preparation of Inocula

The antibiotic susceptibility pattern of isolates of *S.aureus, E.coli* and *Ps.aeruginosa* was carried out using the Kirby-Bauer NCCLS modified disk diffusion technique. The inoculum of each organism was prepared by standardizing overnight nutrient broth (Oxoid) cultures of the isolates using the 0.5 McFarland turbidity standards (CILS, 2005). Sterile cotton swab was dipped into the suspension,

rotated several times and then streaked over the entire surface of already prepared Mueller - Hinton agar (Oxoid) plates and allowed to stand for 15 minutes to allow any excess surface moisture to be absorbed. Antibiotic multidisk (Abtek) comprising Amoxycillin (AMX-25pg), Nitrofurantoin (NIT-300pg), Augmentin (Aug-30pg) Cotrimoxazole (COT-25pg), Gentamicin (GEN-10pg), Nalidixic acid (NAL-30pg), Ofloxacin (OFL-30pg) and Tetracycline (TET-30pg) was then placed on the inoculated plate with a sterile pair of forceps and gently pressed onto the agar surface to provide uniform contact. The plates were then incubated at 37^0C for 18 hours.

3.5.2 Determination of Zones of Inhibition

The diameters of the zones of complete inhibition were measured to the nearest whole millimeter with a ruler held on the back of the inverted Petri dishes. The zone diameters measured around each disk were interpreted on the bases of guidelines published by NCCLS and the organisms were reported as susceptible, intermediate, or resistant to the antimicrobial agents tested (ATCC25922) and *Ps. aeruginosa* (ATCC27853) were used as control strains in the sensitivity tests.

3.6 Determination of Concentration of Heavy Metals

The energy Dispensive X-Ray Fluorescence (EDXRF) of environmental samples with isotopic source (Angeyo, et al., 1998) was used to determine the concentration of some heavy metals in 20 randomly selected samples; 10 samples each from 52 soil samples and 52 wastewater samples.

3.6.1 Sample Preparation

Soil samples were ground manually to powder with an agate mortar and pestle to grain size of less than 125 µm. Pellets of 19 mm diameter were prepared from 0.3 - 0.5g powder mixed with three drops of organic liquid binder and pressed afterwards with a hydraulic press.

The wastewater was acidified with concentrated nitric acid. 1-pyrrolidine dithiocarboxylic acid ammonium salt was then added and allowed to stand for 20-25 minutes, after which it was filtered using a milli-pore membrane filter.

Measurements were performed using an annular 25 mCi Cd as the excitation source, that emits Ag-K X-rays (22. 1 keV) in which case all elements with lower characteristic excitation energies were

accessible for detection in the samples. The system consists further of a silicon or lithium detector, with a resolution of 170 eV for the 5.90 keV line coupled to a computer controlled ADC-card.

Quantitative analysis of the sample was carried out using the Emission Transmission (E-T) method, for which a number of qualification methods have been developed and applied (Tang *et al,* 1986; Makowicz and vanGrieken, 1993, Kump, 1996 Bernasconi *et al.* 1996). These qualification methods provide different approaches to correct the matrix absorption as well as enhancement effects. In this work qualification was carried out using modified version of E-T method (Kump, 1996, Angeyo *et al,* 1998; Funtua, 1999a, Funtua, 1999b) and it involves the use of pure target material (Mo) to measure the absorption factors in the sample.

The Mo target serves as a source of monochromatic X-rays, which are excited through the sample by primary radiation and then penetrate the sample on the way to the detector. In this way, the absorption factor is experimentally determined which the program uses in the qualification of concentration of the elements. In addition, the contribution to the Mo-K peak intensity by the Zr-K is subtracted for

each sample.

Sensitivity calibration of the system was performed using thick pure metal foils (Ti, Fe, Co, Ni, Cu, Zn, Zr, Nb, Mo, Sn, Ta, Pb) and stable chemical compound (K_2CO_3, $CaCO_3$, Ce_2O_3, WO3, THO_2, U_3O_8. The spectra for the sample were collected for 3000s and 2000s with the ^{109}Cd source. The spectra were then evaluated using the AXIL - QXAS program (Bernasconi, 1996). The ^{109}Cd source was used for the analysis of K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ta, W, Ga, As, Se, Pb, Br, Rb, Sr, Th, Y, U, Zr, Nb, and Mo.

3.7 Metal Tolerance Tests

All isolates were tested for tolerance to each of mercury, copper and cobalt to determine their minimum inhibitory concentration against each isolate using the methods of Calomiris *et al.* (1984) and forbes, (1998) as briefly stated below.

3.7.1 Preparation of Inocula

The inocula were prepared using 24 hours broth cultures of the isolates adjusted to 0.5 McFarland turbidity standards. The experimental plates were prepared by supplementing Mueller-Hinton agar (Oxoid) with metal salts to final cationic concentrations of 400,

800, 1600 (µml^{-1}) for Cu^{2+} and Co^{2+} and 2.5, 5.0 and 10 (µgml^{-1}) for Hg^{2+} with micropipettes. The salts used were $CuCl_{2,}$ $CoCl_{2,}$ and $HgCl_{2-}$. The salts agar media were adjusted to a final pH of 7.0-7.2 using sodium hydroxide before pouring onto the plates. After inoculation the plates were incubated for 18 hours at 37^0C and then examined to determine the minimum inhibitory concentration of the isolates.

3.7.2 Determination of Minimum Inhibitory Concentration (MIC)

The works of Calomoris *et al.,* (1984) Vajiheh *et al* (2003) and Alm (2002) were used as reference in the determination of tolerance level of isolates. The lowest concentration of the heavy metals in Mueller Hinton agar, which produced no growth after 18-24 hours of incubation were designated to be MIC of the isolates. As in the case of antibiotic susceptibility, typed strains of S.*aureus* (ATCC29213), *E. coli* (Atcc25922) and *P.aeruginosa* (ATCC27853) were used as control strains in the sensitivity tests.

3.8 Statistical Analysis

Chi-square of association was used to ascertain if there is association between heavy metal tolerance of isolates and their antibiotic

resistance using a three by two table at 5% confidence interval using the formula

$$\chi^2 = \sum_{i=1}^{3} \sum_{j=1}^{2} \frac{(Oij - Eij)^2}{Eij}$$

Correlation coefficient for resistance to antibiotics and tolerance to heavy metals was also calculated at ($p \leq 0.05$).

Chapter Four RESULTS

4.1 Microbiological Analysis

A total of 180 strains of microorganisms consisting of 56 *S. aureus* strains (34 from dumpsites and 22 from wastewater), 55 *E. coli* strains (32 from dump sites and 23 from waste water) and 69 *Ps. aeruginosa* strains (38 from dumpsites and 31 from wastewater) were isolated and characterized using standard procedures. The distribution of isolates in environments is shown in table 1.

Table 1: Distribution of strains in dump sites and wastewater

Organism	No. of dumpsites with Isolates	No. of wastewater with Isolates	Total number of Isolates
S. aureus	34	22	56
E. coli	32	23	55
P. aeruginosa	38	31	69
			Σ = 180

4.2 Antibiotic Sensitivity Testing

All isolated and characterized strains of *S aureus; E. coli* and *Ps. aeruginosa* were tested for sensitivity to gentamicin, augmentin, tetracycline, nalidixic acid, cotrimoxazole, nitrofurantoin, amoxycillin and ofloxacin. Results of the sensitivity are presented in tables 2, 3 and 4. The zones of inhibition are measured in millimeters.

Table 2. Disk diffusion sensitivity results for *S aureus*

Isolate	OFL	AUG	TET	AMX	COT	NIT	GEN	NAL
SS1	30	28	22	24	20	22	20	10
SS2	24	30	32	30	32	26	26	6
SS3	28	22	20	12	0	18	20	0
SS4	30	30	28	26	26	24	26	8
SS5	32	30	28	28	28	28	32	10
SS6	32	34	34	34	30	34	18	0
SS7	30	12	24	4	22	22	24	10
SS8	32	28	28	24	30	22	24	20
SS9	26	30	28	26	26	22	22	0
SS10	32	32	26	28	28	26	20	10
SS11	30	28	22	24	0	22	20	10
SS12	32	26	22	26	6	22	22	8
SS13	24	32	30	32	32	26	26	6
SS14	28	22	12	12	0	18	20	0
SS15	30	12	24	8	22	22	18	12
SS16	32	34	30	30	32	32	28	0
SS17	30	12	24	8	22	22	18	12
SS18	32	28	28	24	30	22	24	20
SS19	28	30	30	28	26	24	24	0
SS20	30	28	22	24	20	22	20	10
SS21	24	30	32	30	32	26	26	6
SS22	28	22	10	12	0	18	20	6
SS23	30	30	28	26	26	24	26	0
SS24	32	30	28	28	26	28	28	10
SS25	32	34	34	34	30	30	32	0
SS26	30	12	24	8	22	22	18	10
SS27	22	28	28	24	30	22	24	18
SS28	26	30	28	26	26	22	26	0
SS29	32	32	26	28	28	26	22	10
SS30	30	28	22	24	4	22	20	10
SS31	32	28	24	22	26	26	22	8
SS32	32	32	30	32	6	22	20	6
SS33	28	22	10	12	0	18	20	0
SS34	30	30	28	28	26	26	24	8

Table 2. cont'd. Disk diffusion sensitivity results for *S aureus*

Isolate	OFL	AUG	TET	AMX	COT	NIT	GEN	NAL
SW1	34	32	30	30	32	32	28	0
SW2	30	12	24	4	22	22	18	0
SW3	32	28	28	24	30	22	24	20
SW4	28	30	30	28	26	24	26	0
SW5	30	28	22	24	20	22	20	10
SW6	30	28	22	24	0	22	20	10
SW7	24	30	32	30	32	26	26	6
SW8	28	22	10	12	0	18	20	0
SW9	30	30	28	26	26	24	26	10
SW10	32	30	28	28	28	28	26	10
SW11	32	34	34	32	30	32	34	0
SW12	30	12	24	4	22	22	20	8
SW13	32	28	28	24	30	22	24	20
SW14	26	30	28	26	22	26	24	0
SW15	26	30	28	26	26	24	22	0
SW16	32	28	28	24	30	22	24	10
SW17	30	12	24	6	22	24	20	10
SW18	32	34	34	28	28	34	34	0
SW19	30	32	28	28	28	30	28	10
SW20	30	30	26	28	26	24	26	8
SW21	28	22	10	12	4	28	20	0
SW22	26	30	32	30	32	26	26	6
CONT	32	32	26	28	38	22	22	12

Table 3. Disk Diffusion Sensitivity Results For *E.coli*

Isolate	OFL	AUG	TET	AMX	COT	NIT	GEN	NAL
ES1	30	10	0	10	6	20	16	18
ES2	20	22	6	22	16	22	18	0
ES3	20	22	8	20	12	28	12	14
ES4	24	10	0	0	0	18	16	20
ES5	24	12	16	10	14	20	14	18
ES6	28	0	0	0	0	0	16	18
ES7	30	20	6	18	12	24	16	22
ES8	30	22	20	8	22	24	14	18
ES9	28	20	18	10	22	18	20	24
ES10	32	18	18	12	20	16	16	22
ES11	30	18	16	8	22	14	18	22
ES12	28	18	16	16	24	20	16	20
ES13	30	10	22	18	4	22	20	0
ES14	26	10	20	12	8	16	22	20
ES15	28	12	20	8	4	14	18	18
ES16	30	14	22	10	6	14	18	18
ES17	30	12	20	8	4	14	20	18
ES18	30	20	18	18	22	18	16	20
ES19	32	22	0	22	18	24	20	24
ES20	30	16	20	12	22	20	18	18
ES21	34	16	0	0	0	18	16	20
ES22	30	22	20	8	4	14	18	18
ES23	28	12	20	8	24	20	18	22
ES24	28	18	16	16	24	24	14	20
ES25	30	20	18	18	22	18	16	20
ES26	28	18	16	8	22	14	18	20
ES27	30	20	18	18	22	18	16	22
ES28	30	10	22	8	8	22	20	6
ES29	26	12	16	12	14	22	14	18
ES30	24	10	16	16	22	18	16	20
ES31	20	22	6	22	16	20	18	10
ES32	26	20	6	18	12	24	16	20

Table 3. cont'd ..Disk Diffusion Sensitivity Results For *E.coli*

Isolate	OFL	AUG	TET	AMX	COT	NIT	GEN	NAL
EW1	20	22	6	22	16	22	18	0
EW2	20	22	8	22	12	28	12	14
EW3	26	22	0	22	16	24	22	24
EW4	26	20	6	18	12	24	16	22
EW5	28	20	0	18	16	20	18	20
EW6	30	20	0	18	16	20	18	22
EW7	32	22	0	22	18	24	20	24
EW8	34	16	0	0	0	18	16	20
EW9	30	10	22	8	4	22	20	0
EW10	24	12	16	10	14	20	14	18
EW11	24	10	0	0	0	18	16	20
EW12	30	22	20	8	24	14	18	22
EW13	28	12	20	8	4	14	18	18
EW14	28	18	16	16	24	20	16	20
EW15	30	18	16	8	22	14	18	22
EW16	30	10	0	10	6	20	16	18
EW17	30	20	18	18	22	18	16	20
EW18	20	22	6	22	16	22	18	0
EW19	20	22	8	20	12	28	12	14
EW20	26	22	0	22	16	24	22	24
EW21	26	20	6	18	12	24	16	22
EW22	28	20	0	18	16	20	18	20
EW23	30	20	0	18	16	20	18	22
CONT	30	16	20	12	22	20	18	18

Table 4. Disk diffusion sensitivity results for *Ps. aeruginosa*

Isolate	OFL	AUG	TET	AMX	COT	NIT	GEN	NAL
PS1	36	24	22	24	26	26	20	26
PS2	30	6	20	0	26	12	18	20
PS3	32	26	26	24	30	24	26	24
PS4	4	0	0	0	0	0	0	0
PS5	34	22	24	24	32	26	22	26
PS6	36	6	26	0	36	36	16	34
PS7	26	18	24	8	0	28	18	22
PS8	30	0	18	0	26	18	18	22
PS9	36	8	4	0	24	12	22	26
PS10	24	0	12	0	0	0	14	4
PS11	28	14	0	0	18	24	18	22
PS12	28	8	20	0	22	14	18	26
PS13	34	6	0	0	0	18	16	22
PS14	40	20	18	20	26	20	16	22
PS15	30	22	0	0	0	14	16	18
PS16	32	20	6	0	18	26	20	24
PS17	26	20	0	18	0	26	20	24
PS18	14	0	0	0	0	0	4	8
PS19	22	0	0	0	0	0	6	18
PS20	18	0	0	0	10	0	0	12
PS21	26	0	0	0	0	0	0	16
PS22	24	0	0	0	18	0	0	14
PS23	30	0	0	0	0	0	0	20
PS24	28	0	8	0	0	0	0	22
PS25	26	0	0	0	0	0	0	16
PS26	26	0	0	0	0	0	0	10
PS27	28	0	0	0	0	0	0	16
PS28	26	0	0	0	22	0	0	16
PS29	26	0	16	0	0	0	0	20
PS30	28	0	0	0	0	0	0	20
PS31	26	0	0	0	0	0	0	16
PS32	30	0	0	0	0	0	0	20
PS33	34	6	0	0	0	18	16	22
PS34	40	20	18	20	26	20	16	22
PS35	30	22	0	0	0	14	16	18
PS36	28	14	0	0	18	24	18	22
PS37	30	0	14	0	0	0	16	0

Table 4. cont'd Disk diffusion sensitivity results for *Ps. aeruginosa*

Isolate	OFL	AUG	TET	AMX	COT	NIT	GEN	NAL
PW1	28	0	0	0	0	0	0	16
PW2	26	0	0	0	0	0	0	10
PW3	24	0	12	0	0	0	14	4
PW4	24	0	0	0	18	0	0	14
PW5	26	0	0	0	22	0	0	16
PW6	30	6	20	0	26	12	18	20
PW7	34	22	24	24	32	26	22	26
PW8	22	0	0	0	0	0	6	18
PW9	34	20	6	0	18	26	20	26
PW10	26	20	0	18	0	26	20	24
PW11	26	18	24	8	0	28	18	22
PW12	36	6	26	0	36	36	16	34
PW13	32	26	26	24	30	24	26	24
PW14	18	0	0	0	10	0	0	12
PW15	36	8	4	0	24	12	22	26
PW16	26	0	0	0	0	0	0	16
PW17	28	0	8	0	0	0	0	22
PW18	26	0	16	0	0	0	0	20
PW19	28	0	0	0	0	0	0	20

Table 4. cont'd Disk diffusion sensitivity results for *Ps. aeruginosa*

Isolate	OFL	AUG	TET	AMX	COT	NIT	GEN	NAL
PW20	30	0	18	0	26	18	18	22
PW21	28	8	20	0	22	14	18	26
PW22	30	0	12	0	0	0	18	0
PW23	14	0	0	0	4	0	6	10
PW24	12	0	0	0	0	0	10	0
PW25	26	0	0	0	0	0	0	16
PW26	30	4	0	0	0	0	0	20
PW27	34	10	0	0	0	18	16	20
PW28	36	20	18	18	24	20	16	22
PW29	30	22	0	0	0	14	16	18
PW30	26	0	16	0	0	0	0	20
PW31	26	0	0	0	22	0	0	16

Interpretative Standards For The Disk Diffusion Tests

Zone of inhibition diameter (mm)

Antibiotic	Susceptible	Intermediate	Resistant
AUG	≥ 20	_	≤ 19*
	≥ 18	14-17	≤ 13
GEN	≥ 15	13-14	≤ 12
NAL	≥ 19	14-18	≤ 13
OFL	≥ 16	13-15	≤ 12
NIT	≥ 17	15-16	≤ 14
TET	≥ 19	15-18	≤ 14
COT	≥ 16	11-15	≤ 10
AMX	≥ 18	14-17	≤ 13

KEY:

AUG - Augmentin

GEN - Gentamicin

NAL - Nalidixic Acid

OFL - Ofloxacin
NIT _ Nitrofurantion

TET - Tetracycline

COT - Contrimoxazole

AMX - Amoxycillin
* For *S. aureus*
(Adapted from NCCLS data (2000).

Figures 1, 2 and 3, represent the percentage resistance of the strains of *S. aureus*, *E. coli* and *P. aeruginosa*. *S.aureus* isolates were sensitive to most of the antibiotics, with 100% sensitivity to ofloxacin, nitrofurantoin and gentamicin. However they showed marked resistance to nalidixic acid (fig.1*). E.coli* isolates were 100% sensitive to ofloxacin, but they showed resistance in a few of the isolates to nitrofurantoin and gentamicin. Resistance to the other antibiotics was higher (fig. 2). *P.aeruginosa* show greater resistance to most of the antibiotics as shown in fig.3. By comparison, *P.aeruginosa* was more resistant to the various antibiotics than *E.coli* and *S.aureus* (fig. 4).

A comparison of resistance by isolates from the two environments (dumpsites and wastewater), shows that higher resistance was shown by isolates from dumpsites. This is illustrated in figures 5, 6, and 7.

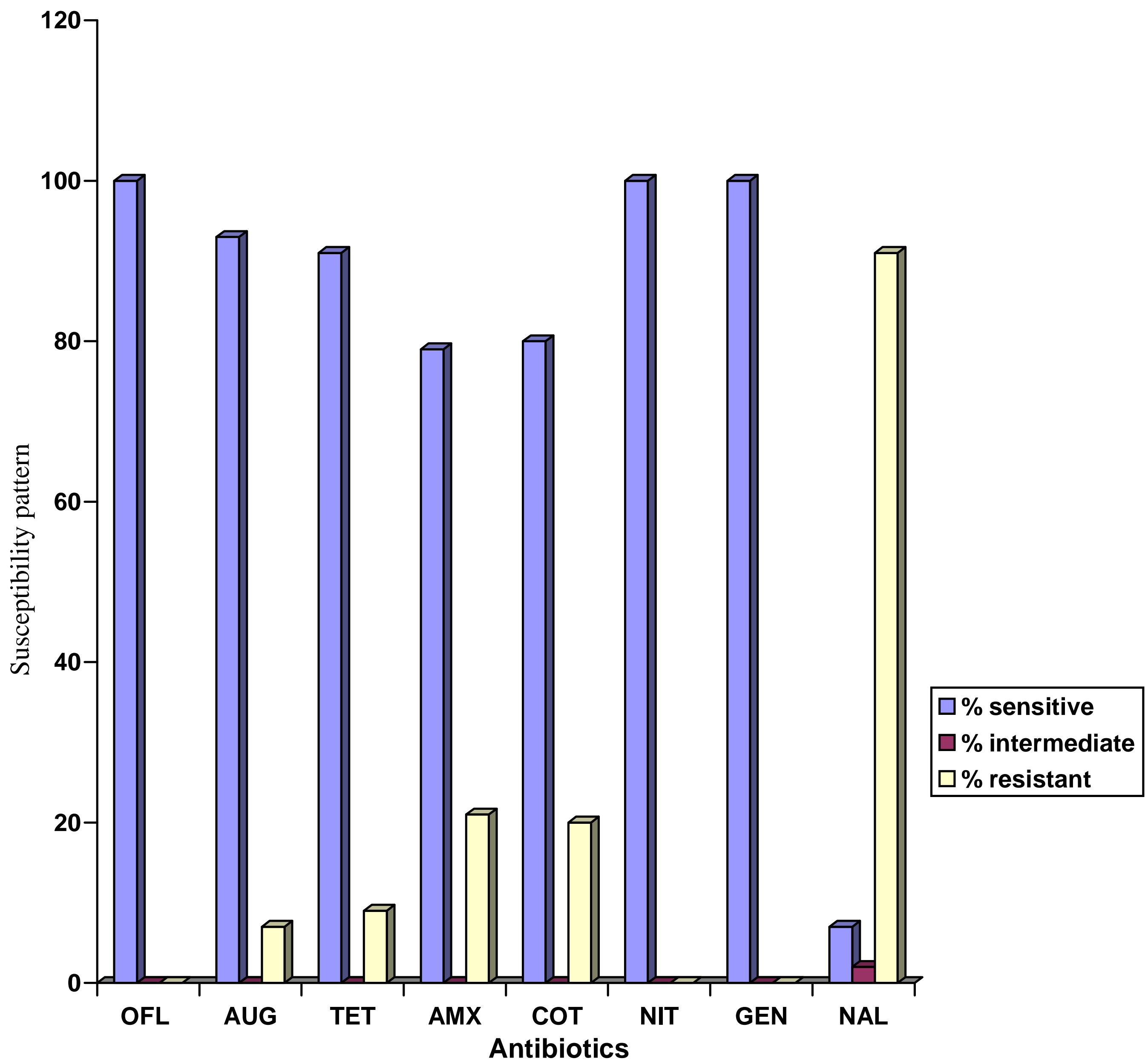


Figure 1: Antibiotic sensitivity pattern of *S. aureus* strains

Note: OFL-ofloxacin; AUG-augmentin; TET-tetracycline; AMX-amoxicillin; COT-cotrimoxazole; NIT-nitrofurantoin; GEN-gentamicin; NAL-nalidixic acid

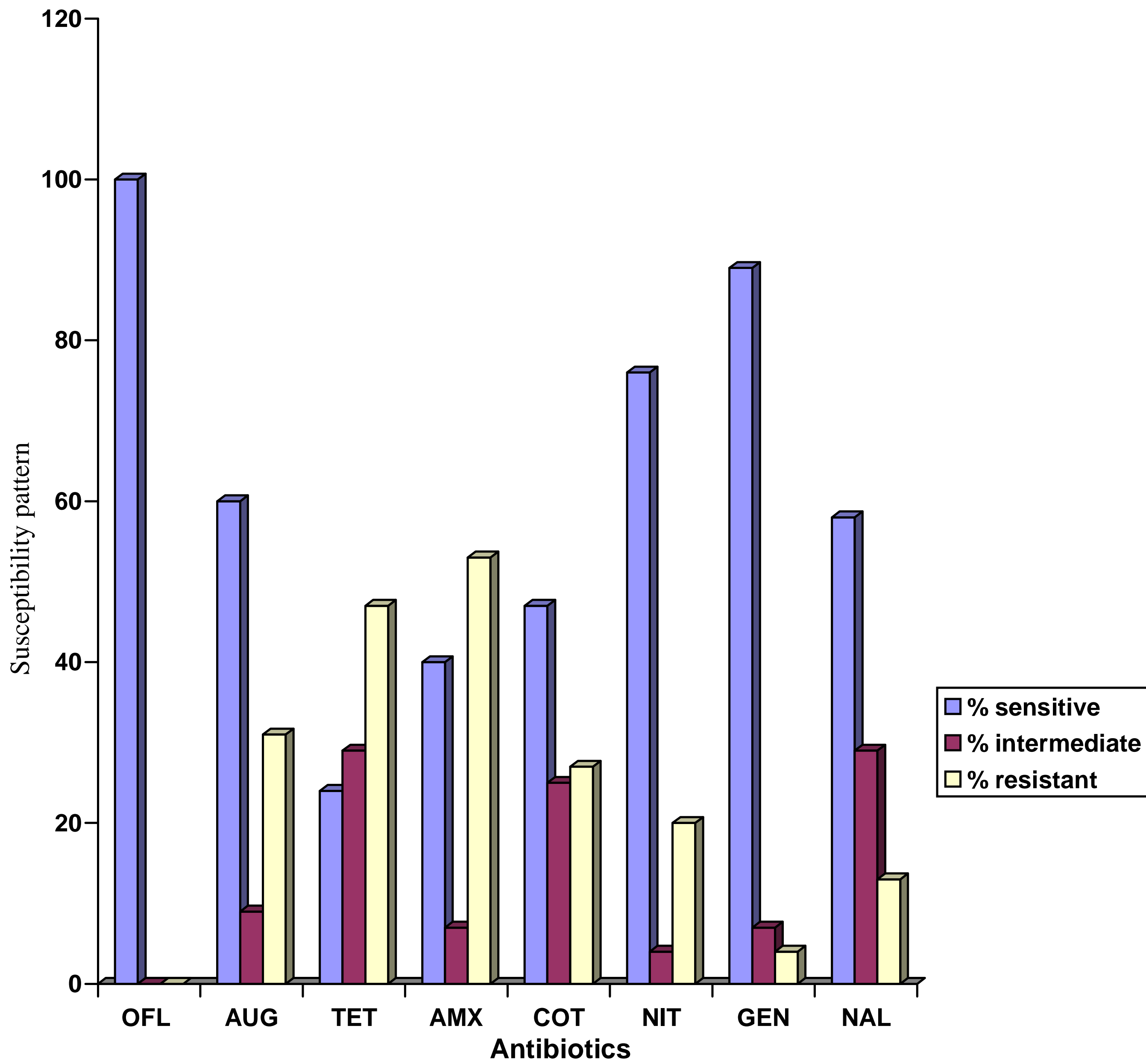


Figure 2: Antibiotic sensitivity results for *E. coli* strains

Note: OFL-ofloxacin; AUG-augmentin; TET-tetracycline; AMX-amoxicillin;

COT-cotrimoxazole; NIT-nitrofurantoin; GEN-gentamicin; NAL-nalidixic acid

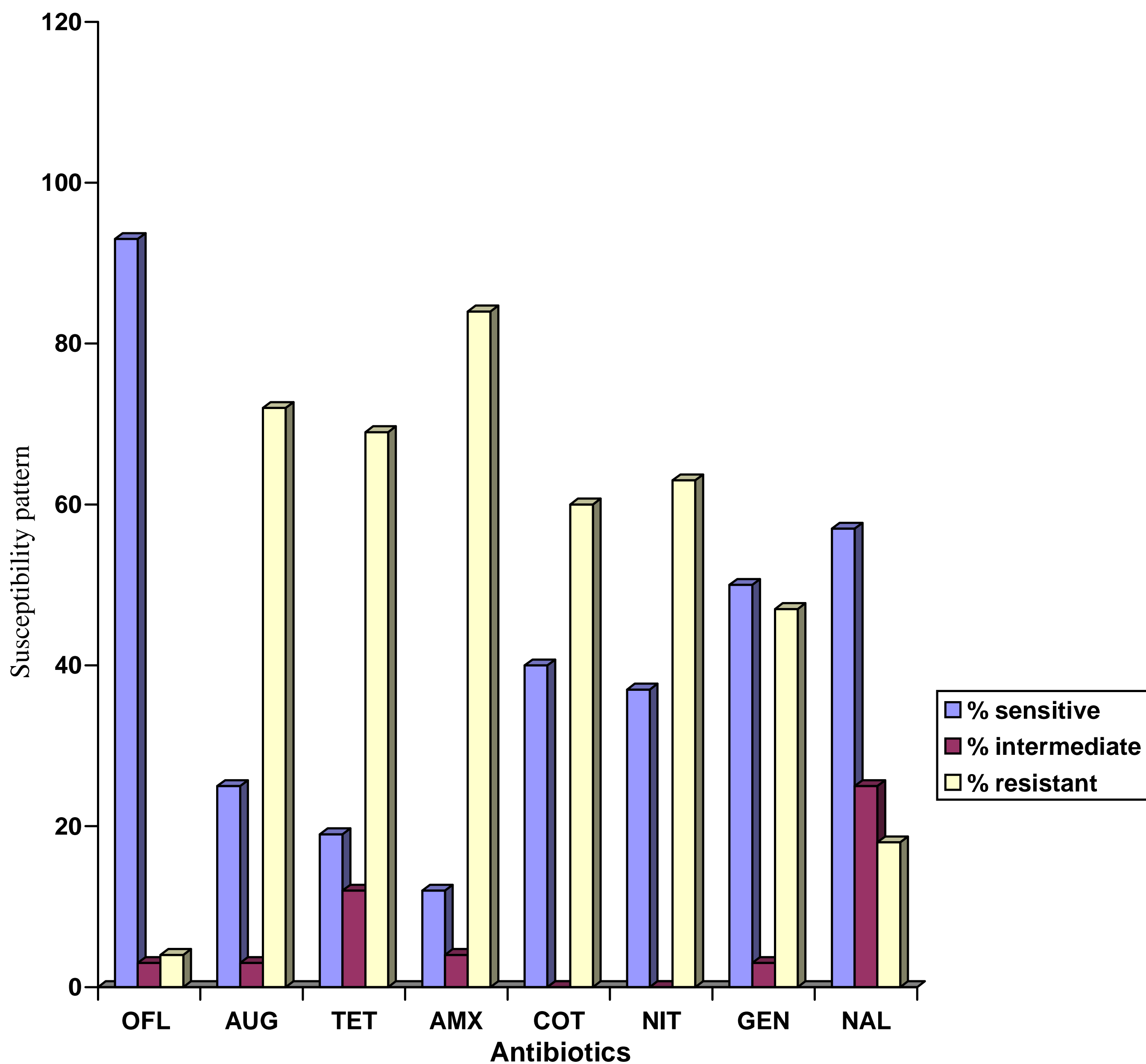


Figure 3: Antibiotic sensitivity results for *Ps. aeruginosa* strains

Note: OFL-ofloxacin; AUG-augmentin; TET-tetracycline; AMX-amoxicillin; COT-cotrimoxazole; NIT-nitrofurantoin; GEN-gentamicin; NAL-nalidixic acid

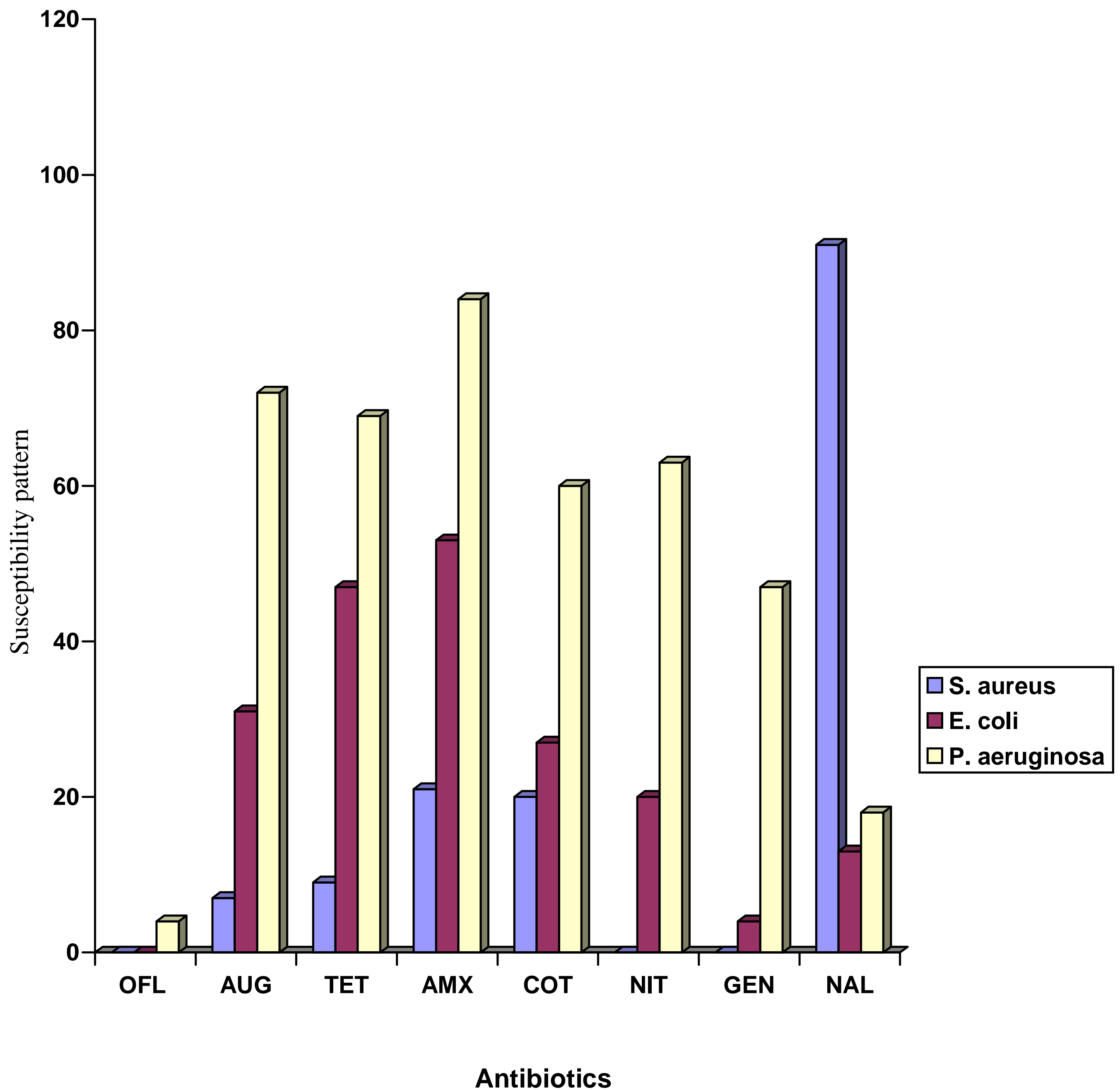


Figure 4: Comparison of percentage resistance by strains

Note: OFL-ofloxacin; AUG-augmentin; TET-tetracycline; AMX-amoxicillin;

COT-cotrimoxazole; NIT-nitrofurantoin; GEN-gentamicin; NAL-nalidixic acid

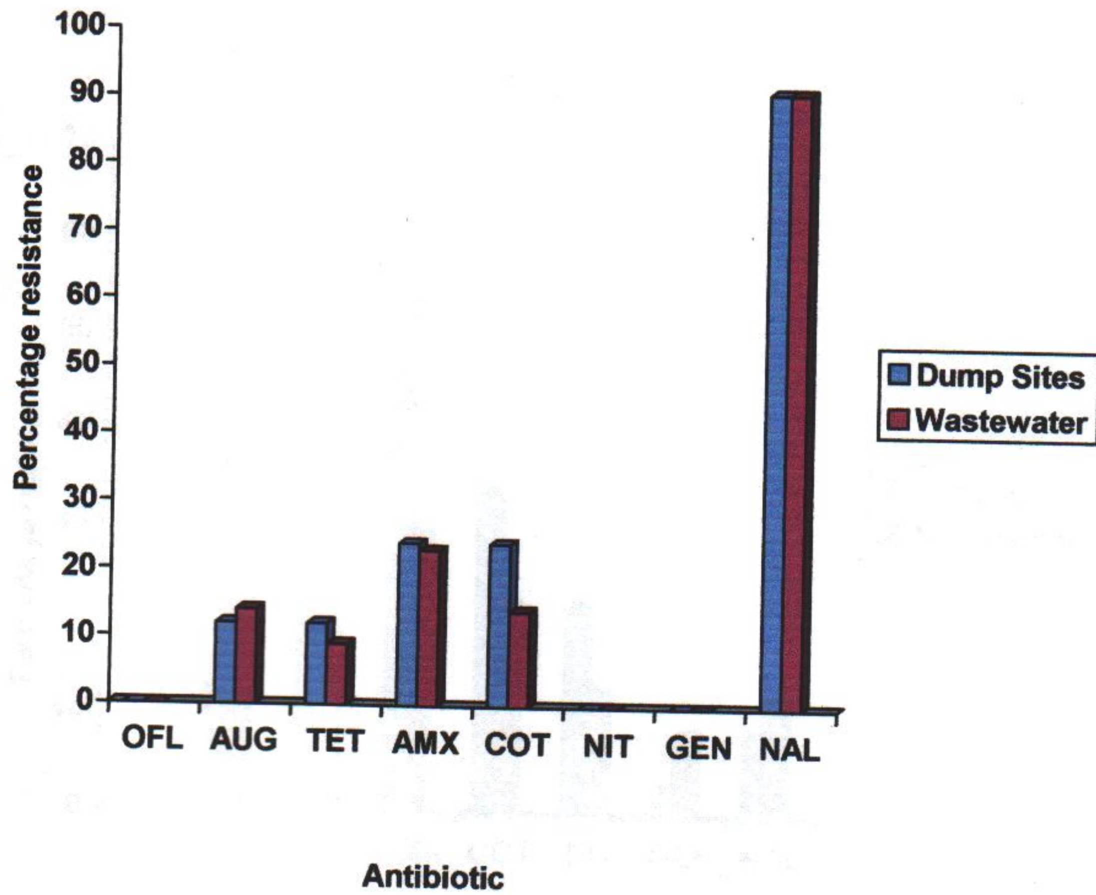
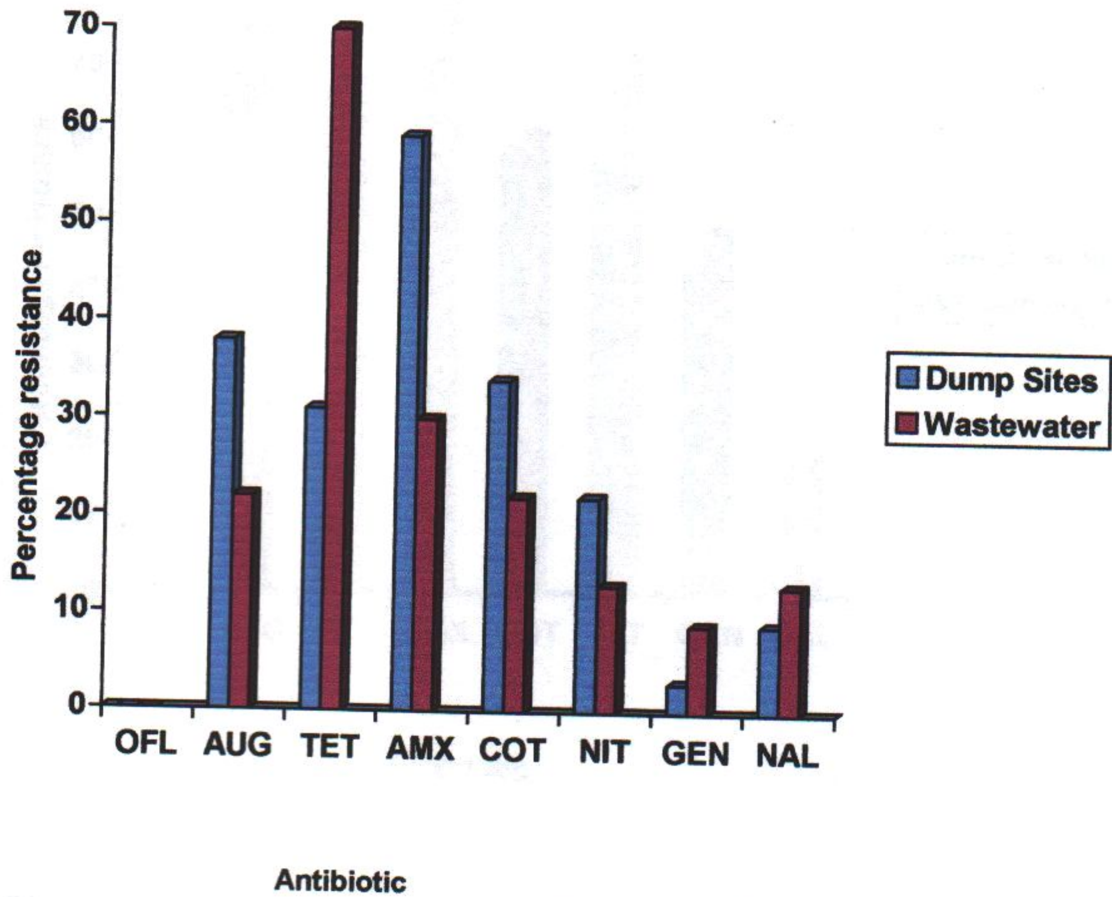


Fig. 5:Comparison of percentage resistance of *S. aureus* Isolates from Dumpsites and wastewater



Antibiotic

Fig. 6: Comparison of percentage resistance of *E. coli* Isolates from Dumpsites and wastewater

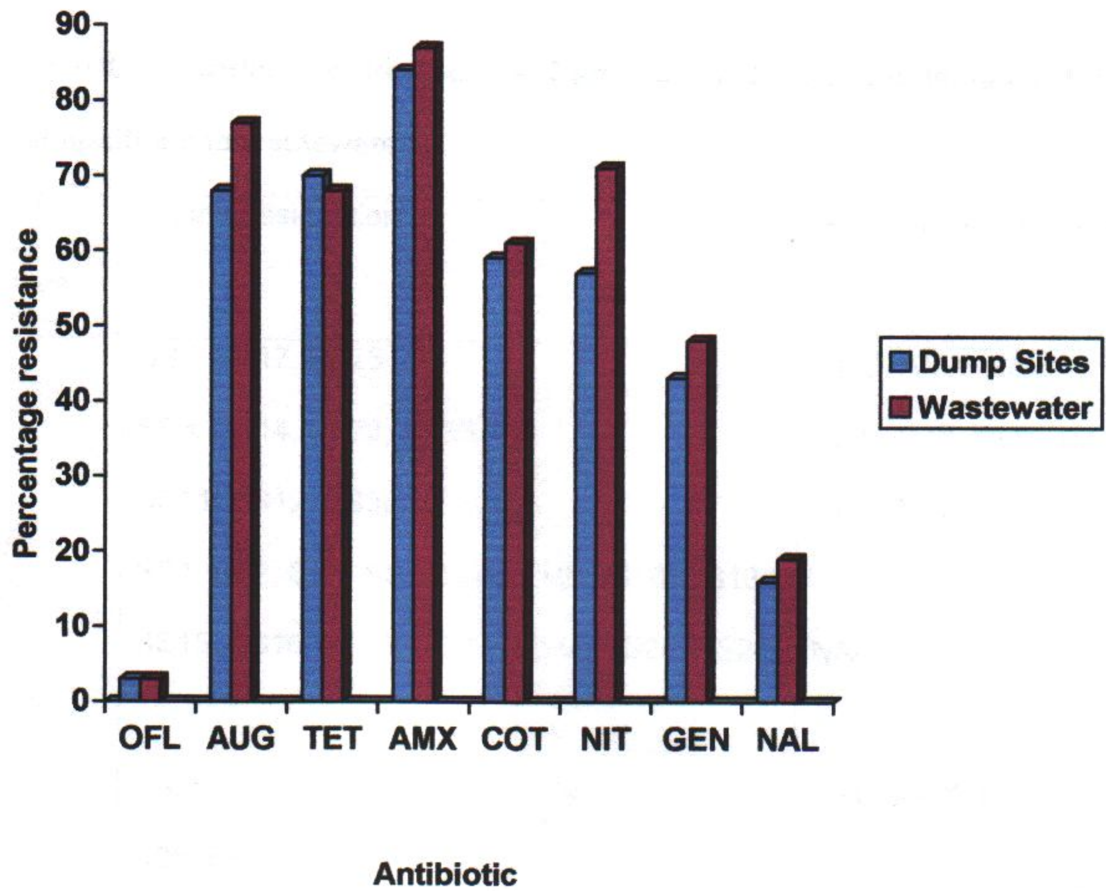


Fig. 7 Comparison of percentage resistance of *Ps. aeruginosa* Isolates from Dumpsites and wastewater

4.3 Patterns of Antibiotic Resistance

Antibiotic resistance patterns of *S. aureus* isolates, *E. coli* isolates and *Ps. aeruginosa* are given in tables 5, 6and 7 respectively. The patterns shown by the three organisms studied were not largely predictable as illustrated in the following tables.

Table 5: Antibiotic Resistance Patterns of *S. aureus* Isolates from Dumpsites and wastewater

Group dumpsites	Isolate designation	Antibiotic resistance pattern
1	SS7, SS17, SS26	AUG, AMX, NAL
2	SS3, SS14, SS12, SS33	TET, AMX, COT, NAL
3	SS11, SS12, SS30, SS32	COT, NAL
4	SS1, SS2, SS4, SS5, SS6, SS9, SS10, SS13, SS15, SS16, SS20, SS21, SS23, SS24	NAL
5	SS25, SS28, SS29, SS31, SS34 SS8, SS18, SS17	SENSITIVE TO ALL
Wastewater		
1	SW2, SW12, SW17	AUG, AMX, NAL
2	SW8, SW21	TET, AMX, COT, NAL
3	SW6	COT, NAL
4	SW1, SW4, SW5, SW7, SW9, SW10, SW11, SW14, SW15, SW18, SW19, SW20, SW22	NAL
5	SW3, SW13	SENSITIVE TO ALL

Key: SS = Isolates from dumpsite

SW = Isolates from waste water

Table 6: Antibiotic Resistance Patterns of *E. coli* Isolates from Dumpsites and wastewater

Group dumpsites	Isolate designation	Antibiotic resistance pattern
1	ES6	AUG, TET, AMX, COT, NIT
2	ES1, ES4	AUG, TET, AMX, COT
3	ES13, ES28	AUG, AMX, COT, NAL
4	ES15, ES17	AUG, AMX, COT, NIT
5	ES14	AUG, AMX, COT
6	ES2, ES31	TET, NAL
7	ES3	TET, GEN
8	ES23, ES29, ES5	AUG, AMX
9	ES8, ES26, ES11	AMX, NIT
10	ES21	TET, AMX, COT
11	ES16, ES22	AMX, COT NIT
12	ES30	AUG
13	ES7, ES19, ES32	TET
14	ES9, ES10, ES20, CONTROL	AMX
15	ES12, ES18, ES24, ES25, ES27	SENSITIVE TO ALL

Table 6 cont: Antibiotic Resistance Patterns of *E. coli Isolates* from Dumpsites and wastewater

Group dumpsites	Isolate designation	Antibiotic resistance pattern
Wastewater		
1	EW9	AUG, AMX, COT NAL
2	EW11, EW16	AUG, TET, AMX, COT
3	EW13	AUG, AMX, COT, NIT
4	EW8	TET, AMX, COT
5	EW10	AUG, AMX
6	EW2, ES19	TET, GEN
7	EW1, EW18	TET, NAL
8	EW12, EW15	AMX, NIT
9	EW3, EW4, EW5, EW6, EW7, EW20, EW21, EW22, EW23	TET
10	EW14, EW17	SENSITIVE TO ALL

Key: ES = Isolates from dumpsite

EW = Isolates from waste water

Table 7: Antibiotic Resistance Patterns of *Ps. aeruginosa Isolates* from Dumpsites and wastewater

Group dumpsites	Isolate designation	Antibiotic resistance pattern
1	PS4	AUG, TET, AMX, COT, NIT, GEN, OFL
2	PS18, PS20, PS26, PS24, PS23, PS21, PS19	AUG, TET, AMX, COT, NIT, GEN, NAL
3	PS31, PS52, PS30, PS27, S25	AUG, TET, AMX, COT, NIT, GEN
4	PS29	AUG, TET, AMX, NIT, GEN
5	PS22	AUG, TET, AMX, NIT, NAL
6	PS37, PS10, PS28	AUG, TET, AMX, NIT
7	PS9	TET, AMX, COT, NIT
8	PS14	TET, AMX, COT
9	PS35	AUG, TET, AMX, COT
10	PS13, PS33	AUG, AMX, NIT
11	PS2, PS12	AUG, AMX
12	PS8, PS6	TET, AMX
13	PS11, PS36, PS16	TET, COT
14	PS17	AMX, COT
15	PS7,PS1,PS3,PS5,PS15,PS34	SENSITIVE TO ALL

Table 7 cont: Antibiotic Resistance Patterns of *Ps. aeruginosa Isolates* from Dumpsites and wastewater

Group wastewater	Isolate designation	Antibiotic resistance pattern
1	PW24	AUG, TET, AMX, COT, NIT, GEN, OFL
2	PW2, PW14, PW23	AUG, TET, AMX, COT, NIT, GEN, NAL
3	PW26, PW1, PW8, PW16,	
	PW17, PW19, PW25	AUG, TET, AMX, COT, NIT, GEN
4	PW3, PW22	AUG, TET, AMX, COT, NIT, NAL
5	PW4, PW5, PW31	AUG, TET, AMX, NIT, GEN
6	PW30	AUG, TET, AMX, GEN
7	PW27	AUG, TET, AMX, COT
8	PW29	TET, AMX, COT, NIT
9	PW15	AUG, TET, AMX, NIT
10	PW21, PW20	AUG, AMX, NIT
11	PW18	AUG, AMX, COT, NIT, GEN
12	PW12, PW20	AUG, AMX
13	PW10	TET, COT
14	PW9	TET, AMX
15	PW11	TET, COT
16	PW28, PW7, PW13	SENSITIVE TO ALL

Key: PS = Isolates from dumpsite

PW = Isolates from waste water

4.4 Antibiotic Resistance and Heavy Metal Tolerance

The relationship between heavy metal tolerance and antibiotic resistance patterns of isolates is shown in tables 8 (for *E. coli* isolates), 7 (for *Ps. aeruginosa* isolates).

Relating heavy metal tolerance with antibiotic resistance, it was found that 27(71%) of *E. coli* isolates that were multiple resistant to antibiotics were also heavy metal tolerant, while 47(80%) of *Ps. aeruginosa* isolates that were multiple resistant to antibiotics were also tolerant to heavy metal used in this study. None of the *S.aureus* isolates that showed multiple resistance to antibiotics was, however, tolerant to any of the heavy metals.

Table 11 shows the overall summary of the relationship among all isolates.

Table 8: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *E. coli*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
ES1	-	-	++	AUG,TET, AMX, COT
ES2	-	-	-	TET, NAL
ES3	-	-	-	TET, GEN
ES4	++	-	+++	AUG, TET, AMX, COT
ES5	-	-	++	AUG, AMX
ES6	-	-	+++	AUG, TET, AMX, COT, NIT
ES7	-	-	-	TET
ES8	++	-	-	AMX, NIT
ES9	-	-	-	AMX
ES10	-	-	-	AMX
ES11	-	-	-	AMX, NIT
ES12	-	-	-	SENSITIVE TO ALL
ES13	++	-	+++	AUG, AMX, COT, NAL
ES14	++	-	+++	AUG, AMX, COT, NIT
ES15	++	-	++	AUG, AMX, COT, NIT
ES16	++	-	++	AMX, COT, NIT
ES17	-	-	++	AUG, AMX, COT, NIT

Table 8 cont: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *E. coli*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
ES18	-	-	-	SENSITIVE TO ALL
ES19	++	-	++	TET
ES20	-	-	-	AMX
ES21	++	-	+++	TET, AMX, COT
ES22	-	-	-	AMX, COT, NIT
ES23	-	-	++	AUG, AMX
ES24	-	-	-	SENSITIVE TO ALL
ES25	-	-	-	SENSITIVE TO ALL
ES26	-	-	-	AMX
ES27	-	-	-	SENSITIVE TO ALL
ES28	-	-	++	AUG, AMX, COT, NAL
ES29	-	-	-	AUG, AMX
ES30	-	-	-	AUG
ES31	-	-	+++	TET, NAL
ES32	-	-	++	TET
CONTROL	-	-	++	AMX

Table 8 cont: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *E. coli*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
EW1	-	-	++	TET, NAL
EW2	-	-	-	TET, GEN
EW3	-	-	-	TET
EW4	++	-	-	TET
EW5	++	-	++	TET
EW6	-	-	++	TET
EW7	-	-	++	TET
EW8	++	-	++	TET, AMX, COT
EW9	++	-	++	AUG, AMX, COT, NAL
EW10	++	-	+++	AUG, AMX
EW11	++	-	++	AUG, TET, AMX,COT
EW12	-	-	++	AMX. NIT
EW13	++	-	-	AUG, AMX, COT, NIT
EW14	-	-	-	SENSITIVE TO ALL
EW15	-	-	+++	AMX, NIT
EW16	++	-	-	AUG, TET, AMX, COT
EW17	-	-	-	SENSITIVE TO ALL

Table 8 cont: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *E. coli*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
EW18	-	-	+++	TET, NAL
EW19	++	-	-	TET, GEN
EW20	-	-	++	TET
EW21	++	-	-	TET
EW22	-	-	-	TET
EW23	-	-	-	TET

Key: ES = Isolates from dumpsite

EW = Isolates from waste water

\- = Sensitive

++ = Moderately tolerant

+++ = Highly tolerant

Table 9: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *Ps. auruginosa*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
PS1	-	-	-	SENSITIVE TO ALL
PS2	++	++	+++	AUG, AMX, NIT
PS3	-	-	-	SENSITIVE TO ALL
PS4	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN, NAL, OFL
PS5	-	-	-	SENSITIVE TO ALL
PS6	-	-	-	AUG, AMX
PS7	-	-	-	AMX, COT
PS8	-	-	-	AUG, AMX
PS9	-	-	-	AUG, TET, AMX, NIT
PS10	++	-	-	AUG, TET, AMX, NIT, NAL
PS11	++	-	++	TET, AMX, NIT
PS12	++	-	+++	AUG, AMX, NIT
PS13	+++	-	+++	AUG, TET, AMX, COT
PS14	++	-	+++	TET, AMX, COT, NIT
PS15	-	-	-	SENSITIVE TO ALL
PS16	++	-	++	TET, AMX
PS17	++	-	-	TET, COT

Table 9 cont: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *Ps. aeruginosa*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
PS18	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN, NAL
PS19	++	-	-	AUG, TET, AMX, COT, NIT, GEN
PS20	+++	-	+++	AUG, TET, AMX, COT, NIT, GEN, NAL
PS21	++	++	+++	AUG, TET, AMX, COT, NIT, GEN
PS22	++	-	+++	AUG, TET, AMX, COT, NIT, GEN
PS23	++	-	-	AUG, TET, AMX, COT, NIT, GEN
PS24	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN
PS25	++	-	-	AUG, TET, AMX, COT, NIT, GEN
PS26	++	-	+++	AUG, TET, AMX, COT, NIT, GEN, NAL
PS27	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN
PS28	+++	++	+++	AUG, TET, AMX, NIT, GEN
PS29	+++	-	++	AUG, AMX, COT, NIT, GEN
PS30	-	-	-	AUG, TET, AMX, COT, NIT, GEN
PS31	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN
PS32	++	-	-	AUG, TET, AMX, COT, NIT, GEN
PS33	-	-	-	AUG, TET, AMX, COT
PS34	-	-	-	SENSITIVE TO ALL

Table 9 cont: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *Ps. aeruginosa*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
PS35	-	-	-	TET, AMX, COT
PS36	-	-	-	TET, AMX
PS37	++	-	-	AUG, TET, AMX, NIT, NAL
PW1	+++	+++	-	AUG, TET, AMX, COT, NIT, GEN, NAL
PW2	+++	++	++	AUG, TET, AMX, COT, NIT, GEN, NAL
PW3	++	-	++	AUG, TET, AMX, COT, NIT, GEN, NAL
PW4	-	-	-	AUG, TET, AMX, COT, NIT, GEN
PW5	++	-	-	AUG, TET, AMX, COT, NIT, GEN
PW6	-	-	-	AUG, AMX, NIT
PW7	-	-	-	SENSITIVE TO ALL
PW8	++	-	++	AUG, TET, AMX, COT, NIT, GEN
PW9	-	-	-	TET, AMX
PW10	-	-	-	TET, COT
PW11	++	-	-	AMX, COT
PW12	-	-	-	AUG, AMX
PS13	-	-	-	SENSITIVE TO ALL
PW14	++	-	++	AUG, TET, AMX, COT, NIT, GEN, NAL

Table 9 cont: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *Ps. aeruginosa*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
PW15	++	-	-	AUG, TET, AMX, NIT
PW16	+++	-	+++	AUG, TET, AMX, COT, NIT, GEN
PW17	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN
PW18	+++	++	+++	AUG, AMX, COT, NIT, GEN
PW19	++	-	-	AUG, TET, AMX, COT, NIT, GEN
PW20	-	-	-	AUG, AMX
PW21	-	-	-	AUG, AMX, NIT
PW22	++	-	++	AUG, TET, AMX, COT, NIT, GEN, NAL
PW23	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN, NAL
PW24	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN, NAL, OFL
PW25	++	-	-	AUG, TET, AMX, COT, NIT, GEN
PW26	+++	++	+++	AUG, TET, AMX, COT, NIT, GEN
PW27	++	-	-	AUG, TET, AMX, COT

Table 9 cont: Heavy Metal Tolerance and Antibiotic Resistance Patterns of *Ps. aeruginosa*

Isolate designation	Heavy metal tolerance			Antibiotic resistance patterns
	Cu	Hg	Co	
PW28	-	-	-	SENSITIVE TO ALL
PW29	+++	++	+++	TET, AMX, COT, NIT
PW30	+++	-	++	AUG, AMX, COT, NIT, GEN
PW31	+++	-	+++	AUG, TET, AMX, NIT, GEN
PW32	++	-	-	SENSITIVE TO ALL

Key: PS = Isolates from dumpsite

PW = Isolates from waste water

\- = Sensitive

++ = Moderately tolerant

+++ = Highly tolerant

4.5 Determination of metal concentration

The results of heavy metal analysis using EDXRF for soils from waste dumpsites and wastewater samples are summarized in tables (10 and (11). Other metals, which occurred rather, irregularly in the samples, are not included in the Tables. They include: Talinium, Serenium, Tubidium, and Strontium. From the results of analysis chromium (2.38×10^3), cobalt (8.37 x 102), copper (6.20 x 102), nickel 5.57 x 102) and lead (3.50 x 102), which are potentially toxic heavy metals occurred with high frequency and in significant concentrations (ppm) in the soil samples. The frequency of these metals is also high in wastewater samples, but the concentrations are not as in the soil samples.

Table 10: Concentrations (in ppm) of various metals in the different soil from dumpsites (A-J)

metal	A	B	C	D	E	F	G	H	I	J
Ca	1.32×10^5	2.2×10^4	4.30×10^4	1.12×10^4	1.60×10^5	1.81×10^5	3.62×10^4	2.25×10^4	5.16×10^4	2.11×10^4
Cr	2.99×10^3	2.14×10^3	1.76×10^3	2.24×10^3	1.98×10^2	9.21×10^2	6.00×10^3	3.13×10^4	1.10×10^4	3.42×10^3
Mn	1.51×10^2	1.50×10^3	2.22×10^3	4.21×10^2	1.15×10^4	2.14×10^3	9.32×10^2	1.51×10^3	1.74×10^3	2.10×10^3
Fe	3.14×10^2	1.28×10^5	1.30×10^5	2.81×10^4	8.13×10^4	4.22×10^4	6.84×10^4	1.60×10^5	1.91×10^4	2.48×10^5
Co	8.67×10^2	8.07×10^5	9.00×10^2	7.97×10^2	8.11×10^2	4.86×10^2	1.21×10^3	6.77×10^2	9.64×10^4	8.67×10^2
Ni	5.15×10^2	5.99×10^2	6.21×10^2	9.10×10^2	5.66×10^2	7.12×10^2	9.98×10^1	4.87×10^2	5.82×10^2	6.14×10^2
Cu	6.07×10^2	6.33×10^2	7.11×10^2	5.92×10^2	6.13×10^2	6.47×10^2	6.12×10^2	5.78×10^2	6.02×10^2	6.06×10^2
Zn	2.51×10^2	3.41×10^2	4.51×10^2	2.64×10^2	2.87×10^2	3.62×10^2	3.99×10^2	4.13×10^2	2.16×10^2	2.24×10^2
Pb	2.97×10^2	4.02×10^2	3.35×10^2	4.13×10^2	4.01×10^2	5.01×10^2	4.43×10^2	2.61×10^2	3.91×10^2	3.50×10^2

Key: Ca - calcium, Cr - chromium, Mn - manganese, Fe - iron, Co - cobalt, Ni - nickel, Cu - copper, Zn - zinc, Pb - lead.

A-J – Represent sample designation for dumpsites soils.

Table 11: Concentration (in ppm) of various metals in the different wastewater samples (A-J)

Metals	A	B	C	D	E	F	G	H	I	J
K	18.10	13.30	15.70	18.10	14.20	13.40	15.60	16.30	19.30	20.10
Ca	8.89	8.37	8.28	7.91	9.23	10.11	8.66	9.12	8.88	9.40
V	2.44	2.36	2.45	2.11	2.67	6.81	7.24	2.37	2.22	2.41
Cr	2.35	1.88	1.79	2.17	2.46	2.12	1.95	2.31	1.78	2.51
Mn	1.36	1.34	1.32	1.28	1.41	1.78	1.52	1.24	1.11	1.46
Fe	1.16	1.15	3.83	2.58	4.13	2.61	1.12	2.69	2.89	3.33
Co	1.03	1.29	1.16	1.32	1.11	1.08	1.23	1.07	1.81	1.37
Ni	0.70	0.67	0.71	0.68	0.73	0.84	0.78	0.92	0.73	0.67
Cu	0.55	0.65	0.60	0.59	0.63	0.57	0.81	0.47	0.57	0.61
Zn	1.39	1.52	1.71	0.47	0.67	0.93	0.39	1.02	0.76	1.44
Pb	0.65	0.88	0.69	0.77	0.83	0.94	1.01	0.79	1.37	0.69

Key: K - potassium, Ca - calcium, V- vanadium, Cr - chromium,

Mn - manganese, Fe - iron, Co - cobalt, Ni - nickel, Cu - copper, Zn - zinc,

Pb – lead

A-J – Represent sample designation for wastewater samples.

4.6 Heavy metal Tolerance Testing

By stringent criteria, the minimum inhibitory concentration (MIC) was designated to be a two-fold dilution of the heavy metal ion concentration, which inhibited the growth of the tested bacteria strains in Mueller Hinton agar. MIC values indicative of metal tolerance were 800µg/ml for c0pper (Cu) and cobalt (Co), and 5.0µg/ml for mercury (Hg). Results for heavy metal tolerance is given in table 12 (heavy metal tolerance of *E.coli)* and table 13 (heavy metal tolerance of *P.aeruginosa*). The isolates which were tolerant to copper and cobalt at 400, 800 and 1600 (µg/ml), were designated (+++), while those that tolerated 400 and 800 (µg/ml), were designated (++). The rest which were either tolerant to 400µg/ml or not at all were designated (-). In the case of mercury, isolates designated (++), were tolerant to 2.5µg/ml and 5.0µg/ml.

Table 12. **Heavy Metal Tolerance Profile Of *E.coli***

Isolate designation	Heavy Metal Tolerance CU	Hg	Co
ES1	-	-	++
ES2	-	-	-
ES3	-	-	-
ES4	++	-	+++
ES5	-	-	++
ES6	-	-	+++
ES7	-	-	-
ES8	++	-	-
ES9	-	-	-
ES10	-	-	-
ES11	-	-	-
ES12	++	-	-
ES13	++	-	+++
ES14	++	-	+++
ES15	++	-	++
ES16	++	-	++
ES17	-	-	-
ES18	-	-	-
ES19	++	-	++
ES20	-	-	-
ES21	++	-	+++
ES22	-	-	-
ES23	-	-	++
ES24	-	-	-
ES25	-	-	-
ES26	-	-	-
ES27	-	-	-
ES28	-	-	+++
ES29	-	-	-
ES30	-	-	-
ES31	-	-	+++
ES32	-	-	++
Control	-	-	++

Table 12. cont'd: **Heavy Metal Tolerance Profile Of *E.coli***

Isolate	Heavy Metal Tolerance		
	CU	**Hg**	**Co**
EW1	-	-	++
EW2	-	-	-
EW3	-	-	-
EW4	++	-	-
EW5	++	-	++
EW6	-	-	++
EW7	-	-	++
EW8	++	-	++
EW9	++	-	-
EW10	++	-	+++
EW11	-	-	++
EW12	++	-	++
EW13	-	-	-
EW14	-	-	-
EW15	++	-	+++
EW16	-	-	-
EW17	-	-	-
EW18	-	-	+++
EW19	++	-	-
EW20	-	-	++
EW21	++	-	-
EW22	-	-	-
EW23	-	-	-

Table 13: Heavy Metal Tolerance Profile Of *P.aeruginosa*

Isolate designation	Heavy Metal Tolerance CU	Hg	Co
PS1	-	-	-
PS2	++	++	+++
PS3	-	-	-
PS4	+++	++	+++
PS5	-	-	-
PS6	-	-	-
PS7	-	-	-
PS8	-	-	-
PS9	-	-	-
PS10	++	-	-
PS11	++	-	++
PS12	++	-	+++
PS13	+++	++	+++
PS14	++	-	++
PS15	-	-	-
PS16	++	-	++
PS17	++	-	-
PS18	+++	++	+++
PS19	++	-	-
PS20	+++	-	+++
PS21	++	++	+++
PS22	++	-	+++
PS23	++	-	-
PS24	+++	++	+++
PS25	++	-	-
PS26	++	-	+++
PS27	+++	++	+++
PS28	+++	++	+++
PS29	+++	-	++
PS30	++	++	++
PS31	+++	++	+++

Cont’d

Isolate designation	CU	Hg	Co
PS32	++	-	-
PS33	-	-	-
PS34	-	-	-
PS35	-	-	-
PS36	-	-	-
PS37	+	-	-
PW1	+++	++	-
PW2	+++	++	++
PW3	++	-	++
PW4	-	-	-
PW5	++	-	-
PW6	-	-	-
PW7	-	-	-
PW8	++	-	++
PW9	-	-	-
PW10	-	-	-
PW11	++	-	-
PW12	-	-	-
PW13	-	-	-
PW14	++	-	++
PW15	++	-	-
PW16	+++	-	+++
PW17	+++	++	+++
PW18	+++	++	+++
PW19	++	-	-
PW20	-	-	-
PW21	-	-	-
PW22	++	-	++
PW23	+++	++	+++
PW24	+++	++	+++
PW25	++	-	-
PW26	+++	++	+++
PW27	++	-	-
PW28	-	-	-
PW29	+++	++	+++
PW30	+++	-	+++
PW31	+++	-	+++
PW32	++	-	-

4.7 Statistics

By comparing the antibiotic resistance and heavy metal tolerance of the isolates, 78% of *E.coli* that were copper tolerant were also multiple antibiotic resistant. While 100% of *Ps.aeruginosa* that were copper tolerant, were multiple resistant to antibiotics. In cobalt, 75% of *E.coli* and 100% of *Ps.aeruginosa* showed similar relationship. This is illustrated in table 14.

Correlation coefficient calculated for the isolates showed positive correlation between antibiotic resistance and heavy metal tolerance as illustrated in table 15.

Table 14: Distribution of Multiple Antibiotic Resistance and Heavy Metal Tolerance of Isolates

Organism	Number of isolates No. resistant to more than one antibiotic	No. copper tolerant	No. copper tolerant and Multi resistant to antibiotic	Percentage of copper tolerants that are multiple resistant
S. aureus,	17 (30%)	0	0	0
E. coli	32 (58%)	18 (33%)	14 (44%)	78%
Ps. aeruginosa	60 (87%)	44 (64%)	44 (73%)	100%

Organism	Number of isolates No. resistant to more than one antibiotic	No. cobalt tolerant	No. cobalt tolerant and Multi resistant to antibiotic	Percentage of cobalt tolerants that are multiple resistant
S. aureus,	17 (30%)	0	0	0
E. coli	32 (58%)	28 (33%)	21 (44%)	75%
Ps. aeruginosa	60 (87%)	32 (64%)	32 (73%)	100%

Organism	Number of isolates No. resistant to more than one antibiotic	No. mercury tolerant	No. mercury tolerant and Multi resistant to antibiotic	Percentage of mercury tolerant that are multiple resistant
S. aureus,	17 (30%)	0	0	0
E. coli	32 (58%)	0	0	0
Ps. aeruginosa	60 (87%)	18 (26%)	18 (30%)	100%

Table 15: Correlation coefficient that indicated positive association

Correlation	Correlation coefficient
MR. to antibiotics and tolerance to Cu	0.99583
MR. to antibiotics and tolerance to Co	0.91826
MR> to antibiotics and tolerance to Hg	0.89757

Key MR - Multiple resistance, CU - copper, Co - cobalt, Hg - Mercury

Chapter Five DISCUSSION

In this study, S. *aureus, E. coli* and *Ps. aeruginosa* strains were isolated from domestically polluted environments of waste dumpsites and wastewater effluents. The presence of these pathogens in these habitats is due to the level of pollution of these environments. *S. aureus* is a pathogen with high degree of adaptability and resistance to environmental stress (Wikipedia, 2007). *E. coli* is a pollution indicator. According to Ivanildo and Richard, (1995), its presence is mainly due to faecal pollution of these environments. *Ps. aeruginosa* is an opportunistic pathogen, largely found in the environment.

It is observed that the frequency of occurrence of *E. coli* and *S.aureus* did not differ much in the overall number of each strain isolated as shown in Table 1; this is probably due to the polluted nature of the environments under study. *P.aeruginosa,* however, occurred more frequently than the other strains; this is the result of their broad environmental distribution made possible by their simple growth requirements.

Figures 1-3, show the percentage antibiotic sensitivity for the environmental strains of *S. aureus, E. coli* and *P. aeruginosa* isolated from dumpsites and wastewater. Many of the isolates showed

resistance to one or more of ofloxacin, augmentin, tetracycline, amoxicillin, cotrimoxazole, nitrofurantoin, gentamicin, and nalidixic acid.

The *S. aureus* strains were largely sensitive to the antibiotics used. Few resistant strains were seen in the antibiotics such as amoxicillin - 12(21%), cotrimoxazole - 11(20%), tetracycline - 5(9%) and augmentin - 4(7%). However, 51(91%) of the isolates were resistant to nalidixic acid. This is likely due to an efflux-dependent quinolone resistance gene present in *S. aureus* according to the findings of levy, (1989). It has been reported that methicillin resistant *S. aureus* may acquire other resistances encoded on plasmid, including β-lactamase production and resistance to trimethoprim (cotrimoxazole) and the aminoglycosides (Report, 1990).

E. coli isolates were more resistant to the antibiotics than the *S. aureus* strains, as follows Amoxycillin (n = 29; 53%), tetracycline (n = 26; 47%) augmentin (n = 17; 31%) cotrimoxazole (n = 15; 27%) and nitrofurantoin (n = 11; 20%). *E. coli* is naturally sensitive to amoxicillin. Resistance to amoxicillin is conferred by a plasmid encoded TEM-1 β-lactamase. Other plasmid encoded β-lactamases are sometimes seen in *E. coli* but about 90% of resistant strains

produce TEM-1. These resistant organisms are usually sensitive to coamoxiclav (augmentin), a combination of amoxycilin and β-lactamase inhibitor clavulanic acid; however, some isolates may be resistant to the combination, due to hyperproduction of TEM-1 β-lactamase (French and Phillips, 1997). This is probably responsible for the higher number of resistant strains of *E. coli* to amoxicillin than augmentin.

Ps. aeruginosa strains were significantly ($P \leq 0.5$) resistant to most of the antibiotics used, such as amoxycilin - 57(84%), tetracycline - (47(69%), nitrofurantoin - 43(63%), cotrimoxazole - 41(60%), gentamicin - 32(47%), nalidixic acid - 12(18%) and ofloxacin - 3(4%). *Ps. aeruginosa* is inherently resistant to β-lactam antibiotics, tetracyclines, chloramphenicol, sulphonamides and nalidixic acid (French and Phillips, 1997). However, resistance to nitrofurantoin, cotrimoxazole, gentamicin and ofloxacin are worthy of note. Resistance to gentamicin and the other antibiotics is acquired. Gentamicin resistance is due to plasmid-mediated aminoglycoside - modifying enzymes, while ofloxacin resistance may be due to a mutation in the DNA gyrase, or a decrease in membrane permeability, or both (Sherman, 2006).

Resistance to amoxicillin occurred in the isolates more than any of the antibiotic agents, with 21% resistance in *S. aureus,* 53% in *E. coli* and 84% in *Ps. aeruginosa.* Although, amoxicillin and augmentin are both β-lactam antibiotics, Augmentin showed greater activity against the isolates, this is probably because augmentin is composed of amoxicillin and the β-lactamase inhibitor - clavulanic acid, giving it greater potency over amoxicillin. The most important mechanism of resistance to β-lactam antibiotics is the production of specific enzymes - β-lactamases. These enzymes bind to β-lactam antibiotics usually via active serine sites, and the cyclic amide bonds of the β-lactam rings are hydrolyzed by the serine hydroxyl group. The open ring forms of the β-lactams cannot bind to their target sites and thus have no antimicrobial activity (Lowy, 2003).

S. aureus has the most clinically significant β-lactamase among Gram-positive cocci, which rapidly hydrolyses benzylpenicillin, and related compounds, but is much less active against the anti-staphylococcal penicillins and most cephalosporins (French and Phillips, 1997). This is probably the reason why resistance to amoxicillin and augmentin is less in *S. aureus* than the Gram-negative strains. Among Gram-negative bacilli the situation is more complex. These organisms produce many different β-lactamases with different spectra of activity,

thus producing high-level resistance (French and Phillips, 1997) as seen in the *E. coli* and *Ps. aeruginosa* strains. Gentamicin proved to be highly effective against the environmental strains of the three pathogens. The percentage resistances were 0% and 4% in *S. aureus.* And *E. coli* respectively. Howeer, *Ps. aeruginosa* showed marked resistance of 47%.

Gentamicin belongs to the aminoglycoside family of antibiotics. Aminoglycosides are highly potent, broad spectrum, bactericidal antibiotics. They act by inhibiting prokaryotic protein synthesis (Davis, 1987; Mazel and Davies, 1999). They induce misreading of the genetic code by binding to the 16S rRNA A-site in bacteria (Carter *et al.,* 2001). The most important mechanism of aminoglycoside resistance is the enzymatic modification of the antibiotic by aminoglycoside modifying enzymes, which are divided according to enzymatic activity into the aminoglycoside acetyltransferase, nucleotidyltransferases, and the phosphoryltransferases (Shaw *et al.,* 1993; Vaculenko and Mobashery, 2003). The genes coding for these enzymes are often transposable and in some cases have been transferred to the chromosome. Changes in surface lipopolysaccharides may increase permeability resistance; this mechanism is involved in some aminoglycoside and tetracycline

resistance in *Ps. aeruginosa.*

In this study, tetracycline resistance was highest in *Ps. aeruginosa* (69%), followed by *E. coli* (47%). *S. aureus,* showed (9%) resistance to tetracycline. Tetracycline resistance can be due to a number of other mechanisms, and is associated with production of a variety of inducible proteins (Chopra and Howe, 1979). Although, tetracycline resistance may result from reduced membrane permeability, failure of the drug to bind properly to 30s rRNA, or because of interference by inducible proteins, it is more commonly caused by active extrusion of the drug from the bacterial cell (Levy, 1993). Rapid efflux of tetracycline from resistant bacteria is associated with production of a family of inner membrane proteins called *Tet*, which may be constitutive or inducible (McMurray *et al.*1980). This form of resistance is usually encoded on transferable plasmid, but may also be chromosomal. In this study, the probability of tetracycline resistance due to this mechanism is higher in *E. coli* and *Ps. aeruginosa* that showed 47% and 69% resistances respectively. Only 9% of *S. aureus,* however, showed resistance to tetracycline.

Resistance to nalidixic acid was 91% in *S. aureus,* 13% in *E. coli* and 18% in *Ps. aeruginosa.* Resistance to nalidixic acid may result from

alteration in surface lipopolysaccharide, but this does not affect susceptibility to the more hydrophilic fluoroquinolones (Nikaido, 1993). This is reflected in the findings in figure 1-3, where resistance to nalidixic acid is higher than resistance to ofloxacin (only 4% in *P. aeruginosa* from figure 3). Although quinolone resistance is usually due to mutations in the target DNA gyrase molecule, some low level resistance in Gram-negative bacteria can be caused by efflux mechanisms. *S. aureus* demonstrated a high level resistance to nalidixic acid in this study (91%). This is likely due to an efflux-dependent quinolone resistance gene in *S. aureus* according to the findings of levy, (1989).

Cotrimoxazole resistance shown by some of the strains of the three isolates may be due to reduced drug entry caused by decreased amounts of specific porin proteins, especially OmpF (Nikaido, 1989). This mechanism may affect several classes of antibiotic drugs simultaneously, producing a broad spectrum of resistance. Acquired resistance to this class of antibiotics (sulphonamide) is usually due to plasmid-mediated synthesis of new dihydrofolate reductases, which are much less susceptible to trimethoprim than natural ones. The resistance genes are also associated with transposons (Nikaido, 1989).

S. aureus strains showed 100% sensitivity to nitrofurantoin, but resistance by some *E. coli* (20%) and *Ps. aeruginosa* (63%) strains is questionable, because NADH or NADPH-linked reductases present in the cells must reduce nitrofurantoin to an active intermediate. One of these enzymes act aerobically and another act anaerobically. Naturally occurring resistance to nitrofurantoin is uncommon, since such strains must lose more than one reductase to be come resistant. It has been asserted that no appreciable resistance to the nitrofurans has occurred after several decades of clinical use probably due to its multiple mechanisms of action (Guay, 2001). The antibiotic sensitivity pattern shown by the various strains in Tables 5, 6 and 7 generally did not show any predictable pattern. This may be due to the diversity of element or factors present in the environment which are responsible for development of resistance ranging from antimicrobial agents, heavy metals, movable genetic elements and the input of already resistant strains into these environments. It is significant to note that 30% of the *S. aureus*, 58% of the *E. coli* and 88% of the *Ps. aeruginosa* isolates were multiple resistant. This implies that, of the strains of these organisms encountered in such environments 30% of *S. aureus*, 58% of *E. coli* and 88% of *Ps. aeruginosa* strains will have multiple resistances to commonly used antibiotics. The risk of

acquiring infection with a multiple resistant strain of these organisms from the environment is therefore probably close to the 70% associated with hospital acquired bacteria infections. (NIAID fact sheet, 2006).

Considering the two environments studied (wastewater and dumpsites), the patterns show that, isolates from dumpsites are more multiple resistant than isolates from wastewater. From table 5, 11 (32%) of *S. aureus* strains from dumpsites were multiple resistant to antibiotic while 6(27%) of the *S. aureus* from wastewater were multiple resistant. For *E. coli* (Table 6) 11 (34%) of dumpsites isolate were resistant to at least three antibiotic while 5 (22%) of wastewater isolates were resistant to at least three antibiotics. This phenomenon may be due to greater diversity of waste on dumpsites than wastewater thus exposing the strains to more stresses and the development of more diverse resistance (NIAID, 2006). This phenomenon, however, is not seen in Table 7 where *Ps.aeruginosa* showed that 25 (68%) from dumpsite were resistant to at least three antibiotics while 23 (74%) of the isolates from wastewater were resistant to at least three antibiotics. This abnormality may not be

unconnected with the high intrinsic resistance among *Ps. aeruginosa* strains as most of them have naturally low susceptibility to antibiotics (Wikipedia, 2007).

In general strains of *Ps. aeruginosa* were resistant to most of the antibiotics with some showing resistance to all the antibiotics used in the study (figure 3). This is because *Ps. aeruginosa* is inherently resistant to many antibiotics. A combination of intrinsic resistances due mainly to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes together with the low permeability of the bacteria cellular envelops and the acquired resistances discussed above are responsible for the high resistance profile in *Ps. aeruginosa* (Wikipedia, 2007).

The energy Dispersive X-ray Fluorescence (EDXRF) analysis of soils and wastewater obtained from dumpsites and exposed drains from homes respectively revealed enormous quantities of heavy metals as shown in Tables 10 and 11. From the results in these tables, heavy metals such as chromium, cobalt, nickel, copper, zinc and lead, had regular frequencies, as they occurred in all samples analyzed. The amount of these metals in the dumpsites is slightly higher than they

occurred in wastewater. This is probably due to the more stable nature of solid waste on dumpsites compared to the varying characteristics of the flowing wastewater in drains, and for the fact that the nature of materials on dumpsites is more diverse, giving room for greater proportion of materials that contain metals in varying quantities. Among the heavy metals detected in the samples, chromium had the highest average concentration in the soil samples while vanadium had slightly higher average value in the wastewater samples. Sources of these heavy metals in the environment include; geological weathering, the use of metals and metal compounds, such as copper compounds in agriculture, lead as anti-knock in gasoline, heavy metals in human and animal excretions, metals released to the air from automobiles which later settles on land, as reported by Ivanildo and Richard, (1994). Although these heavy metals occurred in almost all the samples, the level of heavy metals in samples did not exceed the standard background values of the heavy metals in the environment (U.S.E.P.A, 1992).

The values of lead (3.50 x 102ppm) and chromium (2.38×10^3 ppm) detected in dumpsites exceeded the maximum acceptable concentrations (MAC) of 100pg/g for both elements in 100% of tested samples. The values of zinc also exceeded the MAC of 300ppm in

50% percent of the tested samples. Copper, (6.20 x 102ppm), however, did not exceed the MAC of 1000pg/g, according to the United States Environmental Protection Agency, (1992). It could be concluded that the environments studied are contaminated with lead chromium and zinc. However, the wastewater samples did not show significant concentration of these metals. This may be due to dilution effect on the wastewater, whereas the dumpsites are generally more stable and have greater diversity of waste materials ranging from agricultural wastes, fabrics, rusted metals, nails, metal cans of various products including herbicides, pesticides, dry cell e.t.c; substances which could contribute heavy metals to the dumpsites. However, such diversity of waste material is lacking in wastewater and the constant dilution of wastewater by daily additions may account for the concentrations.

The result of metal tolerance shows that *Ps. aeruginosa* had greater tolerance to all three (Cu, Co and Hg) metal ions tested, with concentration values as high as 1600µg/ml for copper and cobalt, and 5.0 µg/ml for mercury followed by *E. coli* which had tolerance of 800µg/ml for copper and 160µg/ml for cobalt. *S. aureus* did not show tolerance to any of heavy metals tested. Metal tolerance of *Ps. aeruginosa* and *E.coli* have been previously studied. No such

reference is found for *S. aureus.* Vajiheh *et al,* (2003) studied tolerance of *E. coli* of clinical origin against Hg, Cu, Pb, and Cd, and found tolerance level as high as 1750µg/ml for Cu. In study, Alm, (2002), found an MIC of 1000µg/ml for Cu and Co and 10µg/ml for Hg, in environmental strains of *E. coli.*

Tables 12 and 13 shows that Cu and Co were most tolerated, while tolerance to Hg was rather very low and shown only by *Ps. aeruginosa* in spite of the low concentrations used. This indicated that Hg was more toxic than Cu and Co. in a similar study, Mergeay *et al,* (1985), tested the MIC of several different metal ions for *E. coli* on agar medium, and the most toxic metal (with the lowest MIC) was mercury and least toxic metal was Manganese (Mn).

Relating heavy metal tolerance with antibiotic resistance, it was found that 27(71%) of *E. coli* isolates that were multiple resistant to antibiotics were also heavy metal tolerant, while 47(80%) of *Ps. aeruginosa* isolates that were multiple resistant to antibiotics were also tolerant to heavy metal used in this study. None of the *S.aureus* isolates that showed multiple resistance to antibiotics was, however, tolerant to any of the heavy metals. Unlike antibiotic resistance, there are no universally acceptable metal ion concentrations that are used to

designate microbial metal tolerance. Although, the heavy metal concentrations used to define tolerance were deduced for the purpose of this study, references have been made to the works of others (Calomiris *et al*, 1984; Choudhury and Kumar, 1996; Alm, 2002; Vajiheh *et al,* 2003).

From table 8, 9 14 and 15, showing the relationship between antibiotic resistance and heavy metal tolerance, 15(83%) of *E. coli* isolates that were tolerant to copper were also tolerant to cobalt. Out of this combination 10(67%) showed resistance to at least three antibiotics and 8(53%) showed resistance to AUG, AMX and COT in their pattern. The resistance to a combination of AMX and COT was shown in 11(61%) of the *E. coli* isolates that were tolerant to copper (800mg/ml) while 10 (56%) had a combination of AUG and AMX in their pattern and 9(50%) were either singly resistant or had TET resistance in their patter. Similarly 12 (43%) out of cobalt tolerant isolates of *E.coli* showed resistance to AUG, AMX and COT in their pattern, 15(54%) showed TET resistance in their pattern 8(29%) showed a combination of TET, AMX, and COT in their pattern. This pattern is not uniform and may be due to environmental diversity (i.e. diversity of stresses) and other factors not considered in this study.

The *E. coli* isolates that showed high tolerance to cobalt (1600µg/ml) were distinctive in their resistance pattern. Out of 10 strains, 5(50%) showed the same pattern of resistance (AUG, TET, AMX, COT) 2 (20%) showed the pattern (AUG, AMX, COT NAL). In general 8 (80%) of them were resistant to three or more antibiotics. This is a corresponding increase in antibiotic resistance and cobalt tolerance.

In table 9, it is observed that *Ps. aeruginosa* showed greater tolerance to the heavy metals used. At least 20 (29%) of the entire *Ps. aeruginosa* isolates were tolerant to high concentration of copper (1600µg/ml), while 27 (39%) were tolerant to lower concentrations (800µg/ml). Of the isolates that showed high level tolerance 15 (75%) were resistant to AMX, COT, NIT and GEN. all of the isolates 20 (100%) were resistant to COT, NIT and AMX.

Tolerance to mercury is particularly significant because 14 (82%) of the isolates that were tolerant to mercury were resistant to AUG, TET, AMX, COT, NIT and GEN. Mercury is the most toxic heavy metal from studies by Mergaey *et al,* (1985), this is reflected in table 9, because all isolates that were tolerant to mercury were tolerant to copper and cobalt and have correspondingly shown high antibiotic resistance patterns with some resistant to all antibiotic used. The fact

that 39 (91%) of isolates of *Ps. aeruginosa* that had resistance patterns involving four or more antibiotics were also tolerant to two or all of the heavy metals used indicate a strong relationship between heavy metals tolerance and multiple antibiotic resistance. Some of the isolates 16 (23%), however, showed resistance to two or three antibiotics which are predominantly AUG, AMX, and COT in various patterns, among these 3 (13%) were tolerant to either of copper and cobalt or both metals.

This can be explained by the intrinsic resistance of most *Ps. aeruginosa* to antibiotics (Goni - Urriza *et al,* 2000). About 8 (12%) of the isolates however, showed neither resistance patterns nor metal tolerance.

Table 14 which show a summary of the relationship between heavy metal tolerance and multiple antibiotic resistances. 78% of *E. coli* and 100% of *Ps. aeruginosa* strains that were tolerant to copper were also multiple antibiotics resistant. About 75% of *E.coli* and 100% of *Ps.*aeruginosa that were tolerant to cobalt were multiple antibiotics resistant. This indicates a positive correlation between metal tolerance and multiple antibiotic resistances.

Similar result were obtained in a previous study by Calomiris *et al* (

1984), where 24.4% of multiple resistant bacteria isolated from distribution waters were tolerant to two of copper, lead and zinc and 20.9% were tolerant to all three. In a related study, Vajiheh *et al,* (2003) found that 32% Nosocomial isolates of *E.coli* carried a conjugative plasmid (>56.4kb) encoding resistance to heavy metals and antibiotics. By comparison, the control strains showed insignificant resistance to antibiotics, and a minimum tolerance to heavy metals, with only *Ps. aeruginosa* showing tolerance to copper (Tables 5, 6, and 7).

Numerous reports of the effects of heavy metals and other toxic substances on bacteria obtained from soils, sediments and aquatic habitats have been published. In most of these studies bacteria were isolated from locations exposed to industrial pollution with antibiotics and heavy metals (Halling-Sorensen *et al,* 1998; Hernandez *et al,* 1998; Goni-Urriza *et al*, 2000; Kummerer, 2004) and then susceptibility of isolated strains to the toxic substances was studied in pure culture. It has been shown that the presence of heavy metals in the environment provides a selective pressure for antibiotic resistance and heavy metal tolerance as reported in the findings of Calomiris *et al,* (1984); Dhakephalkar and Chopade, (1994); Walsh, (2000).

Though the use of antibiotics in medicine and agriculture clearly stimulates the proliferation of antibiotic resistance as reported by Neu; (1992), it has been found that most antibiotics are readily degraded in the environment, while metals persist and thus represent a long-term selective pressure, meaning that in the environment the danger of inducing antibiotic resistance by heavy metals is more disturbing than antibiotics given their persistence in the environment.

The importance of this finding is even more, considering the fact that these dump sites and wastewater drains are located within residential areas where people live thus bringing the effect of antibiotic resistance closer to human community.

Statistical analysis using chi-square of association with a confidence level of 5% been considered significant in a three by two table (χ^2 = 11.599). There was a strong association between heavy metal tolerance and multiple antibiotic resistances. This agrees with the works of Calomiris *et al.,* (1984), Alm, (2002), and Vajiheh *et al*., (2003).

Correlation coefficient for resistance to antibiotics and tolerance to heavy metals, calculated for the strains against each heavy metal showed significant correlation at ($p \leq 0.05$) between antibiotic

resistance and heavy metal tolerance.

Table 15shows the significant positive correlation values obtained from the correlation of resistance to antibiotics and tolerance to heavy metals.

5.1 Conclusion

The emergence and spread of resistant pathogens is an environmental problem analogous to other problems in the human environment that threatens health in recent times. The frequent misuse of antibacterial agents in clinical practice, both in third world and in the developed nations, and release of enormous quantities of antibiotic agents in agriculture and animal husbandry continue to provide conditions favorable to the selection of resistant bacteria.

The isolation of multidrug-resistant bacteria from the environment out side hospital setting represent a potential threat to the public health, since antibiotic resistance can pass from bacterium to bacterium, and resistant bacterial infection can pass from person to person. It then means that if large numbers of bacteria are resistant to antibiotics, it will be more difficult and more expensive to treat human bacterial infections.

Although some heavy metals are important and essential trace elements, at high concentrations such as those found in many environments today, most can be toxic to microorganisms and humans. Microorganisms have adapted tolerance to the presence of metals. Thus a number of interactions between microorganisms and metals have important environmental and health implications. Some implications are useful, such as the use of bacteria to clean up metal-contaminated sites. Other implications are not as beneficial, as the presence of metal tolerance mechanism may contribute to the increase in antibiotic resistance. Over all it is most important to remember that what we put into the environment can have many effects, not just on humans, but also on the environment and on the microbial community. It is therefore, suggested that the potential impact of chemically polluted, most specifically metal polluted locations on human life may be much greater that the direct effect of the pollution.

5.2 Recommendation

The knowledge about how antibiotics resistance arises, how resistant strains and resistance genes spread in nature and the significance of this for humans and nature is far from complete. There are not enough data available to draw final conclusion especially with respect to the

input of already resistant bacteria into the environment. This topic needs further consideration and investigation.

The location of waste dumpsites near residential homes is potentially dangerous as it increases the risk of contact with pathogenic agents from these polluted environments. Dump sites should therefore be located a distance away from residential areas to reduce this risk.

Adequate waste treatment facilities should be put in place to prevent the release of resistant pathogens in to the environment. Waste effluents should also be channeled properly to treatment plants to avoid runoff into water sources.

REFERENCES

Aarestrup, F.M., Seyforth, A.M., Embrog, H.D. (2001). Effrect of abolishment of the use of Antimicrobial agents for growth on occurrence of Antimicrobial resistance in faecal enterococci from food animals in Denmark. *Antimicrobial Agents and Chemotherapy*, 45: 2054 - 2059.

Alliance for the Prudent use of Antibiotics. (1999). Resistant Organisms: Global impact in Continuum of care. *Royal Society of Medicine,* 220: 1-6.

Alm, E. (2002). Implications of microbial heavy metal tolerance in the environment. *Reviews in Undergraduates Research-Issue 2*.

Alonso, A., Sanchez, P. and Martinez, J.L. (2000). Stenotrophomonas multophilia D457 R contains a cluster of genes from gram-positive bacteria involved in antibiotic and heavy metal resistance. *Antimicrobial Agents and chemotherapy*, 44: 1778 - 1782.

Anderson, S.R. and Sandaa, R.A. (1994). Distribution of tetracycline resistance among Gram-negative bacteria isolated from polluted and unpolluted marine sediments. *Applied and Environmental Microbilogy*, 60 (3): 908 -912.

Angeyo, K.H., Petal, J.P., Mangala, J.M. and Naraya, D.G.S. (1998). Optimization of X-ray fluorescence elemendtal analysis: an example from Kenya. *Applied Radiation Isotopy*. 49: 885 - 891.

Ayres, R.U. (1992). Toxic heavy metals: material cycle optimization. *Process National Academy of Science.* USA, 89: 815 - 820.

Baquero, F., Negri, M.C., morosini., and Blazquez, J. (1998). Antibiotic-selective environments. *Clinical Infectious Diseases* 27: 5-11.

Barberio, C., Pagliai, L., cavalieri. D., and Fani, R. (2001). Biodiversity and horizontal gene transfer in culturable bacteria isolated from activated sludge enriched in nonylphenol ethozylates. Research in Microbiology. 152(1) : 105-112.

Barkay, T., Miller, S.M., and summers, A. O (2003) Bacteria matal resistance from atoms to ecosysterms. FEMS Microbiology Reviaw, 27: 355-384.

Bathe, S., Lebuhn, M., Ellwart, j.w., Wuertz S., and Hauser, M. (2004). High phylogenetic diversity of transconjugants carrying plasmid pJP4 in an activated slugde-derived microbial community. *FEMS Microbiology. Letter.* 253 – 219.

Baur, B., Hanselmann, K., Schlimme, W., and Jenni, B. (1996). Genetic transformation in freshwater *Escherichia coli* is able to develop natural competence. *Applied and Environmental Microbiology,* 62: 3673 – 368.

Beaber, J. W., Hochhut, B., and Waldor, M. K. (2004). SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature,* 427: 72 - 74.

Beard, S.J., Hashim, R., Hernandez, J., Hughes, M., and Poole, R.K. (1997). Zinc (II) tolerance in *Escherichia coli* K-12: Evidence that the znt A gene (0732) encodes a cation transport ATpase. *Molecular microbiology*, 25(5): 993-891.

Bernasconi, G.B., Barnford, S.A., Dosan, B., Haselberger, N., Marcowicz, A., Mahmoud, A. and Valcovic, V. (1996). Applicanility of annular source excited system in quantitative XRF analysis. *X-Ray Spectrum,* 23: 65-70.

Bhattacherjee, J.W., Pathak, S., Gaur, A. (1988). Antibiotic Resistance And Metal Tolerance of Coliforrn Bacteria Islated From

Gomati River Water at Lucknow City. *Journal of General and Applied Microbiology,* 34: 391-399.

Bjorkran, J., Kralikova, K., Kremery. V. and Schafer, V. (2001). Bacteriophages transducing antibiotic resistance from a cluster of lysogenic strains of Pseudomonas aeruginosa isolated from patients. *Journal of chemotherapy,* 13: 331-333.

Boon, P.I., and Cattanach, M. (1999). Antibiotic resistance of native and faecal bacteria isolated from rivers, reservoirs and sewage treatment facilities in Victoria, southeastern Australia. *Letters in Applied Microbiology,* 28(3): 164-168.

Bouma, J. E., Lenski, R. E. (1988). Evolution of bacteria/plasmid association. *Nature,* 335: 351-352.

Calomiris, J., Amstrong, J. L. and Seidler, R.J. (1984). Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. *Applied and Environmental Microbiology,* 47(6): 1238-1242.

Carter, A.P., Cemons, W.M., Brodersen, D.E., Morgan-Warren, R.J., Hartsch, T., Wimberly, B.T. and Ramakrishnan, V. (2001). Crystal structure of an initiation factor bound to the SOS ribosomal subunit science, 291: 498-501.

Centre for Disease Control and prevention (CDC). (2000). A public health action plan to combat Antimicrobial resistance, Interagency Task Force on Antimicrobial Resistance. Workshop Report; *Available at: www.cdc.gov/drugresistance/actionplan*. .

Cheesbrough, M. (2000). District laboratory Practice in Tropical countries. Part 2. Cambridge University Press, UK.

Cheetham, B.F., and Katz, M.E. (1995). A role for Bacteriophages in the evolution and transfer of bacterial virulence determinants. *Molecular Microbilogy*, 18:201-208.

Chopra, I. and Howe, T.G.B. (1978). Bacterial resistance to the tetracyclines. *Microbiological Reviews*, 42: 707 -724.

Choudhury, P., and Kumar, R. (1996). Association of metal tolerance with multiple antibiotic resistance of enteropathogenic organisms isolated from coastal regions of deltaic Sunderbans. *Indian Journal of Medical Research*, 104: 148-151.

Clinical Institute for Laboratory Standards (CILS). (2005). Performance Study for Antimicrobial susceptibility. 15th International Summit. *CILS Document* 25:M100-515.

Cooksey, D.A (1993). Copper uptake and resistance in bacteria. *Molecular Microbilogy*, 7: 1-5.

Cooksey, D.A. (1994). Molecular mechanisms for copper resistance and accumulation in bacteria. *FEMS Microbiology Reviews*, 14:381-386.

Cookson, B. and Phillips, I. (1988). Epidemic Melhicillion resistant Staphylococcus aureus. *Journal of Antimicrobial chemotherapy* 21:57-65.

Davis, B.D. (1987). Mechanism of bactericidal action of aminoglycosides. *Microbiology Reviews,* 51: 341-350.

Davison, J. (1999). Genetic exchange between bacteria in the environment. *Plasmid,* 42:73-91.

De Souza, M., Nair, S., Bharathi, P.A., and chandramohan, D. (2006). Metal and antibiotic resistance pattern of bacteria population isolated from sea water. *Online Journal of microbiology.* *<http://www.springerlink.com/content/U22q827tml3161v71/>*.

Dhakephalkar, P.K. and Chopade, B.A. (1994). High levels of multiple metal resistance and its correlation in Acinetobacter. *Biometals*, 7: 67-74.

Donen, A.K., Torsvik, V.L., Goksoyr, J., and Top, E.M. 91998). Effect of mercury addition on plasmid incidence and gene mobilizing capacity of bulk soil. *FEMS Microbilogy Ecology*, 27:381-394.

Erah P.O. Akujieze C.N. and Oteze G.E. (2002): The quality of Groundwater in Benin City. *Tropical Research*; 1(2): 75-82.

Falahi-Ardakani, A. (1984). Contamination of environment with heavy metals emitted from automatives. *Ecotoxicology and Environmental safety*, 8:1520161.

Finland, M., Jones, W., and Barves, M.W. (1959). Occurrence of serious bacterial infectious since the introduction of antibacterial agents. *Journal of the American Medical Association* 170: 2188-2197.

Forbes, B.A. (1998). Enterobacteriaceae. Baily and Scott's Diagnostic Microbiology Baltimore, *Mosby*. Pp509-526.

Fouace, J. (1981). Mixed cultures of Staphylococcus-Aureus-Some Observations concerning Transfer of antibiotic Resistance. *Anneles De Microbiologie*, 132: 375-386.

Fourest, E., Canal, C., and roux, J.C. (1994). Improvement of heavy metal biosorption by mycelial dead biomasses (Rhizopus arrhizus, Mucor meihei and Penicillium chrisiogenum): pH control and cationic activation, *FEMS Microbilogy. Review*, 14:325-332.

French, G.L. and Phillips, I. (1997). Resistance. In: O'Grady, F., Finch, R.G., Lambert, H.P. and Greenwood, D. (eds.). Antibiotic and Chemotherapy: Anti-infective Agents and their use in Therapy. Longhand, Singapore, 23-43.

Frischer, M.E., Stewart, G.J., and Paul. J.H. (1994). Plasmid Transfer of Indi genous Marine Bacteria-Population by Natural Transformation. *FEMS Microbiology Ecology*, 15:127-135.

Funtua, I.I. (1999a). analysis of Nb-Ta ores by energy dispersive X-ray fluorescence spectrometry. *Journal of Trace Microprobe Technology*, 17(2): 189-195.

Funtua, I.I. (1999b). Application of transmission-emission method in EDXRF for the detection of trace elements in geological and biological materials. *Journal of Trace Microprobe Technology,* 17(3):293-297.

Gadd, G.M., and White, C. (1993). Microbial treatment of metal pollution-a working biotechnology? *Trends in Biotechnology*, 11: 353-359.

Garbarino J.R; Hayes, H.C; Roth, D.A; Terry, R.C. and Taylor, H.E. (1995). Heavy metalsa in the Mississippi River. http;//pubs. Usgs.gov/cir/circ1133/heavy-metals.html.

Goel, M.K. (1994) Biotechnology: An Overview.
www. Foa.Om/docrep/field/003/AC279E18. him

Goni-Urriza, M., Capdepuy, M., Arpin, C., Raymond, N., Caumette, P., and Quentin,C. (2000). Inpact of an urban effluent on antibiotic resistance of riverne enterbactehaceae and Aeromonas spp. *Applied and Enviromental Microbiology*, 66(1): 125-132.

Guardabassi, L., Peterson, a., Olsen, J.E., AND Dalsgaard, A. (1998). Antibiotic resistance in Acinetobacter spp. Isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. Applied and *Environmental Microbiology*, 64(9): 3499-3502.

Guay, D.R. (2001). An update on the role of nitrofurans in the management of urinary tract infections. Review. *Drugs,* 61(3): 353-364.

Gupta, s.d., Lee, B., Camacaris, J., and Wu, H.C. (1995). Identification of cut Cand cutF (nipE) genes involved in copper tolerance in Escherichia coll. *Journal of Bacteriology*, 1779(15): 4207-4215.

Haley, R.W., Hightower, A.W., and Khabbaz, R.F. (19820. The emergence of methicillin-resistant Staphylococcus aureus infections in United States hospitals. *Annals of Internal Medicine,* 97;297-308.

Halling-Soresen, B., Nors-Nielsen, S., Lanzky, P.F., Holten-Lutzhoft, H.C., and Jorgensen, S.E. (1998). Occurrence, fate and effects of pharmaceuticial substances in the enviroment- a review. *Chemosphere,* 36;357-393.

Hamiton, W.D., Lenton, T.M. 919980. Spora and Gaia: how microbes fly with their clouds. *Ethol Ecological Evolution*, 10: 1-16.

Hernandez, A., MELLADO, R.P., AND Mellado, T.M. (1998). Metal accumulation and vanadium-induced multidrug resistance by environmental isolates of Escherichia hermannii and Enterobacter cloacae. *Applied and Enviromental Microboilogy*,64(11): 4317-4320.

Heuer, H.E., Krogerrecklenfort, E.M.H.,Wellington, S., Egan, J.D.V. Elsas, L.V., Overbeek, J.M., Collard, G., guillaume, A.D., kara goun, T.L. Nikolakopoulou, and Smallaz, K. (2002). Gentamicin resistance genesin environmental bacteria: prevalence and transfer. *FEMS Microbilogy and Ecology*, 42:289-302.

Hooper, D.C. (2001). Emerging mechanism of fluoroquinolone resistance. *Emerging infectious Disease*, 7:311-341.

House of Lords (UK) (1998). House of Lords select committee on science and technology. 7th report. The stationary office, London

.

Huys, G., Gevers, D., Temmerman, R., Cnockaert, M., Denys, R., Rhodes, G., Pickup, R., McGann, P., Hiney, M., Smith, P, and

Swings, J. (2001). Comparison of the Antimicrobial tolerance of oxytetracycline resistant hetertrophic bacteria isolated from hospital sewage and freshwater fishfarm in Belgium. *Systematic and Applied Microbiology*. 24(1): 122-130.

Hyders, S.L. and Streitfeld, M.M. (1978). Transfer of Erythromycin resistance from clinically isolated lysogenic strains of streptococcus-py genes via their Endogenous Phage. Journal of Infectious Diseases, 138:281-286.

Ivanildo, H. and Richard, H. (1995). Water pollution, *www.ilo.org/encyclopedia/?print&nd=857100180*

Iversen, A., Kuhn, I., Franklin, A and Mollby, R. (2002). High prevalence of vancomycin-resistant enterococci in Swedish sewage. *Applied and Environmental Microbiology*, 68(6): 2838-2842.

Jones, J.G., Gardener, S.E., simon, B.M. and Pickup, R.W. 91986). Antibiotic resistant bacteria in Windermere and two remote upland tarns in the English Lake District. *Journal of applied Bacteriology*, 60, 443-453.

Kadiiska, M.B., Mason, R.P., Dreher, K.L., Costa, D.L. and Ohio, A.J. (1997). Invivo evdence of free radical formation in the rat lung after exposure to an emission source air pollution particle. *Chemical Resources Toxicology*, 10:1104-1108.

Kasprzak, C.T. and Cafferkey, M.T. 91984). Re-emergence of methicillin-resistant Staphylococcus aureus causing severe infection. *Journal of infection*, 9:6-16.

Keane, C.T. and Cafferkey, M.T. (1984). Re-emergence of Methicillin- resistant Staphylococcus aureus causing severe infection. Journal of infection, 9:6-16.

Khachatourians, G. (1998). Agricultural use of antibiotics and evolution and transfer of antibiotic-resistant bacteria. *Canadian Medical Association Journal*, 159:1129-1139.

Kolpin, D.W., Furlong, E.T. Meyer, M.T.., Thurman, E.M., Zaugg, S.D. Barber, L.B. and Buxton, H.T. streams, 1999-2000: A national reconnaissance. *Environmental Science and technology,* 36: 1202-1211.

Kummerer, K. (2004). Resistance in the environment. *Journal of Antimicrobial Chemotherapy*, 54(2):311-320.

Kump, P. (1996). QAES, Quantitative analysis of environment samples. *Instruction Manual*, Ljblijana.

Lawrence J. G. (2000). Clustering of antibiotic resistance genes: beyond the selfish operon. *American society of microbiology News*, 66(5):281-286.

Leff, L.G.; Dana, J.R; McArthur, J.V.; Shimkets, L.G. (1993). Detection of tn5-like sequence in kanamycin resistant stream bacteria and environmental DNA. *Applied and Environmental Microbiology,* 59:417-421.

Levy, S.B. (1989). Evolution and spread of tetracycline resistance determinants. *Journal of Antimicrobial Chemotherapy*, 36:695-703.

Levy, S.B (1983). Active efflux Mechanism for antimicrobial resistance determinates. Journal of Antimicrobial Chemotherapy, 24:1-3.

Lewis, R. (1995). The Rise of Antibiotic-Resistant Infection U.S. food and Drug Administration. *Consumer Magazine* 1-4.

Liebert, C.A., Hall, R.M., and Summers, A.O. (1999). Transposon Tn21, flagship of the floating genome. *Microbiology and Molecular Biology Reviews*, 63: 507-522.

Lin, C and Olson, B.H. (1995). Occurrence of cop-like resistance genes among bacteria isolated from water distribution system. *Canadian Journal of Microbiology*, 59(11): 642-646.

Lowy. D.F. (2003). Antimicrobial resistance; the example of Staphylococcus aureas. *Journal of clinical Investigation*, 11: 1265-1273.

Lorenz, M.G. and Wackmagel, W. (1994). Bacterial Gene-Transfer by Natural Geneti-Transformation in the Environment. *Microbiological Reviews*, 58: 563-602.

MacDougall, C., Harpe, S.E., Powell, J.P., Johnson, C.K. Edmond, M.B. and Polk, R.E. (2005). Pseudomonas aeruginosa, Staphylococcus aureus, and Fluoroquinolone use. *Emerging Infectious Diseases*, 11(8): 1197-1204.

Mackie, T.J., (1989). Mackie and McCartney; Practical Medical Microbilogy. Thirteenth edition, 2. *Churchill Livingstone*, London. ISBN 0-443-02332-8.

Madany, I.M., and Raveendran, E. (1992). Polycyclic hydrocarbons, nickel and vanadium in air particulate matter in Bahrain during the birning of oil fields in Kuwait. *Science Total Environment,* 116: 281-289.

Man, F.X., Banin, A., Su, Y., Monts, D.L. Plodinec., n M.J., Kingery, W.I. and Triplett (2002). Industrail age anthropogenic inputs metals into the pedosphere. *Naturwissenschaften*, 89: 497-504.

Markowicz, A.A. and Van Grieken., R.E. (1993). Quantification in XRF analysis of intermediate thickness samples, In: Van Grieken and Markowicz (eds*). Handbook of X-ray spectroscopy, Marcel Dekker Inc*. New York, 339-358.

Martinez, J.L. (2000). Mutation frequencies and antibiotics resistance. *Mini review. Antimicrobial Agent and Chemotherapy, 44: 1771 – 2777*

Mazel, D., and Davies. (1999). Antibiotic resistance in microbes. *Cellular Molecular Life Science.* 56: 742-754.

McArthur, J. V. and Tuckfield, R. C. (2000). Spatial patterns in antibiotic resistance among stream bacteria; Effects of industrial pollution. *Applied and Environmental Microbiology,* 66: 3722 - 3726.

McMurry, L., Petrucci, R.E., Levy, S.B. (1980). Active efflux of tetracycline encoded by four genetically different

tetracyclineresistance determinants in *Escherichia coli. Proceedings of the National Academy of Science, USA,* 24: 544-551.

Meageay, M., Nies, D., Schlegel, H.D., Gents, J., Charles, P. and van Gijsegem, F. (1985). *Alcaligens eutrophus* CH34 is a facultative chemolithotroph with plasmid-borne resistance to heavy metals. *Journal of Bacteriology,* 162: 328-334.

Miranda, C. D., and Castillo, G. (1998). Resistance to antibiotics and heavy metais of motile aeromonads from Chilean freshwater. *The Science of the Total Environment,* 224(1-3): 167-176.

Nation constitute of Allergy and Infectious Diseases (NIAID) (2006). The Problem of Antibiotic Resistance/NIAID Fact Sheet. 1-6. http://www.niaid.nih.gov/factsheets/antimicro.htm

National committee for clinical laboratory standards (NCCLS) (2000). Performance standards for Antimicrobial disk susceptibility test-approved standard (document mz-a7). 7th ed. NCCLS, Pennsylvania, USA.

Neu, .H.C. (1992). The crisis in antibiotic-Resistance. *Science, 257:* 1064-1073.

Nielsen, K.M., vanWeelret, D.M., Berg.T.N., Bones, A.M., Hagler, A.N., and vanElsas, J.D. (1997). Natural transformation and availability of transforming DNA to Acinetobacter calcoaceticus in soil microcosms. *Applied and Environmental Microbiology, 63:* 1945-1952.

Nies, D.H. (1999). Microbial heavy metal resistance. *Applied Microbiology and Biotechnology,* 51: 730-750.

Nies, D.H., and Silver S. (1995). lon efflux system involved in bacteria metal resistances. *Journal of Industrial Microbiology,* 14:186-199.

Nikaido, H. (1989). Outer membrane barrier as a mechanism of Antimicrobial resistance. *Antimicrobial Agents and Chemotherapy,* 33: 1831-1836.

Nikaido, H. (1993). Transport across the bacterial outer membrane. *Journal of Bioenergetics and Biomembranes,* 25: 581-589.

Nikaido, H. (1996). Multidrug efflux pumps of gram - negative bacteria. *Journal of Bacteriology,* 178: 5853 - 5859.

Nikaido, H. (1998). Evolutionary origins of multidrug and drug - specific efflux pumps in bacteria. *FASEB journal,* 2: 265 - 274.

Nriagu, J.O. and Pacyna, J.M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature,* 333: 134-139.

O'Brien, Thomas, F. (2002). "Emergence, spread and environmental effect of antibiotic resistance: How use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else". *Clinical Infectious Diseases,* 334: 78-84.

Osborn, AM., Bruce, K.D., Strike, P., and Ritchie. D. A. (1993). Polymerase chain reaction-restriction fragment length polymorphism analysis shows divergence among *mer* determinants from Gram-negative soil bacteria indistinguishable by DNA-DNA hybridization. *Applied and Environmental Microbiology.* 59:4024-4030.

Osborn, A.M., Bruce, K.D., Strike, P., and Ritchie, D.A. (1997). Distribution diversity and evolution of the bacteria mercury resistance (mer) operon. *REMA microbiology Review,* 19: 239-262.

Owusu-Yaw, J., Cohen, M.D., Fernando, S.Y., and Wei, C. I. (1990). An assessment of the genotixicity of vanadium. *Toxicology Letter,* 50: 327-336.

Papadopoulou, B., and Courvalin, P. (1988). Dispersal in Campylobacter spp.; of Apha-3, a kanarnycin resistance determinant from Gram-positive Cocci. *Antimicrobial Agents and Chemotherapy,* 32: 945-948.

Parveen, S., Muphee, R.l., Edmiston, L., Kaspar, C.W., Portier, K.M. and Touplin, M.L. (1997). Association of multiple antibiotic resistance profiles with point and non-point source of *Eschehchia coli* in apalachicola Bay. *Applied and environmental Microbiology,* 63: 2607-2612.

Paul, J.H., Frischer, M.E., and Thurmond. J.M. (1991). Gene-Transfer in marine water column and sediment microcosms by natural plasmid transformation. *Applied and Environmental Microbiology,* 57: 1509-1515.

Paulsen, I.T., Brown, M.H., and Skuray, R.A. (1996). Proton dependent multidrug efflux systems. *Microbiology Review,* 60: 515-608.

Perron, K., Caille, O., Rossier, C., vanDelden, C., Dumas, J.L., and Kohler, T. (2004). XzcR-CzcS, a two-component system involved in heavy metal and cabapenem resistance in *Pseudomonas aeruginosa. Journal of Biological Chemistry,* 279: 8761-8768.

Phillips, I. and Shannon, K.P. (1997). Anti-infective agents and their use in therapy. In antibiotic and chemotherapy. (Francis, O., Roger, G.F., Harold, P.L., and David, G. Eds). Longman, Singapore.

Pickup, R.W., Mallinson, H.E.R., Rhodes, G., and Chatfield, L.K. (1997). A novel nickel resistance determinant found in sewage associated bacteria. *Microbiai Ecology,* 33: 230-139.

Rasmussen, L. D., and Sorensen, S. J. (1998). The effect of long term exposure to mercury on the bacteria community in marine sediment. *Current Microbiology,* 36:291-297.

Report of a combined Working Party of Hospital Infection Society and British Society for Antimicrobial Chemotherapy. (1990). Revised guidelines for the control of epidemic methicillin-resistant

Staphysococcus aureus. *Journal of Hospital Infection,* 16: 351-377.

Rice, L.B. (1998). Tn916 family conjugative tansposons and dissemination of Antimicrobial resistance determinants. *Antimicrobial agents and Chemotherapy,* 42: 1871-1877.

Richmond, M.H., and Hohn, M. (1964). Co - transduction by staphylococcal phage of genes responsible for penicillinase sythesis + resistance to mercury salts. *Nature,* 202: 360-367.

Roane, M.T. and Kellogg, S.T. (1996). Characterization of bacterial communities in heavy metal contaminated soils. *Canadian Journal of Microbiology,* 42: 593-603.

Sabry. S.A., Ghozlan, H.A., and Abonzeid, D.M. (1997). Metal tolerance and antibiotic resistance patterns of a bacterial population isolated from seawater, *Journal of Applied Microbiology,* 82: 245-252.

Saier, M.H., Paulsen, I. T., Sliwinski, M.K., Pao, S.S., Skurray, R.A., nd Nikaido, H. (1998). Evolutionary origin of multidrug and drug-specific efflux pumps in bacteria. *FASEB Journal*, 12: 265-274.

Salyers, A.A., Shoemaker, N.B., Stevens, A.M., and Li, L.Y. (1995). Conjugative Transposons- an Unusual and Diverse Set of Intergrated Gene- Transfer Eeiements. *Microbiological Reviews, 59:* 579-588.

Sanger, D.M., Holland, A.E., and Scott, G.I. (1999). Tidal creek and salt marsh sediments in south Carolina coastal estuaries: I. distribution of trace metals. *Archives of Environmental Communication and Toxicology, 37:* 447-457.

Santini, J.M., Stolz J.F., and Macy, J.M. (2002). Isolation of a new arsenate respiring bacterium - physiological and phylogenetic studies.

Geomicrobiology Journal, 19: 41-52.

Schaefler, S. (1989). Methicillin resistant strains of *Staphylococcus aureus* resistant to quinolones. *Journal of Clinical Microbiology,* 27: 335-336.

Selvaratnam. S., and Kumberger, J.D. (2004). Increased frequency of drug-resistant bacteria and conforms in an Indian creek adjacent to farmland amended with treated sludge. *Canadian Journal of Microbiology,* 50(8): 653-656.

Shanon, D.C. (1981). Antibiotic resistance in *Staphylococcus aureus. Journal of Hospital Infection,* 2: 11-36.

Shaw, K.J. Rather, P.N., Hare, R.S. and Miller, G.H. (1993). Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside- modifying enzymes. *Microbiology Reviews,* 57: 138-163.

Sherman, M. (2006). An Overview of Antibiotic Resistance *US Pharm*. 31(1): HS-24-HS-28.

Silva, J.; Castillo, G.; Callejas, L; Lopez, H.; Olmos, F. (2006). Frequency of transferable multiple antibiotic resistances amongst coliform bacteria isolated from a treated sewage effluent in Antofagasta, Chile. *Electronic Journal of Biotechnology,* 12: 1-7.

Silver, S. (1996). Bacterial resistance of toxic metal ions - a review. *Gene*. 179: 9-19.

Silver, S., and Phung, L. T. (1996). Bacterial heavy metal resistance: new surprises. *Annual Review Microbiology.* 50: 753-789.

Smalla, K., Haines, A.S., Jones, K., Krongerrcklenfort, E., Heuer, H., Schloter, M., and Thomas, C.M. (2006). Increased aboundance of incP-16 plasmid and mercury resistance genes in mercury-polluted river sediments: first discovery of IncP-ld Plasrnid with a complex rner transposon as the sole accessory element. *Applied and Environmental Microbiology,* 72(11): 7253-7259.

Smit, E., Wolters, A., and vanElsas, J.D. (1998). Self-Transmissible mercury plasmids with gene-mobilizing capacity in soil bacterial populations: influence of wheat roots and mercury addition. *Applied and Environmental Microbiology,* 64: 1210-1219.

Sobecky, P.A. (1999). Plasmid ecology of marine sediment microbial commuity. *Hydrobiologia,* 401: 9-18.

Summers, A. O. (2002). Generally overlooked fundamentals of bacterial genetics and ecology. *Clinical Infections diseases,* 34: 585-592.

Summers, A. O., Wireman, J., Virmy, M. J., Lorscheider, F.L., Marshall, B., Levy, S. B., Bennett, S., and Billard, L. (1993). Mercury release from dental Silver fillings provokes an increase in mercury - resistant bacteria in oral and intestinal floras of primate. *Antimicrobial Agents and chemotherapy,* 37: 825 - 834.

Tang, S.M., Kurnp, P., Yap, C.T. and Bilal, M.G. (1986). Calculation of relative fluorescence intensity for annular-source geometry by Monte Carlo method. *X-Ray spectrum,* 15: 289-293.

Tietze, E., Tschape. H., and Voigt, W. (1989). Characterization of new resistance plasmids belonging to incompatibility group IncQ. *Journal of Basic Microbiology,* 29: 695-706.

Touch, A., Schluter, A., Bischoff, N., Goesmann, A., Meyer, F., and Puhler, A. (2003). The 79, 370~bp conjugative plasmid pB4 consist of

an IncP-1R bacbone loaded with a chromate resistance gene pair, the oxacillinase gene bla (NPS-I), and a tripartite antibiotic efflux system of the resistance-nodulation-division family. *Molecular Genetics and Genomics,* 268: 570-584.

U.S Environmental Protection Agency. (1992). Heavy metals in Mississippi River. *U.S. Geological Survey Circular*. 1133.

Unz, R.F., and Shuttlework, K.L. (1996). Microbial Mobilization and Immobilization of Heavy Metals. *Current Opinions in Biotechnology,* 30; 249-254.

Vajiheh, K., Naser, B. and Giti, E. (2003). Antimicrobial, heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in a hospital in Isfahan, Iran. *African Journal of Biotechnology*, 2(10): 379-383.

Vakulenko, S.B., and Mobashery, S. (2003). Versatility of aminoglycosides and prospects for their future. *Clinical Microbiology Review,* 16: 430-450.

Vilanova. X., Manero A., Cerda-cuellar, M. and Blanch, A.R. (2004). The composition and persistence of faecal coiiforms and enterococcal populations in sewage treatment plant. *Journal of Applied Microbiology,* 96(2): 279-288.

Volesky, B. (1994). Advances in Biosorption of metals: selection of biomass types. *FEMS Microbiology Reviews,* 14: 291-302.

Volesky, B., and Holan, Z.R. (1995). Biosorption of heavy metals. *Biotechnology Progress,* 11: 235-250.

Walsh, C. (2000). Molecular mechanisms that confer antibacterial drug resistance. *Nature,* 406: 775-781.

Westen, D.P. (1996). Environmental considerations in the use of antibacterial drugs in aquaculture. In Aquaculture and water resources management. (Baird, D. J., Beveridge, M. C. M., Kelly, I. A Eds). *Blackwell science*. Oxford.

Wikipedia, the free encyclopedia. (accessed 2007). Antibiotic Resistance.
<http://en.wikipedia.org/wiki/wikipedia/antibiotic_resistance>

Williams, S. (2002). Antibiotic resistance: Not just for people anymore. *Journal of Young Investigators,* 3: 1-4.

Wireman, J., Liebert, C. A, Smith, T., and Summer, A. O. (1997). Association of mercury resistance with antibiotic resistance in gram -negative feacal bacteria of primates. *Applied and environmental Microbiology* 63: 4494-4503.

Wise, M. G., Shimkets, L.G., Wheat, C. and McArthur, J.V. (1996). Temporal variation in genetic diversity and structure of a lotic population of *Burkholderia (Pseudomonas) cepacia. Applied and Environmental Microbiology,* 62: 1558-1562.

Witte, W., Klare, I. and Werrer, G. (1999). Selective pressure by antibiotics as feed additions. *Infection,* 27: 35-37.

Wommack, K. E., and Colwell, R.R. (2000). Virioplankton viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews,* 64: 69-114.

World Health Organisation (2001). WHO global strategy for containment of Antimicrobial resistance. Geneva.

Yurieva, O., Kholoddii, G. Minkhin, L, Golenko, Z., Kalaeva, E., Mindiin, S., Nikiforov, V. (1997). Intercontinental spread of promiscuous mercury resistance transspossons in environmental bacteria. *Molecular Microbiology,* 24: 321-329.