CULTURE-DEPENDENT AND CULTURE-INDEPENDENT MOLECULAR ANALYSIS OF SELECTED ELECTRONIC-WASTE DUMPSITES IN LAGOS AND IBADAN, NIGERIA AS POTENTIAL RESERVOIR OF ANTIMICROBIAL RESISTANCE

BY

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CERTIFICATION

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I dedicate this research project to the loving memory of my late sister Ehiaghe Victory Ikhimiukor, an energetic, brilliant young lass who left this world suddenly at young age. I am grateful for finally fulfilling the promise I made to you before your untimely passing.

ABSTRACT

Antimicrobial Resistance (AR) in bacteria is currently one of the greatest threats to global health. Antimicrobial resistance research has primarily focused on hospitals, but recent information points to the environment as important platform for the proliferation of AR due to selection pressure from anthropogenic pollutants such as Heavy Metals (HMs). Electronic waste (E-waste) is an important source of environmental contamination with HMs in developing countries, which could lead to proliferation of AR in the bacterial flora of E-waste dumpsites. However, few studies have investigated E-waste dumpsites as reservoir of AR. Therefore, this study was aimed at investigating selected E-waste dumpsites in Lagos and Ibadan, Nigeria as reservoirs of AR.

Fifteen soil (Lagos=9, Ibadan=6) and 24 water (Lagos=15, Ibadan=9) samples from three E-waste dumpsites in Lagos and two in Ibadan were processed for isolation of bacteria using standard methods. The HMs content of the samples were determined using inductively coupled plasma-optical emission spectroscopy. Isolated bacteria were tested for co-resistance to metals and ten antibiotics using spot inoculation and disc-diffusion, respectively. Selected bacteria showing co-resistance to metals and antibiotics were subjected to whole genome sequencing to determine genetic basis of metal and antibiotic resistance. The abundance of selected Antibiotic Resistance Genes (ARGs) (*sul1*, *sul2*, *dfrA1*, *tetA*, $bla_{\text{CTX-M-1}}$) and mobile genetic element *intI1* in the metagenomic DNA of the samples were measured by qPCR to determine the level of E-waste dumpsite contamination with ARGs. Data were analysed using linear regression and Principal Component Analysis (PCA) at $\alpha=0.05$ to examine the relationship between HMs concentrations and ARG abundance.

Eighty-four metal and antibiotic resistant bacteria identified as *Escherichia coli* (n=66), *Enterobacter* (n=10), *Citrobacter* (n=6), *Kluyvera* (n=1) and *Leclercia* (n=1) species were isolated from the soil and water samples. Heavy metals (Cu, Pb, Zn, Fe, Ni, Al, Co, Se and Cd) concentration in the water were beyond permissible limits set for drinking water quality by the Standards Organization of Nigeria. Ninety-four percent of the strains were multidrug resistant. Forty-nine different ARGs conferring resistance to tetracycline, aminoglycosides, trimethoprim, sulphonamides, β-lactams, fosfomycins, phenicols and Macrolide-Lincosamides-Stretogramines were identified. Detected genes were *tetA/C/D*, *strA/B*, *aadA*, *aac(6')Ib-cr*, *dfrA1*, bla_{TEM}, bla_{MIR}, bla_{OXA}, bla_{CMY}, bla_{ACT}, $bla_{\text{CTX-M-15}}$, *sul1/2/3*, *qnrS1*, *qnrB*, *qepA*, *fosA*, *catA/B*, *cmlA1* and *mphA*. Metal-resistance genes *arsB*, *pcoABDRS*, *silABCPRS*, *merA* and plasmids of the Col, IncF, IncY, IncR, IncI and IncR groups were identified. The ARG/*intI1* abundance (copy number/gram) in soil ranged from $1.09\text{x}10^{6}$–$1.23\text{x}10^{8}$ (*sul1*), $1.48\text{x}10^{6}$–$9.53\text{x}10^{7}$ (*sul2*), $1.33\text{x}10^{5}$–$3.19\text{x}10^{7}$ (*dfrA1*), $1.25\text{x}10^{5}$–$1.10\text{x}10^{6}$(*tetA*),$9.90\text{x}10^{4}$–$2.08\text{x}10^{5}$($bla_{\text{CTX-M-1}}$) and $6.73\text{x}10^{6}$–$8.76\text{x}10^{7}$ (*intI1*). Abundance (copy number/100ml) for water samples ranged from $1.07\text{x}10^{5}$–$2.61\text{x}10^{8}$ (*sul1*), $4.75\text{x}10^{4}$–$1.47\text{x}10^{8}$ (*sul2*), $1.73\text{x}10^{4}$–$1.12\text{x}10^{8}$ (*dfrA1*), $8.80\text{x}10^{3}$–$1.28\text{x}10^{6}$(*tetA*),$4.69\text{x}10^{4}$–$5.67\text{x}10^{6}$($bla_{\text{CTX-M-1}}$) and $1.05\text{x}10^{4}$–$2.61\text{x}10^{7}$(*intI1*). Linear regression and PCA confirmed positive ($0.28 \geq r \leq 0.78$) relationships between HM contamination and ARGs abundance in soil and water samples. A higher correlation ($0.60 \geq r \leq 0.78$) occurred between *intI1* and HMs, while poor correlations were observed with Co, Se and Cd.

The selected electronic waste dumpsites in Lagos and Ibadan were reservoirs of antimicrobial resistant bacteria with heavy metals playing a role in the proliferation of antimicrobial resistance.

Keywords: Heavy metals pollution, Antibiotic resistance, Whole genome sequencing, Quantitative PCR

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TABLE OF CONTENTS

APPENDICES

LIST OF TABLES

LSIT OF FIGURES

LIST OF PLATE

CHAPTER ONE

INTRODUCTION

1.1 Electronic waste

The term electronic waste (E-waste) is generally used to denote electrical electronic equipment or devices which does not fulfil the needs of the initial purchaser anymore (Peralta and Fontanos, 2006). They are also known as end-of-life electronic equipment(Basel Action Network, 2011) and has become a generic name to label all waste comprising of electrically driven components which, although valuable, may pose environmental and health hazards (Asiimwe and Åke, 2012). Due to the rapid inclusion and implementation of Information and Communications Technology (ICT) in virtually all aspects of our daily living, the E-waste phenomena has experienced a huge boom in the last decades(Basel Action Network, 2011). Currently, it has been rated to be amongst the quickest rising waste streams globally, resulting in a growth rate of 3 to 5% per year, this growth rate is reported to be three magnitudes higher than the growth rate of estimated global normal municipal solid waste (MSW) (Ogungbuyi *et al.*, 2012).

1.2 Classification of electronic wastes

Electronic waste comes in numerous varieties, hence an apt classification of all E-waste is difficult, however, three main categories of E-waste constituting about 75% (33.66 Mt of 44.7 Mt) of global E-waste by weight includes the following(United Nations University, 2017):

Small equipment (such as microwaves, fans and other ventilation machines, bread toasters, electric kettles, electric shaving clippers, electronic calculating devices, radio devices, video cameras, electronic toys, small electrical and electronic tools, vacuum cleaners, laptop computers, small medical devices).

Big equipment (including cloth-washing machines, dish-washers, electric cooking stoves, large printing and photocopying machines).

Temperature exchange equipment (including refrigerators, air cooling machines and heat pumps).

Electronic wastes has over a thousand diverse constituents, including both precious and harmful materials, their physical and chemical components makes them distinctive from other forms of municipal and industrial waste (Okorhi *et al.,* 2018). Their heterogeneous nature is a testament to their widespread production and consumption globally.

1.3 Overview of the global production of E-waste

According to the United Nations Environment Programme – UNEP, the estimated global production of E-waste was placed between twenty to fifty million metric tonnes per year (20 to 50 MMT/year) (UNEP, 2006). This represents about 1 – 3 percent of the total municipal waste produced globally (1636 MMT/year) (OECD, 2013). According to reports from the United Nations University (UNU), about 44.7 MMT of waste electronic equipment were produced in 2016, representing about 8% increase of 3.3 Mt between 2014 and 2016 (United Nations University, 2017). Correspondingly, this figure is expected to keep rising as specialists have predicted a 17% increase in E-waste generationfrom 41.4 million Mt in 2014 to 52.2 million Mt by 2021, thus, making Electronic waste the fastest rising domestic waste stream all over the world (Balde *et al.*, 2017). In respect to its generation by country, the highest per capita producers of E-waste globally are Australia, New Zealand, (at 17.3 kg per inhabitant), Russia (16.6 kg per inhabitant) and Americas (11.6 kg per inhabitant) (United Nations University, 2017).In Africa, the overall E-waste generated was projected to be 1.9 million tonnes in 2014, which interpreted to an average of about 1.9 kg of E-waste accumulated per person(United Nations University, 2017). Leading producers on the continent includes Egypt at 0.37 million Mt, Republic of South Africa at 0.35 million Mt and Federal Republic of Nigeria at 0.22 million Mt (Sthiannopkao and Wong, 2013). However, due to poor information on collection rates on the continent, it is likely that these figures are much less than what is actually obtainable.

Despite the enormous generation rates, only a meagre twenty percentarising from all E-waste produced is acknowledged to bere-assembled and reprocessed or recycled (United Nations University, 2017). Factors such as lack of adequate recycling facilities, high labour costs, and poor environmentally friendly regulations has hampered adequate recycling of E-waste, as a result, the producing (rich) countries are more likely not to recycle E-waste (Cobbing, 2008; Gweme *et al.*, 2016). Rather, E-waste generated are either dumped or buried in landfills or distributed to low and

middle income (developing and underdeveloped) countries, where they accumulate and constitute a serious problems to the ecosystem (Cobbing, 2008; Lewis 2010; Gweme *et al.*, 2016).

1.4 Electronic waste dumpsites in Nigeria

Most of the electrical electronic equipment used in Nigeria is imported, with used or second-hand EEE or E-waste being as high as 70% (Figure 1) (Lewis, 2010; Ogungbuyi *et al*, 2012). In 2010, at least 100,000 tons of E-waste was recorded to have been illegally importedinto the country, including about 2.4 million pieces of second-hand computer screens (Ogungbuyi *et al.*, 2012; Nnorom *et al.*, 2013). Additionally, the Basel Action Network (2011) estimates the penetration rate of E-waste in Nigeria to be 4.4kg per inhabitant. The infiltration of huge amount of E-waste into the country poses lots of challenges to its management because its generation increases faster than the ability to effectively manage it (Ogungbuyi *et al.*, 2012; Omole *et al.*, 2015). Thus, E-waste are found mostly in uncontrolled dumpsites where activities such as dismantling and recycling through pyrolytical processes by scavengers are carried out with little or no regard for safety of individuals, the surrounding community or the environment (Cobbing, 2008; Ogungbuyi *et al.*, 2012; Nnorom *et al.*, 2013). These activities further aids in the release of potential carcinogens and neurotoxins into the ecosystem (Olafisoye *et al.*, 2013). In addition, Nigeria's informal electronic waste sector has seen significant growth over the years, currently amassing a workforce of over a 100,000 people, processing half a million tonnes of discarded appliances every year (Ogungbuyi *et al.,* 2012; UNEP, 2019). These army of crude E-waste processors mostly carryout illegal and unsustainable extraction of precious heavy metals which are abundant in E-waste (Basel Action Network, 2011; Nnorom *et al.*, 2013). Hence, the handicapped status of electronic waste control system in Nigeria is therefore seen as a serious environmental problem and threat to public health (Achi *et al.*, 2013).

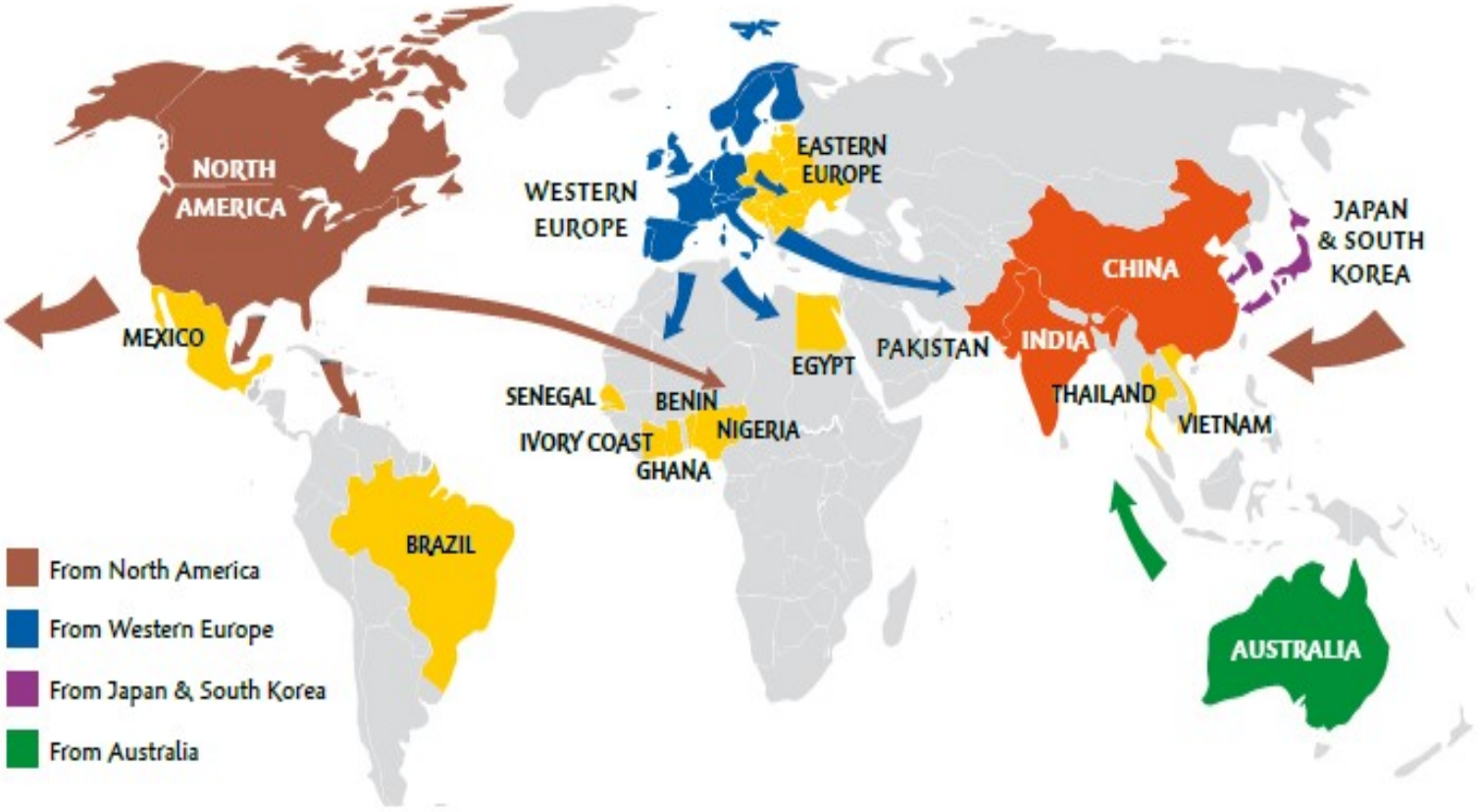

Fig. 1.1. Illustration of the flow of E-waste trade (legal and illegal) across the globe (Adapted from Lewis, 2010

1.5 Heavy metal pollution and electronic waste dumpsites

According to the UNU, the estimated value of recoverable resources inherent in E-waste is projected at about fifty-fivebillions in Euros (Balde *et al.*, 2017). Heavy metals are abundant and valuable components present in E-waste. For instance, as high as seven percent (7%) of all the gold in the world might be present in E-waste (UN Environement, 2019). A private computer may contain up to 4g of Gold (Au) and other valuable but toxic heavy metals which includes Silver (Hg), Lead (Pb), Cadmium (Cd) and Chromium (Cr) (Hilty, 2005). Similarly, cathode ray tubes which are integral parts of computer monitors may comprise up to 8-20% of Pb, which could be as much as 0.68-2.72kg of lead(Nnorom *et al*., 2013). Funds obtained from the sale of precious metals from E-waste has made heavy metal extraction processes such as open burning, acid leaching to be common and lucrative activities within electronic waste dumpsites(Asiimwe and Åke, 2012; Ogungbuyi*et al.*, 2012; Gangwar *et al*., 2019). However, crude metal extraction processes only yields about 25% recovery efficiency, in comparison to the 95% obtained from modern recycling systems using integrated smelters (Schluep *et al.*, 2013). These unsustainable metal extraction procedures are reported to exacerbate the input of heavy metals (HMs) into, including its subsequent pollution of, the environment surrounding E-waste dumpsites (Ha *et al.*, 2009;Asiimwe and Åke, 2012; Olafisoye*et al.,*2013; UNEP, 2019).

1.6 Heavy metals, antibiotic resistance and E-waste dumpsites

Currently, the problem of antimicrobial resistance imposes a great menace to global public health, as the prevalence of high level of resistance to antimicrobials worldwide frustrates efforts in effectively treating bacterial diseases (WHO, 2019). Evidences indicating that the evolution and proliferation of antibiotic resistance (AR) in bacterial strains and communities may be likely initiated by selection pressure exerted by anthropogenic pollutants like heavy metal, has been on the rise(Chen *et al.*, 2015a; Nguyen *et al.* 2019). Since the 1970s, there has been a build-up of studies examining the connections between heavy metals contamination and the prevalence of antimicrobial resistance (Sütterlin *et al.*, 2018). Heavy metal exposure force bacteria strains to evolve tolerance and resistance mechanisms for survival in metal contaminated natural ecosystems(Bengtsson-Palme*et al.*, 2018). Such heavy metal tolerance mechanisms have been described to promote bacterial resistance to several

antibiotics through co-selection mechanisms(Romero *et al*., 2017; Sütterlin *et al*., 2018; Zhang *et al*., 2018a). This suggests that heavy metal polluted environments like E-waste dumpsites may contain bacteria strains harbouring metals and antibiotics co-resistance(Seiler and Berendonk, 2012; Olafisoye*et al.*, 2013; Di Cesare *et al.,* 2016; Knapp *et al.*, 2017).

Furthermore, cross contamination of soil and aquatic ecosystems by heavy metals via leachate from E-waste dumpsites have been reported previously(Wang and Guo, 2006; Olafisoye *et al.*, 2013). For instance, downstream of an E-waste dumpsite was discovered about 0.4 mg/l of Pb (which is 8 times more than the allowable limit required in water meant for drinking, 0.05 mg/l) in the receiving river water located in China(Wang and Guo, 2006). Ha *et al.* (2009) also described soils at an electronic waste salvaging area within the city of Bangalore, India to contain up to 39 mg/kg of Cd, 957 mg/kg of Sn, 180 mg/kg of Sb,49 mg/kg of Hg, 2850 mg/kg of Pb, and 2.7 mg/kg of Ni. These values were about a hundred times higher than values measured in control soils obtained from a near site within the same town. Consistent with this, reports by Olafisoye *et al.* (2013) at the Alaba E-waste dumpsite in Lagos, Nigeria, revealed elevated concentrations of metals in water and plants surrounding the dumpsite. Due to the established linkthat exists between environmental pollution with heavy metals to aid in the development of antibiotic resistance in bacterial strains,including the potential forfurther contamination of other soil and water ecosystems with heavy metals from these E-waste dumpsites,therefore, makes these E-waste dumpsites a potential public health threat worthy of further investigation.

1.7 Justification of the study

Despite the increasing rate of E-waste accumulation in Nigeria and their potentials as a source of environmental contamination with heavy metals, and increasing evidence linking metal tolerance in bacteria with the development and proliferation of antibiotic resistance, very little is known about the potential of E-waste dumpsites as hotspots for the evolution and proliferation of antibiotic resistance in environmental bacteria in Nigeria. It is against this background that this study was carried out to examine the roles of E-waste dumpsites as potential reservoir of bacteria harbouring co-resistance to metals and antibiotics using molecular biology tools.

1.8 Aim of the study

The study is designed to investigate the prevalence of co-resistance to metals and antibiotics in the bacterial flora of selected E-waste dumpsites in Lagos and Ibadan, south-western Nigeria, using culture-dependent and culture-independent molecular analysis.

1.9 General objectives of the study

The aim of the study was implemented through the following objectives:

1. To establish heavy metal contaminations status of the selected dumpsites by determining the concentrations of heavy metals in soil and water samples collected from the selected E-waste dumpsites.
2. To investigate the pattern of phenotypic resistance to metals and antibiotics among enterobacterial strains isolated from the selected E-waste dumpsites.
3. Use Whole Genome Sequencing (WGS) to investigate the genetic determinants of co-resistance to metals and antibiotics among the isolated Enterobacteriaceae strains.
4. Use Real-Time quantitative PCR (qPCR) to quantify selected antibiotic resistance genes (ARGs) in the metagenomic DNA fraction of samples and correlate the ARG abundance with measured heavy metal (HM) concentrations to estimate the likely contribution of HMs to the development of resistance.

1.10 Significance of the study

This study will serve as the first systematic investigation of the roles played by metal pollutants from E-waste dumpsites in the evolution and dissemination of antibiotic resistance in environmental bacteria in Nigeria. This would be important in highlighting the important role of these dumpsites as environmental reservoirs of antibiotic resistance and will be very helpful in combating the rising threat of environmental antibiotic resistance in Nigeria and other developing countries. The information obtained from this study would also be useful in making a case for efficient management of E- waste in Nigeria.

CHAPTER TWO

LITERATURE REVIEW

2.1 Global threat of antimicrobial resistance

Today, antimicrobial resistance (AMR) poses a great danger to the sustainability of modern medicine as well as to public health worldwide(WHO, 2019). The magnitude of the crisis is manifested in the increasing number of common diseases such as those transmitted sexually, infections of the urinary and respiratory systems and more are becoming untreatable or increasingly difficult to treat. In tandem, carrying-out life-saving medical procedures is becoming much more risky than ever before (Ross and Katz, 2015). As a result, drug-resistance related diseases currently results in a minimum mortality of about 700,000 people annually (Review on Antimicrobial Resistance, 2014). In the coming years, this trend is projected to worsen to an estimated annual mortality of ten (10) million by 2050 (Review on Antimicrobial Resistance, 2014; Ross and Katz, 2015). Asides the mortality rate associated with the global drug-resistance problem, a secondary effect is expected to cause a global financial crisis resulting in extensive damage to the world economy, thereby, forcing millions of people into extreme poverty (Ross and Katz, 2015). Thus, the Interagency Coordinating Group on Antimicrobial Resistance (IACG) called for the urgent implementation of organised and ambitiously bold steps to prevent the disastrous fallout of the antimicrobial drug resistance crisis (Ross and Katz, 2015).

The AMR crisis is expected to have varying impacts on different parts of the world (Figure 2.1). In terms of mortality per population size, Africa is expected to suffer greatest from the current AMR crisis with an estimated mortality of over four million people by 2050 as illustrated in Figure 2.1 (Review on Antimicrobial Resistance, 2014). According to Tadesse *et al.* (2017), over 40 % of the countries in Africa do not have any AMR-related data, and the level of resistance has greatly increased as commonly prescribed antibiotics are quickly failing. Key challenges with AMR on the

continent includes; weak regulations governing the circulation and prescription of antibiotics

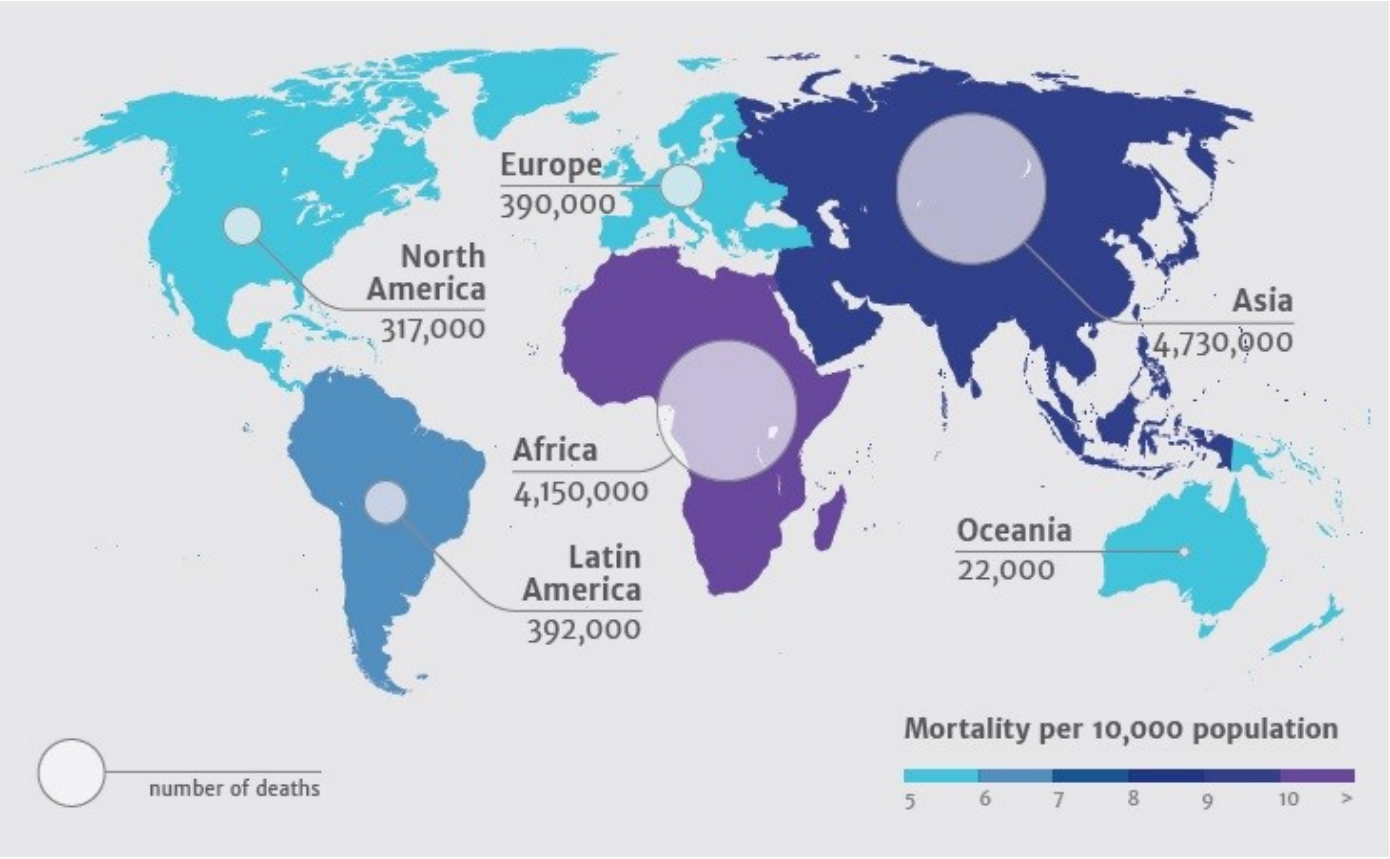

Fig. 2.1. Predicted world mortality rate attributed to antimicrobial resistance by 2050. Africa is expected to be heavily impacted by AMR (Adapted from Review on Antimicrobial Resistance, 2014)

availability of inferior and counterfeit antimicrobials, poor political will-power to implement comprehensive policies to address the AMR crisis and the presence of poor of AMR monitoring system, weakened capacity of laboratories to undergo effective AMR investigation, experimentation and reporting resulting particularly from the unavailability of basic laboratory equipment, chemicals, consumables and technical know-how(Ndihokubwayo *et al.*, 2013).

Although several efforts have been made by different stakeholders in pursuit of sustainable solutions to the antimicrobial drug-resistance crisis, it is generally agreed that a comprehensive understanding of the communal nature and the interconnected roles played by living organisms (human-beings and animals), food and the natural environment, famously tagged as the "One Health" strategy is most suitable in tackling this problem(Collignon *et al*., 2018; Collignon and Beggs, 2019). One Health is a multisectoral collaborative effort to attain optimal health for humans, domestic animals, wildlife, plants and the environment (McEwen and Collignon, 2018). The One health theory was first propounded by Balfour Eve in 1943 (Balfour 1943; Ramakrishnan *et al.*, 2019), when she first pointed out that: *'the health of soil, plant, animal and man is one and indivisible'*. This means that sickness of any one domain of the tetrad can affect the overall health of all domains (Vieweger and Döring, 2014). In view of the one health approach, AMR research in the recent past has grown beyond the clinical environment to focus on different environment matrices as a vital platform supporting the development and proliferation of resistant bacteria species and pathogens (Bengtsson-Palme *et al.*, 2018).

2.2 The natural environment as progenitors of antimicrobial resistance

In the past, the fight against antibiotic resistance was largely limited to clinical settings, with the assumption that these areas were solitary hotspots aiding the evolution and proliferation of AMRresulting from the wide usage of antibiotics in the treatment of infection therein (Bengtsson-Palme *et al.*, 2018). However, resistance seen in the clinical settings today often bear close similarity to what has long been observed in the environment (Allen *et al.*, 2010). However, this is not shocking as the bulk of the antibiotics being used today were products obtained from microorganisms which evolved resistance long before the era of medicinal antibiotic use, and are

commonly found within the chemical repertoire of soil microbes (Abraham and Chain, 1940; Clardy et al., 2007; Hoskisson, 2016; Bush, 2018).

Early reports proposed that antibiotic producers may be the primary sources of theresistanceproblem circulating in the clinic today (Benveniste and Davies, 1973). For instance, various aminoglycoside-inactivating enzymes such as 6' amino group of kanamycin A and B, gentamicin and neomycin and the 2'amino group of the hexose ring of gentamicin which were present in *Streptomyces kanamyceticus* and *Streptomyces spectabilis*bore close similarity to those found in antibiotic resistant Gram negative bacteria (Benveniste and Davies, 1973). In tandem, the initial acquisition of the extended spectrum beta-lactamase (ESBLs) $bla_{\text{CTX-M}}$ from environmental *Kluyvera* species which later emerged in the clinical environmentduring the 1990s was discovered to be the principal enzyme to efficiently hydrolyze expanded spectrum cephalosporin antibiotics at levels reported to be of clinical significance (Livermore *et al.*, 2007). Currently, the $bla_{\text{CTX-M}}$ are reported to have expanded into five (5) major groups including, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-9}}$ and$bla_{\text{CTX-M-25,}}$ with each of the groups having several members (Boyd *et al.* 2004). They have quickly developed to being the most dominant ESBLs in Enterobacteriaceae, and are still evolving novel variants (Ramadan *et al.*, 2019). Recently, novel CTX-M variants were isolated and characterized amongst *E. coli* strains from a medical facility in Egypt, they are $bla_{\text{CTX-M-14.2}}$ and $bla_{\text{CTX-M-15.2}}$, sharing similar close amino acid sequence with $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ respectively (Ramadan *et al.*, 2019).

Similarly, results following *in silico* and molecular analysis of the environmental *Shewanella* spp. genomes determined these strains as the progenitors and reservoir of $bla_{\text{OXA-48}}$-like genes. (Tacão *et al.*, 2018). The $bla_{\text{OXA-48}}$ are members of the class D β-lactamases and have since produced more than a few variants which share comparable enzymatic profiles, they include $bla_{\text{OXA-162}}$, $bla_{\text{OXA-181}}$, $bla_{\text{OXA-204}}$, $bla_{\text{OXA-232}}$, $bla_{\text{OXA-244}}$, $bla_{\text{OXA-245}}$, $bla_{\text{OXA-370}}$, $bla_{\text{OXA-436}}$, $bla_{\text{OXA-438}}$ and $bla_{\text{OXA-484}}$ (Lutgring *et al.*, 2018). Studies have revealed $bla_{\text{OXA-48}}$ to be carried on plasmids and are now widely disseminated amongst members of the Enterobacteriacea(Poirel *et al.*, 2012; Mathers *et al.*, 2013). Also, the $bla_{\text{OXA-48}}$ producing Enterobacteriaceae have been increasingly associated with several hospital outbreaks around the world including those which occurred in France (Cuzon *et al.*, 2011), China (Guo *et al.*, 2016), Algeria (Loucif *et al.*, 2016), Slovenia (Pirš *et al.*, 2019), Netherlands(Dautzenberg *et al.*, 2014),

Germany (Kola *et al.*, 2015), Spain (Robustillo-Rodela *et al.*, 2017), Croatia (Bedenić *et al.*, 2018), and many more which reported occurrences of the ARG (Poirel *et al.*, 2012).

Another member of the environmental *Shewanella*, *Shewanella algae* have been reported to be progenitors of the plasmid mediated quinolone resistance gene, *qnrA*(Poirel *et al.*, 2005). This gene was however, first reported occurring in plasmid (pMG252) from clinical isolates of *Klebsiella pneumoniae*, protecting the bacterial DNA gyrase from inhibition by the fluoroquinolone antibiotic, ciprofloxacin (Martínez-Martínez*et al.*, 1998). The plasmid mediated quinolone resistance genes (PMQR) currently includes six *qnr* genes encoding gyrase-protection repetitive peptides, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS* and *qnrVC*(Robicsek *et al.*, 2006; Fonseca and Vicente, 2013). These *qnr* determinants have been identified worldwide and are increasingly found in Enterobacteriaceae strains (Guan *et al.*, 2013). According to Spellberg and Doi (2015), quinolone resistance rates in community associated Enterobacteriaceae have skyrocketed in recent years to about >10% to 30% in the United States and about >50% in many parts of the world (Spellberg and Doi, 2015). The origin of clinically important ARGs from the environmental bacteria is a strong indicator of the potential of the natural ecosystem to be important reservoirs aiding the evolution and proliferation of AMR.

2.3 Ecology-evolutionary perspective of antimicrobial resistance

In the natural ecosystems, microorganisms are usually the first recipients of the negative effect of environmental perturbations or stressors which threatens their continuous survival. Because stress-induced response proteins can be phenotypically inherited, these stressors triggers a physiological response in bacteria, eliciting the transcription of a several explicit and extremely regulated adaptive reactions to improve microbes' survival in the presence of stressors (Lin and Kussell, 2016; Poole, 2017; Roemhild and Schulenburg, 2019). Further, these stressors impact or influence the natural selection and/or evolution of microbial populations to favour the advancement of one group of organisms (tolerant/resistant) over the other (susceptible) in a phenomenon known as selection pressure (Händel *et al.*, 2016).

2.4 Antibiotics selection pressure as drivers of evolution

The proliferation of microbial resistance to antimicrobials seen inthe natural environs today is reported to be linked partly to environmental pollution of antimicrobials which are known to exert significant selection pressure on exposed microorganisms. The development of AMR through antibiotic selection pressure are major players in global AMR and have been elucidated in several published literatures (Muziasari *et al*., 2014; Le *et al*., 2016; Danner *et al*,. 2019). In the past 70 years, antibiotics misuse occurring in different spheres of human life has imposed huge selection pressure on the community bacterial populations. Worldwide, the annual usage of antibiotics exceeds 100,000 tonnes, however, the therapeutic use of commercially produced antibiotics in human illnessesand diseases represents below half of its total application(Allen *et* al., 2010; Bernier and Surette, 2013; Van Boeckel *et al*., 2014). Their use, including their abuse is prevalent in other processes including, as growth promoters and prophylactic treatments in animal husbandry and aquaculture, for example, amoxicillin and erythromycin have been widely reported to be used in treating animal diseases and in promoting animal growth and enhance their feed efficiency(Allen *et al*., 2010; Shistaand Curle, 2014). Also, antibiotics are commonly used to control plant disease in agriculture and for therapeutic/prophylactic use in household pets (Harada and Asai, 2010; Shistarand Curle, 2014). In addition, several studies have implicated antibiotic pollution in the increase of both ARGs and their bacterial hosts in different environmental platforms (Devarajan *et al.*, 2015; ; Le *et al.*, 2016; Danner *et al.*, 2019; Khan*et al.*, 2019). Antibiotics entering the environment could persist in land and aquatic ecosystems, the selective pressure enforced by these pollutants on bacteria could diminish the efficacy of drugs used in treatment of human illnesses, particularly when resistance spreads to clinically relevant bacteria(Davies and Davies, 2010; Shistarand Curle, 2014; Zhu *et al*., 2013; Amador *et al*., 2019). Antibiotics are thus important pollutants in the natural environment.

2.5 Roles of anthropogenic pollutants in antimicrobial resistance

Like antibiotics, there is growing evidence which indicates that anthropogenic pollution likely fosters the development and proliferation of microbial resistance to antibiotics (Poole, 2017; Bengtsson-Palme*et al.*, 2018). Although microbial populations are frequently exposed to selection pressure from antimicrobials in both clinical and environmental settings, a striking difference is that microbes in clinical settings are exposed to one to a few antibiotics at a time, whereas, microbes in the

environment are much likely to be exposed to a combination of polluting toxicants at a time (Chait *et al.*, 2012). When released into the environment pollutants can linger for months or years due to their structure (Gren, 2012). Thus, pollutants in the environment may be available to exert selection pressure on exposed microbes over a longer time period. As a result, environmental microbes that are constantly exposed to selection pressure from anthropogenic pollutants are often forced to develop various coping mechanisms to overcome the toxic effects of pollutants.

Investigations aimed at determining roles played by anthropogenic pollution towards the evolution and proliferation of ARGs and their respective bacterial hosts within bacterial populationsfrom various ecological niches has been carried out (Di Cesare *et al.*, 2016; Knapp *et al.*, 2017; Zhang *et al.*, 2018b).Also, studies have suggested that the dissemination of bacteria species which are resistant to antibiotics and the respective genetic determinants conferring such resistance in the natural environment may be imposed by selective pressures from pollutants on the exposed bacteria community(Seiler and Berendonk, 2012; Romero *et al.*, 2017; Bengtsson-Palme*et al.,* 2018). Thus, it could be hypothesized that the incessant dosing of pollutants into the natural environment couldsupport the propagation of bacterial species exhibiting resistance phenotypes to antibiotics in the community flora, causing an ecological shift in favour of microbial species virtually different from what was known several decades ago (Levy, 1997). Certain anthropogenic pollutants have been reported to promote resistance to antimicrobials in the natural environment.

2.5.1 Selected anthropogenic pollutants and antimicrobial resistance in the natural environment

Generally, the natural ecosystem serves as a hub for the exposure of bacterial species to a plethora of antimicrobial agents, however anthropogenic pollution activities have exacerbated their exposure to higher and more toxic doses of these antimicrobials. Available theoretical and experimental evidences in scientific literature has linked the following common environmental pollutants with the propagation and dissemination of AMR in the environment

2.5.1.1 Biocides

Biocides are extensively employed in the disinfection of appliances and facilities. They include as disinfectants, quarternary ammonium compounds (QACs), antiseptics, preservatives, which were formulated to be harmful to microbes, but has over the years shown declining efficacy on pathogenic bacteria (Stickler and Thomas, 1980; Griffiths *et al.*, 1997; SCENIHR, 2009; Wales and Davies, 2015). Experiments have shown that selective pressure from biocide exposure, significantly impacts the bacterial community structure and may be responsible for raising low-level or reduced antibiotic sensitivity in microorganisms and proliferation of resistant strains (Wales and Davies, 2015; Murray *et al.*, 2019). For instance, experiments involving treatments of wastewater community bacteria with the biocide, benzalkonium chloride resulted in complete loss of 18 bacterial species and persistence of 5 bacterial genera including *Citrobacter, Escherichia, Klebsiella, Morganella* and *Pseudomonas*(Murray *et al.*, 2019). Five chromosomally encoded benzalkonim chloride resistance genes were found within the strains, including *acrE/envC*, *acrF/encD*, *cpxA*, *cpxR*, *adeT1* and *abeS* (Murray *et al.*, 2019).

Other biocide resistance genes belonging to the QACs that have been well described in Gram negative bacteria include *qacE*, *qacΔ1*, *qacF*, *qacG*, *gacH*(Kücken *et al.*, 2000). Amongst these bacterial groups, the *qacΔ1* are the most widespread, they operate as partially deleted but functional efflux pumps (Kücken *et al.*, 2000; Amos *et al.*, 2018). The *qacΔ1* are reportedly found fused together with the sulphonamide resistance gene*sul1* in the 3′- conserved section of class 1 integrons alongside other ARGs(Romero *et al.*, 2017; Amos *et al.*, 2018; Murray *et al.*, 2019). Their physical linkage on integrons might infer that they could foster antibiotic resistance via co-resistance mechanism (Romero *et al.*, 2017; Amos *et al.*, 2018).

In tandem, experiments by Amos *et al.* (2018) on a riverine environment impacted by discharge effluents from a wastewater treatment plant revealed 89% of the class 1 integron carrying bacterial isolates to be resistant to biocides, of which 75% contained a biocide resistance gene (*qacE*, *qacEΔ1*, *qacH* and *qacI*) alongside ARG, $bla_{\text{CTX-M}}$. Further conjugation experiments revealed 42% of the QAC resistant donor strains were successful in transferring their QAC resistance determinants along with class 1 integrons and the $bla_{\text{CTX-M}}$ gene to recipient *E. coli* strain. This experiment demonstrated the mobilization of mobile genetic element (MGE) and ARG resulting from selection pressure from QACs.

Similarly, large scale bioinformatic analysis involving 2522 bacterial whole genome sequences and 4582 plasmid genomes across several environmental matrices revealed strong connections of genes specifying resistance to several types of biocides including alcohols, acids, and peroxides, to co-occur with ARGs conferring resistance determinants against an extensive variety of antibiotic classes such as the β-lactams, aminoglycosides, amphenicols, sulphonamides and tetracyclines (Pal *et al.*, 2015), thus, indicating possible co-selection.

2.5.1.2 Pesticides/Herbicides

The use of chemical pesticides and herbicides in controlling pests and weeds respectively in agriculture and other processes has been a common occurrence for several decades(Curutiu *et al.*, 2017; Ramakrishnan *et al.*, 2019). Like antibiotics, resistance to pesticides arose soon after their large scale use (Sparks and Nauen, 2015). The implication of this is that, many pests which are resistant to pesticides are also vectors for some human pathogens (Curutiu *et al.*, 2017; Ramakrishnan *et al.*, 2019).

Usually, before their introduction into the environment, the toxicity of these pesticides/ herbicides to microbes are established, however, the sub-lethal effects of these chemicals on microbes are rarely determined (Kurenbach *et al.*, 2015). According to Kurenbach *et al.* (2015), sub-lethal doses of several commercial herbicides such as dicamba,2,4-dichlorophenoxyacetic acid and glycophosphate on exposed *E. coli* and *Salmonella enterica* induced changes in response to antibiotics, ampicillin, ciprofloxacin, chloramphenicol, kanamycin and tetracycline, such that killing curves recorded antibiotics minimum inhibitory concentrations (MICs) to increase up to 6 fold. Also, in the study of Shafiani and Malik, (2003), 64 bacterial strains (*Pseudomonas*,*Azotobacter* and *Rhizobium* species) isolated from soils which had been irrigated with wastewater were assayed for their susceptibilities to a number of pesticides including endosulfan, carbofuran, and malathion, and to several antibiotics including, chloramphenicol, tetracycline, amoxycillin and doxycycline. Results revealed the strains to be tolerant to varying concentrations of the pesticides and also display resistance to one or more of the antibiotics. Studies have similarly described bacterial strains displaying phenotypic co-tolerance to pesticides and antibiotics(Kleiner*et al.*, 2007; Naphade *et al.*, 2012). Although some evidences of possible pesticide co-selection of antibiotic resistance exists, additional research

towards elucidating the mechanisms mediating such resistance to antibiotics were necessary.

Hence, results following a recent research by Armalytė *et al.* (2019) observed that low level antibiotic resistance to β-lactam, aminoglycosides, tetracycline, erythromycin and rifampicin antibiotics in the soil microbial flora from traditional and organic farms in Lithuania were due largely to the existence of multiple efflux pumps associated with the Resistance Nodulation Devision and ATP binding cassettes families. Similarly, the role played by the inducer *soxS* of an RND efflux pump AcrAR-Tol in causing increased resistance to selected antibioticswhen exposed to sub-lethal concentrations of chemical herbicides was investigated. Results confirmed increased resistance of *E. coli* to chloramphenicol and kanamycin when simultaneously exposed to dicamba and glyphosate respectively (Kurenbach *et al.*, 2015). Thus, indicating possible cross-resistance mechanisms in AMR.

Due to the recalcitrant nature of chemical pesticides and herbicides, their introduction into environments that are already polluted with low or below-lethal doses of antibiotics may cause the combined concentrations of both antimicrobial agents to be ample enough in selecting for antimicrobial resistant strains (Shafiani and Malik, 2003; Curutiu *et al.*, 2017). Short term evolutionary experiments by Kurenbach *et al.* (2018) involving combinations of various herbicide and antibiotics revealed that under specific experimental settings, co-exposure increased the development of antibiotic resistance (ie., increase in the minimum inhibitory concentrations - MIC) in microbes, irrespective of whether the concentration of the herbicide alone raises or lowers the MIC of the drug. This phenomenon was credited to the influence of the herbicides to have an effect on the MIC and/or on the minimum selective concentration of an antibiotic pairing (antibiotic and herbicide). This results also suggests that microbes may attain resistance in the natural environment in amountsthat are considerably greater than what is obtained under laboratory conditions.

2.5.1.3 Polycyclic aromatic hydrocarbons (PAHs)

The ubiquity of PAHs pollutants in the environment have been reported to be capable of posing significant threats to the health of human, animals and the environment (Adenij*et al.*, 2018; Gorovtsov*et al.*, 2018). A recent review focussing on the roles of

PAHs on the development of antimicrobial resistance amongst indigenous microbial flora of polluted environment has been well described (Gorovtsov *et al.,* 2018). Here, Gorovtsov*et al.* (2018) explained that PAH polluted soils are likely selective medium for antibiotic resistant bacteria as the level of ARG expressions in such medium is much higher. For instance, a previous study revealed that significant correlations exists between heavy metal and antibiotic resistance with antibiotic resistance and hydrocarbon degradation capability of bacterial isolates (*Pseudomonas corrugata* BBB2, *Pseudomonas veronii* CSGG7 and *Rhodococcus erythropolis* BGN2) from diesel contaminated soil samples (Máthé *et al.*, 2012). PAH tolerant bacterial isolates from highly anthropogenic PAH impacted environments have been reported to often display elevated tolerance toboth heavy metals and antibiotics (Máthé *et al.* 2012). However, mechanism of PAH enrichment of antibiotic resistance are still unclear.

Nonetheless, metagenomic profiling of PAH contaminated soils of a petrochemical plant in China established contamination of the soils with ARGs specifying resistance to tetracycline, sulphonamides, macrolide, fluoroquinolones, chloramphenicol, β-lactams, aminoglycosides, fosfomycin and others. Further detailed profiling showed that over 70% of ARGs were dominated by gene encoding efflux pumps linked to the extrusion of cyclic-ring shaped antibiotics such as fluoroquinolones and acriflavine (Chen *et al.*, 2017). Many characterized efflux pumps which function to expel organic pollutants out of the cell of bacteriaalso function as multi-drug efflux pumps driving microbial resistance to several antibiotics(Martinez *et al.*, 2009; Blanco *et al.*, 2016). For instance, EmhABC, an important RND family extrusion pump in *Pseudomonas fluorescens* strain cLP6a has been described to discharge hydrophobic antibiotics (chloramphenicol, tetracycline) and PAHs including phenanthrene from the bacterial cells (Hearn *et al.*, 2003). However, the localization of genes which encode these efflux pumps on mobile genetic elements (MGEs) is currently not well described. Nonetheless, correlation analysis by Sun *et al.* (2015) which investigated the interaction between mixed pollutants (including phenanthrene, pentachlorophenol, sulfadizine and roxithromycin) and ARGs (*sul1*, *sul2*, *ermA* and *ermB*) and the class one integron integrase, *intI1*, showed significant correlations between pollutants and ARG/*intI1* abundance. This results suggested a direct/indirect impact of the pollutants on soil ARG/*intI1* proliferation.

2.5.1.4 Heavy metals

Heavy metals including zinc (Zn), copper (Cu), lead (Pb), iron (Fe), aluminium (Al) and nickel (Ni) are ubiquitous in the environment, due mostly from anthropogenic pollution (Nies, 1999; Mohammed *et al.*, 2011). Heavy metal pollution studies have examined the roles of heavy metals contamination to the proliferation of antibiotic resistance in the natural environment (Berendonk *et al.*, 2015; Poole, 2017). Following activities which contaminate or pollute the natural environment with heavy metals, these metals can accumulate to dangerous levels which can trigger various mechanisms that are accountable for antibiotic resistance co-selection in bacterial species(Chen *et al*., 2015b; Poole, 2017; Zhang *et al*., 2018b). Mechanisms of heavy metals co-selection via cross-resistance and co-resistance have been well described (Fang *et al.*, 2016; Poole, 2017). Further detailed information on heavy metals, their sources and factors affecting their selection in the natural environment are discussed later in this chapter (from section 2.7).

The diverse and non-specific application of the various pollutants earlier discussed could provide a continuous and persistent selection pressure necessary for the maintenance and propagation of resistant strains in polluted ecosystems (Davies and Davies, 2010). This evolutionary selection pressure has become enormous and unprecedented worldwide as a result of large scale release of these pollutants into natural ecosystems (Wales and Davies 2015). Importantly, there are evidences linking the development of these adaptive evolution mechanism to the co-selection of antibiotic resistance traits among bacteria species from polluted natural ecosystems(Khan*et al.*, 2019; Murray *et al*., 2019; Roemhild and Schulenburg, 2019).

2.6 Co-selection of antibiotic resistance

Di Cesare *et al.* (2016) explained bacterialco-selection mechanism to involve the concomitant evolutionary selection of more than one resistance gene, notwithstanding whether the bacterial host of such genes are exposed to a single selective stressor. This phenomenon has been efficient in explaining the occurrence of some ARGs even when there exists no selection pressure from the corresponding antibiotic(s) (Martinez, 2009). The incorporation of resistance mechanisms against antibiotics and anthropogenic pollutants, such as heavy metals has been described to be key players promoting the obstinacy and perseverance of antibiotic resistance in various environmental matrices(Stepanauskas *et al.*, 2006; Poole, 2017; Zhang *et al.*, 2018b).

Zhang *et al.* (2018a) carried out copper shock experiments on the bacterial communities in drinking water to ascertain the outcome of the roles of copper metal in the evolution of bacterial resistance to antibiotic in the drinking water microflora. Results showed that bacterial resistance were considerably increased to antibiotics such as vancomycin, sulfadiazine, lincomycin, rifampicin, erythromycin, kanamycin upon exposure to 10mg/l and 100mg/l of copper. Further, there was a marked enrichment of heavy metal and antibiotic resistance genes, including *aadA*, *aac(6')I1*, $bla_{\text{ACC-1}}$, $bla_{\text{CTX-M}}$, *cueD*, *oprD*, and *merD*, by at least one fold following timed exposures (at 6, 12, 18, and 24 hrs) to the copper shock. The experiment revealed that copper played a part in the overall enrichment of AMR in the potable water.

Further, research studies on the likely outcome of the spread of resistance genes (including *sul1*, *sul2*, *sul3*, *qepA*, *qnrA*, *qnrB*, *qnrD*, *qnrS*, *tetA*, *tetB*, *tetW*, *tetQ*, *tetO*, *ermB* and *ermC*) when exposed to selection pressure conferred by heavy metals pollution in a catchment scale in China were determined (Xu *et al.*, 2017). Multivariate Redundancy analysis (RDA) revealed that the measured concentrations of the heavy metals (Ni, Cu, Zn, Cd and Hg) in theXiangjiang and Haihe river samples showed meaningful correlation relationships with the absolute gene abundances (copy number/ml) of *sul3*, *tetA*, *qepA*, *qnrA* and *tetM*. Additionally, bivariate correlation analysis showed strong positive correlations of ARGs, *qnrA* and *qnrB* with metal resistance gene *czcD* at R=0.819 and 0.959 for *qnrA* and *qnrB* respectively, where $p<0.0001$. This indicates that *czcD* may co-select for *qnrA* and *qnrB* if the genes are co-localized on the same mobile element in the metal tolerant and antibiotic resistant bacteria.Similar results were obtained in heavy metal (Cd, Pb, Hg, As, Cu, Zn) contaminated agricultural soils where the normalized ARG (*sul1, sul2 tetA, tetA* and *tetM*) and mobile element (*intI1*) abundances were determined. However, results from RDA showed Zn to be the major contributor to the proliferation of sulfonamides *sul1*and *sul2* and the class 1 integrons *intI1* in the soil bacteria population. Co-selection mechanisms are either coupled via cross-resistance (physiologically) and/or via co-resistance (genetically)(Baker-Austin*et al.*, 2006).

2.6.1 Cross-resistance mechanisms

Here, resistance genes responsible for resistance to a particular chemical substance also mediates resistance to another unrelated chemical substance(Baker-Austin *et al.*,

2006). This phenomenon refers to resistance to several antimicrobial agents mediated by a single molecular mechanism (Colclough *et al.*, 2019). It may occur when different antimicrobials attacking a similar target triggers a shared mechanisms which causes cell death, or it may occur when different antimicrobials share a similar path to access their corresponding targets(Baker-Austin *et al*., 2006; Romero *et al*., 2017;Colclough *et al.*, 2019). Here, the antimicrobial compounds may be structurally dissimilar, a typical example is the case of metals and antibiotics(Seiler and Berendonk, 2012). Cross-resistance is usually mostly mediated by multi-drug efflux pumps (Blanco *et al.*, 2016). These pumps belong to ancient and well-conserved proteins which cause decreased susceptibility of the organism to toxins by speedy expulsion of these toxins from out of the cell (Webber and Piddock, 2003; Martinez, 2009; Blanco *et al.*, 2016). However, since efflux pumps were selected long before the use of antibiotics in treating human illnesses, its well-described role nowadays as antibiotic resistance determinants may only represents a secondary function (Blanco *et al.*, 2016).

Several multidrug resistance (MDR) efflux pumps arereported to mediate both intrinsic, acquired and phenotypic resistance to several toxic materials in both Gram negative and positive bacteria species(Mosolygó *et al.* 2019; Nolivos *et al.* 2019). In addition, theseMDR efflux pumps have been experimentally proven to carry out cross-resistance, for example, *Pseudomonas aeruginosa* encoding an MexGHI–OpmD efflux pump caused an increase in the bacterial resistance to metal (vanadium) and antibiotics (ticarcillin/clavulanic acid) in contrast with the bacterial mutants lacking MexGHI–OpmD pump (Aendekerk *et al.*, 2002). Another well-studied RND bacterial efflux pump conferring resistance to multiple antimicrobials, including antibiotics, metals, dyes, biocides are the AcrAB–TolC pumps, they are increasingly described in Gram negative species (Baucheron *et al*., 2004; Li*et al.*, 2015a; Nolivos *et al*., 2019).

2.6.2 Co-resistance mechanisms

Another type of bacterial co-selection mechanism, co-resistance, takes place when multiple (2 or more) genetically linked genes conferringdifferent resistance phenotypes are co-localized on the same mobile genetic element (MGE)(Chapman, 2003). Here, the physical linkage of genes on MGEs such as plasmids, integrons and integrative and conjugative elements (ICEs) can result in co-selection of resistance toboth metals and antibiotics in organisms where this occurs (Chapman, 2003; Li*et al.*, 2015b; Fang *et*

al., 2016; Zhai *et al*., 2016). The genetic linkage of heavy metals and antibiotics resistance genes on plasmids have been reported to occur in diverse environments(Li*et al.*, 2015b; Zhai *et al*., 2016; Wu *et al*., 2018). Furthermore, the 'cad cluster' reported by Pal *et al.* (2015) in plasmids obtained from different environments and bacterial taxa showed the co-localization of resistance determinants specifying resistance to metals (cadmium and zinc - *cadD*) and antibiotics (aminoglycosides and macrolides) on the same MGE. In another study, analysis of twenty-five (n=25) IncH12 plasmids from *E. coli* strains isolated from animals in Chinese farms revealed co-existence of genes specifying elevated tolerance to copper (*pco* operon) and silver (*sil* operons) alongside *oqxAB*/$bla_{\text{CTX-M}}$ and other ARGs such as *aac(6')-Ib-cr* (n=18/25), *floR* (n=16/25), *rmtB* (n=6/25), *qnrS1* (n=13/25) and *fosA* (n=2/25), contributing to elevated MICs of Cu and Ag. The plasmids contained a Tn7-like transposon, further phylogenetic analysis showed that the Tn7-like transposons might have played a vitalfunction in the transference of the heavy metal resistance determinants and ARGs among genus of the Enterobacteriaceae(Fang *et al.*, 2016).

Phenotypic evidences expatiating on the vital role of plasmids in co-resistance of AMR was carried out by Ghosh *et al.* (2000).Here, plasmid curing experiments revealed that *Salmonella abortus equi* which were initially heavy metals (Cd, Ni and Ar, Cr, Hg and Pb) and antibiotics (ampicillin, kanamycin, penicillin G, oxacillin, vancomycin and erythromycin) resistant became susceptible to all the metals and antibiotics (ampicillin and kanamycin) (Ghosh *et al.*, 2000). Hence, indicating that resistance determinants to the metals and antibiotics were borne on the eliminated plasmids. The phenotypic changes towards the propagation of susceptible phenotypes following plasmid curing experiments underscores the invaluable roles played by plasmids in augmenting microbial persistence in anthropogenic polluted environments. Since certain plasmid types comprise an efficient machinery for controlling their replication in new hosts, they therefore represent important mechanism in the transfer of novel genes that can aid co-resistance of antibiotic resistance in the ecosystem.

2.7 Heavy metals and their sources in the environment

Heavy metals is a generic term used to refer are a group of metals or metalloids with atomic density that is more than 4000 kgm^3 or at least 5 times the atomic density of water (Ferguson, 1990). Heavy metals are not biodegradable, but are persistent

contaminants which are able to accumulate in the tissues of living things (Gochfeld 2003). Although heavy metals exist at natural background levels from mineral weathering, anthropogenic activities has exacerbated their input in the environment to levels that have detrimental effects on living organisms and pose ecological threats (Kimiran-Erdem, 2015). Anthropogenic input of metals in the environment is associated primarily with rapid industrialization. Routes of their input into the environment includes, but are not limited to, mine tailings, smelting, agriculture, burning of coal and fossil fuels,fertilizers application, animal manures, sewage sludge, traffic emissions, urban effluents, pesticides, electronic waste dumps, irrigation of wastewaters, petrochemical spillages (Baldé *et al*., 2017; Shaheen and Iqbal, 2018). Activities involving the extensive usage of heavy metals further supports the ubiquity of metals in diverse environmental matrices. For instance, early efforts to control the effects of microbial growth utilized metals such as copper sulphate as plant fungicides and mercuric salts against disease and infections (Aditi *et al.*, 2015). Metals have also been applied as food supplements in controlling animal infections and as growth enhancers(Zhu *et al*., 2013).

2.8 Heavy metal toxicity and microbial tolerance

Depending on their level of accessibility or bioavailability to microbes, heavy metals can have deleterious effect such as damage to microbial cell membranes, alteration of the specificity of enzyme,disruption of cellular functions, DNA structure damage and imposition of oxidative stress on microbes, thereby adversely affecting growth of microbes, their morphology and various biochemical processes, ultimately causing a decrease in microbial biomass and the community diversity (Bruins *et al.*, 2000; El-Sayed, 2016).

Regardless of the detrimental impacts of heavy metals on microbial metabolism, microbes have evolveda number of mechanisms in order to evade the toxic effects of the metals and rather use them for their (microbes) respiration (Nies, 1999; Nies, 2003). Tolerance mechanisms to heavy metals may evolve in bacteria via accessory genes and become part of the genetic repertoire of the microbe (Gómez-Sanz *et al.*, 2013). Such mechanisms may either be intrinsic and/or genetic adaptation mechanism (Wuertz and Mergeay, 1997). Known heavy metal tolerance mechanisms include; intra- and extra-cellular complex formation/ sequestration/ biosorption (Harrison *et al.*,

2007; Ikhimiukor and Adelowo, 2018), use of active efflux pumps (Nies and Silver, 1995),enzymatic detoxification, metal exclusion by use of cell permeability barrier and decrease in the susceptibilities of heavy metal ions onthe target cell organelles and further conversion of the metal ions to a favourable and a lesser toxic ionic compound(Nies, 1999; Seiler and Berendonk, 2012). One or a combination of these processes enables microbes to be more tolerant and carry out their activities or functions in environments contaminated with heavy metals.

In contrast to antibiotic resistant bacteria, heavy metal tolerant bacteria poses no direct threat to human health but can rather be employed in restoration of contaminated environments (Máthé *et al.*, 2012; Atieno *et al.*, 2013; Ikhimiukor and Adelowo, 2018). However, they can become a risk when mechanisms conferring resistance to heavy metals concomitantly co-selectsfor antibiotic resistance in the same bacterium thus having the ability to constitute a possible hazard to public healthiness (Martins *et al.*, 2014; Chen *et al.*, 2015b).

2.9 Co-selection of antibiotic resistance driven by heavy metals

Concerns about the potentials of heavy metals to indirectly select for antibiotic resistance in bacterial communities began a long time ago (Koditschek and Guyre, 1974; Sütterlin *et al.*, 2018). As a result of the strong correlations that occurs between microbial tolerance to heavy metals and antibiotic resistance, their inter-relationship has thus developed to be a critical area of study over the years (De Rore *et al.*,1994; El-Sayed, 2016). The co-contamination of these two antimicrobials have been reported to occur in diverse environments(Looft *et al*., 2012;Devarajan *et al*., 2015; Zhou *et al*., 2016). Their presence in diverse environments have been increasingly linked to the upsurge in the occurrence and proliferation of antibiotic resistant microorganism (Zhou *et al*., 2016; Poole, 2017). In line with this, the study ofPeltier *et al.* (2010) examined the propensity of Zn to enhance antibiotic resistance in microorganism present in activated sludge bioreactors, the results showed that the exposure to zinc and oxytetracycline augmented the occurrence of antibiotic resistance in the microbial community. Similarly, Ni and Cd were reported to raise the frequency of bacterial antibiotic resistance in freshwater microbial community impacted by antibiotics and metal contaminants (Stepanauskas *et al.*, 2006). Several other reports of the linkage between resistance to metals and antibiotic is summarized in Table 2.1.

It has also been proposed that the observed increase in microbial resistance to antibiotics in metals and antibiotics co-contaminated environments may be a result of the metals acting as an enrichment and growth enhancer to the indigenous microbial community already bearing ARGsor that the co-contamination of both antimicrobials may induce resistance in bacteria previously sensitive to antibiotics (Chen *et al.*, 2015b). In contrast to the persistent and bio-accumulating nature of heavy metals in the environment, antibiotics are "pseudo-persistent", such that their persistence in the environment is primarily dependent on their continuous introduction owing to their short half-lives (Hernando *et al.*, 2006; Stepanauskas *et al.*, 2006). Since metals are non-biodegradable, their persistence in contaminated environments even at relatively low concentrations have been described to be capable of inducing bacterial antibiotic resistance (Chen *et al.*, 2015b). This scenario creates much more appreciation of the roles of heavy metals in contaminated environments in driving the selection of antibiotic resistance. This also suggests that antibiotic resistance induced by metal selection pressure might therefore be very common among various microbial species in ecosystems contaminated with heavy metals (Chen *et al.*, 2015b). This development is particularly worrisome when ARGs are then transmitted to bacteria strains of clinical importance via mobile genetic elements(Gómez-Sanz *et al*., 2013; Flach *et al*., 2015;Fang *et al*., 2016;Domínguez *et al*., 2019).

Owing to latest improvements in bacteria genomics and the availability of complete sequences of bacterial and plasmids genomes, the investigation of co-localization and comprehensive profiling of the types and subtypes of metals and ARGs occurring together on same MGEs and those occurring on the chromosomes of diverse bacteria species is possible (Pal *et al*., 2015; Fang *et al*., 2016). Plasmid and metagenomic DNA sequencing experiments reveals that metals commonly found in the environment such as Cu, Ag, Co, Ni, As, Sb, Cd, Fe, Zn, Hg play important roles as theyhave the potentials of fostering the co-selection of bacteria strains to become resistant to a number of antibiotic classessuch as sulfonamides, beta-lactams, amphenicols, tetracyclinesand aminoglycosides (Pal *et al.*, 2015). Furthermore,Poole (2017) reported the enrichment of antibiotic resistance resulting from genetic linkage between resistance determinants of Cu and Zn metals and those of antibioticsoccurring on either the chromosomes or plasmid of microbes isolated from several reported metal contaminated environments (Table 2.2). The transfer of metals and ARGs co-localized

on mobile genetic elements to other bacterial species via any of the bacterial horizontal gene transfer methods would greatly support the development and propagation of resistance to both antimicrobials by bacteria species in the natural ecosystem.

Furthermore, there have been postulations concerning the importance of acquired resistance (via mutations and resistance genes) and the probable fitness cost which could be conferred on the selecting bacterial strain and bacterial community(Hall and Corno, 2014; Holmes *et al*., 2016). The implication of this is that, co-localization of resistance determinants to metals and antibiotics on same MGEs would meanthat a selection pressure for retaining one will consequently support the spreading and relativeabundance of the other(Ghosh *et al*., 2000; Li*et al.*, 2017). Findings have however suggested that a costless resistance can be preserved in a bacterial population in the presence of very little concentrations of either one of the selecting toxicant(Allen *et al*., 2010; Andersson and Hughes, 2010; Murray *et al*., 2019).

Therefore, heavy metals in metal contaminated environments may provide sufficient selection pressure to maintain a cost-free resistance in the bacterial communities. This has the potentials to promote the prolonged existence of ARGs in the natural ecosystem and work against their removal from the microbial community (Pal *et al.*, 2015), thereby further elevating the significance and complexity of the ecological roles of under-appreciated metal contaminated environments. It is therefore safe to hypothesize that heavy metal contaminated environments could serve as potentially important hotspots or pools for the evolution and proliferation of bacteria species resistant to heavy metal and antibiotics and their associated resistance genes. In this regard E-waste dumpsites may therefore represent an under-appreciated metal contaminated environment which deserve further considerations as a likely hotspot or pool of antibiotic resistance determinants.

Table 2.1. Heavy metals co-selection of antibiotic resistance and associated antibiotic resistance genes (adapted from Gorovtsov *et al.*, 2018)

Metal	Antibiotics	Antibiotic resistance genes	Reference
Nickel	β-lactams, aminoglycosides, tetracycline, vancomycin	β-lactams, aminoglycosides, tetracycline, vancomycin resistance genes, not specified	Hu *et al.*, 2017
Cadmium	Tetracycline, sulfadiazine, roxithromycin	*tetM, tetX, sul1, sul2*	Ye *et al.*, 2016
Copper, Zinc	Aminoglycoside, tetracycline, polypeptides, chloramphenicol, sulfonamides	*strB, strA, sulI, tetA, cmxA*	Zhou *et al.*, 2016
Zinc	Sulfamethazine	*sul1, sul2, dfrA7*	Duan *et al.*, 2018
Copper, Zinc	Tetracyclines sulfonamides	*tetA, tetG, tetW, sul1, sul2 and IntI1*	Lin *et al.*, 2016
Copper, Mercury,	Tetracyclines sulphonamides	*sul1, sul2, tetM, tetW, tetQ, tetO, tetT, tetB/P*	Zhou *et al.*, 2017
Arsenic, Cobalt, Copper, Mercury, Manganese,Nickel, Lead, Selenium, Uranium, Vanadium, Zinc	β-lactams, tetracyclines, sulfonamides	bla_{TEM}, bla_{CTX}, bla_{SHV}, bla_{OXA}, *sul2, sul3, tetM, tetW, tetB, tetC, tetD, tetA, tetE, tetG, tetK, tetL, tetM, tetO, tetS, tetA(P), tetX, tetQ*	Knapp *et al.*, 2017
Mercury, Nickel, Zinc	Amoxicillin, ampicillin, vancomycine, tetracycline	ND	Sinegani and Younessi, 2017

Table 2.2. Genetic Linkage of Metal and Antibiotic Resistance Genes in Bacteria from human and animal sources (adapted from Poole, 2017).

Metal	Metal genes	Antibiotic genes	Location	Organism	Reference
Copper	*mco, copA*	*ermT, tetL, dfrK, ermC*	Plasmid, evidence of co-transfer	MRSA (livestock, human)	Gómez-Sanz *et al.* (2013)
Copper	*pcoA–D, silABC*	*bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *bla*$_{OXA-1}$, *aac(60)-Ib-cr, aadA2, tetA, dhfrXII, sul1, mphR-mxr-mphA, qacED1*	Plasmid	*Escherichia coli, Klebsiella pneumoniae* (human	Sandegren *et al.* (2012)
Copper	*copA, mco*	*aadD, ermB, dfrK, tetL, apmA*	Plasmid	MRSA (livestock)	Feßler *et al.* (2017)
Copper	*pcoA–E*	*aac(60)-Ib-cr, oqxAB, rmtB*	Plasmid, evidence of co-transfer	*E. coli* (livestock)	Fang *et al.* (2016)
Copper	*pcoA–D, silA–E*	*bla*$_{TEM-1}$, *strAB, sul2, tetB*	Chromosome	*Salmonella typhimurium* (human)	Mourão *et al.* (2015)
Copper	*pcoE, pcoS*	*bla*$_{TEM-1}$, *blaSHV-1, aac (60)-Ib, strAB, aac3, dfrA19, sul1, qacED1, catA2, ereA, arr2*	Plasmid	*K. pneumoniae* (human)	Zhai *et al.* (2016)
Copper	*Sil*	*bla*$_{OXA-1}$, *blaSFO-1, aadA, aacA4, armA, tetA, mrx-mphA, msrE-mphE, sul1, qacED1, catB4*	Plasmid	*Leclercia adecarboxylata*	Sun *et al.* (2016)
Copper	*cusS, pcoE*	*bla*$_{TEM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *dhfrA14, strAB, aac(60)-Ib, aadA1, sul2, catA1, , catB3*	Plasmid	*S. typhimurium*	Kariuki *et al.* (2015)
Copper	*silABC, pcoS, pcoE*	*bla*$_{ACC-1}$, *bla*$_{VIM-1}$, *aacA4, aadA1, strAB, qacED1, sul1*	Plasmid	*E. coli*, *S. typhimurium* (livestock)	Falgenhauer *et al.* (2017)
Copper	*copB*	*tetA, tetW*	Plasmid, evidence of co-transfer	*Bacillus megaterium* (aquatic)	Xu *et al.*(2017)
Copper	*copA, copB*	*tetA*	Plasmid, evidence of co-transfer	*Pseudomonas aeruginosa* (aquatic)	Martins *et al.* (2014)
Zinc	*czrC*	*mecA*	Chromosome	MRSA	Cavaco *et al.* (2010)
Zinc	*Cad*	*mphC, msrA, aph(30)-IIa*	Plasmid	Not specified	Pal *et al.* (2015)
Zinc	*czcD*	*qnrA, qnrB*	Plasmid, evidence of	*Shewanella oneidensis*	Xu *et al.* (2017)

	co-transfer	(aquatic)

CHAPTER THREE

METHODOLOGY

3.1 Study areas

The city of Lagos and Ibadan are situated in the south-western part of Nigeria (Fig 3.1). Ibadan is the capital of Oyo State and is estimated to have over 3.5 million inhabitants. It is the largest city in West Africa, covering an area of approximately 35,743 km^2 and lies between latitude 4° N and 14° N and longitude 3 and 15° E (Odunaike *et al.*, 2008a; World Population Review, 2019a). Ibadan has been reported to have several informal E-waste recycling dumpsites (Adesokan *et al.*, 2016; Ohajinwa *et al.*, 2016). In contrast, Lagos State is projected to have over 21 million inhabitants, making it the most populated city in Sub-Saharan Africa (SSA). It has a land mass of 3577km^2, and lies between longitude 2° 42'E and 3° 42'E and latitude 6° 22' N and 6° 52'N (Odunaike *et al.*, 2008b; World Population Review, 2019b). Lagos is the location of Nigeria's major ports and is reported to receive an estimated 500,000 tonnes of used electronic equipment annually. Similarly, a number of the biggest electronic markets in West Africa are located in this city (Basel Action Network 2011; Ogungbuyi *et al.*, 2012).

3.2 Sampling areas

Due to the culture of poor waste segregation in Nigeria, most dumpsites containing E-waste also contain other forms of municipal waste co-disposed in the same dumpsite. However, for the purpose of this study, dumpsites that were solely observed (visually) to contain electronic waste were chosen. E-waste dumpsites in Lagos (located in Alaba International Market, Aswani Market and Ikeja Computer Village) and Ibadan (located in Ogunpa and Arulogun) were selected for the study. Electronic wastes found in these areas were typically discarded in heaps on the selected dumpsites (Figure 3.2). Underground and surface water sources were found in close proximity to these dumpsites. A description of each of the sampling site is given below;

Fig. 3.1. Map of Nigeria showing Lagos and Oyo States (Ibadan).

3.2.1 Alaba International market, Lagos (Longitude 6.4617260, Latitude 3.1915253)

Commonly referred to as the hub of electronics market in Nigeria and West Africa, the market is located at the Ojo area of Lagos State. The Alaba International Market has more than 5000 shops excluding attachments and sub-shops with more than two million business transactions daily (Awoniyi, 2016). Within this market there are large electronic waste dumpsites consisting of several heaps of discarded/end of life electronics (Plate 3.1). Virtually all manner of electronics parts can be found in this dumpsite, including computers, television sets, fans, refrigerators, washing machines and so on. Dismantling and open burning of electrical components is a very common occurrence in the dumpsites. Public water sources including two hand-dug wells (both about 100meters away from the E-waste dumpsite) and a river receiving effluent from the dumpsite (about 50 meters away from the dumpsite) were observed.

3.2.2 Aswani Market (Longitude 6.5397082, Latitude 3.3321592)

The market is situated at Isolo, along Oshodi-Apapa Expressway, Lagos. Several pockets of electronic waste dumps exists within the vicinity of the market. In one of the dumpsites, electronic parts that were seen accumulated in heaps includes microwave ovens, television sets, washing machines, electric fans, printers, air conditioners and so on (Plate 3.1). Dismantling and open burning are common activities done in this dumpsite. A borehole is found approximately 30 meters from the dumpsite.

3.2.3 Ikeja Computer Village, Lagos (Longitude: 6.5936469, Latitude:3.3424968)

This computer market is located in Ikeja, Lagos and it has been described as the largest computer product and other associated goods market in the Sub-Saharan Africa. The market boasts of having over 3,000 shops located therein (Lawal, 2019. Electronic wastes generated from the computer village are accumulated in dumps (Longitude: 6.5936469*,* Latitude:3.3424968*)* and typically comprise of computers, phones, keyboards, printers and photocopiers. A borehole is found about 50 meters from the dumpsite.

Plate 3.1. A cross-section of E-waste dumpsites from sampling sites. Plates A and B are E-waste dumpsites at the Alaba International Market. Plate C is from the Aswani Market. Plate D is E-waste dumpsite at the Ikeja computer village

3.2.4 Ogunpa, Ibadan (Longitude 7.3838136, Latitude 3.8844049)

This area receives and accumulates electronic scraps obtained across Ibadan town. A major river, "Ogunpa river" is located downstream of the E-waste dumpsite. Electronics scraps found accumulated therein consists majorly of television sets, printers, radio sets and computer parts.

3.2.5 Arulogun, Ibadan (Longitude 7.4736458, Latitude 3.9219324)

This area consists majorly of refrigerator repair shops. Damaged refrigerators are discarded and accumulated outside the shops. Two hand-dug wells are located close to the refrigerator dumpsite.

3.3 Sampling and sample collection

Sampling was carried out three times during the rainy season and dry season (Table 3.1). Sampling in the rainy season were done between December 2016 to February 2017, whereas sampling in the dry season was carried out in August 2017. Samples which include soil and water (surface water and ground water) were collected following methods described by the Industrial Waste Resource Guideline (IWRG) by the Environmental Protection Agency (EPA) (IWRG, 2009; EPA, 2014). The soil samples were derived from five (5) random positions in each of the sampling sites and pooled to form a composite sample. Sub-surface soil was obtained using a clean hand shovel to dig a depth of 10-15 cm into the ground, and the soils transferred into sterile sampling bags. Garden soil which served as the control soil sample were collected from farm lands at the International Institute for Tropical Agriculture (IITA) as described above. Water samples were collected into clean 1L capacity plastic containers. Water obtained from hand-dug wells were collected using "water drawing buckets" and poured into sample bottles, whereas water from boreholes were obtained by opening the taps of storage tanks into the sample bottles. Samples from surface waters (rivers) were collected below the surface, away from the river bank. On the field, the samples were preserved in ice packs before being refrigerated at 4°C in the laboratory until the onset of experimentation.

Table 3.1. Sampling locations, coordinates and dates of sampling

Location	Coordinates (Long., Lat.)	1st Sampling	2nd Sampling	3rd Sampling
Lagos		15 Dec,	1 Feb,	10 Aug,
Alaba International Market	(6.461, 3.191)	2016	2017	2017
Ikeja Computer Village	(6.593, 3.342)			
Aswani market	(6.539, 3.332)			
Ibadan		03 Jan,	05 Feb,	17 Aug,
Ogunpa market	(7.383, 3.884)	2017	2017	2017
Arulogun	(7.477, 3.923)			

3.4 Determination of physicochemical parameters of soil and water samples from E-waste dumpsites

Measurements of the various physicochemical parameters of the soil and water samples collected from the dumpsites in Lagos and Ibadan were determined using standard and classical titrimetric methods of United States Environmental Protection Agency (USEPA, 1996) and American Public Health Association (APHA) and American Water Works Association (AWWA) (APHA and AWWA, 2012). The procedures for the determination of each parameter are highlighted as follows:

3.4.1 Determination of pH

The pH meter (Jenway 3510) was standardized using standard buffer solutions of pH 4 and 7. The water samples were poured into a 200 ml beaker and pH measurement were taken by inserting the pH probe into the samples. The readings were taken and repeated three times and recorded accordingly. The soil samples were first separated in a 6 mm sieve, next 20 ml of distilled water was poured into the beaker containing the 20 g sieved soil. The contents was stirred using a sterile glass rod to obtain a slurry and allowed to stand for 1 hour. The pH readings were taken in triplicates and recorded accordingly.

3.4.2 Determination of electrical conductivity and total dissolved solids (TDS)

This was carried out using a Jenway 4510 conductivity/TDS meter. The metre was calibrated in a standard solution (1413µS). Preparation of the standard solution was carried out by introducing 0.746 g of dried Potassium Chloride (KCl) to 1L of double distilled water. Next, the cell of the conductivity/TDS meter were immersed into water samples in a 200 ml beaker and the results were recorded.

3.4.3 Determination of Soil Texture (Particle size)

This was carried out using the hydrometer method. Soil samples were sieved in a 2 mm mesh. Distilled water (10 ml) and calgon respectively were added to 50 g of sieved soil. Calgon was prepared by mixing 10g of sodium hexametaphosphate to 90 ml of distilled water. Next, the mixture of soil and calgon were placed on a shaker and allowed to rotate at 150rpm for 1 hour. The content was transferred into a 1500 ml measuring cylinder then topped up with distilled water to reach the 1300 ml mark. Next, 200 µl of iso-amyl alcohol was added. A stirrer was used to mix the content of

the measuring cylinder, a hydrometer was inserted 20 secs after mixing and the readings were recorded.

3.4.4 Determination of Total Nitrogen

This was carried out by the preparation of two reagents, N1 and N2. Reagent N1 was prepared by dissolving 34 g of sodium salicylate, 25 g of sodium citrate, 25 g of sodium tartate and 0.12 g sodium of nitroprusside in 1 Litre of double distilled water. Next, reagent N2 was prepared by dissolving 30 g of sodium hydroxide and 10ml sodium of hypochlorite into 1 Litre distilled water. A Stock solution containing 11.793 g of ammonium sulphate in 1 L distilled water was prepared. The acid digested samples (procedures given in section 3.5.1) were diluted 1:9 (v/v) with distilled water. 0.2 ml of the diluted samples were placed into clean test tubes where 5.0 ml of reagent N1 added and vortexed. Next, 5.0 ml of reagent N2 were also added to the mixture and vortexed. The solution was then allowed to stand for 2 hrs and absorbence at 650nm was measured. The nitrogen content in the samples was determined using the formula below;

$$\text{N\%} = \frac{(a-b) \times y \times 100}{1000 \times w \times al \times 1000}$$

where;

a = concentration of Nitrogen in solution,

b = concentration of Nitrogen in the blank,

y = total volume at the end of the experiment

w = weight of dried sample

al = aliquot of the solution taken

3.4.5 Determination of Total Phosphorus

Acid digested samples (50 ml) were added into a 200mlglass conical flask. Next, 1 ml of 11 N sulfuric acid (prepared by gently adding 310 ml of sulphuric acid into 600 ml distilled water, the solution was then made up to 1000 ml by adding distilled water) was introducedinto the digested sample solution and left to stay for 5 mins. Next, 0.4 grams of ammonium persulfate was added and mixed frequently with a sterile glass rod while boiling for about 30 to 40 mins or until a final volume of about 10 ml of solution is attained. The solution was then cooled and diluted to approximately 40 ml. Next, 2 ml of ascorbic acid solution (Prepared by dissolving 60 g of ascorbic acid in 1

litre of distilled water already containing 2 ml of acetone) was introduced to make-up the last ingredient to the solution. The solution was mixed and the absorbance was measured at 650 nm with a spectrophotometer and determine the phosphorus concentration was then determined from the standard curve.

3.5 Determination of heavy metal content of samples

Heavy metals analysis of the samples was conducted using Inductively Coupled Plasma Optical Electron Spectroscopy (ICP-OES) as described in the USEPA Method 6010b (USEPA, 1996). Heavy metals analysed include Lead (Pb), Copper (Cu), Zinc (Zn), Cadmium (Cd), Cobalt (Co), Selenium (Se), Nickel (Ni), Chromium (Cr), Aluminium (Al), Iron (Fe) and Manganese (Mn).

3.5.1 Acid digestion of soil samples

The samples were spread on clean polythene bags and allowed to air dry for 24 hrs. The air-dried samples were sieved using a sieve with pore size of 0.2 mm to obtain granular particles. The sieved fine particles were dried out overnight in an oven at 110°C. Acid digestion of samples was carried out by adding 10 ml of 1:1 HNO_3. The mixture was heated to 95^{o}C ± 5^{o}C and then refluxed for 10 to 15 mins without boiling. Next, 5 ml of concentrated nitric acid was introduced again and then the mixture refluxed for 30 mins. The mixture was cooled to about 40°C, then 2 mland 3 ml of of water and 30% H_2O_2respectively were added followed by stirring. Aliquots of H_2O_2(1 ml) was added with warminguntil effervescence was minimal. Next, 10 ml conc. HCl was introduced into the sample digestate and heated to 95^{o}C ± 5^{o}C for 15 mins and then allowed to cool. The digestate was then filtered by means of a Whatman No. 41 filter paper and collected in sterile 50 ml polypropylene centrifuge tubes.

3.5.2 Acid digestion of water samples

The water samples were first filtered by means of a 0.45 µm Whatman No. 41 filter paper and collected in a 500 ml Erlenmeyer flask. Immediately after filtration, 20 ml of the filtrate was acidified with 20 ml of HNO_3 to attain a pH <2. The filtrate is collected in 50 ml polypropylene centrifuge tubes.

3.5.3 Inductively coupled plasma optical emission spectroscopy (ICP-OES) Analysis

Upon ignition of the plasma, the instrument was allowed to thermally stabilize for 30 to 60 mins prior to calibration. After plasma stabilization, the instrument profile for the various heavy metals was verified and the spectrometer calibrated according to the manufacturer's recommended procedures. Calibration was done using a blank and the working calibration standards for the various heavy metals. Next, the initial calibration verification standard (ICV) were analysed to obtain a percent recovery within ±10%. Then the initial calibration blank (ICB) was analysed and concentration confirmed to be less than the reporting limit for each heavy metals to be analysed. Other standard solutions including the water and soil reporting limit standard, stock interference check standard (ICS), continuing calibration verification (CCV), and continuing calibration blank (CCB) standard were analyzed. This was followed by analysis of the blank, laboratory control standards and the digested samples. Further a CCV/CCB was run after every 10 samples.

The quantification of the metals in the sample was calculated thus;

$$concentration\ of\ metal\ in\ sample\ mg/kg = \frac{A \times V}{F \times W} \times DF$$

where:

A = mg/l (or µg/l) of metal in processed sample from read-out

F = concentration unit factor

V = Final volume of the processed sample

W = Weight in of sample (in grams)

DF = Dilution factor

3.6 Determination of total-culturable heterotrophic bacteria count (THBC)

This was determined using spread plate technique on Mueller Hinton Agar (MHA) (Oxoid Ltd.). The soil (1 g) and water (1 ml) samples respectively were used in a ten-fold serial dilution in normal saline (0.85% NaCl). Aliquots (100 µl) of serial dilutions (10^{-3}, 10^{-5} and 10^{-7}) were spread using a sterile glass spreader on MHA in Petri-dishes and allowed to incubate at 35°C for 24 hrs. Plating was done in triplicates. Bacterial colonies growing on the MHA containing petri-dishes were enumerated and totalled and further used to estimate the colony forming unit (CFU) as either CFU/g or CFU/ml for soil and water samples respectively.

$$CFU/ml\ (g)\ =\ \frac{mean\ of\ total\ bacteria\ count\ \times dilution\ factor}{volume\ plated}$$

3.7 Determination total-culturable background metal tolerant bacteria count

This was carried out as reported by Oriomah *et al.* (2015). MHA was supplemented with 50 µg/ ml of the Copper (Cu), Zinc (Zn) and Lead (Pb) used as $CuSO_4$, $ZnSO_4$ and $Pb(CH_3COO)_2$ respectively. Stock solutions of the metal was prepared using the method of Narasimhulu*et al.* (2010). Both metals and the culture media were sterilized separately in an autoclave for 15 mins at 121°C and a pressure of 1.05g/cm^2. The metals solution were then poured into molten agar (45°C) containing magnetic beads. The mixture was placed on a magnetic stirrer and stirred before pouring into sterile Petri-dishes.Aliquots (100 µl) of the serially diluted samples (10^{-3}, 10^{-5} and 10^{-7}) were spread on the metal supplemented MHA plates. The metal supplemented agar plates were then incubated at 35°C for 24 hrs. After incubation, the colonies were counted and the counts were expressed as percentage of total count on plates without metals (THBC).

3.8 Isolation of bacteria from metal supplemented MHA

Morphologically distinct colonies from the previous experiment (section 3.7) were picked and sub-cultured onto fresh heavy metal supplemented MHA plates by repeated streaking on overnight prepared MHA preparedin an attempt to obtain uncontaminated cultures. All plating experiments were carried out in triplicates. The pure cultures of the bacteria strains were keptat 4°C on slants containing Nutrient agar(NA) and also on glycerol broth (containing Nutrient broth and 15% glycerol) at -20°C for further studies.

3.9 Isolation of Enterobacteriaceae on eoisin-methylene blue (EMB) agar

Aliquots (100 µl) of serially diluted samples (10^{-3}, 10^{-1}) were spread on overnight prepared EMB agar. Next, the EMB plates containing the spread diluents were placed in an incubator at 35°C for 24 hrs. Randomly selected colonies, including suspected *E. coli* appearing as green metallic sheen were purified on fresh EMB agar plates. All plating experimentations were carried-out in replicates, either duplicates or triplicates. The pure cultures of purified strains were storedat 4°C on NA slants and also in glycerol broth at - 20°C.

3.10 Determination of heavy metals minimum inhibitory concentrations of the isolated bacteria

The bacteria isolates were screened on MHA medium supplemented with heavy metals (Cu, Zn and Pb) to determine their minimum inhibitory concentration (MICs). This was done in accordance to methods describedearlier(Narasimhulu *et al.*, 2010), with minor changes. Overnight cultures (18-24 hrs) of the bacterial isolates were spot-inoculated on the metal supplemented media and allowed to incubate at 35°C. The isolates were exposed to incremental concentrations of the heavy metals in the media (50 - 1000µg/ml). The inoculated heavy metal supplemented plates were inspected daily for bacteria growth until 72 hrs. The presence or lack of bacterial growth on the plates were recorded. The metal concentration at which bacteria failed to show any observable growth on the medium was taken as the MIC(Singh *et al.*, 2014).

3.11 Antibiotic susceptibility testing

The susceptibilities of the metal tolerant bacteria isolates to several clinically relevant antibiotics were determined using the agar disc diffusion technique described by the Clinical and Laboratory Standards Institute (CLSI, 2017).Choice of antibiotics used was based on the CLSI recommended antimicrobial agent for determination of susceptibilities for Enterobacteriaceae strains. Antibiotics used for the assay included the following; Aminoglycosides (gentamicin 10 µg, kanamycin 30 µg), Beta-lactams [third generation cephalosporins (cefpodoxime 30 µg, ceftazidime 30 µg) and carbapenems (ertapenem 10 µg, imipenem 10 µg and meropenem 10 µg)], Fluoroquinolones (ciprofloxacin 5 µg), Foliate inhibitors (sulphamethoxazole/trimethoprim 23.75/1.25 µg), Phenicols (florfenicol 30 µg) and Tetracyclines (tetracycline 30 µg) (Oxoid Ltd.).

3.11.1 Preparation of 0.5 McFarland Standard

A 0.5 Mcfarland solution represents roughly 1.5x 10^8 bacterial cells per ml. This solution was prepared following procedure described by Chapin and Lauderdale (2003). A 0.5 ml of 1.175% $BaCl_2$. $2H_2O$ (1.175g of $BaCl_2.2H_2O$ + 100 ml of deionized water) was introduced dropwise into 85 ml of 1% H_2SO_4 in a 100 ml Erlenmeyer flask with constant swirling. The solution was filled-up to 100 ml by adding 1% H_2SO_4. A magnetic bead was placed in the solution and placed in the

magnetic stirrer for 5 mins. The solution was visibly examined to certify it free of visible clumps.

3.11.2 Susceptibility testing

Cultures of the isolates (18 – 24 hrs) were picked from NA plates using sterile swab sticks and inoculated into a 2 ml normal saline solution (0.85% NaCl) in test tubes. The bacterial suspension were mixed well and diluted (where necessary) with saline to conform to 0.5 McFarland turbidity(Chapin and Lauderdale, 2003). With the use of the sterile swab sticks, bacterial suspensions were spread evenly on MHA plates in duplicates. Sterile forceps were used to put the antibiotic-impregnated discs on the already bacterial inoculated MHA plates. The inoculated MHA plates containing antibiotic discs were then incubated at 35^{o}C for 24 hrs. Clear zones denoting the inhibition of bacterial growth (if any) surrounding each antibiotic disc were measured using a metre rule. Interpretation of the measured zones of inhibition to represent either susceptible, intermediate or resistant were ascertained by using the CLSI zone diameter interpretation standards for Enterobacteriaceae(CLSI, 2017)

3.12 Extraction of bacterial genomic DNA

Bacterial genomic DNA extraction was carried out using the Zymo Quick-DNA™ Fungal/Bacterial Miniprep Kit. Firstly, the bacterial cells were sub-cultured on NA plates and incubated overnight at 35°C. Further, single colonies were picked from the overnight cultures and inoculated into 30 ml Nutrient broth and incubated overnight at 35°C. The overnight culture in Nutrient broth were centrifuged at 7,500 rpm for 10 mins to harvest the cells. The supernatant was then discarded 200 µl of isotonic buffer was used to re-suspend the cells and transferred into a ZR BashingBead™ Lysis Tube (0.1 mm and 0.5 mm). BashingBead™ Buffer (750 µl) was added to the tube. The tubes were fastened tightly in a Disruptor genie™ bead beater and run at the highest speed for ≥ 5 mins. The ZR BashingBead tubes were then placed in a microcentrifuge (Eppendorf^{TM} Bench Top Centrifuge) and spinned at 10,000 x g for 1 minute. Next, 400 µl of the supernatant was added into a Zymo-Spin™ III-F Filter in collection tubes and was centrifuged at 8,000 x g for 1 min. Genomic Lysis Buffer (1,200 µl) was introduced into the filtrate in the collection tubes. The mixture (800 µl) was thenplaced into a Zymo-Spin™ IICR Column inside a collection tube and the mixture was spin at 10,000 x g for 1 min in a microcentrifuge. The flow through was discarded out from

the collection tube and this step repeated byusing another 800 µl of the mixture. A DNA Pre-Wash Buffer (200 µl) was introducedinto the Zymo-Spin™ IICR Column in a new collection tube and spinned at 10,000 x g for 1 min in a microcentrifuge. The genomic DNA Wash Buffer (500 µl) was introduced into the Zymo-Spin™ IICR Column and spinned at 10,000 x g for 1 min. The Zymo-Spin™ IICR Column was then transferred to sterile 1.5 ml Eppendorf microcentrifuge tubes and a 100 µl of DNA Elution Buffer added directly to the column matrix. This was spinned at 10,000 x g for 30 secs to elute the DNA. The DNA was run on a Nanodrop spectrophotometer and an agarose gel electrophoresis to check quantity and quality as described below.

3.13 Gel Electrophoresis

Extracted DNA were examined using a 1% agarose Gel. One (1) gram of agarose powder (DNA Agar, SERVA) was weighed and poured in 50 ml of 1x TAE buffer (50X Tris-Acetate buffer, Rotiphorese®) (containing 20 ml of 50X TAE stock solution added to 980 ml distilled water) in autoclave bottles. The bottles containing the mixture were microwaved for 2-5 mins until the suspended agarose powder was fully dissolved. The solution was kept at room temperature and allowed to cool to about 50°C after which its content were poured carefully(avoiding the formation of bubbles) into a gel tray with the gel combs well placed in the tray. The gel in the gel tray was allowed to set for 15-20 mins until it had completely solidified. Once solid, the gel combswere carefully removed to avoid any damage to the gel. Next, the gel in the gel tray were transferred into a gel box containing 1X TAE buffer which covered the entire gel. Each of each of the DNA samples (2 µl) were mixed with Two microlitres of loading dye (6x MassRuler, Thermo Scientific, Schwerte, Germany). The mixture was carefully added into the wells and the voltage machine turned on and set to run at 100V for 1 hr. The gel was then carefully removed and placed inside an Ethidium bromide bath (500 ml of distilled water + 5 drops of ethidium bromide) in a fume hood. This was allowed to stain for 10 – 15 mins, after which the gel was rinsed in a distilled water bath and visualized using a GelDoc TM XR+ (BioRad Laboratories Inc., USA).

3.14 Determination of DNA quality and quantity by NanoSpectrophotometry

A Peqlab NanoDrop Spectrophotometer (ND-1000, Erlangen, Germany) connected to a computer was used to determine DNA quality and quantity based on the

manufacturer's guidelines. The Nanodrop pedestal were carefully cleaned with laboratory wipes moistened with distilled water. The Nanodrop computer application was started and DNA protocol selected to measure the nucleic acid concentration at 280 nm absorbance. Two microlitres (2 µl) of RNase-free water were loaded on the pedestals and run to calibrate the machine. The pedestal arm was lifted and cleaned with laboratory wipes. Aliquots (2 µl) of elution buffer (blank) that was used in eluting the DNA, was loaded on the Nanodrop pedestal and run to calibrate the machine. The pedestals were cleaned again with laboratory wipes, and 2 µl of each of the DNA samples were loaded and measured. The DNA quantitation in ng/ µl and a graph of the absorbance 260/280 were generated and readings were recorded.

3.15 Enterobacterial Repetitive Intergenic Consensus Polymerase Chain reaction (ERIC-PCR)

This method of bacterial fingerprinting distinguishes members of the Enterobacteriaceae using primers derived from ERIC sequences (Wilson and Sharp, 2006). The ERIC primer sequencesused in this study were obtained from the works ofVersalovic *et al.* (1991) (Table 3.2). All PCR reactions were carried under the PCR Workstation (PEQLab, Erlangen, Germany). PCR reactions comprised of 12.5 µl reaction volumes containing 6.25 µl Red*Taq* DNA Polymerase, 2X Master mix (VWR®, Dresden, Germany), 0.25 µl of 0.2 µM concentration of the respective ERIC primer (Microsynth, Balgach, Germany), 2 µl DNA template and 3.75 µl nuclease free water (Roth ®, Karlsruhe, Germany). The PCR cycle was as follow: initial denaturation at 95°C for 2 mins, followed by 30 cycles of denaturation at 95°C for 30 secs, annealing at 49°C for 1 minute, and extension at 72°C for 30 secs, with final extension at 72°C for 5 mins.

A 4 µl volume of the PCR products were mixed with 4 µlloading dye (6x MassRuler, Thermo Scientific, Schwerte, Germany) and carefully added into the wells of 1.5% agarose gels. Four microlires (4 µl) of a Quick Load - 100bp molecular ladder (New England Biolabs, Frankfurt, Germany) was added to the first wells of the agarose gels. The gels were run at 80V for 80 mins. Gels were stained in ethidium bromide for 10-15 mins and rinsed in clean water baths and then visualized using a GelDoc TM XR+ (BioRad Laboratories Inc., USA).Dendograms showing phylogenetic relationships between the Enterobacteriaceae strains based on ERIC sequence clusters were created using the GelClust software, method employed for clustering ERIC sequences was

theUnweighted Pair Group Method Arithmetic Mean (UPGMA) using the Dice similarity coefficient (Khakabimamaghani *et al.*, 2013).

3.16 Whole genome sequencing

Sequencing of the bacterial isolates' whole genomes was done in accordance to protocols earlier described by Adelowo *et al.* (2018a). Library preparation and sequencing of the strains was carried-out at the Institute for Biological Interfaces (IBG5), Karlshule Institute of Technology, Karlshule, Germany.

3.16.1 DNA extraction

Genomic DNA from the bacterial isolates were extracted following protocols of the Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany). The Bacterial cells were harvested from 18-24 hrs culture in Luria Bertani Broth by centrifuging at 7,500 rpm for 10 mins. The bacterial cell pellets were re-suspended in 180 µl of buffer ATL and 20 µl of proteinase K as introduced into the suspension. The mixture was vortexed and then incubated at 56°C for 3 hrs in a Thermomixer (Eppendorf R mixer, 2.0 ml block, Hamburg, Germany) to allow for the complete lysis of the bacterial cells. The mixture was vortexed for 15 secs and 200 µl of Buffer AL was added to the samples. Following incubation, the mixture was vortexed briefly and 200 µl ethanol (96–100%) was introduced and the vortexing repeated. The mixtures were then transferred by pipetting into a DNeasy Mini spin column already placed inside a 2ml collection tube and centrifuged at 8000 rpm for 1 min. The resulting flow-through was discarded and 500 µl Buffer AW1 was introducedinto the spin column followed by centrifugation for 1 min at 8000 rpm. The flow-throughs were again discarded and 500 µl Buffer AW2 was introducedinto the DNeasy spin column in new Collection tubes and centrifuged for 3 mins at 14,000 rpm (this step was necessary to dry the DNeasy membrane to avoid contaminants in the final product). The DNeasy Mini spin columns were then placed in steriles 1.5 ml Eppendorf tubes and 100 µl of elution Buffer AE were added directly onto the DNeasy membrane followed by incubation for 1 min at room temperature. The tubes were then centrifuged for 1 min at 8000 rpm to elute genomic DNA.

3.16.2 Determination of DNA quantity and quality

A Quibit fluorometer (Invitrogen, Schwerte, Germany) was used to measure DNA quantity (ng/ µl). DNA concentrations measured using a Qubit fluorometer (ThermoFisher®). The procedure used in measuring DNA concentration for WGS analysis is as follows;

Working solutions containing (1× n) µl Quant-iT Reagent and (199×n) µl Quant-iT Buffer were homogenized in 0.5 ml PCR tubes by vortexing for 3 secs, where n = number of samples + 2 (standard solutions). Further, two standard solutions were made by mixing190 µl of Working Solution and 10 µl of Standard #1 (0 ng/µl) and the second containing 190 µl of Working Solution + 10 µl of Standard #2 (10 ng/µl). Tubes containing standard solutions were vortexed and inserted into the Qubit fluorometer and used to calibrate the machine. After calibration, 1µl of DNA sample and 199µl of the Working Solution were loaded unto clean tubes, vortexed briefly and inserted into the Qubit fluorometer. The readings were recorded. A Peqlab NanoDrop Spectrophotometer (ND-1000, Erlangen, Germany) was used in measuring the absorbance at 260nm following the procedures earlier described (section 3.14).

3.16.3 DNA Library preparation

A Covaris S220 sonication device (Covaris Inc. Massachusetts, USA) was used to shear about 100 pg – 500ng of the purified genomic DNA of each isolate. Settings for sonication are as follows: 55 s, 175 W, 5% Duty factor, 200 cycles of burst, 55.5 µl input volume. Sequencing libraries were prepared using the NEBNext Ultra II FS DNA Library prep kit, containing sample purification beads (New England Biolabs, Frankfurt, Germany). The Ultra II FS enzyme and reaction buffer were thawed and vortexed to mix. Both solutions were placed on ice. Volumes of DNA (26 µl), Ultra II FS Enzyme (7 µl) and Ultra II FS Reaction Buffer (2 µl) were added to a 0.2 ml PCR tubes and vortexed briefly before spinning in a microcentrifuge. The samples were then transferred into a pre-heated Thermocycler with program; 5–30 mins at 37°C, 30 mins at 65°C and held at 4°C.

3.16.4 Adapter ligation and Library enrichment

To each of the FS reaction mix described above, the following components were added; 30 µl NEBNext Ultra II Ligation Master, 1 µl NEBNext Ligation Enhancer and 2.5 µl of NEBNext ® Multiplex Oligos for Illumina® (96 Unique Dual Index Primer Pairs) and adaptor ligation carried out following the instructions of the manufacturer.

The mixtures were pipetted up and down 10 times to homogenize and the tubes were carefully placed on a thermocycler and cycling conditions for amplification were as follows: 98°C for 30 secs, 5 cycles of 98°C for 10 secs, 65°C for 75 secs, 65°C for 5 mins. The library quality were assessed on a Bioanalyzer by running 1 µl of the libraries on a DNA High Sensitivity Chip.

3.16.5 Sequencing of reads

The libraries were sequenced on an Illumina® HiSeq machine by using paired-end approaches with 301 cycles per read. Raw sequences were subjected to adapter clipping and quality trimming using Trimmomatic (Bolger *et al.*, 2014), and processed reads were then assembles using SPAdes v3.13.0(Bankevich *et al.*, 2012). The quality of the assemblies and the taxonomic placements of the genomes were evaluated with CheckM v1.0.4 (Parks *et al.*, 2015). Further scaffolding of assemblies was carried out with the aid of the multi-draft based scaffolder (MeDuSa) (Bosi *et al.*, 2015). Annotations of assemblies were done using Prokaryotic Genome Analysis Pipeline (PGAP) from the National Centre for Biotechnology Information (NCBI) (Tatusova *et al.*, 2016).

3.17 Bioinformatics analysis of Whole Genome Sequence (WGS) assemblies

Bioinformatics analysis was carried-out using either FASTA, FASTQ or GENBank file format of bacterial whole genome assemblies. This was done using publicly available databases, pipelines and software as described below;

3.17.1 Determination of acquired antibiotic resistance genes

With the aid of the genome assemblies as a query file, ARGs present in the bacterial genomes were identified on the ResFinder 3.2 database (https://cge.cbs.dtu.dk/services/ResFinder/) (Zankari *et al.*, 2012) of the bacterial analysis pipeline of the Centre for Genomic Epidemiology (Thomsen *et al.*, 2016) and the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/) (Jia *et al.*, 2017).

3.17.2 Determination of heavy metals resistance genes

Experimentally confirmed metal resistance genes were downloaded from the Antibacterial Biocide and Metal Resistance Genes Database – BacMet (http://bacmet.biomedicine.gu.se/) (Pal *et al.*, 2014). The downloaded databases were used as a template to run a local BLAST (basic local alignment search tool) search

against the fasta genome assemblies (query sequences) using the BioEdit Sequence Alignment Editor version 7.2.5 (Hall, 1999). BLAST results showing percentage similarity of ≥ 85% were manually confirmed by examining the annotated sequences files from the GenBank.

3.17.3 Determination of plasmid replicon types

The *in silico* detection of plasmid replicon types was carried out using the PlasmidFinder 2.0 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) of the Centre for Genomic Epidemiology (Carattoli *et al.*, 2014). Fasta files of the assemblies were uploaded unto the database for curation and results returned in terms of percentage similarity to known plasmid replicon types.

3.17.4 Bacterial Multi-Locus Sequence Type (MLST) and calling of Single Nucleotide Polymorphisms (SNPs) in the bacterial genomes

The genome assembly files of the sequenced strains in FASTA format were uploaded to the MLST 2.0 database (https://cge.cbs.dtu.dk/services/MLST/) of the Centre for Genomic Epidemiology (Larsen *et al.*, 2012). The MLST configurations were determined by selecting the appropriate bacterial species that were being analysed. MLST of *E. coli* strains were confirmed using the Enterobase database (http://enterobase.warwick.ac.uk/) (Zhou *et al.*, 2019). Calling of SNPs was done using the CSIPhylogeny 1.4 database (https://cge.cbs.dtu.dk/services/CSIPhylogeny/) of the Centre for Genomic Epidemiology (Kaas *et al.*, 2014). Phylogeny was determined and the tree rooted using NCBI GenBank reference sequences including *Escherichia coli* K-12 MG1655 (NC_000913.3), *Enterobacter cloacae* subsp. cloacae ATCC 13047 (CP001918.1) and *Citrobacter freundii* CFNIH1 (CP007557.1).

3.18 Quantitative determination of antibiotic resistance genes and mobile genetic element (MGE)in the metagenomic DNA samples from sampling sites.

This was carried out according to methods earlier described by Adelowo *et al.* (2018b). Determination of the copy numbers of selected ARGs and MGE in the metagenomic DNA fraction of samples from E-waste dumpsites by qPCR is described below.

3.18.1 Metagenomic DNA extraction

Metagenomic DNA of the soil and water samples obtained from the E-waste dumpsites were extracted with the aid of the Qiagen DNeasy PowerSoil Kit and Qiagen DNeasy PowerWater Kit (Hilden, Germany) respectively, following the manufacturers' instructions. A brief description of the extraction protocols is given below;

3.18.1.1 Metagenomic DNA extraction from soil samples

Soil samples (0.5 g) were introduced into the PowerBead tubes and gently vortexed. Pre-warmed (60°C) Solution C1 (60 µl, contains SDS and several cell disruptive materials necessary for the total lysis of the bacterial cells) were introduced to the tube containing samples and vortexed briefly. PowerBead tubes were fastened tightly in a horizontal position in a Vortex Adapter (FastPrep-24™ MP Biomedicals™ Eschwege, Germany)and vortexed at the highest speed for 10 mins for thorough mixing and lysis of the bacterial cells. The tubes were placed in a microcentrifuge and spinned at 10,000 x g for 30 secs.After centrifugation, the tubes were transferred into fresh 2 ml collection tubes. Solution (250 µl) C2 (optimized for the removal of inhibitors for the mixture contains reagent that aid in the precipitation of non-DNA organic and other inorganic materials such as humic materials, cell debris and proteinaceoussubstances) was introduced and brief vortexing for 5 secs. The tubes were centrifuged at 10,000 x g for 1 min and then about 600 µl of the supernatant was transferred to fresh2 ml collection tubes. Next, 200 µl of Solution C3 (a second reagent optimized for removal of inhibitors from the mixture) was added, vortexed briefly and centrifuged at 10,000 x g for 1 minute. Taking care to avoid the pellets, about 750 µl of supernatants were transferred to fresh 2ml collection tubes and 1200 µl Solution C4 (a solution with high conc. of saltswhich allows DNA to bind efficiently with the silica membrane) was introduced to the supernatants and briefly vortexed for 5 secs. About 675 µl of the supernatants were placed onto MB spin columns (containing silica membrane) and centrifugation was done at 10,000 x g for 1 min and the resulting flow-through discarded. Aliquot (500 µl) of Solution C5 (a DNA wash solution with ethanol as its primary ingredient which function to further purify the DNA that is already binded to the silca membrane filter) was added to the spin columns and centrifugation carried out for 30 secs at 10,000 x g. The resulting flow throughs were put away and spin columns centrifuged for 1 min at 10,000 x g. The spin columns were placed carefully in clean 2 ml Eppendorf tubes and 50 µl of Solution C6 (sterile DNA elution buffer) was introduced to the centre of the silica membrane filter. The tubes were centrifuged for

30 secs at 10,000 x g. The spin columns were put-away, aliquots of the extracted DNA were made in separate microcentrifuge tubes and put in storage at –80°C. A Peqlab NanoDrop Spectrophotometer (ND-1000, Erlangen, Germany) was used to determine the quality and quantity of the metagenomic DNA.

3.18.1.2 Metagenomic DNA extraction from water samples

The water samples (100 ml) were filtered using membrane filters (pore size 0.22µM) fixed in a filter funnel securely attached to a vacuum pump. Sterile forceps were used to pick up the filter membranes at opposite edges into a cylinder with the top side facing inward. The filter papers were placed in a 5 ml PowerWater DNA Bead Tube. Aliquot (1 ml) of pre-warmed (55°C) Solution PW1 (an inhibitor removal and lysing reagent) was introduced into the tubes. The tubes were fastened tightly to a Vortex Adapter (FastPrep-24^{TM} MP Biomedicals^{TM} Eschwege, Germany) and vortexed at maximum speed for 5 mins before being centrifuged at 4000 x g for 1 min. The resulting supernatant were transferred to fresh 2 ml collection tube and centrifuged at 13,000 x *g* for 1 min. While avoiding pellets, the supernatants were transferred to fresh 2 ml collection tubes and 200 µl of Solution IRS (another inhibitor removal reagent which functions to remove organic and inorganic contaminants) was added and briefly vortexed. Again, the tubes were spinned in a microcentrifuge at 13,000 x *g* for 1 min and the resulting supernatants were pipetted into fresh clean 2 ml collection tubes. Next, 650 µl of Solution PW3 (a high-concentration salt solution which to mediates the binding of DNA to the silica membrane) was added and vortexed briefly. Then, 650 µl of the supernatants were transferredunto the spin columns and centrifugation done at 13,000 x *g* for 1 min and the resulting flow-throughs discarded. This step was repeated until all the supernatants had been processed. The MB spin columns were placed in fresh 2 ml collection tubes and 650 µl of Solution PW4 (a DNA wash solution with alcohol as its primary ingredient) was pipetted into the columns. Next, the contents were centrifuge at 13,000 x g for 1 min and the resulting flow-throughs were put away. Ethanol (650 µl, to remove PW4 solution) was added and centrifuge at 13,000 x *g* for 1 min. The resulting flow-throughs were put away and the spin column were spinned again at 13,000 x *g* for 2 mins. The spin columns were placed into a fresh 2 ml Eppendorf tubes and 100 µl of Solution EB (sterile elution buffer) was introduced to the middle of the white filter membrane in the column and again spinned at 13,000 x *g*

for 1 min. The MB spin columns were discarded, aliquots of DNA were made in separate Eppendorf tubes and put in storage at –80°C. A Peqlab NanoDrop Spectrophotometer (ND-1000, Erlangen, Germany) was used to determine the quality and quantity of the freshly extracted metagenomic DNA.

3.18.2 Purification of antibiotic resistance genes, *intI1* and *uidA* standards for qPCR

Genes coding for 16S rRNA, *sul1*, *sul2*, *tetA*, *dfrA1*, $bla_{\text{CTX-M-1}}$, *intI1* and *uidA* were amplified from strains EC1, EC2, EC12, EC25, EC47, EC68, EC75 and EC83 respectively by using conventional PCR oligonucleotide primers (The source of each primer pair are presented in Table 3.2). The PCR reaction mix comprised of a 100 µl reaction volume in 0.2 ml PCR tubes (Labsolut, Th.Geyer®, Hamburg, Germany) containing 50µl Red*Taq* DNA Polymerase 2X Master mix (VWR®, Dresden, Germany), 2µl of 0.2µM concentration of each primers (Microsynth, Balgach, Germany), 16 µlof the DNA template and 30 µl of nuclease free water. The PCR cycling condition were as follow: initial denaturation at 95°C for 2 mins, followed by 35 cycles of denaturation at 95°C for 30 secs and extension at 72°C for 30 secs, with final extension at 72°C for 5 mins. Annealing temperatures and time for each primer is given in Table 3.2. A 4 µl volume of the PCR amplicons or products were mixed with 4 µlloading dye (6x MassRuler by Thermo Scientific, Schwerte, Germany) and added to the wells of the 1.5% agarose gel. Molecular ladder (4 µl,Quick Load - 100bp ladder by New England Biolabs, Frankfurt, Germany) was added to the first and last wells of the agarose gels. The gels were run at 100V for 60 mins. Next, the gels were placed in an ethidium bromide bath and allowed to stain for 10-15 minsbefore being washed in clean water baths and visualized using a GelDoc TM XR+ (BioRad Laboratories Inc., USA).

The remainder of the PCR amplicons were purified using the Nucleospin ® Gel and PCR Clean-up kit (Machery-Nagel, Düren, Germany)in accordance to the manufacturer's instructions; briefly 96 µl of the PCR amplicons were mixed with 192 µl of Buffer NTI and the samples pippetted into NucleoSpin® Gel and PCR Clean-up columns in a clean 2 ml collection tube and then centrifugation was done for 30 secs at 11,000 x g. The resulting flow-through were put away and 700 µl of Buffer NT3 was pipetted into the NucleoSpin® Gel and PCR Clean-up columns followed by centrifugation for 30 s at 11,000 x g. The resulting flow-throughs were discarded and

the silica membranes dried by centrifugation at 11,000 x g for 1 min. The NucleoSpin® Gel and PCR Clean-up columns were placed in clean 1.5 ml Eppendorf tubes, and the DNA eluted with 15 µl of elution buffer. The tubes were then allowed to incubate for 1 min at ambient temperature and then centrifuged again for 1 min at 11,000 x g. A NanoDrop Spectrophotometer (Peqlab ND-1000) was used in measuring the quantity and quality of the purified PCR product.

3.18.3 qPCR quantification of *E. coli* (*uidA*), antibiotic resistance genes and *intI1* in soil and water samples

The samples were prepared in a Holten LaminAir – HV Mini Class 100 Cabinet (Thermo Fisher Scientific, Schwerte, Germany). All the reagents, template DNA and standards were placed on ice during the process of sample preparation. For each of the samples, the template DNA were diluted tenfoldin an attempt to make compensations for the probable effects of PCR inhibitors that may be present in the samples.

Two real time qPCR protocols involving SYBR green-based detection (for *sul1*, *sul2*, *tetA*, *dfrA1tetA* and *uidA*) and TaqMan-based detection (for $bla_{\text{CTX-M-1}}$) were carried out. Amplification using the SYBR green based real-time qPCR reaction consisted of a 12.5 µl reaction volume which contained 6.25 µl SYBR green (KAPA SYBR FAST qPCR Master Mix 2X), 2 µl template DNA. Optimized primer concentrations for SYBR green based real time qPCR were 200nM for 16S rRNA, *sul2* and *uidA*, 100nM for *sul1*, *dfrA1* and *tetA* and 400nM for *IntI1* (The sources of each primer pair used in this study are presented in Table 3.2). The qPCR cycling conditions were 95˚C for 2 mins, then 40 cycles of 95˚C for 20 secs, 20 secs at the corresponding melting temperatures and 72˚C for 20 secs.

Amplification of $bla_{\text{CTX-M-1}}$ was carried out following protocols described by Colomer-Lluch *et al.* (2011), involving a real-time reaction mixture consisting of 6.25 µl TaqMan® Environmental Master Mix 2.0 (Applied Biosystems®, Darmstadt Germany), 4.63 µl nuclease free water (Roth ®, Karlsruhe, Germany), 2 µl of template DNA, 0.125 µl of CTX-M probe and 0.25 µl of 200nM concentration of each primers (Table 3.2). The qPCRcycling conditions were 50˚ for 2 mins (1 cycle), 95˚C for 15 mins (1 cycle), 94˚C for 15 secs and 60˚C for 1 min (45 cycles).

The reaction mixtures were carefully pipetted into 96 well PCR plates (Biozym Scientific GmbH) in triplicates. The plates were sealed and spinned on a PCR plate Spinner (VWR ®, Dresden, Germany) for 30 secs and qPCR was run on a StepOne

Plus Cycler (Applied Biosystems, Darmstadt, Germany). The number of DNA copies in the purified genes (standards) were determined using the formular;

$$CN = (c \times 6.022 \times 10^{23}) / 660 \times N$$

where;

c = measured DNA concentration (μg/ μl) and

6.022×10^{23} = Avogadro's constant

660 = average mass of 1 bp of dsDNA

N = fragment length of DNA template

3.19 Statistical analysis

All data obtained in this study were recorded into Microsoft Office Excel spread sheets and analyzed with the Statistical Package for Social Sciences (SPSS) v25.0. The mean of the measured concentration of metals and other physicochemical parameters were compared using analysis of variance (ANOVA) at α = 0.05% significance level. The relationships between heavy metal concentrations and antibiotic resistances genes in samples from E-waste dumpsites were studied by using bivariate (Linear regression) and multivariate (Principal component analysis) with the Pearsons correlation coefficient (r) on R statistical software version 3.5.3 (RStudio Inc.). Observed positive correlation coefficients were grouped as either very weak (0.00–0.19), weak (0.20–0.39), moderate (0.4–0.59), strong (0.60–0.79) or very strong (0.80–0.99), with a *P* significance of <0.05(Laffite *et al*., 2016;Knapp *et al*., 2017; Romero *et al*., 2017).

Table 3.2. Oligonucleotide primers used in this study and annealing conditions

Target gene	Primer name	Oligonucleotide sequence (5′ to 3′)	Amplicon size (bp)	Conventional PCR annealing conditions	qPCR annealing conditions	Reference
16S rRNA	519F	CAG CMG CCG CGG TAA TWC	500	55°C for 1 min.	60°C for 20 secs	Wang and Qian (2009)
	909 R	CCG YGA ATT CMT TTR AGT				
uidA	Eco-F	CTGCTGCTGTCGGCTTTA	205	60°C for 30 secs	60°C for 20 secs	(Kaushik *et al.*, 2012)
	Eco-R	CCTTGCGGACGGGTAT				
bla$_{CTX-M-1}$	CTX-M-1 F*	ACGTTAAACACCGCCATTCC	356	60°C for 1 min	-	Colomer-Lluch *et al.* (2011)
	CTX-M-1 R*	TCGGTGACGATTTTAGCCGC				
	CTX-M-1 F**	ACCAACGATATCGCGGTGAT	101	-	60°C for 1 minute	Colomer-Lluch *et al.* (2011)
	CTX-M-1R**	ACATCGCGACGGCTTTCT				
	CTX-M-1 probe**	6FAM – TCGTGCGCCGCTG-MGBNFQ				
dfrA1	dfrA1-F	TTC AGG TGG TGG GGA GAT ATA C	150	62°C for 40 secs	55°C for 20 secs	Muziasari *et al.* (2014)
	dfrA1-R	TTA GAG GCG AAG TCT TGG GTA A				
ERIC sequences	ERIC R	ATGTAAGCTCCTGG GGATTCAC	-	49°C for 1 min.		Versalovic *et al.* (1991)
	ERIC F	AAGTAAGTGACTGGGGTGAGCG	-			
sul1	sul1 F	CGGCGTGGGCTACCTGAACG	433	68°C for 1 min	64°C for 20 secs	Kerrn *et al.* (2002)
	sul1 R	GCCGATCGCGTGAAGTTCCG				
sul2	sul2 F	GCGCTCAAGGCAGATGGCATT	293	68°C for 1 min	61°C for 20 secs	Kerrn *et al.* (2002)
	sul2 R	GCGTTTGATACCGGCACCCGT				
tetA	tetA F	GCTACATCCTGCTTGCCTTC	210	55°C for 1 min	56°C for 20 secs	Ng *et al.* (2001)
	tetA R	CATAGATCGCCGTGAAGAGG				
intI1	intI1 F	GGGTCAAGGATCTGGATTTCG	500	57°C for 1 min	60°C for 20 secs	(SON 2015)
	intI1 R	ACATGCGTGTAAATCATCGTCG				

*CTX-M-1 primers used in conventional PCR

**CTX-M-1 primers used in qPCR.

CHAPTER FOUR

RESULTS

4.1 Samples obtained from study sites

A total of thirty-nine (39) samples from the five (5) Electronic waste dumpsites in Lagos (n=3) and Ibadan (n=2) were obtained during the three sampling campaigns. The samples were soil (n = 15) and water (n = 24). The sources of the water samples were; rivers (n=2), hand-dug wells (n=4) and boreholes (n =2). A description of the sample types, sampling sites and their respective short codes which will be used as reference subsequently in this thesis are presented in Table 4.1.

4.2 Measured physicochemical properties of soil samples from E-waste dumpsites

The physicochemical properties of soils from the selected E-waste dumpsite are shown in Table 4.2. Garden soil from IITA (International Institute for Tropical Agriculture, Ibadan) were used as control sample, and were analysed alongside the soil samples from E-waste dumpsites. With use of One-way ANOVA, the measured physicochemical parameter occurring within each sample were examined to determine more similar or divergent samples across the different sampling periods (Table 4.2). In Table 4.2, variables in columns with dissimilar superscripts refers to significantly different samples at 95% confidence interval (CI).

The pH range of the samples were acidic, 6.62 (IKJ2), to alkaline, 8.22 (OS1), whereas pH of Garden soil was alkaline (8.40). The pH of the samples had a Standard Error Mean (SEM) of 0.14, there was no significant difference between the pH of E-waste dumpsite soil samples and the Garden soil ($p<0.05$). Further, the Ca^{2+} content varied greatly amongst the soil samples ($p<0.05$), with AL1 having the highest concentration at 150.81 Cmol/kg and the lowest in ARU2 at 13.21 Cmol/kg. Amongst the soil samples, the Ca^{2+} of Garden soil (25.23 Cmol/kg) was only higher than OS1 (22.17 Cmol/kg), ARU2 (13.21 Cmol/kg), OS2 (22.56 Cmol/kg) and OS3 (23.03 Cmol/kg). On the other hand, all the soil samples had lower Mg^{2+} content compared to

Table 4.1. Description of the samples and their respective short codes used in this study.

Types of samples	Source of samples	Locations	short codes
Soil	Soil	Alaba International Market, Lagos	AL
Water	River	Alaba International Market, Lagos	AR
Water	Hand-dug well	Alaba International Market, Lagos	AW1
Water	Hand-dug well	Alaba International Market, Lagos	AW2
Soil	Soil	Aswani Market, Lagos	AS
Water	Borehole	Aswani Market, Lagos	ASB
Soil	Soil	Aswani Market, Lagos	IKJ
Water	Borehole	Aswani Market, Lagos	IKB
Soil	Soil	Arulogun, Ibadan	ARU
Water	Hand-dug well	Arulogun, Ibadan	UW1
Water	Hand-dug well	Arulogun, Ibadan	UW2
Soil	Soil	Ogunpa market, Ibadan	OS
Water	River	Ogunpa market, Ibadan	OR

Table 4.2. Measured physicochemical parameters of soil samples

Parameters	Source	pH	%N	%OC	% P	%SAND	%SILT	%CLAY	Ca	Mg	K (Cmol/kg)	Na
AL1	Alaba market	6.890^{a}	0.226	0.906^{b}	6.790^{l}	74.000	11.000	15.000	150.810^{b}	7.820^{b}	0.230^{b}	2.760^{cd}
AS1	Aswani market	6.970^{a}	0.061	0.422^{b}	23.550^{e}	72.000	13.000	15.000	65.960^{i}	3.520^{c}	0.350^{b}	3.000^{cd}
IKJ1	Ikeja computer village	7.450^{a}	0.078	0.717^{b}	36.14^{c}	72.000	13.000	15.000	77.720^{h}	4.310^{c}	0.320^{b}	2.400^{cd}
ARU1	Arulogun	7.060^{a}	0.041	0.189^{b}	12.560^{j}	72.000	12.000	14.000	41.410^{k}	2.630^{e}	0.160^{b}	2.250^{cd}
OS1	Ogunpa market	8.220^{a}	0.001	0.070^{b}	17.780^{gh}	73.000	14.000	13.000	22.170^{l}	2.870^{d}	0.160^{b}	2.960^{cd}
AL2	Alaba market	7.500^{a}	0.192	1.034^{b}	2.440^{m}	73.000	13.000	16.000	112.630^{c}	8.780^{b}	0.250^{b}	2.090^{cd}
AS2	Aswani market	7.410^{a}	0.177	0.923^{b}	13.810^{j}	73.000	12.000	15.000	79.500^{g}	3.960^{c}	0.670^{b}	3.690^{c}
IKJ2	Ikeja computer village	6.620^{a}	0.199	0.932^{b}	64.660^{b}	71.000	12.000	17.000	57.860^{j}	4.750^{c}	0.620^{b}	2.440^{cd}
ARU2	Arulogun	7.500^{a}	0.039	0.072^{b}	66.680^{a}	71.000	16.000	13.000	13.210^{m}	2.770^{e}	0.780^{b}	7.880^{b}
OS2	Ogunpa market	7.950^{a}	0.001	0.053^{b}	19.200^{fg}	75.000	12.000	13.000	22.560^{l}	2.730^{e}	0.150^{b}	2.480^{cd}
AL3	Alaba market	7.870^{a}	0.121	0.707^{b}	15.550^{i}	77.000	10.000	13.000	97.460^{e}	4.900^{c}	0.210^{b}	1.670^{d}
AS3	Aswani market	7.670^{a}	0.040	0.391^{b}	28.450^{d}	73.000	12.000	15.000	85.980^{f}	3.710^{c}	0.300^{b}	2.090^{cd}
IKJ3	Ikeja computer village	7.540^{a}	0.082	0.762^{b}	9.950^{k}	73.000	12.000	15.000	109.590^{d}	8.310^{b}	0.350^{b}	1.860^{cd}
ARU3	Arulogun	7.350^{a}	0.012	0.147^{b}	15.590^{i}	73.000	12.000	15.000	79.090^{h}	4.230^{cde}	0.510^{b}	2.360^{cd}
OS3	Ogunpa market	7.690^{a}	0.001	0.110^{b}	20.560^{f}	77.000	10.000	13.000	23.030^{l}	2.170^{f}	0.140^{b}	2.170^{cd}
Garden soil	IITA	8.40 ^{a}	0.362	5.201^{a}	17.21h^{i}	60.000	8.000	32.000	25.23^{a}	11.10^{a}	1.900^{a}	1.621^{a}
SEM	-	0.14	0.12	0.21	2.6	0.87	0.28	1.00	8.38	3.74	0.67	0.52

Mean of the variables occurring on the same column with dissimilar superscripts are significantly different ($p < 0.05$) using one way ANOVA

%N = Total Nitrogen, %OC = Total organic carbon, %P = Total phosphorus, OC = Organic Carbon, N = Total Nitrogen, P = Available Phosphorus, IITA = International Institute for Tropical Agriculture, SEM = Standard Error Mean

Numbers at the end of the sample codes appearing as either 1, 2 or 3 represents samples obtained during the 1st, 2nd and 3rd sampling campaigns respectively.

the control sample (11.10 Cmol/kg). The highest concentration was observed in sample AL2 (8.78 Cmol/kg) and lowest concentration in OS3 (2.17 Cmol/kg). There were significant differences in the concentrations of K^+ in soil samples when compared with the control soil (1.90 Cmol/kg) but were not significantly different amongst the soil samples (excluding the control). The concentrations of K^{2+} occurred between 0.78 Cmol/kg (ARU2) and 0.14 Cmol/kg (OS3).

The same pattern of occurrence of K^+ in the soil samples were also observed with the organic carbon content (%OC) where %OC in the E-waste dumpsite soils were different significantly in comparison to the control soil sample (5.21%) but weren't significantly different amongst the soil samples (excluding the control). The highest %OC was observed in AL2 (1.034%) and lowest in OS2 (0.053%). All the soil samples had Sodium (Na) concentrations higher than the control (1.621 Cmol/kg). The highest was observed in sample ARU2 (7.88 Cmol/kg) and the lowest in AL3 (1.67 Cmol/kg). On the other hand, the nitrogen content (% N) of the control sample (0.362%) was higher than the E-waste soil samples, ranging from 0.226% (AL1) to 0.001 (OS1, OS2 and OS3). Also, 46.67% of the E-waste soil samples had higher concentrations of total phosphorus (%) which ranged between 1.03 (OS1) to 3.87 (ARU2) compared to the control (17.21%). The soil texture revealed the samples to be sandy soils (71 to 77%), in contrast to the higher clay content of the garden soil (60% sandy and 32% clay).

4.3 Measured physicochemical properties of water samples from E-waste dumpsites

Differences in concentrations of the quantified physicochemical parameters of water samples from E-waste dumpsites were observed across the samples and sampling periods. Using One-way ANOVA variations occurring between the water samples to determine significantly different or similar samples were determined at 95% confidence interval (Table 4.3).

The pH of the water samples ranged from acidic, 5.1 (IKB1), to neutral 7.2 (AR2). All the samples from boreholes were slightly more acidic than water from other sources, with pH ranging from 5.1 (IKB1) to 6.0 (ASBIII). Forty-five percent (45%) of the water samples had pH values below the limits set by the Standards Organization of Nigeria (SON 2015) and the Environmental Protection Agency (EPA 2001) drinking

water quality (6.5 – 8.5). About 25% of the water samples, mostly from river sources had NH_4concentrations above EPA recommended limits of 4.0ppm, no NH_4 limit were provided by SON. The highest NH_4 concentration occurred in water samples from rivers AR1 (76.65 ppm), AR2 (46.14 ppm) and AR3 (25.33 ppm), whereas NH_4 was not detected in hand-dug well samples AW1III and AW2III.

A regression curve between Electrical Conductivity (EC) and TDS gave a strong positive linear and highly significant (r = 0.99) relationship at 99.96% confidence interval (Fig. 4.1). Indicating an increase of one parameter led to a corresponding increase of the other. Sample AW2II had the highest reading for both parameters (TDS – 1755 mg/l, EC – 3502 µS/cm), whereas IKB3 had the lowest reading for both parameters (TDS – 92 mg/l, EC – 185 µS/cm). Similarly, 54% of the samples exceeded EC and TDS recommended limits by SON and EPA.

The mineral content such as Ca (Calcium) was highest in AW1III (119.31 ppm) and lowest in ASB1 (5.20 ppm) whereas Mg (Magnesium) was highest in ARW2I (20.29 ppm) and lowest in IKB3 (0.82 ppm). The concentrations of these minerals were more in water samples from hand-dug wells in comparison to water samples from rivers and boreholes. The SON and EPA did not set any limit for Ca and Mg in water samples. Also, the measured concentrations of Ca and Mg in the water samples in the form of $CaCO_3$ and $MgCO_3$ respectively showed that water samples from hand-dug wells were the most hard-water group, with the highest concentrations (Total hardness = $CaCO_3$ + $MgCO_3$) observed in hand-dug well water samples AW1III (356.18 mg/l) followed by AW2III (259.79mg/l), whereas the lowest hardness was observed in borehole water samples IKB3 (21.83 mg/l) and ASB1 (27.74 mg/l).

All the water samples in this study had sodium (Na) content below recommended limits (200 ppm) for drinking water quality by regulatory bodies (SON and EPA). Sample AW21 had the highest Na content (91.86 ppm) whereas it was lowest in IKB3 (14.55 ppm). Similarly, concentration of NO_3 in the water sample were below permissible limit by SON (50 ppm), however, NO_3 was highest in sample AW1II (35.05 ppm) and lowest in OR1 (3.28 ppm). Further, compared to permissible limits set by the EPA for PO_4 (0.70 ppm), only the surface water samples, AR (AR1 = 8.96, AR2 = 13.18 and AR3 = 3.74 ppm) and OR1 (2.01 ppm) had PO_4 above 0.70 ppm.

Table 4.3. Measured physicochemical parameters of water samples

Sample ID	Source	mean pH	TDS (mg/l)	Elect. Cond. (µS/cm)	Ca (ppm)	Mg (ppm)	Hardness (mg/l)			PO_4 (ppm)	NH_4 (ppm)	NO_3 (ppm)	Na (ppm)
							$CaCO_3$	$MgCO_3$	Total				
AR1	Alaba river	7.100^{a}	910.000^{g}	1825.000^{g}	14.010^{m}	8.810^{ghi}	4.000^{u}	36.270^{j}	40.270^{q}	8.960^{b}	76.650^{a}	6.170^{k}	53.190^{h}
AW1I	Alaba well 1	6.500^{abc}	1179.000^{d}	2334.000^{d}	41.050^{e}	17.870^{b}	102.490^{g}	73.590^{b}	176.080^{e}	0.140^{e}	1.940^{hi}	35.810^{a}	30.060^{op}
AW2I	Alaba well 2	7.100^{a}	1657.000^{c}	3314.000^{c}	16.230^{l}	8.160^{hi}	40.510^{o}	33.620^{k}	74.140^{n}	0.020^{e}	1.940^{hi}	32.300^{cde}	91.860^{b}
ASB1	Aswani borehole	5.900b^{cde}	258.000^{u}	517.000^{u}	5.200p	3.590^{m}	12.980^{t}	14.760^{o}	27.740^{t}	0.010^{e}	3.090^{fgh}	31.850^{cde}	30.060^{op}
IKB1	Ikeja borehole	5.100^{e}	300.000^{s}	612.000^{s}	10.320^{n}	2.660^{m}	25.780^{r}	10.940^{p}	36.720^{r}	0.010^{e}	2.480^{fgh}	32.180^{cde}	35.010^{m}
ARW1I	Arulogun well1	6.200^{abcd}	608.000^{l}	1220.000^{l}	20.680^{j}	9.210^{gh}	51.640^{l}	37.940^{u}	89.580^{l}	0.060^{e}	1.780^{hi}	32.820^{cde}	74.180^{d}
ARW2I	Arulogun well 2	6.200^{abcd}	650.000^{k}	1302.000^{k}	34.200^{fg}	20.290^{a}	85.400^{f}	83.540^{a}	168.940^{f}	0.140^{e}	2.500^{fgh}	33.690^{bc}	53.190^{h}
OR1	Ogunpa river	6.900^{a}	689.000^{j}	1378.000^{j}	18.450^{k}	12.810^{de}	46.070^{m}	52.770^{f}	98.840^{k}	2.010^{d}	20.040^{d}	3.280^{l}	77.390^{c}
AR2	Alaba river	7.200^{a}	1755.000^{a}	3511.000^{a}	17.710^{k}	10.270^{fg}	44.220n	42.280^{h}	86.500^{m}	13.180^{a}	46.140^{b}	15.490^{h}	70.630^{e}
AW1II	Alaba well 1	6.500^{abc}	1147.000^{e}	2295.000^{e}	50.260^{d}	13.980^{cd}	125.510^{d}	57.570^{f}	183.080^{d}	0.030^{e}	12.840^{e}	35.050^{ab}	40.770^{k}
AW2II	Alaba well 2	7.200^{a}	1749.000^{b}	3502.000^{b}	20.680^{j}	11.410^{ef}	51.640^{l}	47.000^{g}	98.640k	0.010^{e}	2.480^{fgh}	31.270^{def}	67.110^{f}
ASB2	Aswani borehole	5.400^{de}	336.000^{q}	673.000^{q}	12.530^{m}	3.350^{m}	31.290^{q}	13.810^{o}	45.100^{p}	0.010^{e}	2.440^{fgh}	32.330^{cde}	32.680^{n}
IKB2	Ikeja borehole	5.400^{de}	272.000^{t}	546.000^{t}	10.320^{n}	1.890^{mn}	25.780^{r}	7.780^{q}	33.560^{s}	0.070^{e}	1.780^{hi}	32.190^{cde}	29.090^{pq}
ARW2II	Arulogun well1	6.500^{abc}	563.000^{m}	1130.000^{m}	32.690^{g}	17.360^{c}	81.620^{g}	71.480^{c}	153.100^{g}	0.000^{e}	1.260^{hi}	33.210^{de}	46.710i
ARW1II	Arulogun well 2	6.300^{abcd}	487.000^{o}	975.000^{o}	15.490^{l}	7.200^{ij}	38.670^{p}	29.670^{l}	68.340^{o}	0.080^{e}	2.380^{gh}	31.300^{def}	55.390^{g}
OR2	Ogunpa river	6.700^{ab}	465.000^{p}	854.000^{p}	34.820^{f}	13.760^{cd}	65.720^{i}	42.910^{h}	108.630^{j}	0.380^{de}	1.200^{hi}	12.920^{i}	76.620^{c}
AR3	Alaba river	7.000^{a}	777.000^{h}	1550.000^{h}	69.010^{c}	10.190^{gh}	172.310^{c}	41.950^{h}	214.250^{c}	3.740^{e}	25.330^{c}	23.800^{g}	22.430^{r}
AW1III	Alaba well 1	6.900^{a}	702.000^{i}	1406.000^{i}	119.310^{a}	14.150^{cd}	297.920^{a}	58.260^{f}	356.180^{a}	0.010^{e}	0.000^{i}	33.170^{cde}	31.040^{o}
AW2III	Alaba well 2	7.100^{a}	1090.000^{f}	2184.000^{f}	79.330^{b}	14.990^{cd}	198.080^{b}	61.710^{e}	259.790^{b}	0.000^{e}	0.000^{j}	31.24^{fg}	42.500^{j}
ASBIII	Aswani borehole	6.000^{abcd}	169.000^{x}	391.000^{x}	23.670^{i}	2.040^{mn}	59.090^{j}	8.410^{q}	67.500^{o}	0.000^{e}	4.290^{f}	13.390^{i}	23.990^{r}
IKB3	Ikeja borehole	5.700^{cde}	92.000^{y}	185.000^{y}	7.390^{o}	0.820^{no}	18.460^{s}	3.380^{r}	21.830^{t}	0.000^{e}	1.670^{hi}	15.810^{h}	14.550^{s}
ARW1III	Arulogun well1	5.900^{abcde}	306.000^{r}	617.000^{r}	31.180^{h}	13.780^{cd}	77.850^{h}	56.750^{e}	134.590^{h}	0.330^{de}	1.560^{hi}	31.690^{def}	41.520^{jk}
ARW2III	Arulogun well 2	6.000^{abcd}	235.000^{w}	473.000^{w}	22.920^{i}	5.270^{k}	57.230^{k}	21.710^{n}	78.940^{n}	0.030^{e}	1.830^{hi}	30.260^{f}	36.690^{l}
OR3	Ogunpa river	6.600^{abc}	238.000^{v}	477.000^{v}	41.050^{e}	5.740^{jk}	102.490^{f}	23.630^{l}	126.130^{i}	0.000^{e}	0.000^{i}	16.030^{h}	27.730^{q}
EPA	-	6.5-8.5	500.000^{n}	1000.000^{n}	NA^{q}	NA^{o}	NA^{v}	NA^{s}	NA^{u}	0.700^{f}	4.000^{fg}	10.000^{j}	200.000^{a}
SON	-	6.5-8.5	500.000^{n}	1000.000^{n}	NA	NA	150	NA	NA	NA	NA	50^{l}	200.000^{a}
SEM	-	0.150	56.150	112.080	3.030	0.670	7.670	2.690	9.410	0.370	2.000	1.170	1.200

Mean of the variables occurring on the same column with dissimilar superscripts are significantly different ($p < 0.05$) using one way ANOVA

SEM = Standard Error Mean, Ca = Calcium, Mg = Magnesium, NA = not available, TDS = Total Dissiolved Solids,

Roman numerals or numbers at the end of the sample codes (I, II, III or 1, 2, 3) represents samples obtained in the first, second and third sampling campaigns respectively

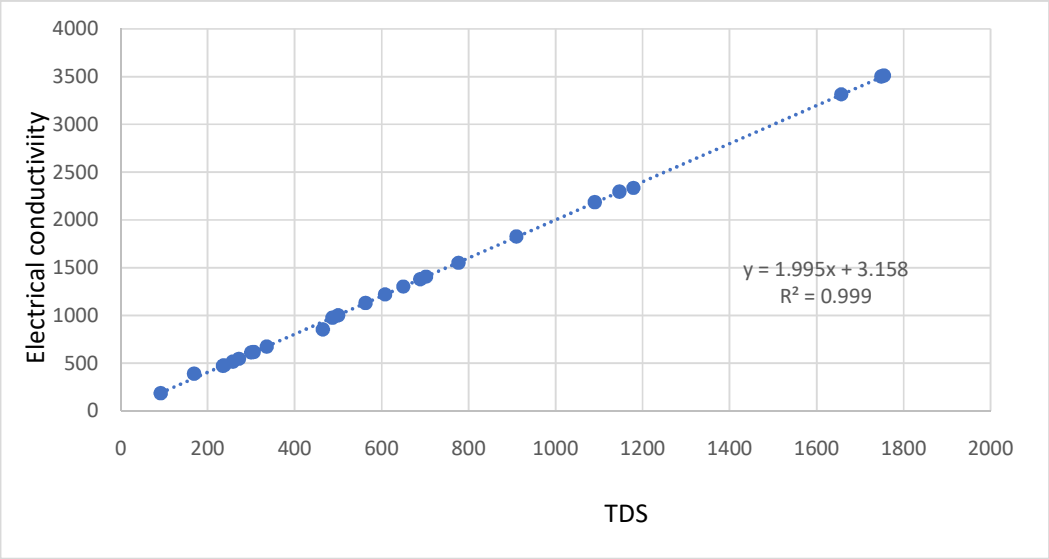

Fig. 4.1. Regression curve between Electrical conductivity and TDS which shows that as EC increases, TDS also increase.

The regression equation: $y = 1.9956x + 3.158$ ($R^2 = 0.9996$).

4.4 Measured heavy metal content of soil and water samples from E-waste dumpsites

The concentrations of eleven heavy metals comprising of Cu, Zn, Pb, Mn, Al, Fe, Co, Cr, Cd, Se and Ni were analysed in soil and water samples (raw data available in Appendix I and II). Heatmaps graphically showing the relationship between heavy metals concentrations (rows) in the samples (columns) are presented in Fig. 4.2 (water samples) and Fig. 4.3 (soil samples). Dendograms clusters in the figures depicts similarities between the samples and heavy metals.

Water quality standards set by the SON were used as controls in analysing metal contamination in the water samples. Concentrations of Aluminium (Al) and Iron (Fe) were the highest in all the samples. Highest concentration of Al was found occurring in samples AR2 (69.68 mg/l), AR3 (75.52 mg/l) and UW2III (95.34 mg/l) which were 348.4, 377.6 and 476.7 times respectively above limits set by the SON (0.2 mg/l). Whereas, highest concentration of Fe measured in OR3 (59.18 mg/l), UW2III (54.88 mg/l) and ASB (49.39 mg/l) were 118.36, 109.76 and 98.78 times respectively above permissible limits by SON (0.5 mg/l). Also, highest concentration of Cd (AR1, AR2 – 0.54 mg/l), Cu (AR3 – 0.33 mg/l), Cr (AR1 – 16.26 mg/l) and Zn (IKB2 – 3.6 mg/l) were 54, 33, 32.52 and 18 times respectively above SON permissible limits for drinking water. SON provided no permissible concentration for Cobalt and Selenium in water (SON 2015). However, concentration of Co occurred between 0.52 mg/l (OR2) and 2.82 mg/l (AR1) while Se was 0.42 mg/l (UW2I) to 4.88 mg/l (AR1). The concentrations of Pb was however below level of detection in samples AW1III, AW2III ASBIII and IKB3. Generally, the mean heavy metals abundance followed the pattern of Al> Fe> Cr> Co> Ni> Se> Pb> Cd> Mn> Zn> Cu in the water samples (Fig. 4.2).

In the soil samples, heavy metals were present in several magnitudes above what was found in the control sample (IITA garden soil)(Fig. 4.3). Concentrations of Ni (AL2), Fe (ARU2) and Mn (AL2) were 483.87, 23.02 and 7.45 times respectively above the concentration found in the garden soil. Among the metals assayed, aluminium concentrations were the highest in all the samples, ranging from 1493 mg/kg (AS3) to 8194 mg/kg (AL2), whereas its concentration in the control soil was 50 mg/kg. Soil samples from the Alaba International Market, Lagos, AL1, AL2 and AL3 had the highest level of copper contamination at 190.21, 296.66 and 102.47 mg/kg

respectively, whereas it was least in samples from Ibadan, ARU2, OS2 and OS3 at 4.08, 2.70 and 2.84 mg/kg respectively. Zinc concentration were highest in AL3 (1164.68 mg/kg) and least in IKJ (143.22 mg/kg), whereas Zn was present in garden soil at 10.70 mg/kg. Pb concentrations were highest in samples from Lagos, reaching concentrations of 1554.8 mg/kg, 1372mg/kg and 1360.2 mg/kg in AL2, AS1 and AL3 respectively. However, Pb, and other metals such as Co and Cd were below limit of detection in the control garden soil. The concentrations of Se (AS1, IKJ1, ARU1, OS1, AS2, IKJ2, ARU2, AL3, AS3, IKJ3 and ARU3), Cd (AS1, IKJ1, ARU1, AS2, IKJ2, ARU2AS3, IKJ3 and ARU3) and Co (AL1 and AL3) were below detection limit. Generally, heavy metals abundances followed pattern of Al> Fe> Zn> Pb> Ni> Cr> Mn> Cu> Cd> Se> Co in soil samples and (Fig. 4.3).

4.5 Cultivable background metal tolerant bacteria

The total culturable metals (Cu^{2+}, Pb^{2+} and Zn^{2+}) tolerant bacteria strains isolated during the three sampling campaigns are expressed as a percentage of the total heterotrophic bacteria counts (THBC) in Fig. 4.4, 4.5 and 4.6. The samples displayed varying bacteria counts in the presence of heavy metals (50 µg/ml) across the three (3) sampling periods.

During the first sampling period, highest population of the total culturable Zn^{2+}, Cu^{2+} and Pb^{2+} tolerant bacteria were obtained in samples AR (67.13% at 1.69×10^{7} cfu/ml), ASB (88.24% at 8.25×10^{6} cfu/ml) and AS (92.85% at 6.50×10^{6} cfu/ml) and respectively, whereas lowest population were observed with UW2 (10.0% at 5.00×10^{2} cfu/ml), ASB (7.8% at 7.00×10^{5} cfu/ml) and ARU (23.06% at 1.55×10^{6} cfu/ml) and for Cu^{2+}, Zn^{2+} and Pb^{2+} and respectively (Fig. 4.4).

During the second sampling period, highest population of the total culturable Cu^{2+}, Zn^{2+} and Pb^{2+} tolerant bacteria were observed in samples IKJ (82.58% at 6.40×10^{6} cfu/ml), IKJ(63.23% at 4.90×10^{6} cfu/ml) and ARU (97.61% at 2.05×10^{6} cfu/ml), whereas lowest counts were observed with AW1 (17.07% at 7.50×10^{5} cfu/ml), UW2 (37.5% at 1.50×10^{3} cfu/ml) and UW1 (11.11% at 5.00×10^{4} cfu/ml) for Cu^{2+}, Pb^{2+} and Zn^{2+} respectively (Fig. 4.5).

During the third sampling period, highest population of the total culturable Cu^{2+}, Pb^{2+} and Zn^{2+} were obtained in samples AW2 (89.80% at 2.20×10^{6} cfu/ml), OR (95.45% at 1.05×10^{6}cfu/ml) and AR (64% at 2.40×10^{6}cfu/ml) respectively, whereas lowest

counts were observed in sample UW1 at 10% (1.50×10^5 cfu/ml), 20% (3.00×10^5 cfu/ml) and 13% (2.00×10^5 cfu/ml) for Cu^{2+}, Pb^{2+} and Zn^{2+} respectively (Fig. 4.6).

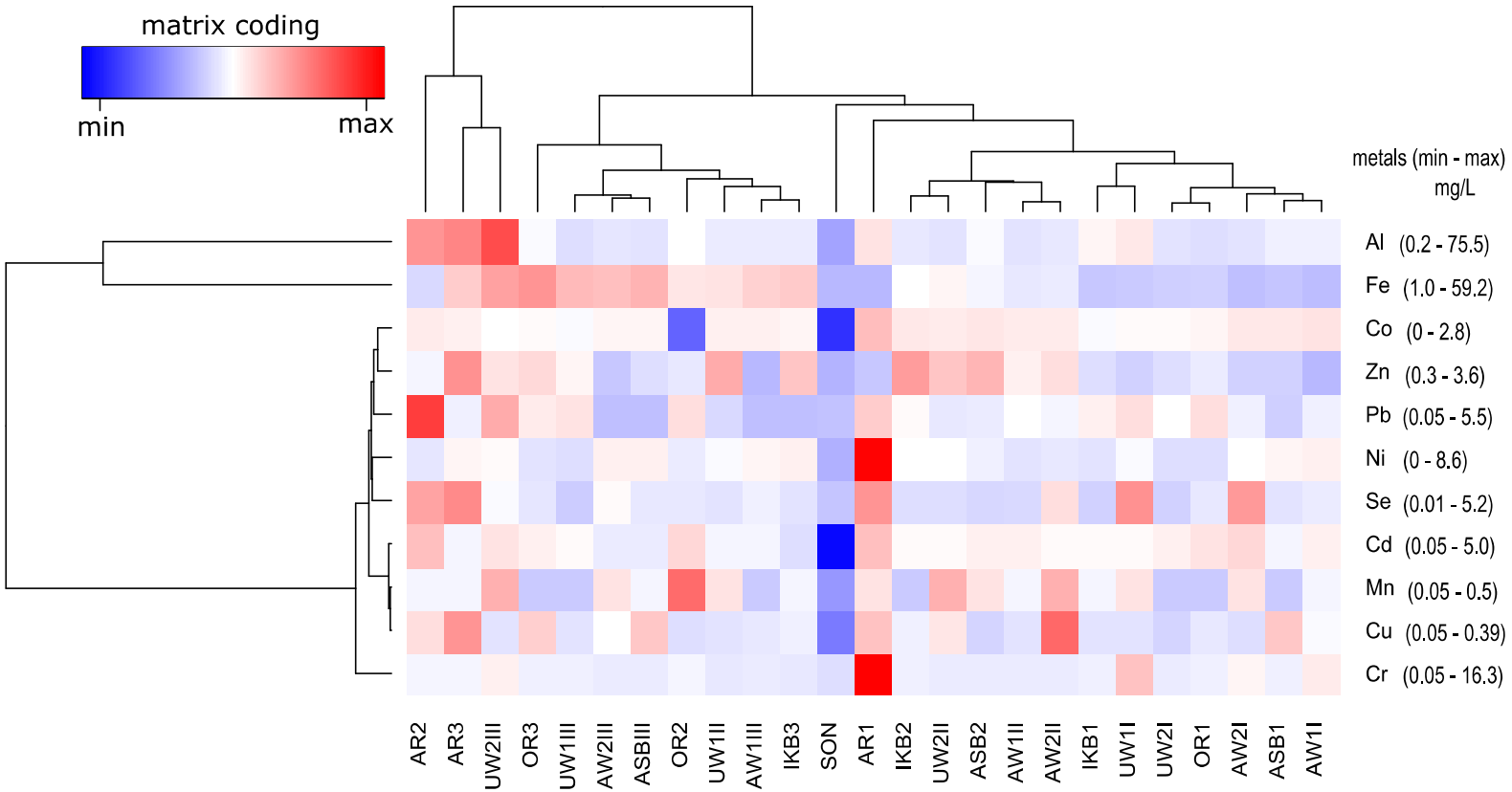

Fig. 4.2. Heatmap of heavy metal (rows) concentrations in each water sample (columns). Dendrograms represent hierarchical clustering of water samples and heavy metal.

SON: Standards organization of Nigeria permissible limits for potable water (SON, 2015).

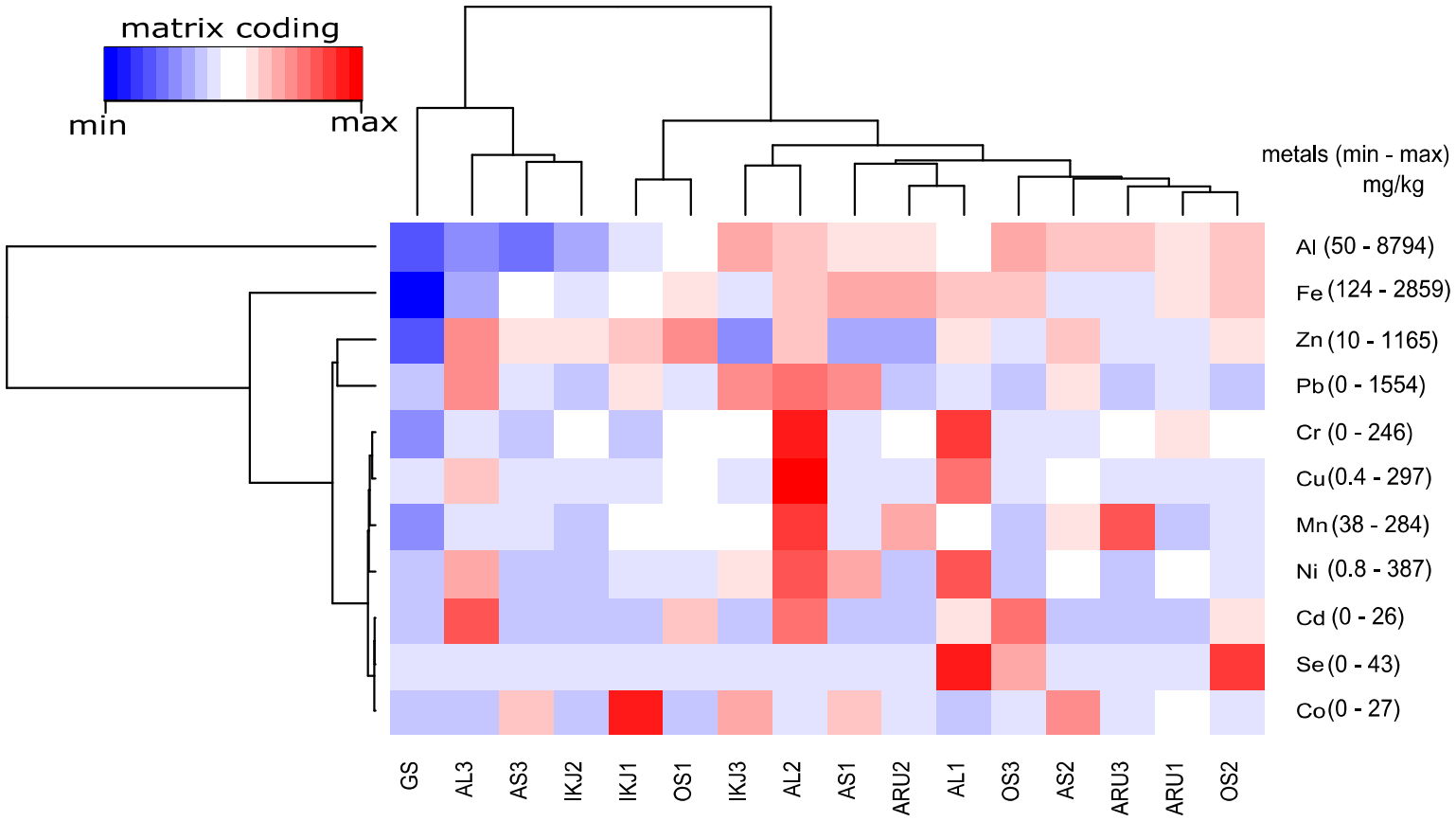

Fig. 4.3. Heatmap of heavy metals (rows) concentrations in each soil sample (columns). Dendrograms represent hierarchical clustering of soil samples or heavy metals

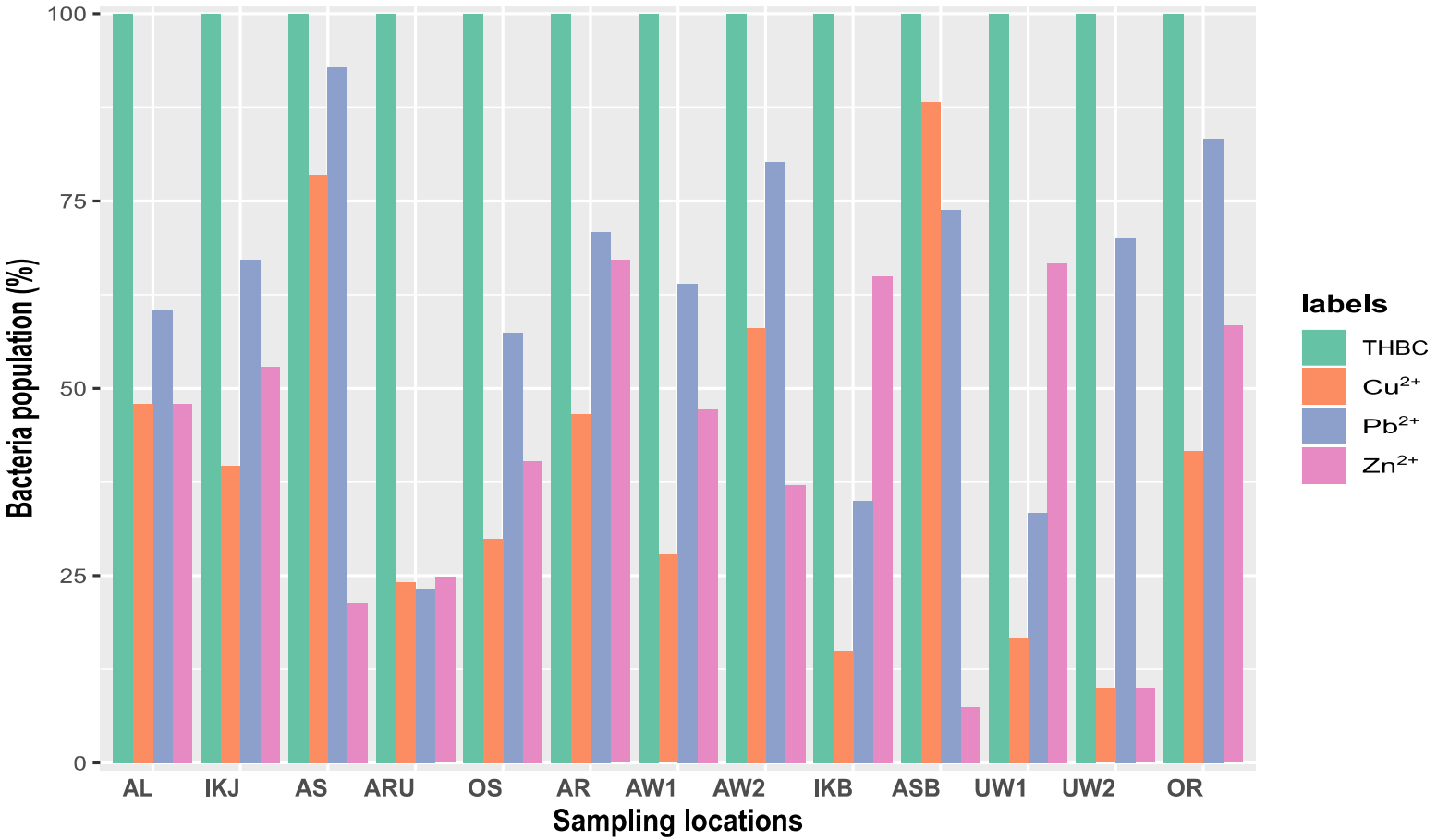

Fig. 4.4. Percentage of metal (Cu^{2+}, Pb^{2+} and Zn^{2+} 50 µg/ml) tolerant bacteria relative to the Total Heterotrophic Bacteria Count of the soil (AL, IKJ, AS, ARU and OS) and water (AR, AW1, IKB, ASB, UW1, UW2 and OR) samples from the E-waste dumpsites during the 1st sampling period.

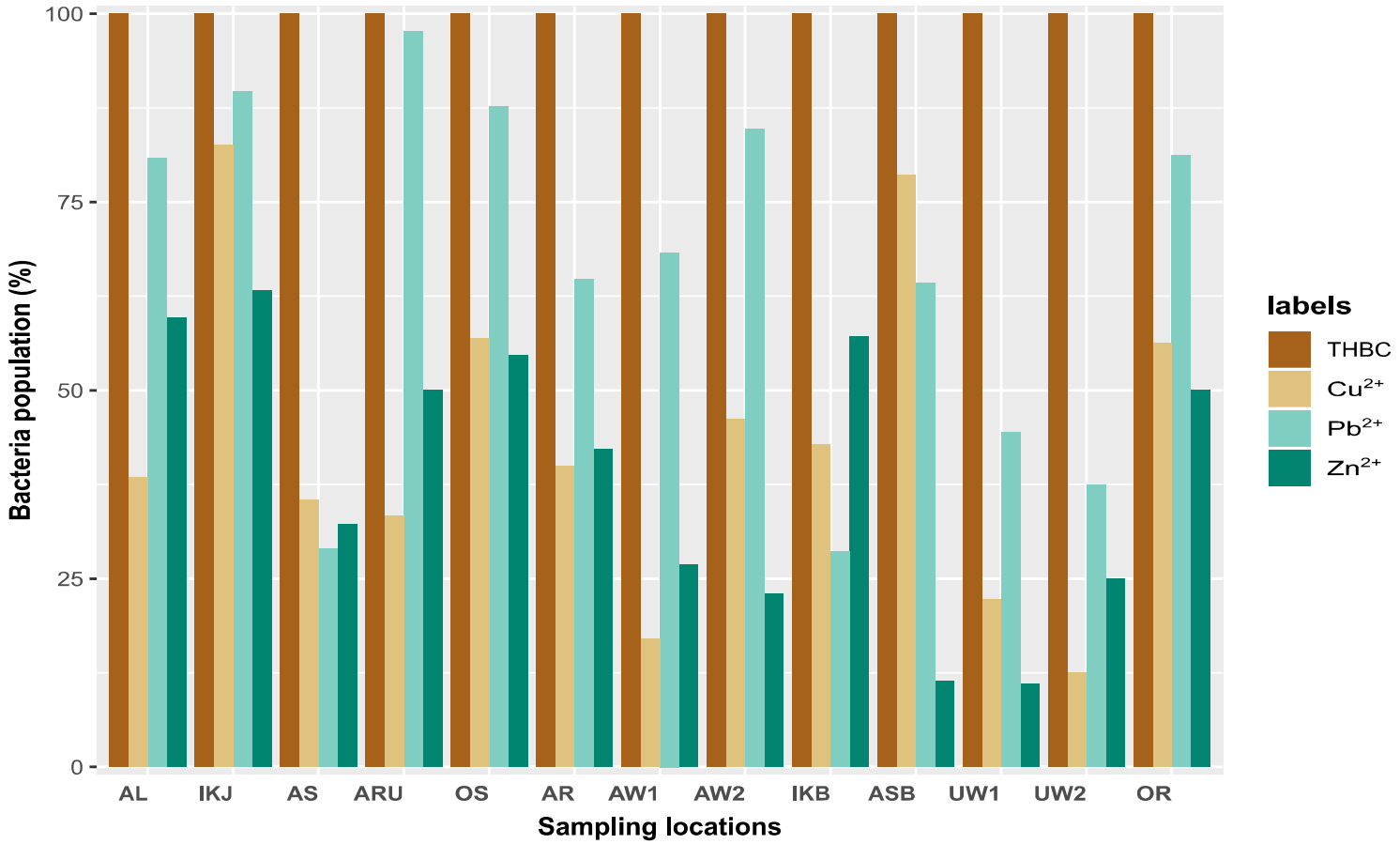

Fig. 4.5. Percentage of metal (Cu^{2+}, Pb^{2+} and Zn^{2+} 50 µg/ml) tolerant bacteria relative to the Total Heterotrophic Bacteria Count of the soil (AL, IKJ, AS, ARU and OS) and water (AR, AW1, IKB, ASB, UW1, UW2 and OR) samples from the E-waste dumpsites during the 2nd sampling period.

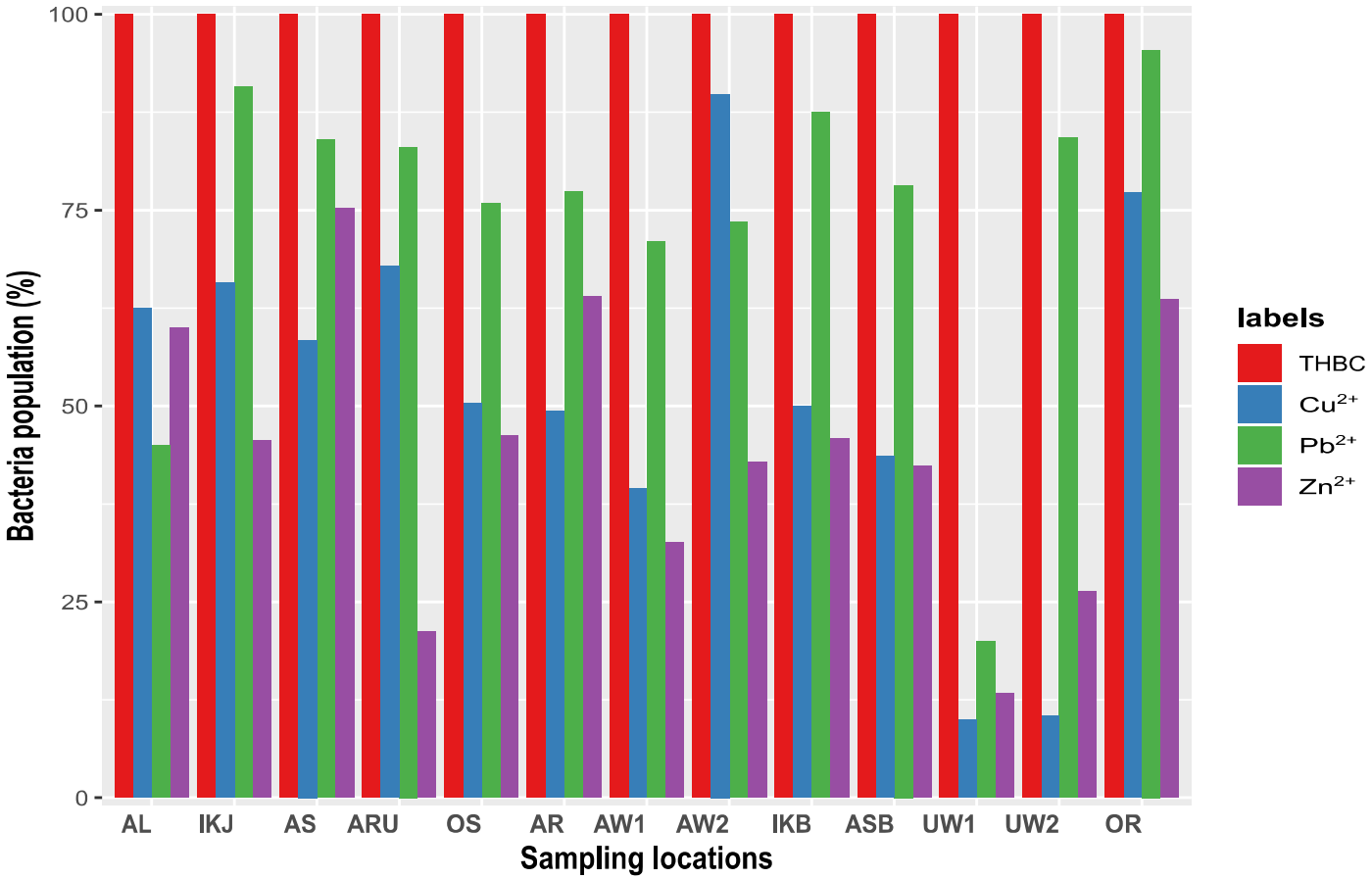

Fig. 4.6. Percentage of metal (Cu^{2+}, Pb^{2+} and Zn^{2+} 50 µg/ml) tolerant bacteria relative to the Total Heterotrophic Bacteria Count of the soil (AL, IKJ, AS, ARU and OS) and water (AR, AW1, IKB, ASB, UW1, UW2 and OR) samples from the E-waste dumpsites during the 3rd sampling period.

4.6 Bacteria isolated from soil and water samples

An overall 434 bacteria were isolated on metals (Cu, Pb and Zn) supplemented agar plates from all the samples in this study. Of the 434 strains isolates, 120, 116 and 114 were isolated from Cu, Pb and Zn supplemented plates respectively. The distribution of the metal tolerant isolates obtained from from each sample is presented in Table 4.4. In addition, 84 Enterobacteriaceae strains including *Escherichia coli* (n=66), *Enterobacter* spp. (n=10), *Citrobacter* spp. (n=6), *Kluyvera* sp. (n=1) and *Leclercia* sp. (n=1), were isolated without metal selection on EMB agar plates (Table 4.5). Further research were carried out on the Enterobacteriaceae strains.

4.7 Heavy metals (Cu^{2+}, Pb^{2+} and Zn^{2+}) tolerance profiles of the enterobacterial isolates

All the Enterobacteriaceaetolerated up to 200 µg/ml of Cu^{2+}, Pb^{2+} and Zn^{2+}. At 400 µg/ml, all strains except *E. coli* EC17 (susceptible to Cu^{2+}), were tolerant to the metals. Further increase in the metals concentrations led to rise in the number of susceptible strains (Fig. 4.7). At 600 µg/ml of Cu^{2+} and Zn^{2+}, 67.90% and 84.50% of the strains respectively were tolerant. A similar trend was observed at 800 µg/ml of the metals, where 21.43% and 45.23% of the enterobacterial isolates were tolerant to Cu^{2+} and Zn^{2+}. However, Cu^{2+} at 1000 µg/ml was toxic for the growth of all the enterobacterial isolates, whereas, 22.60% of the isolates were tolerant to Zn^{2+} at 1000 µg/ml. At 1100 µg/ml of Cu and Pb metals, there was no observable growth among the enterobacterial isolates. On the other hand, Pb^{2+} had no effect on the growth of all the strains even at concentrations up to 1100 µg/ml.

Table 4.4. Number of bacterial strains isolated from metal (50 µg/ml) supplemented MHA plates

Sample	Number of bacteria isolated on heavy metal supplemented MHA plates		
	Cu	Pb	Zn
AL	10	11	10
AR	9	10	9
AW1	8	8	9
AW2	8	7	9
AS	11	10	10
ASB	9	9	8
IKJ	11	8	10
IKB	10	8	8
ARU	9	10	9
UW1	8	10	9
UW2	9	8	7
OS	10	9	9
OR	8	8	7
Total	120	116	114

Table 4.5. Enterobacteriaceae isolated from different samples collected from the E-waste dumpsites.

Isolate name	Isolate code	Location	Isolate name	Isolate code	Location
Escherichia coli	EC1	AR	*Escherichia coli*	EC78	AR
Escherichia coli	EC2	AR	*Escherichia coli*	EC79	AR
Escherichia coli	EC3	AR	*Escherichia coli*	EC80	AR
Escherichia coli	EC4	AR	*Escherichia coli*	EC81	AR
Escherichia coli	EC5	AR			
Escherichia coli	EC6	AR	*Escherichia coli*	EC15	AW1
Escherichia coli	EC7	AR	*Escherichia coli*	EC16	AW1
Escherichia coli	EC8	AR	*Escherichia coli*	EC17	AW1
Escherichia coli	EC9	AR	*Escherichia coli*	EC84	AW1
Escherichia coli	EC10	AR			
Citrobacter freundii	EC11	AR	*Escherichia coli*	EC36	OS
Escherichia coli	EC12	AR	*Escherichia coli*	EC37	OS
Escherichia coli	EC13	AR	*Escherichia coli*	EC38	OS
Escherichia coli	EC14	AR	*Escherichia coli*	EC39	OS
Escherichia coli	EC18	AR	*Escherichia coli*	EC40	OS
Escherichia coli	EC19	AR	*Escherichia coli*	EC41	OS
Escherichia coli	EC20	AR	*Enterobacter* sp.	EC64	OS
C. freundii	EC21	AR	*Escherichia coli*	EC65	OS
Escherichia coli	EC22	AR			
Escherichia coli	EC23	AR	*Escherichia coli*	EC42	OR
Escherichia coli	EC24	AR	*En. roggenkampii*	EC43	OR
Escherichia coli	EC25	AR	*Escherichia coli*	EC44	OR
Escherichia coli	EC26	AR	*Escherichia coli*	EC45	OR
Escherichia coli	EC27	AR	*Escherichia coli*	EC46	OR
Escherichia coli	EC28	AR	*C. portucalensis*	EC47	OR
Escherichia coli	EC29	AR	*Escherichia coli*	EC48	OR
Escherichia coli	EC30	AR	*C. portucalensis*	EC49	OR
Escherichia coli	EC31	AR	*Enterobacter* sp.	EC50	OR
Escherichia coli	EC32	AR	*Kluyvera* sp.	EC51	OR
Escherichia coli	EC33	AR	*En. roggenkampii*	EC52	OR
Escherichia coli	EC34	AR	*En. kobei*	EC53	OR
C. freundii	EC35	AR	*Escherichia coli*	EC54	OR
Escherichia coli	EC66	AR	*Escherichia coli*	EC55	OR
Escherichia coli	EC67	AR	*Escherichia coli*	EC56	OR
Escherichia coli	EC68	AR	*Enterobacter* sp.	EC57	OR
Escherichia coli	EC69	AR	*Leclercia* sp.	EC58	OR
Escherichia coli	EC70	AR	*Escherichia coli*	EC59	OR
Citrobacter sp.	EC71	AR	*En. roggenkampii*	EC60	OR
Escherichia coli	EC72	AR	*En. roggenkampii*	EC61	OR
Escherichia coli	EC73	AR	*Enterobacter* sp.	EC62	OR
Escherichia coli	EC74	AR	*En. hormaeche*	EC63	OR
Escherichia coli	EC75	AR	*Escherichia coli*	EC82	OR
Escherichia coli	EC76	AR	*Escherichia coli*	EC83	OR
Escherichia coli	EC77	AR			

Enterobacteriaceae were isolated from samples originating from two sampling sites (Alaba international market and Ogunpa)

En. – *Enterobacter*

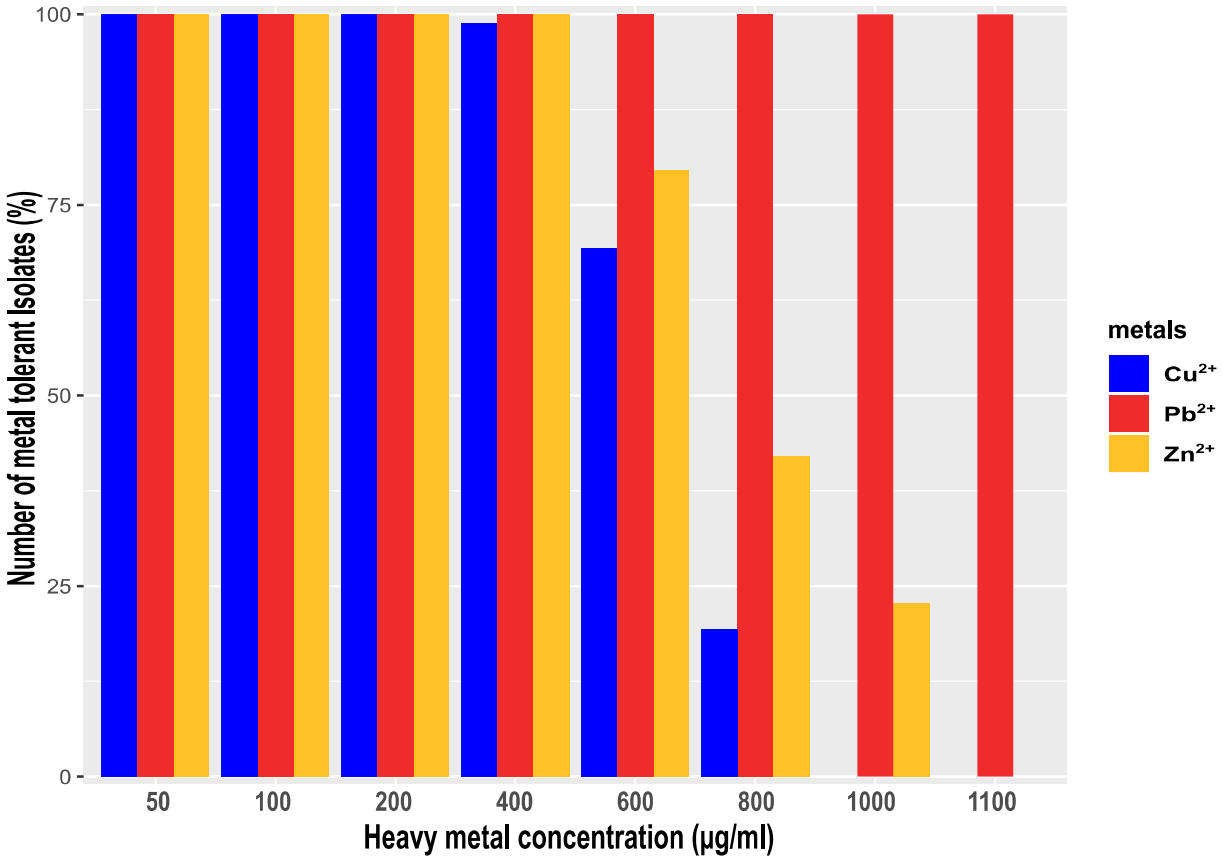

Fig. 4.7. Metal tolerance profile of isolated Enterobacteriaceae strains

4.8 Antibiotic resistance profiles of the enterobacterial strains

CLSI zone diameter breakpoints for Enterobacteriaceae (CLSI, 2017), were used to group the strains into three groups: resistant, intermediate or susceptible (Fig. 4.8). Results showed most of the strains were able to resist the toxicity of the third-generation cephalosporin, cefpodoxime (91.67%), followed by florfenicol (76.20%) and ceftaxidime (64.29%). On the other hand, gentamicin was most active against the bacterial strains with 11.90% showing resistance and 72.62 % susceptible to the drug. Closely followed by gentamicin were ertapenem, ciprofloxacin and meropenem with 13.1%, 17.86% and 25% resistance respectively. Levels of resistance to the other drugs were 45.24% for kanamycin and 53.57% for ciprofloxacin and sulphamethoxazole/Trimethoprim respectively. *Escherichia coli* EC80 (from AR) was however susceptible to nine antibiotics and showed intermediates resistance to cefpodoxime antibiotics. Two strains, *Escherichia coli* EC4 and *Escherichia coli* EC6 were phenotypically resistant to all the test drugs used in susceptibility testing in this study. In total, 89.29% of the strains were multidrug resistant (showing resistance tomore than one class of antibiotics).

The Enterobacteriaceae strains showed distinct variety of phenotypic antibiotic resistance pattern. Overall, 46 antibiotic resistance phenotypes were observed amongst the strains (an average of 1.82 strains per resistance phenotype) (Table 4.6). The most common phenotype, shared by 10.71% of the strains was combined resistance to tetracycline, florfenicol, sulfamethoxazole/trimethoprim, ceftazidime and cefpodoxime (TET, FFC, SXT, CAZ, and CPD).

4.9 Enterobacterial Repetive Intergenic Consensus Fingerprinting

The ERIC profiles of Enterobacteriaceae strains isolated from AR (Fig. 4.9, 4.10 and 4.11), OR (Fig. 4.12), OS (Fig 4.13) and AW1 (Fig. 4.14) are presented. Dendograms differentiated the strains and clustered them according to their similarity index. Interpretation of Dendograms by GelClust showed that the Enterobacterial isolates were largely non-clonal replicates.

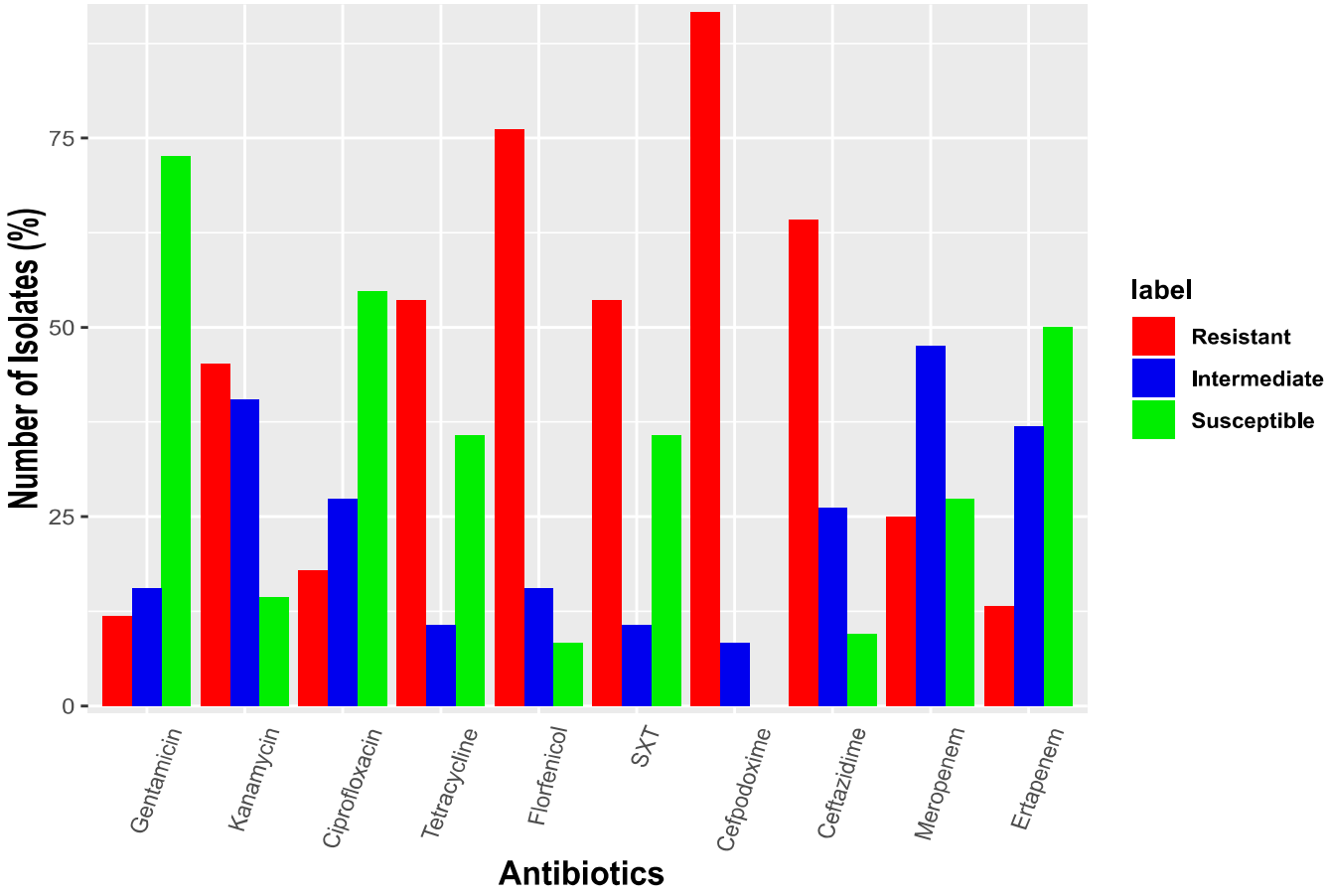

Fig. 4.8. Antibiotic resistance profiles of the enterobacterial strains (n=84) to ten clinically relevant antibiotics with different mechanisms of action

Table 4.6. Phenotypic pattern of antibiotic resistance among the enterobacterial isolates

s/n	Strain	Phenotypes	MAR index
1	EC80	-	0
2	EC52, EC61	FFC	0.1
3	EC16, EC30,	CPD	0.1
	EC32, EC45, EC62, EC63	CAZ, CPD	0.2
5	EC51, EC53,	KAN, FFC	0.2
6	EC18, EC41, EC77	FFC, CPD	0.2
7	EC57	KAN, CPD	0.2
8	EC36	CPD, MEM	0.2
9	E17,	KAN, CPD, ETP	0.2
10	EC31, EC33,	TET, FFC, SXT	0.2
11	EC26	CAZ, CPD, MEM	0.3
12	EC40, EC73, EC81,	TET, SXT, CPD	0.3
13	EC1	KAN, CAZ, CPD	0.3
14	EC60	KAN, SXT, CPD	0.3
15	EC44	SXT, CAZ, CPD	0.3
16	EC54, EC74, EC75,	TET, FFC, CPD	0.3
17	EC71, E82, E83	FFC, CAZ, CPD	0.3
18	EC50	FFC, CPD, CAZ, MEM	0.4
19	EC59	KAN, FFC, CAZ, CPD	0.4
20	EC29,	KAN, CIP, FFC, CPD	0.4
21	EC37, EC47, EC49, EC78	TET, FFC, SXT, CPD	0.4
22	EC34, EC66, EC76	TET, SXT, CAZ, CPD	0.4
23	EC3	KAN, TET, FFC, SXT,CPD	0.5
24	EC19	KAN, CIP, TET, FFC, CPD	0.5
25	EC8	KAN, TET, FFC, CAZ,CPD	0.5
26	EC35, EC55,	KAN, FFC, SXT, CAZ, CPD	0.5
27	EC9, EC14, EC38, EC39, EC42,EC46, EC70 EC72, EC79	TET, FFC, SXT, CAZ, CPD	0.5
28	EC10	GEN, KAN, FFC, CAZ, CPD	0.5
29	EC23, EC24, EC27, EC58,	KAN, FFC, CAZ, CPD, MEM	0.5
30	EC69,	KAN, TET, FFC, CAZ, CPD	0.5
31	EC48,	FFC, CAZ, CPD, MEM, ETP	0.5
32	EC20, EC21	KAN, TET, FFC, SXT, CAZ, CPD	0.6
33	EC1,	KAN, FFC, SXT, CAZ, CPD, ETP	0.6
34	EC64, EC69, EC70	FFC, SXT, CAZ, CPD, MEM, ETP	0.6
35	EC2	KAN, CIP, TET, FFC, SXT, CPD	0.6
36	EC28	CIP, TET, FFC, SXT, CAZ, CPD	0.6
37	EC5,	GEN, KAN, TET, FFC, SXT, CAZ, CPD	0.7
38	EC25, EC65	CIP, TET, FFC, SXT, CAZ, CPD, MEM	0.7
39	EC43	KAN, TET, FFC, CAZ, CPD, MEM, ETP	0.7
40	EC12	KAN, CIP, TET, FFC, SXT, CAZ, CPD	0.7
41	EC15, EC84	KAN, FFC, SXT, CAZ, CPD, MEM, ETP	0.7
42	EC11, EC67	GEN, KAN, CIP, TET, FFC, SXT, CAZ, CPD	0.8
43	EC13	KAN, CIP, TET, FFC, SXT, CAZ, CPD, MEM	0.8
44	EC7, EC22, EC56,	GEN, KAN, CIP, TET, FFC, SXT, CAZ, CPD, MEM	0.9
45	EC68	GEN, KAN, CIP, TET, SXT, CAZ, CPD, MEM, ETP	0.9
46	EC4, EC6	GEN, KAN, CIP, TET, FFC, SXT, CAZ, CPD, MEM, ETP	1.0

Where; KAN – Kanamycin, FFC – Florfenicol, GEN – Gentamicin, TET – Tetracycline, SXT – Sulphamethoxazole/Trimethoprim, CIP – Ciprofloxacin, CAZ – Ceftazidime, CPD – Cefpodoxime, MEM – Meropenem, ETP – Ertapenem.

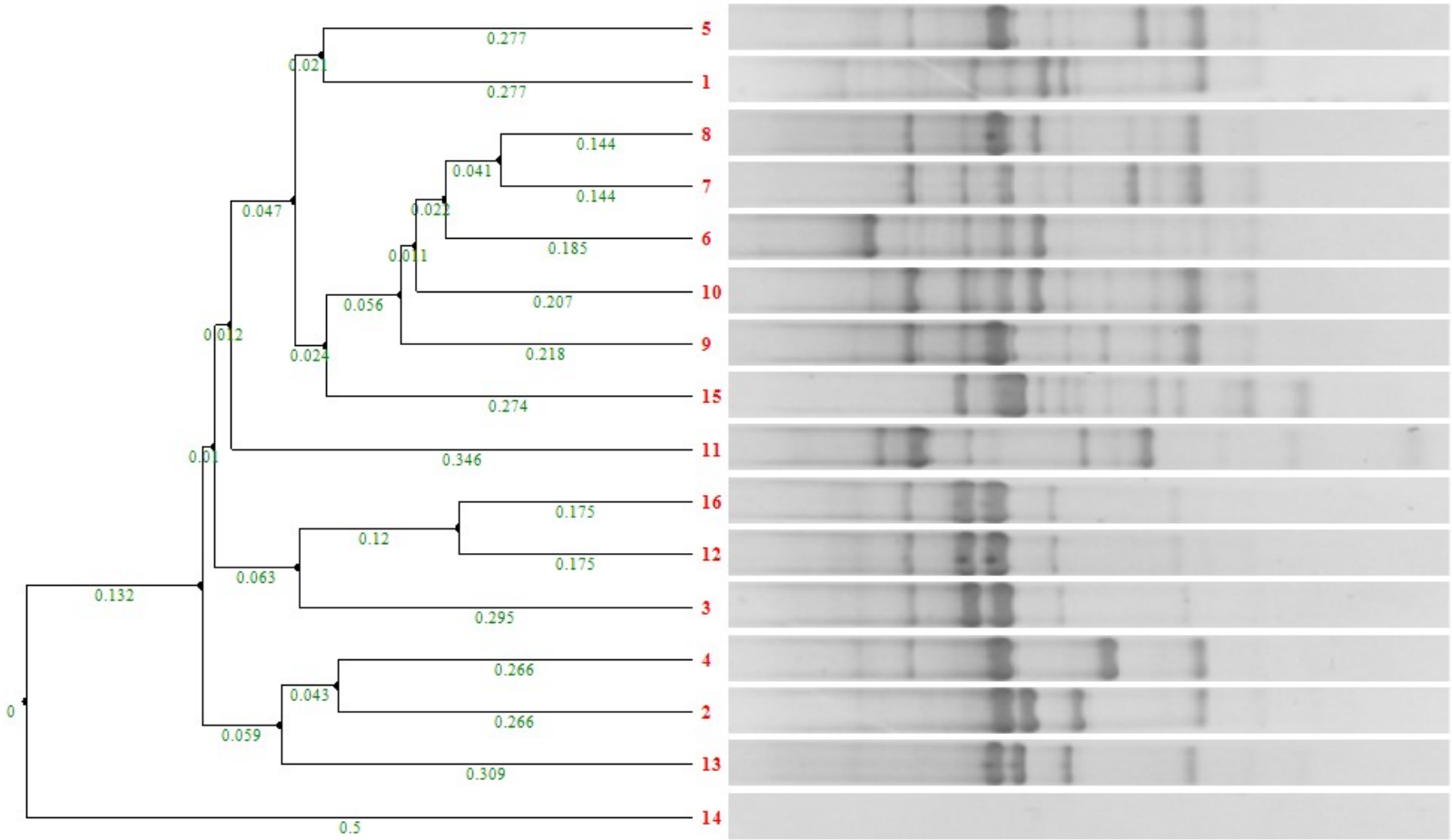

Fig. 4.9. Enterobacterial Repetitive Intergenic Consensus profiles of the first sixteen Enterobacteriaceae strains from AR

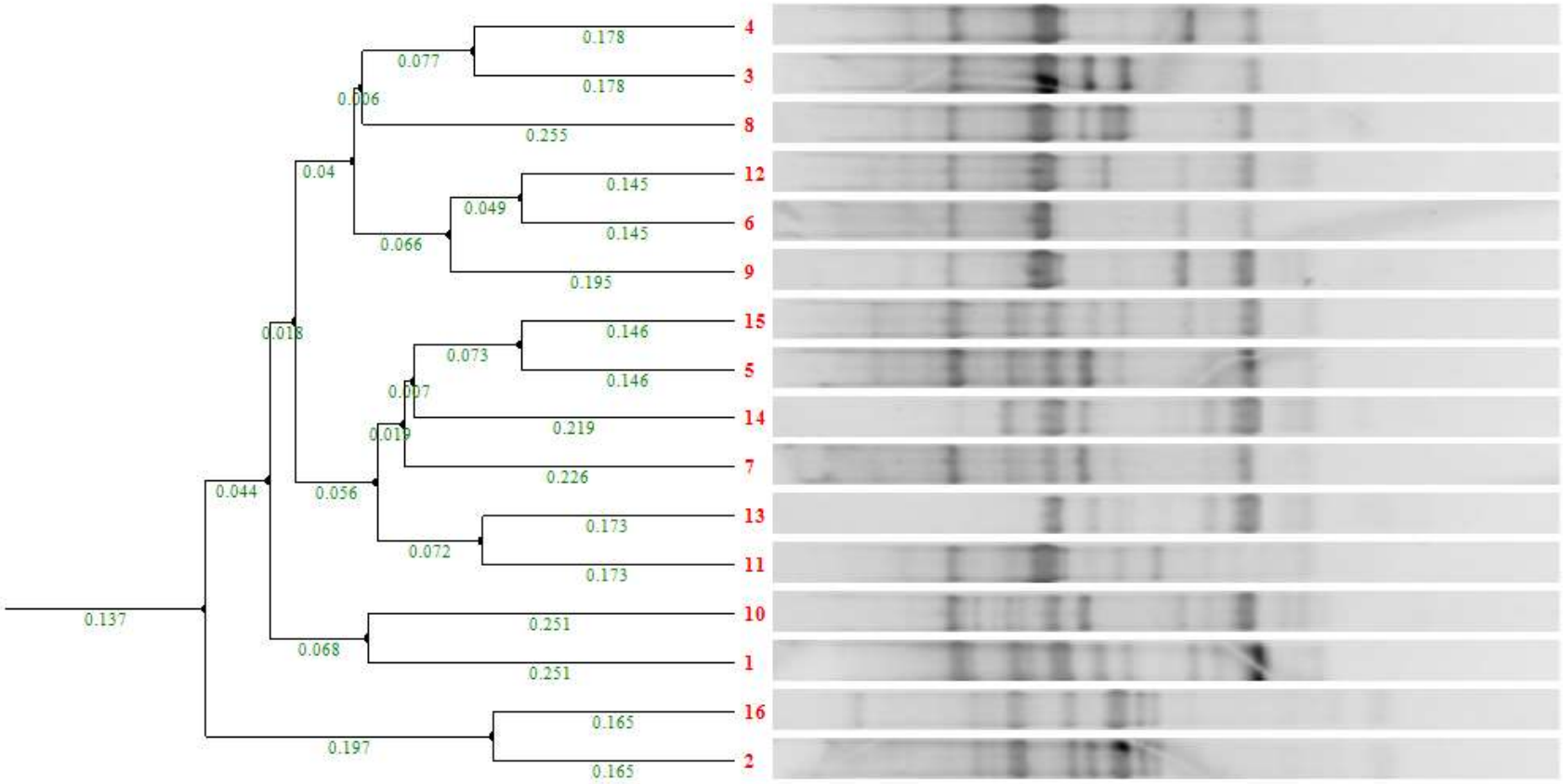

Fig. 4.10. Enterobacterial Repetitive Intergenic Consensus profiles of the Enterobacteriaceae strains from AR (strains 17 to 32 from AR). The strain names and other metadata of the dendograms are provided in Appendix XI.

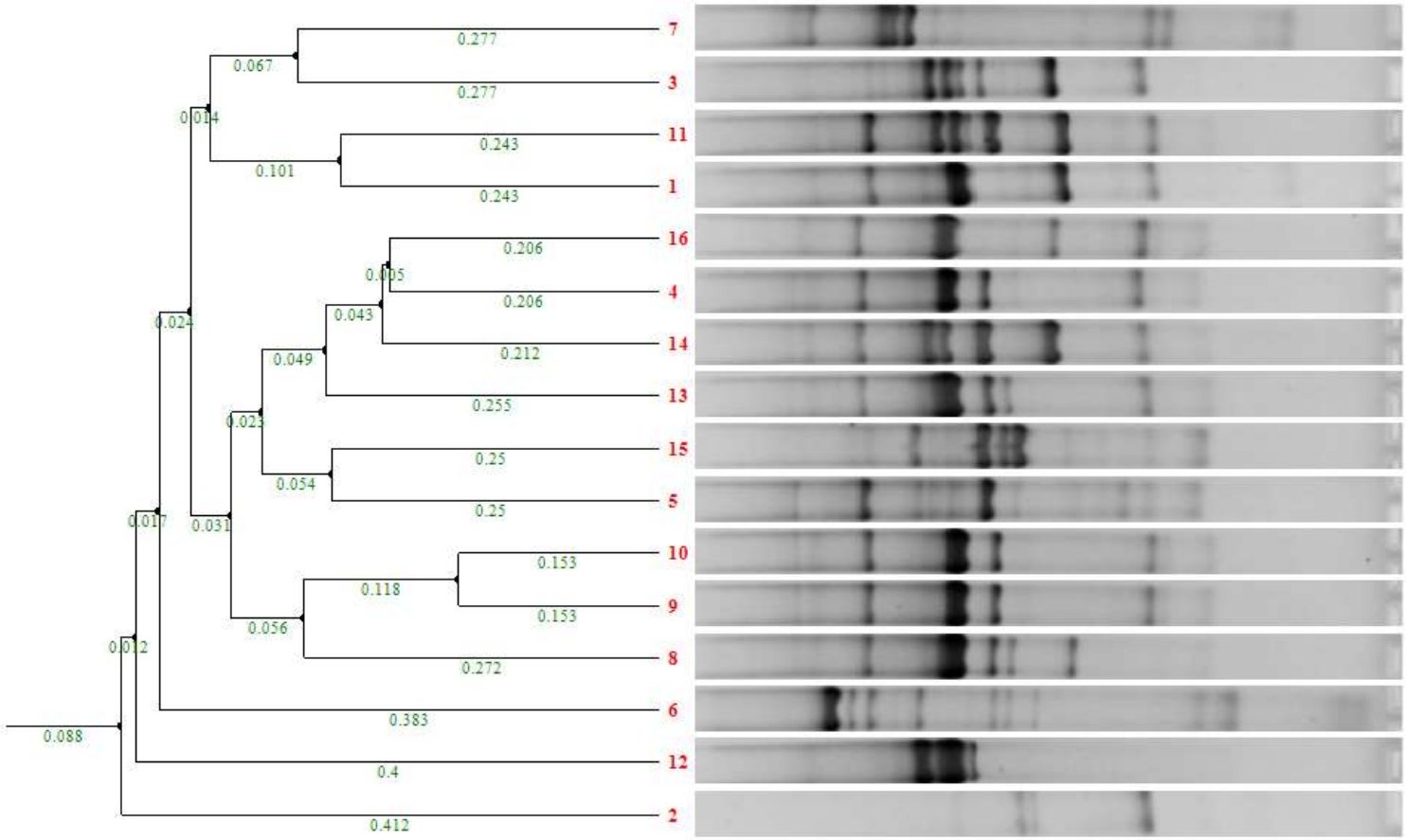

Fig. 4.11. Enterobacterial Repetitive Intergenic Consensus profiles of the Enterobacteriaceae strains from AR (strains 33 to 48 from AR). The strain names and other metadata of the dendograms are provided in Appendix XI.

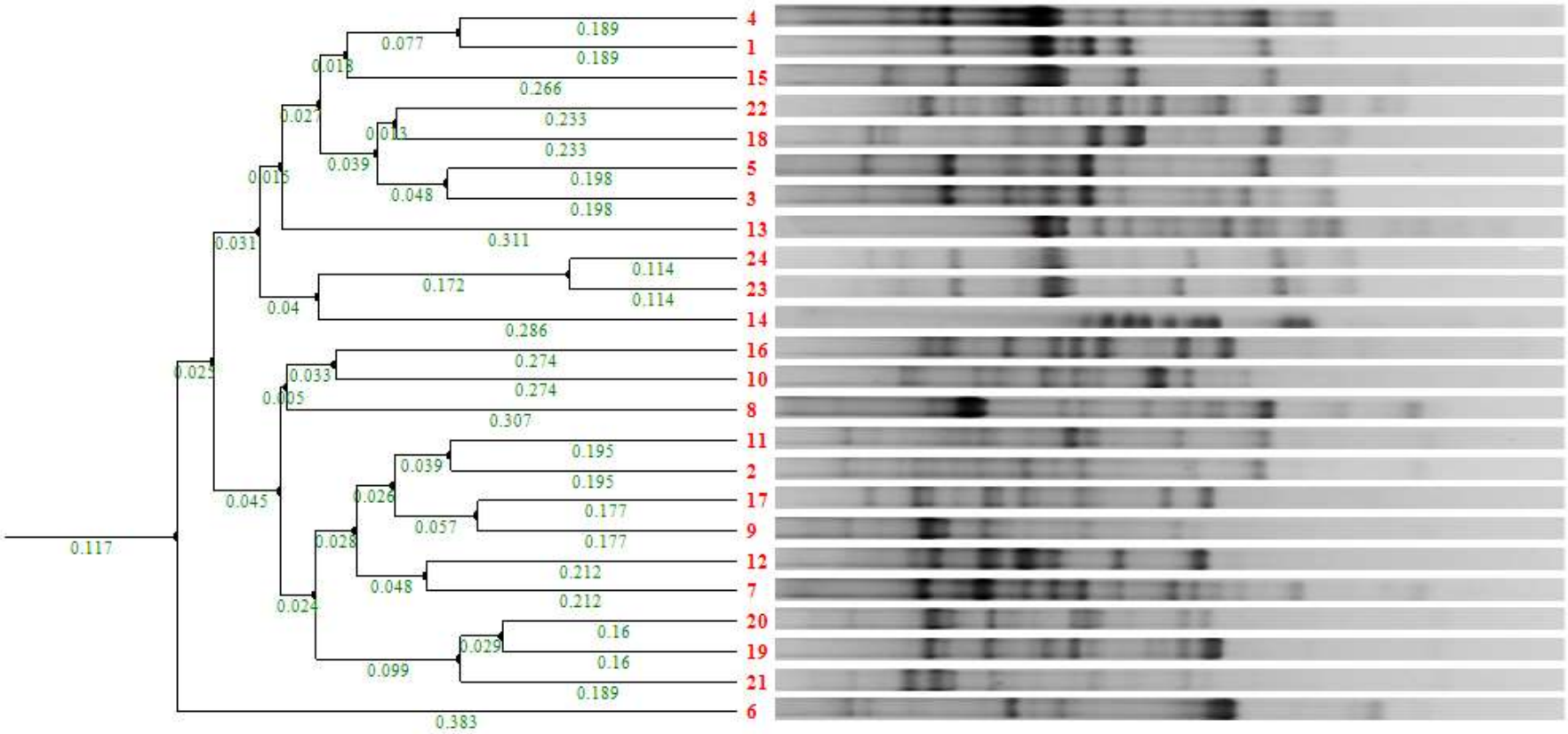

Fig. 4.12. Enterobacterial Repetitive Intergenic Consensus profiles of Enterobacteriaceae strains from OR showing ERIC profiles of the 24 strains isolated from OR. The strain names and other metadata of the dendograms are provided in Appendix XI.

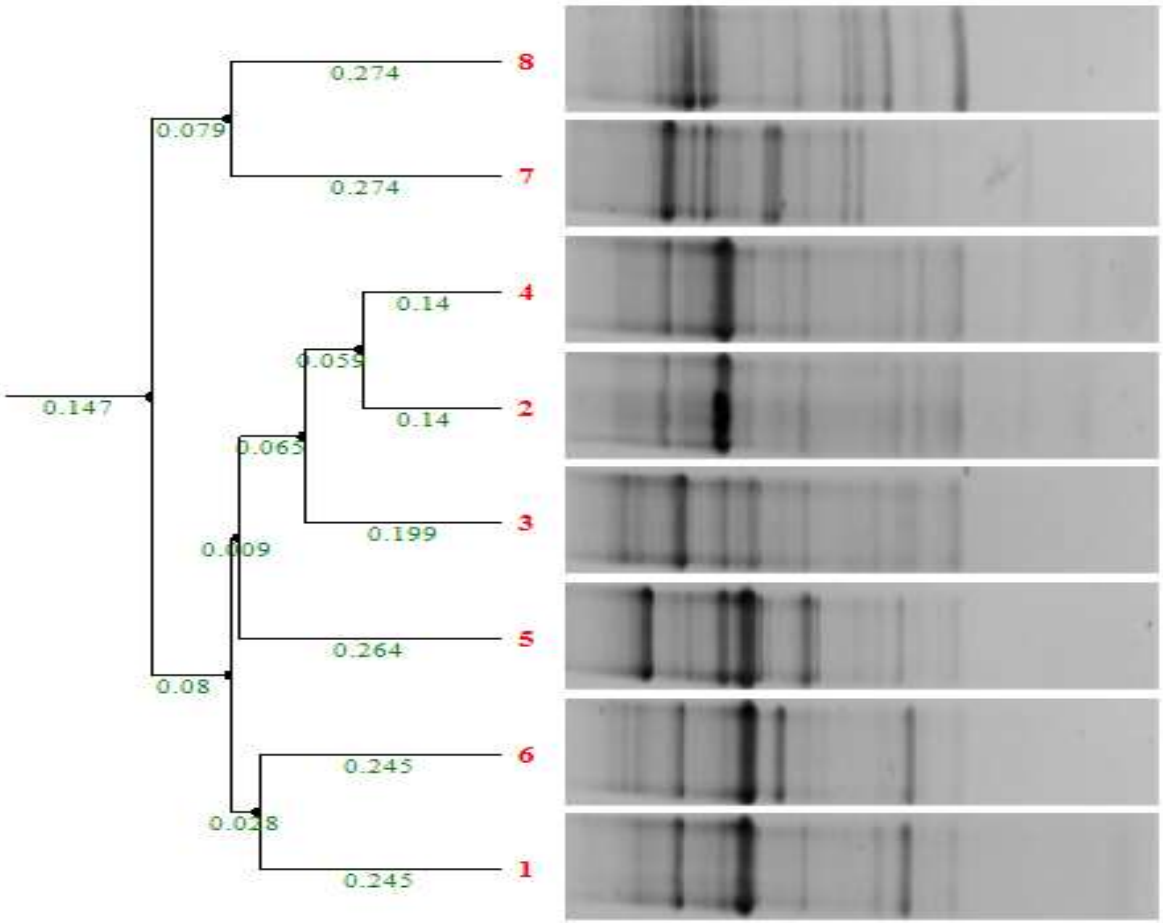

Fig. 4.13. Enterobacterial Repetitive Intergenic Consensus profiles of Enterobacteriaceae strains from OS showing ERIC profiles of the 8 strains isolated from OS. The strain names and other metadata of the dendograms are provided in Appendix XI.

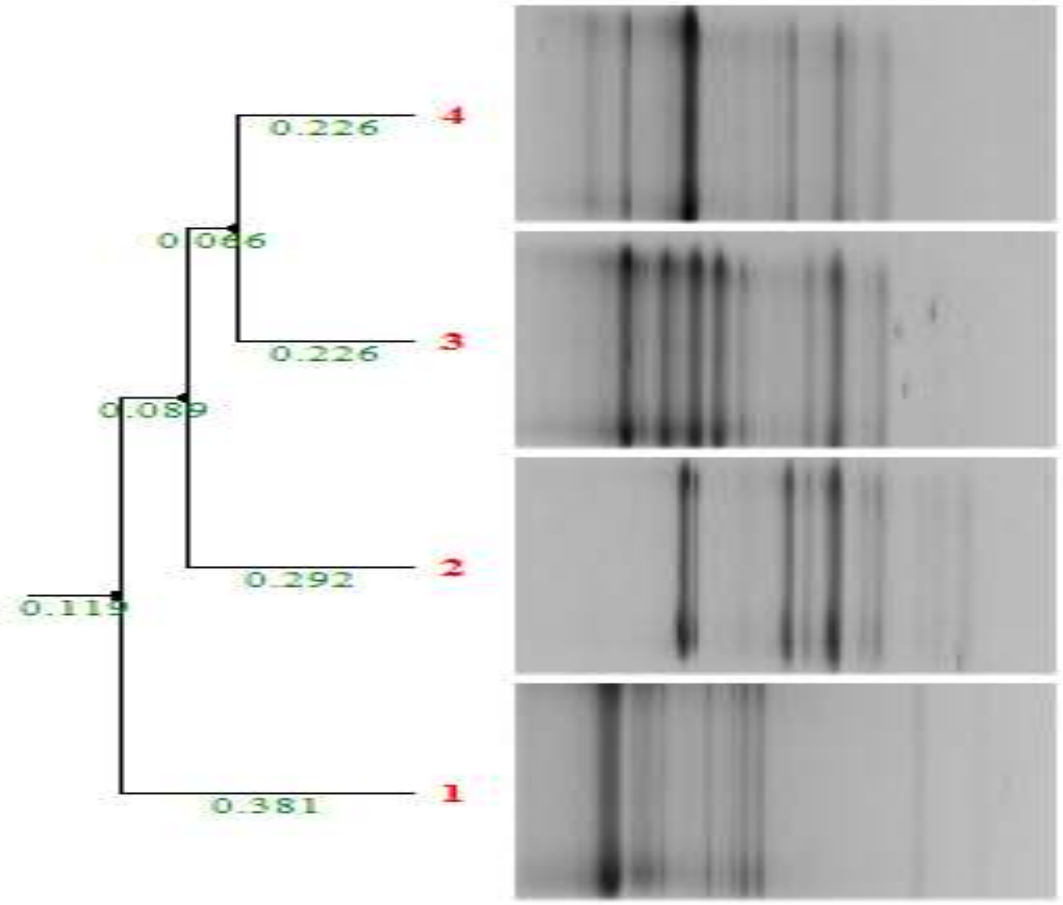

Fig. 4.14. Enterobacterial Repetitive Intergenic Consensus profiles of Enterobacteriaceae strains from AW1 showing ERIC profiles of the 4 strains isolated from AW1. The strain names and other metadata of the dendograms are provided in Appendix XI.

4.10 Multi-Locus Sequence Types

Using the Achtman's 7-gene MLST scheme (*recA*, *purA*, *gyrB*, *fumC*, *adk*, *mdh*, and *icd*), a sum of 33 different Sequence Types (STs) were detected amongst the sequenced *E. coli* strains (Fig. 4.15). The *E. coli* strains belonged to nine (9) clonal complexes (CC), which included CC10, CC206, CC398, CC226, CC38, CC522, CC156, CC165 and CC394. The clonal complex CC10 were the largest represented by the following STs; ST-10, ST-215, ST-167, ST-1721, ST-207, ST-218 and ST-8677. Other clonal complexes included the following STs, CC206 (ST-206), CC398 (ST-398) CC226 (ST-226), CC38 (ST-38), CC522 (ST-541), CC156 (ST-156), 165 (ST-165) and CC394 (ST-394) (Appendix XII).

The most common sequence types were ST-10 and ST-215 having 10 and 8 *E. coli* as members respectively. Five novel *E. coli* sequence types were detected among the isolates and curated on Enterobase. These sequence types include ST-9428 (*E. coli* EC6), ST-9815 (*E. coli* EC31), ST-9816 (*E. coli* EC42), ST-9817 (*E. coli* EC69, EC73 and EC75) and ST-9897 (*E. coli* EC70). The sequence types of the *Citrobacter freundii* were ST-116 (*C. freundii* EC11) and ST-104 (*C. freundii* EC21 and *C. freundii* EC35). Currently, the Centre for Genomic Epidemiology has no MLST database for the classification of the other species of *Citrobacter*, *Enterobacter*, *Kluyvera* and *Leclercia* obtained from this study.

4.11 Phylogeny and Genome comparison of the Enterobacteriaceae isolates by SNP

Genetic variation occurring within the *E. coli* strains are presented as Single Nucleotide Polymorphism (SNP) based phylogenetic tree in Fig. 4.16. SNP trees were created and rooted using *Escherichia coli* k12 MG1655 as reference. A total of 3328437 variant positions were found in all the *E. coli* genomes. SNP pair counts of 114756 was used to infer the tree (Fig. 4.16). The smallest and largest SNPs difference was observed between *E. coli* ST-10 isolates (in a pairwise genome comparison) was 0 and 11495. Strains EC25 and EC16 which showedSNP difference of 0 were obtained from samples AR and AW1 respectively. For the ST215, the detected smallest and largest SNPs differences between the isolates (in apairwise genome comparison) was 4

(between EC17 and EC26) and 11722 (EC19 and *E.coli* k12). EC8 and EC19were the most

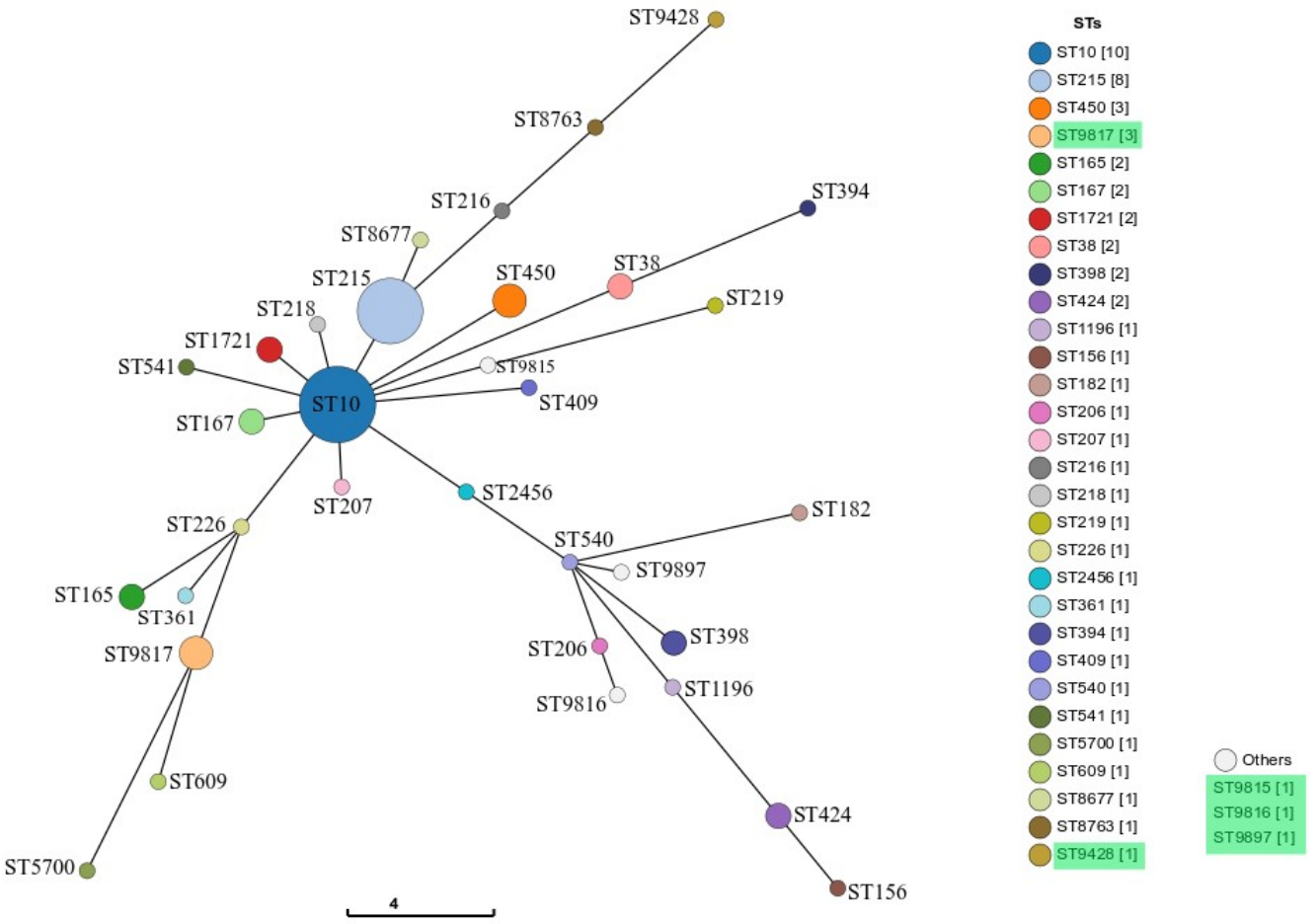

Fig. 4.15. Grape Tree of MLST profiles of the *E. coli* strains. Tree shows evolutionary relationship of the various sequence types of *E. coli* strains. All STs appear to be close or distant relatives of ST10. Novel STs are highlighted in light green rectangular boxes.

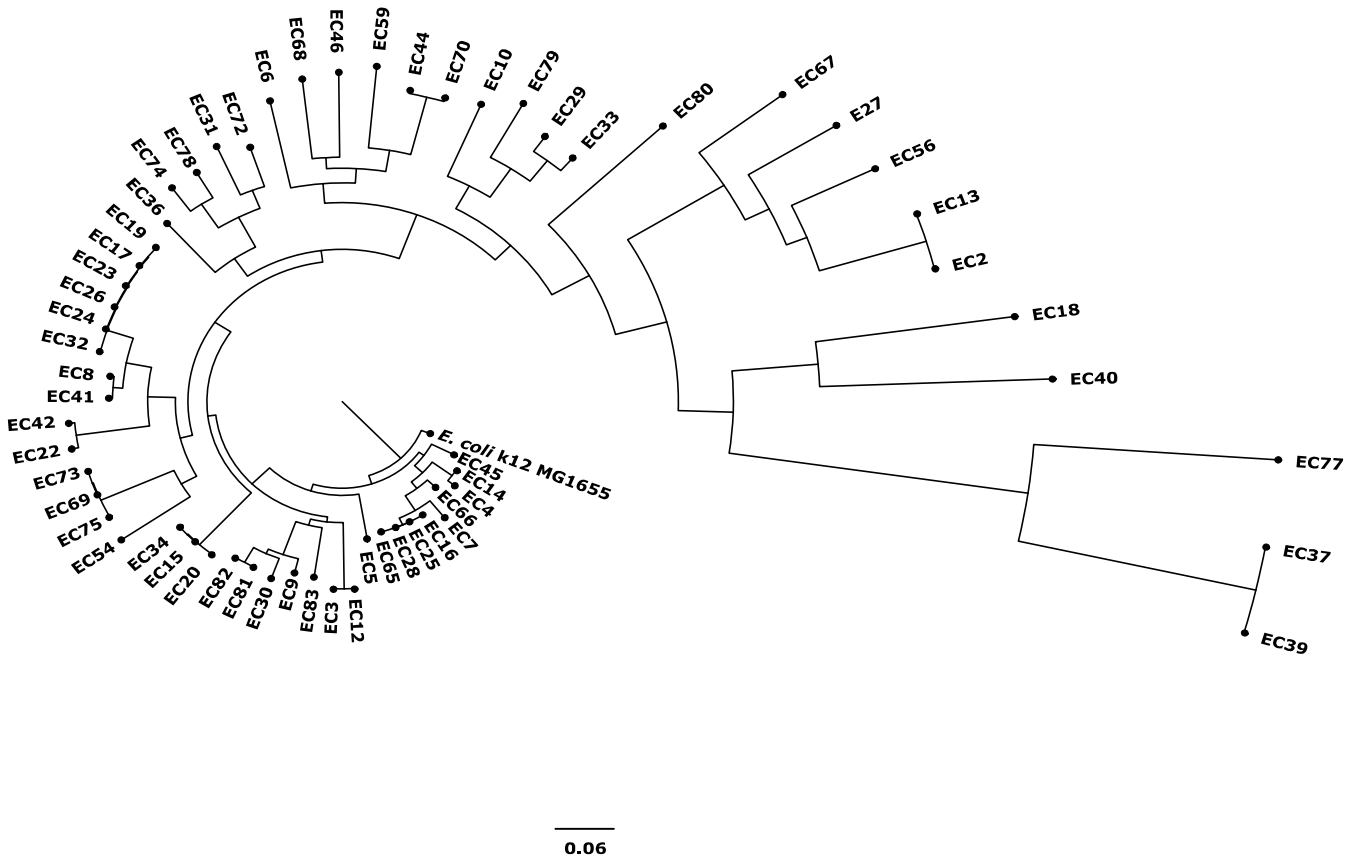

Fig. 4.16. Single nucleotide polymorphism (SNP) genotyping of the *E. coli* strains. The tree was rooted using *Escherichia coli* k12 MG1655. Bootstrap is 100% at all the nodes. Approximate distance between sequences is represented by horizontal bar.

divergent amongst the *E. coli* ST215 from this study having SNP pair count of4209. Strains from clonal complex CC10, including ST10 (EC5, EC7, EC16, EC25, EC28, EC45, EC65 and EC66) and ST167 (EC4 and EC14) were the closest members to the wild type *E. coli* k12 MG1655, with SNPs pair count ranging from 3435 (EC66) to 13409 (EC68).

SNP based phylogenetic trees for *Enterobacter* spp. and *Citrobacter* spp. were rooted using *Enterobacter cloacae* subsp. cloacae ATCC 13047 and *Citrobacter freundii* CFNIH1 as reference strains respectively. Of the 10 *Enterobacter* nucleotide sequences, 316481 variant positions were found in all the analysed genomes, while SNP pair counts of 10655 were used to infer the evolutionary tree (Fig. 4.17). Amongst the *Enterobacter* strains, the observed minimum and maximum SNPs difference in a pairwise genome comparison was 9 (between EC61 and EC64) and 5543 (between EC60 and EC63). Similarly, the 7 *Citrobacter* nucleotide sequences showed 3679365 variant positions when mapped against the reference genome, SNP pair counts of 115255 were used to infer the evolutionary tree (Fig. 4.18). The observed minimum and maximum SNPs difference amongst the *Citrobacter* strains in a pairwise genome comparison was 4 (between EC47 and EC49) and 76572 (between EC11 and EC71).

4.12 Antibiotic resistance genes in the genomes of the enterobacterial isolates

A total of 51 ARGs specifying resistance to 10 antibiotic classes were observed in 98.79% of the sequenced Enterobacteriaceae strains (Fig. 4.19). All the sequenced strains except *Kluyvera* sp. EC51 possessed at least one ARG. The frequency of occurrence of the resistance genes in the *Enterobacter*iacae strains were, tetracyclines; *tetA* (75%), *tetB* (5.36%)), *tetC* (1.79%), *tetD* (1.79%), aminoglycosides; *strA* (41.07%), *strB* (44.64%), *aadA1* (17.86), *aadA2* (16.07%), *aadA5* (8.93%), *aadA8b* (1.79%), *aadA24* (3.57%), *aac(3)-IId* (7.14%), fluoroquinolone and aminoglycoside; *aac(6')Ib-cr* (3.57%), trimethoprim; *dfrA1* (5.36%), *dfrA5* (1.79%), *dfrA7* (1.79%), *dfrA12* (16.07%), *dfrA14* (33.93%), *dfrA15* (3.57%), *dfrA17* (10.71%), β-lactams; $bla_{\text{TEM-1B}}$ (57.14%), $bla_{\text{TEM-1C}}$ (5.35%), $bla_{\text{MIR-1}}$ (5.36%), $bla_{\text{MIR-3}}$ (1.79%), $bla_{\text{MIR-5}}$ (3.57%), $bla_{\text{MIR-6}}$ (1.79%), $bla_{\text{OXA-1}}$ (14.29%), $bla_{\text{CMY-100}}$ (3.57%), $bla_{\text{CMY-129}}$ (3.57%), $bla_{\text{CMY-135}}$ (1.79%), $bla_{\text{HERA-8}}$ (1.79%), $bla_{\text{ACT-7}}$ (1.79%), $bla_{\text{CTX-M-15}}$ (1.79%),

sulphonamides; *sul1* (28.57%), *sul2* (57.14%), *sul3* (14.29%), quinolones; *qnrS1* (28.57%), *qnrB7* (3.57%),

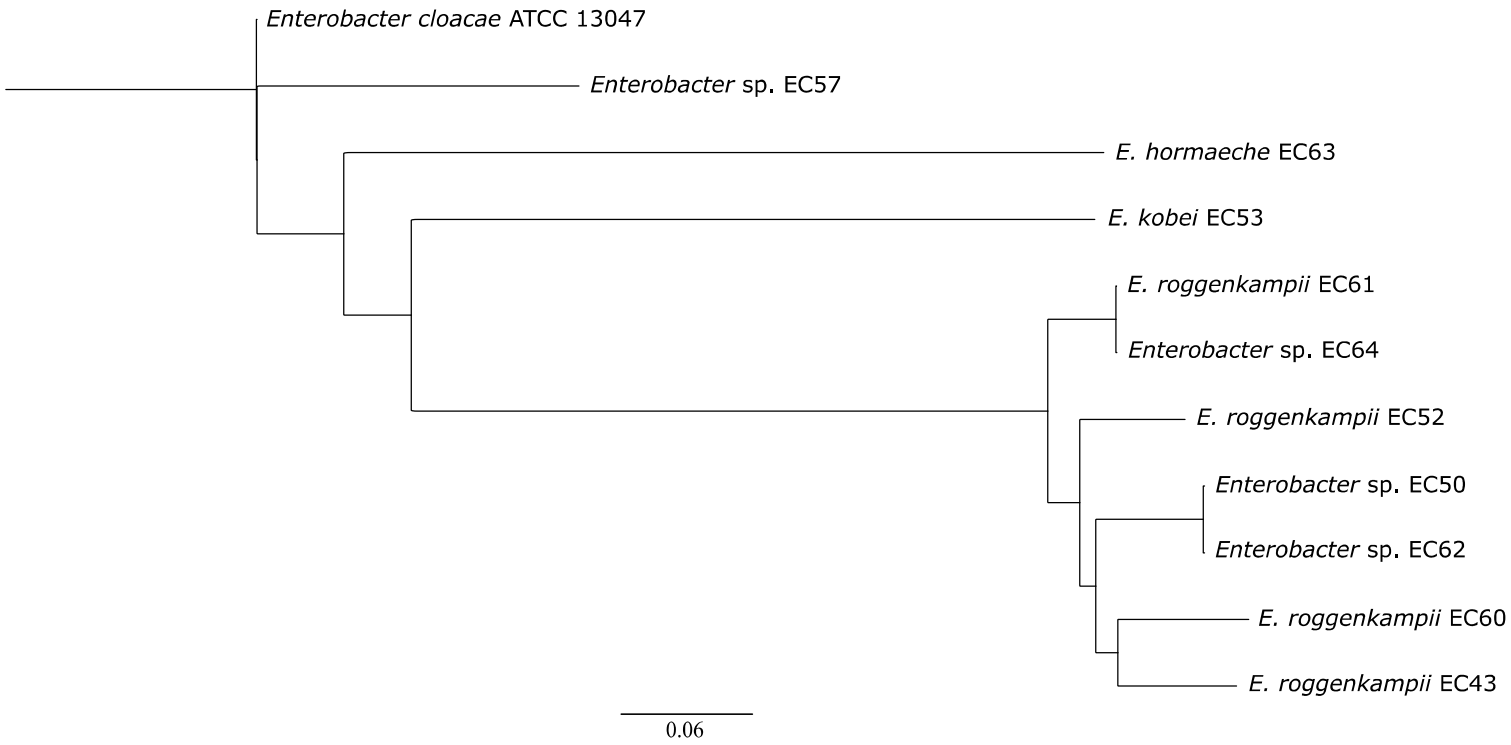

Fig. 4.17. Single nucleotide polymorphism (SNP) genotyping of the *Enterobacter* strains. The tree was rooted using *Enterobacter cloaceae* ATCC 13047. Bootstrap is >92% at all the nodes. Approximate distance between sequences is represented by horizontal bar.

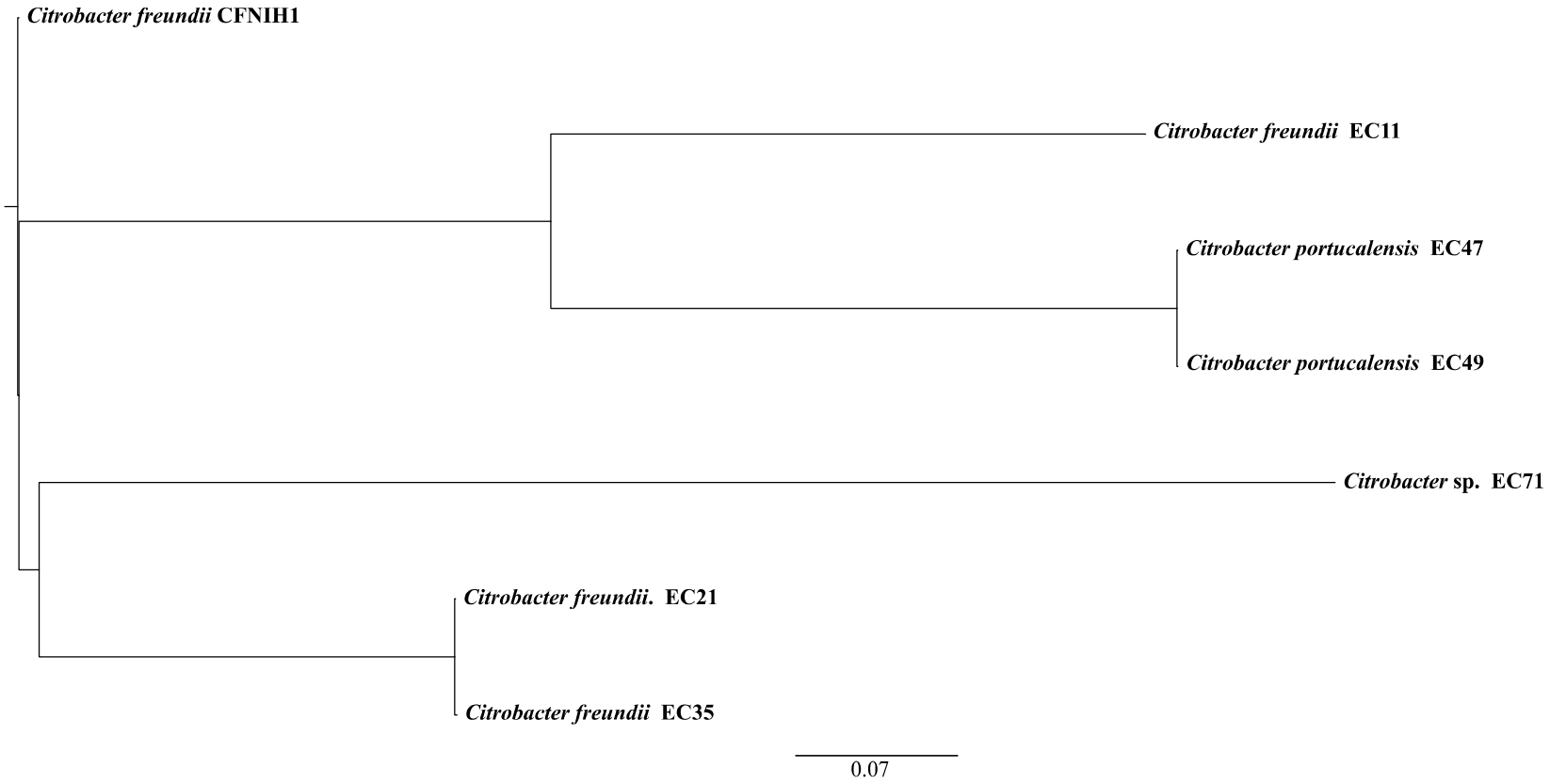

Fig. 4.18. Single nucleotide polymorphism (SNP) genotyping of the *Citrobacter* strains. The tree was rooted using *Citrobacter freundii* CFNIH1. Bootstrap is >100% at all the nodes. Approximate distance between sequences is represented by horizontal bar

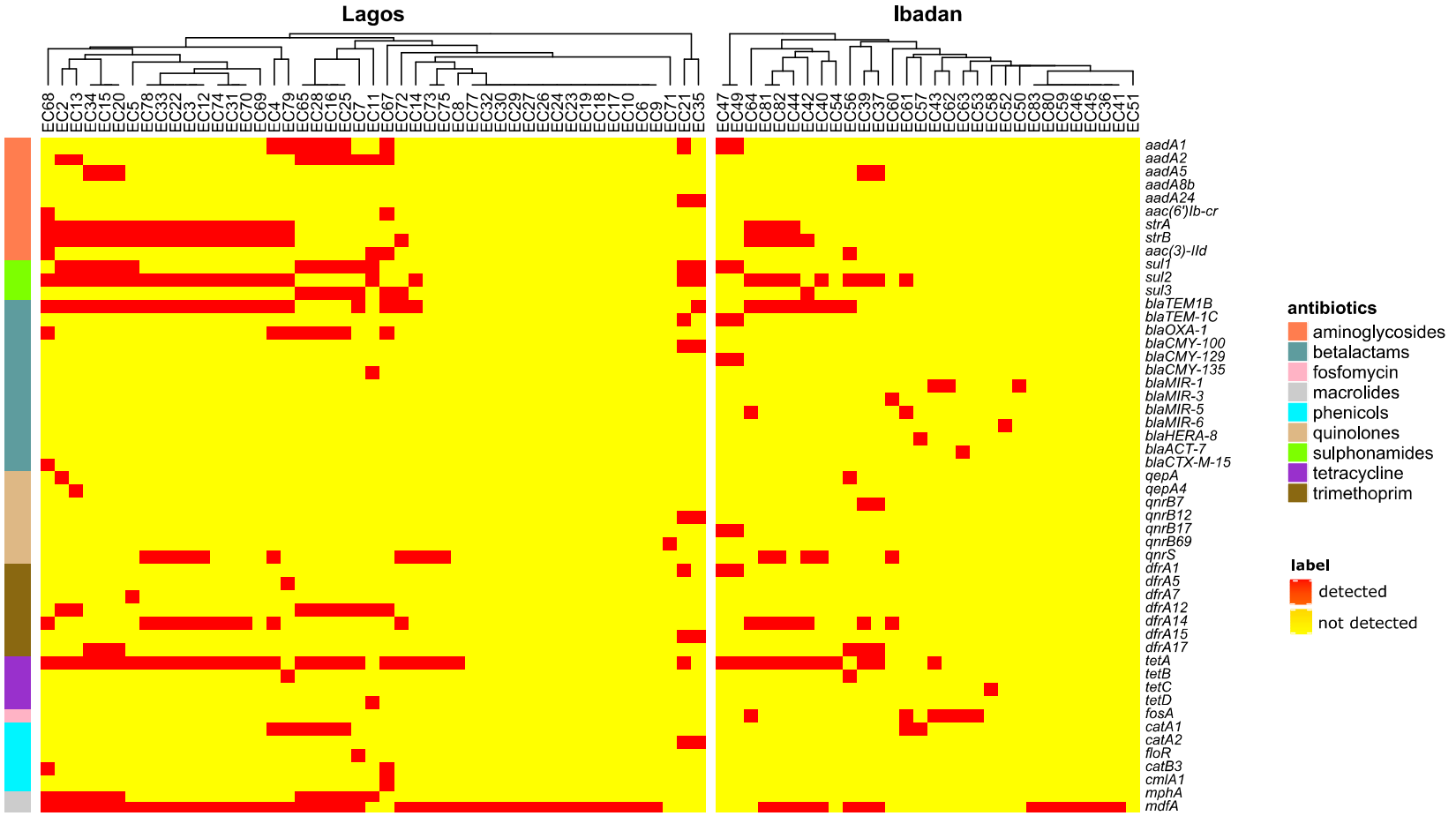

Fig. 4.19.Presence of acquired antibiotic resistance genes conferring resistance to various classes of antibiotics in the Enterobacteriaceae strains isolated from E-wasted dumpsites in Lagos and Ibadan. Hierarchical clustering was done using Euclidean clustering method.

qnrB12 (3.57%), *qnrB17* (3.57%), *qnrB69* (1.79%), *qepA* (1.79%),*qepA4*(1.79%) fosfomycin; *fosA* (12.5%), phenicols; *catA1* (14.29%), *catA2* (3.57%),*catB3* (3.57%), *cmlA1* (1.79%), *floR*(1.79%) and macrolides; *mph(A)* (21.43%) and *mdf(A)* (75.64%) (Appendix XIII).

The macrolide resistance gene *mdf(A)* and tetracycline resistance gene *tetA* were the most frequent ARG, detected in 59 and 42 strains respectively. The *tet* variants, *tetC* and *tetD* were observed to occur only in *Leclercia* sp. EC58 and *C. freundii* EC11 respectively. *C. freundii* EC11 was the sole carrier of $bla_{\text{CMY-135}}$ whereas, *Citrobacter freundii* strains EC21 and EC35 were the sole carriers of *catA2*, $bla_{\text{CMY-100}}$ and *aadA24*. These strains (EC21 and EC35) also harboured *qnrB12* together with *C. portucalensis* EC47 and *C. portucalensis* EC49. However, the resistome of EC21 and EC35 differed, as EC21 additionally harboured *dfrA1*, *tetA*, $bla_{\text{TEM-1C}}$ and *aadA1* which were absent in EC35. The β-lactamases $bla_{\text{TEM-1C}}$ and $bla_{\text{CMY-129}}$ were only found in *C. portucalensis* EC47 and *C. portucalensis* EC49.

The bla_{MIR} β-lactamases were observed to be associated strictly with the *Enterobacter* strains. $bla_{\text{MIR-1}}$ was carried by *E. roggenkampii* EC43, *Enterobacter* sp. EC50 and *Enterobacter* sp. EC62, $bla_{\text{MIR-5}}$ was found in *E. roggenkampii* EC61 and *Enterobacter* sp. EC64 whereas $bla_{\text{MIR-3}}$ and $bla_{\text{MIR-6}}$ were present on *E. roggenkampii* EC60 and *E. roggenkampii* EC52 respectively. Other β-lactamases, $bla_{\text{CTX-M-15,}}$ $bla_{\text{ACT-7}}$ and $bla_{\text{HERA-8}}$ were found occurring only in *Escherichia coli* EC68, *E. hormaeche* EC63 and *Enterobacter* sp EC56 respectively.

4.13 Detected heavy metal resistance determinants among the Enterobacterial isolates

Following local NCBI blast, a plethora of genes conferring resistance mechanisms to metals were discovered in the genomes of the sequenced strains. They comprised of genes specifying resistance to metals including arsenic, cobalt, cadmium, copper, iron, lead, manganese, mercury, nickel, silver, tellurium and zinc. Furthermore, a heatmap showing the distribution of these metal resistance genes in the Enterobacteriaceae strains is presented in Fig. 20.

The resistance genes and frequency of occurrence in the sequenced strains include; *arsA* - arsenic pump driving protein (23.07%), arsenic resistance protein *arsB* and *arsH* at

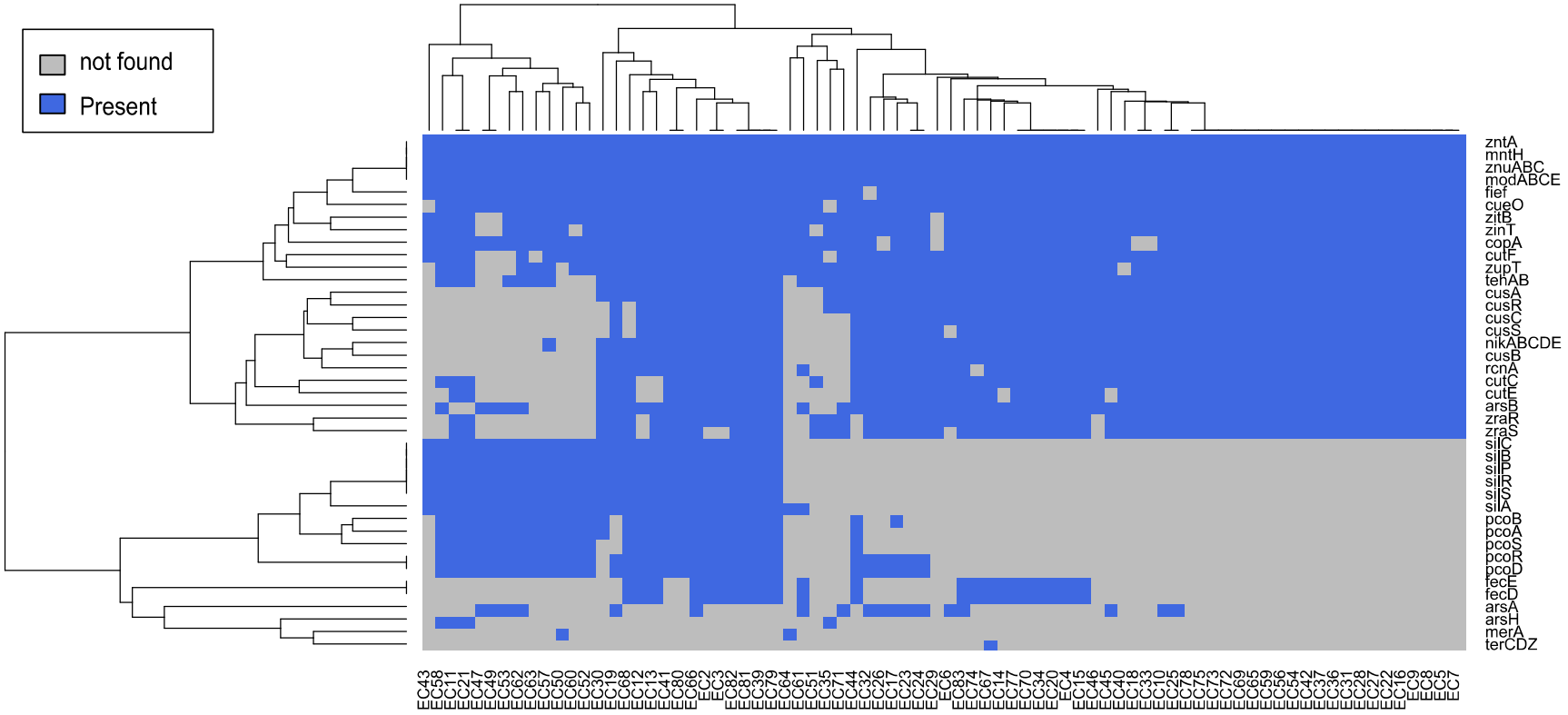

Fig. 4.20. Presence of heavy metals resistance genes among the Enterobacterial strains specifying resistance to various types of heavy metals. Blue boxes indicate their presence whereas grey boxes indicate absence. Hierarchical clustering was done using UPGMA.

85.90% and 5.13% respectively. The *arsH* gene occurred solely in *Citrobacter freundii* strains EC11, EC21, EC35 and *Leclercia* sp. EC 58. The nickel and cobalt efflux gene, *rcnA* was present in 76.92% of the strains, whereas the nickel resistance genes cluster, *nikABCDE* was resent 78.21% of the strains. Divalent metal cation (Mn^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} and Cu^{2+}) uptake system *mntH*, was present in all the strains. The entire copper resistance gene cluster of *pcoABDRS* and *cutCEF* were present in 32.05% and 74.36% of the strains respectively, whereas, copper resistance protein *copA* and periplasmic copper detoxification protein, *CueO*, were respectively present in 94.87% and 97.44% of the sequenced strains respectively. Copper and silver resistance efflux pump gene cluster, *cusABCRS*, were present in 74.36% of the sequenced strains. Similarly, the silver efflux gene cluster *silABCPRS* were detected in 34.62% of the sequenced strains.

A number of zinc associated resistance genes including *zitB* (Zn^{2+}), *znuABC* (Zn^{2+}), *zraSR* (Zn^{2+} and Pb^{2+}), *zinT* (zinc, cadmium, mercury or nickel), *zntA* (zinc lead, cadmium and mercury) and *zupT* (zinc, cadmium and copper) were detected in 96.15%, 100%, 75.64%, 92.30%, 100% and 92.30% of the strains respectively. Further, *merA* which confers bacterial resistance to mercury were present in *Enterobacter* sp. EC50 and *Enterobacter hormaeche* EC64, whereas tellurium, *terCDZ* was present only in *E. coli* EC67. Resistance genes to tellurite, *tehAB* (91.05%), molybdenum, *modABCE* (100%) and iron, *fieF* (98.72%) were also present among the strains.

4.14 Co-presence of ARGs and heavy metal resistance genes (HMRGs) in the genomes of the sequenced isolates

All the sequenced strains possessed multiple HMRGs. In tandem all the ARG harbouring strains possessed multiple metal resistance genes (98.79%). *E. coli* EC12 harbouring ARGs *strA*, *strB*, *sul2*, $bla_{\text{TEM-1B}}$, *qnrS1*, *dfrA14*, *tetA* and *mdfA* also harboured the metal resistance genes *arsB*, *mntH*, *rcnA*, *copA*, *pcoABDRS*, *cusABCRS*, *cueO*, *silABCPRS*, *nikABCDE*, *zntA*, *zitB*, *zinT*, *znuABC*, *zupT*, *tehAB*, *modABCE*, *fecDE*, and *fieF*. Also, *Citrobacter portucalensis* EC47 harbouring ARGs *aadA1*, *sul1*, $bla_{\text{CMY-129}}$, $bla_{\text{TEM-1C}}$, *qnrB17*, *dfrA1*, and *tetA* also carried metal resistance genes *arsA*, *arsB*, *mntH*, *copA*, *pcoABDRS*, *cueO*, *silABCPRS*, *zntA*, *znuABC*, *modABCE* and *fieF*. Similarly, *Enterobacter* sp EC64 harboured *strA*, *strB*, *sul2*, $bla_{\text{MIR-5}}$, $bla_{\text{TEM-1B}}$,

dfrA14, *tetA*, *fosA* and metal resistance genes *mntH*, *copA*, *cueO*, *cutF*, *silA*, *zntA*, *zitB*, *zinT*, *znuABC*, *zupT*, *modABCE*, *fieF* and *merA*.

4.15 Plasmid Replicon Types

A total of 92.31% (72) of the sequenced strains carried at least one plasmid replicon type, with 80.55% of the strains carrying more than one plasmid replicon type. The plasmid replicons were randomly distributed in the strains across the various sampling sites. The frequency of occurrence of the plasmid replicon types among the sequenced strains are shown as nested boxes in Fig. 4.21. The most frequently occurring plasmid types amongst the strains were the Col plasmids present in 66.67% (48) of the strains, however, the IncF (52.78%) plasmids were the most diverse, represented by 16 replicons randomly distributed in the Enterobacteriaceaestrains. The replicons include IncFIA, IncFIA(HI1), IncFIB(K), IncFIB(pHCM2), IncFIB(pECLA), IncFIB(pB171), IncFIB(pCTU3), IncFIB(pQil), IncFIB(AP001918), IncFIC(FII), IncFII(pRSB107), IncFII, IncFII(pSE11), IncFII(pECLA), IncFII(29) and IncFII(Yp). Other plasmid types present in the strains were IncY (29.17%), IncR (23.61%), IncI (5.56%), IncQ (5.56%), IncX1 (4.17%), IncH (4.17%), TrfA (4.17%), p0111 (2.78%), IncB/O/K/Z (1.39%), IncN (1.39%).

Strains such as EC10, EC17, EC23, EC26, EC27, EC30, EC32, EC36, EC41, EC51, EC80 and EC83 which possessed no acquired antibiotic resistance genes, however carried a plethora of plasmid replicon types (Appendix XIV). Plasmid type, TrfA, were carried only by *Citrobacter* strains, EC21, EC35 and EC71, whereas the IncQ plasmids were associated primarily with *E. coli* strains, EC4, EC56, EC68 and EC79. All the IncX1 plasmid replicon were found together with ColRNA1 plasmids in *E. coli* strains EC13, EC42 and EC72, while the IncN and IncB/O/K/Z plasmid replicons were present only in *Enterobacter* sp. EC62 and *E. coli* EC2 respectively. The p0111 plasmid replicon group were present only in *E. coli* EC10 and *E. coli* EC22.

4.16 qPCR quantification of *E. coli* (*uidA*) in soil and water samples

Quantitative PCR enumeration of *E. coli* abundance using the *uidA* as gene marker in soil (copy number/gram) and water samples (copy number/100 ml) showed *E. coli* was below level of quantification in 56% of the samples including hand-dug wells (AW1II, AW1III, AW2I, AW2II, AW2III, UW1III, UW2I, UW2II and UW2III), boreholes

(ASB1, ASB2, IKB1, IKB2 and IKB3) and soil (AL1, AL2, AS2, AS3, OS1, OS2, OS3 and ARU3). However, in water samples where *E. coli* was quantified, *uidA* absolute

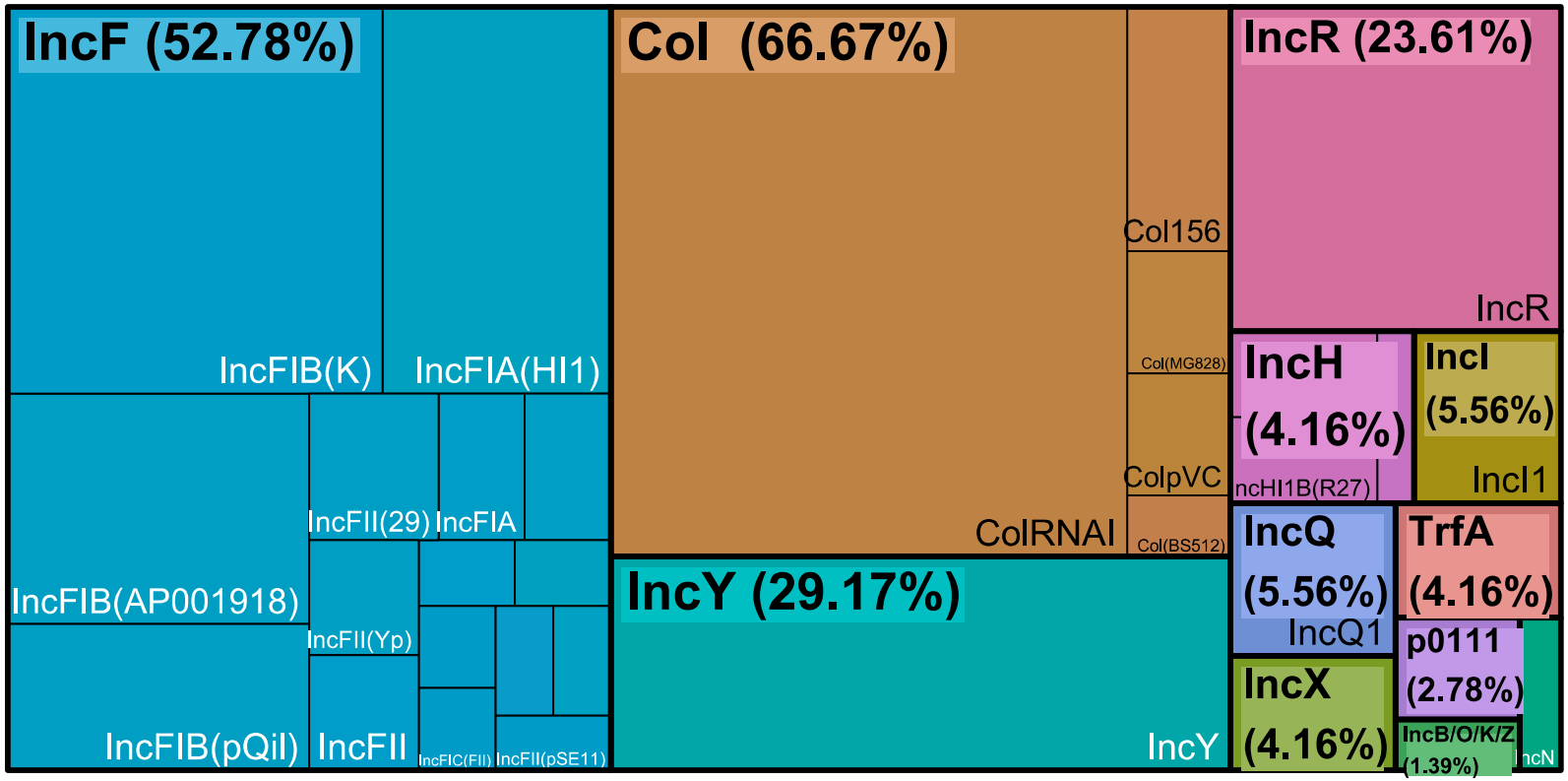

Fig. 4.21. Occurrence of different plasmid replicon types in the bacteria strains (n=72). Six (6) strains had no plasmid. Sizes of nested boxes represent frequency of occurrence of the plasmids in the strains.

gene abundance ranged from $2.02\times 10^5 \pm 4.33\times 10^4$ (AR3) to $2.35\times 10^4 \pm 1.06\times 10^4$ (AW1I). Similarly, absolute *uidA* abundance ranged from $1.51 \times 10^5 \pm 1.31\times 10^4$ (IKJ3) to $5.66\times 10^4 \pm 2.14\times 10^3$ (AS1) in the soils. Furthermore, the 16S relative (normalized) abundances of *uidA* in all the samples in which the gene was quantified ranged from 10^{-4} to 10^{-5}, indicating *E. coli* was present as 1 in 10000 and 1 in 100000 bacteria respectively in the samples. The absolute abundance (± standard deviation) of 16S rRNA and *uidA* and relative abundance of *uidA* in samples in which the gene quantified are presented in Table 4.7.

4.17 qPCR quantification of ARGs and *intI1* in soil and water samples from E-waste dumpsites

The absolute abundances of ARGs and *intI1* are calculated and presented as either copy numbers per gram (soil samples) or copy numbers per 100 ml (water samples). Each of the samples showed unique and varying copy numbers of the quantified genes. Quantitative PCR (qPCR) efficiencies were 88.10% for 16S rRNA, 92.02% for *intI1*, 94.85% for *sul1*, 99.85% for *sul2*, 84.38% for *dfrA1*, 96.73% for *tetA* and 93.61% for $bla_{\text{CTX-M-1}}$ in the soil samples, whereas in the water samples, qPCR efficiencies were 82.41% for 16S rRNA, 92.48% for *intI1*, 90.22% for *sul1*, 91.75% for *sul2*, 83.87 for *dfrA1*, 97.89% for *tetA* and 95.01% for $bla_{\text{CTX-M-1}}$. The quantified abundance of 16S rRNA, ARGs and *intI1* in the samples from the sampling sites is described below. The absolute abundance ± standard deviated of the quantified genes in water and soil samples are presented in Appendix XVI and XVII respectively

Table 4.7. Absolute (± standard deviation) and relative (16S normalized) abundance of *E. coli* (*uidA*) in the soil and water sample from E-waste dumpsite. *E. coli* was below level of quantification in 56% of the samples.

S/No	Sample	Type of sample	16S rRNA gene abundance	Absolute *uidA* abundance	Relative abundance (*uidA*/16S rRNA) of *uidA*
1	AL3	Soil	$1.90 \times 10^8 \pm 9.32 \times 10^6$	$7.39 \times 10^4 \pm 1.90 \times 10^3$	3.90×10^{-4}
2	AR1	River	$1.32 \times 10^9 \pm 1.03 \times 10^8$	$8.57 \times 10^4 \pm 1.39 \times 10^3$	6.50×10^{-5}
3	AR2	River	$2.88 \times 10^9 \pm 1.03 \times 10^8$	$5.69 \times 10^4 \pm 1.69 \times 10^4$	1.98×10^{-5}
4	AR3	River	$8.26 \times 10^9 \pm 5.08 \times 10^8$	$2.02 \times 10^5 \pm 4.33 \times 10^4$	2.45×10^{-5}
5	AW1I	Hand-dug well	$2.33 \times 10^8 \pm 2.70 \times 10^6$	$2.35 \times 10^4 \pm 1.06 \times 10^4$	1.01×10^{-4}
6	AS1	Soil	$2.42 \times 10^8 \pm 2.38 \times 10^7$	$5.66 \times 10^4 \pm 2.14 \times 10^3$	2.33×10^{-4}
7	ASB3	Borehole water	$2.13 \times 10^8 \pm 2.31 \times 10^6$	$2.91 \times 10^4 \pm 3.98 \times 10^3$	1.36×10^{-4}
8	IKJ1	Soil	$5.92 \times 10_8 \pm 1.02 \times 10^8$	$1.30 \times 10^5 \pm 1.29 \times 10^4$	2.20×10^{-4}
9	IKJ2	Soil	$2.85 \times 10^8 \pm 6.38 \times 10^7$	$9.36 \times 10^4 \pm 1.37 \times 10^4$	3.29×10^{-4}
10	IKJ3	Soil	$6.47 \times 10^8 \pm 4.58 \times 10^7$	$1.51 \times 10^5 \pm 1.31 \times 10^4$	2.34×10^{-4}
11	OR1	River	$5.83 \times 10^8 \pm 3.30 \times 10^7$	$4.88 \times 10^4 \pm 1.11 \times 10^4$	8.37×10^{-5}
12	OR2	River	$4.05 \times 10^8 \pm 5.75 \times 10^6$	$5.59 \times 10^4 \pm 9.88 \times 10^3$	1.38×10^{-4}
13	OR3	River	$2.10 \times 10^8 \pm 4.41 \times 10^6$	$5.01 \times 10^4 \pm 1.37 \times 10^4$	2.38×10^{-4}
14	ARU1	Soil	$1.97 \times 10^8 \pm 9.66 \times 10^6$	$1.38 \times 10^5 \pm 2.09 \times 10^4$	7.00×10^{-4}
15	ARU2	Soil	$5.44 \times 10^8 \pm 3.98 \times 10^7$	$7.43 \times 10^4 \pm 6.36 \times 10^3$	1.37×10^{-4}
16	UW1I	Hand-dug well	$2.69 \times 10^8 \pm 2.24 \times 10^7$	$2.96 \times 10^4 \pm 7.31 \times 10^3$	1.10×10^{-4}
17	UW1II	Hand-dug well	$2.95 \times 10^8 \pm 9.99 \times 10^6$	$3.72 \times 10^4 \pm 3.67 \times 10^3$	1.26×10^{-4}

4.18 Absolute abundance of ARGs and *intI1* in samples from Alaba international market E-waste dumpsite

4.18.1 Absolute gene abundance in sample AL

16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA* genes were quantified in samples obtained in the 3 sampling periods, however, $bla_{\text{CTX-M1}}$ was below level of quantification in all the samples analysed. The log transformed copy number of 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA* per 1 gram of AL are presented in Fig. 4.22. The highest gene copy numbers ± standard deviation was observed with *sul2* and *sul1* at $3.17 \times 10^7 \pm 4.20 \times 10^5$ and $2.75 \times 10^7 \pm 8.02 \times 10^5$ respectively during the 3rd sampling period, whereas, *intI1* ranged from $7.52 \times 10^6 \pm 2.29 \times 10^5$ to $1.17 \times 10^7 \pm 1.04 \times 10^6$, *dfrA1* ranged from $1.20 \times 10^6 \pm 6.91 \times 10^5$ to $2.77 \times 10^6 \pm 9.65 \times 10^5$. The lowest gene copy was observed with *tetA* which ranged from $1.47 \times 10^5 \pm 2.04 \times 10^4$ to $6.07 \times 10^5 \pm 5.40 \times 10^4$.

4.18.2 Absolute gene abundances in sample AR

16S rRNA, *intI1*, *sul1, sul2*, *dfrA1*, *tetA* and $bla_{\text{CTX-M-1}}$ were quantified in sample AR obtained in the 3 sampling periods. The log transformed mean copy number of 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA* per 100 ml of sample AR are presented in Fig. 4.23. The highest mean gene copy numbers ± standard deviation of *intI1*, *sul1*, *sul2*, *dfrA1* and $bla_{\text{CTX-M-1}}$ were $2.61 \times 10^7 \pm 5.32 \times 10^5$, $2.61 \times 10^8 \pm 3.05 \times 10^6$, $1.16 \times 10^8 \pm 1.62 \times 10^7$, $1.12 \times 10^8 \pm 5.06 \times 10^6$ and $1.08 \times 10^5 \pm 8.01 \times 10^4$respectively, observed during the second sampling period, whereas *tetA*ranged from $1.28 \times 10^6 \pm 8.66 \times 10^4$ to $9.62 \times 10^4 \pm 1.42 \times 10^4$.

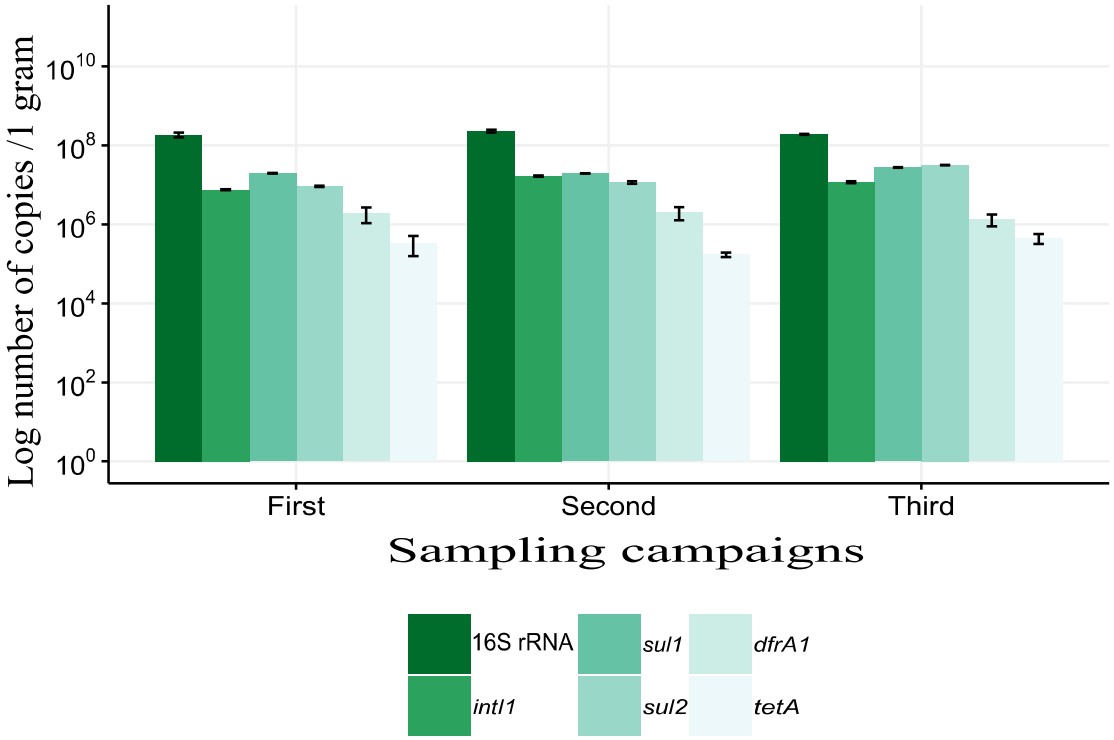

Fig. 4.22. Mean absolute abundance (copy number/ 1 gram) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1* and *tetA* in the metagenomic DNA of soil sample AL.

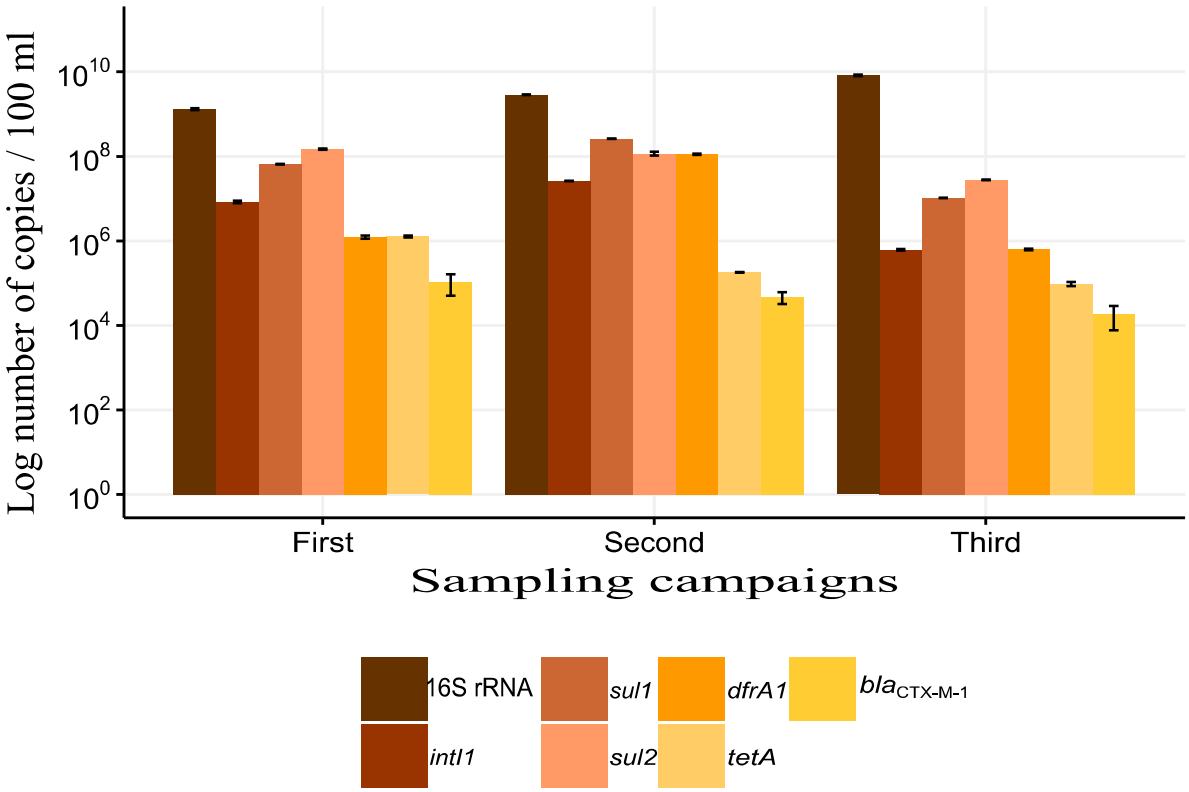

Fig. 4.23. Mean absolute abundance (copy number/ 100 ml) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1*, *tetA and* $bla_{CTX-M-1}$ in the metagenomic DNA of water sample AR.

4.18.3 Absolute gene abundances in sample AW1

The β-lactamase $bla_{\text{CTX-M-1}}$ was below level of quantification in the samples collected from AW1. The log transformed mean number of copies of 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA* per 100 ml of sample AW1 are presented in Fig. 4.24. Abundance of *intI1* and *sul1* were very similar across the 3 sampling periods. The absolute mean concentration ± standard deviation of *intI1* and *sul1* during the sampling periods was $4.52 \times 10^5 \pm 4.09 \times 10^4$ and $2.59 \times 10^6 \pm 3.94 \times 10^5$ respectively. The mean copy numbers ± standard deviation of *sul2*, *dfrA1* and *tetA* ranged from $4.10 \times 10^7 \pm 9.12 \times 10^6$ to $2.29 \times 10^6 \pm 1.54 \times 10^4$, $8.87 \times 10^5 \pm 2.26 \times 10^4$ to $7.17 \times 10^4 \pm 7.59 \times 10^3$ and $4.68 \times 10^4 \pm 1.17 \times 10^4$ to $2.23 \times 10^4 \pm 3.25 \times 10^3$ respectively.

4.18.4 Absolute gene abundances in sample AW2

16S rRNA, *intI1*, *sul1, sul2* were quantified in sample AW2 obtained during the 3 sampling periods. However, *dfrA1* was below level of quantification in the second sampling period, whereas *tetA* could only be quantified in the 3rd sampling period. The β-lactamase $bla_{\text{CTX-M-1}}$ was below level of quantification in the samples collected from AW2. The log transformed mean copy number ± standard deviation of *intI1*, *sul1, sul2*, *dfrA1* and *tetA* per 100 ml of sample AW2 are presented in Fig. 4.25. Mean copy numbers ± standard deviation of *intI1*, *sul1* and *sul2* ranged from $7.27 \times 10^5 \pm 2.85 \times 10^4$ to $5.93 \times 10^4 \pm 2.45 \times 10^3$, $6.04 \times 10^6 \pm 3.20 \times 10^5$ to $1.82 \times 10^5 \pm 7.28 \times 10^4$ and $2.81 \times 10^7 \pm 1.48 \times 10^6$ to $1.90 \times 10^6 \pm 1.08 \times 10^6$ respectively. Tetracycline resistance gene, *tetA* had a mean copy number of $2.49 \times 10^5 \pm 2.80 \times 10^5$ in the 3rd sampling period, whereas *dfrA1* was $1.08 \times 10^5 \pm 1.14 \times 10^3$ and $1.79 \times 10^5 \pm 1.02 \times 10^4$ during the 1st and 3rd sampling periods.

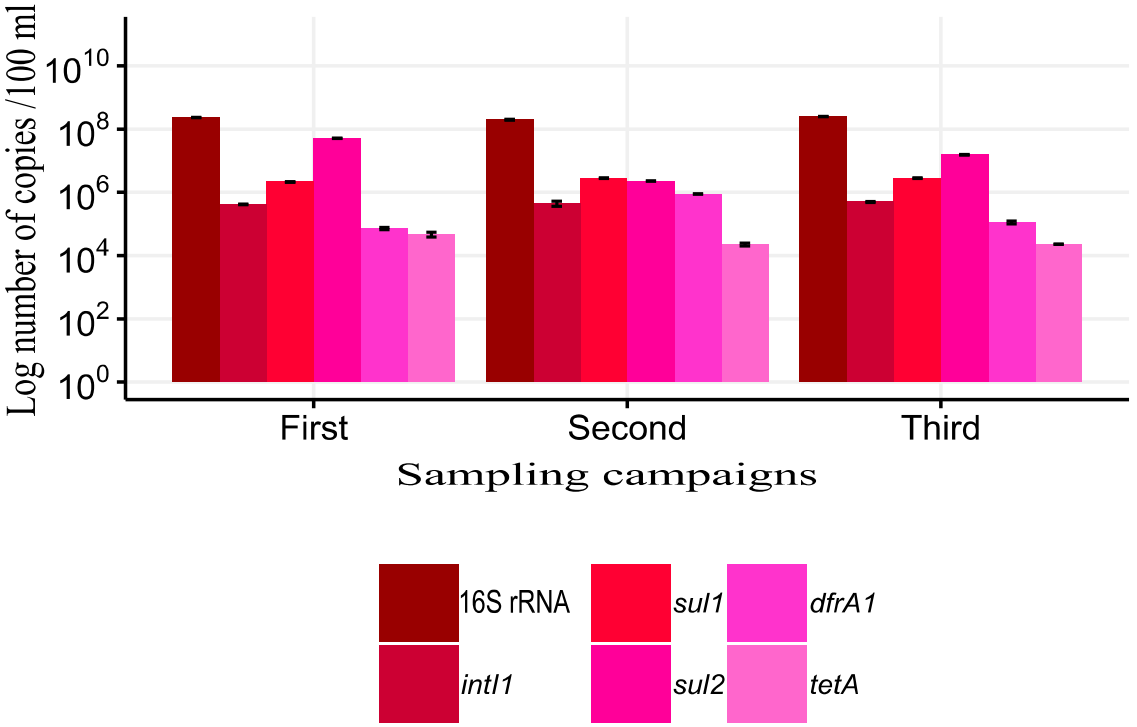

Fig. 4.24. Mean absolute abundance (copy number/ 100 ml) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1* and *tetA* in the metagenomic DNA of water sample AW1.

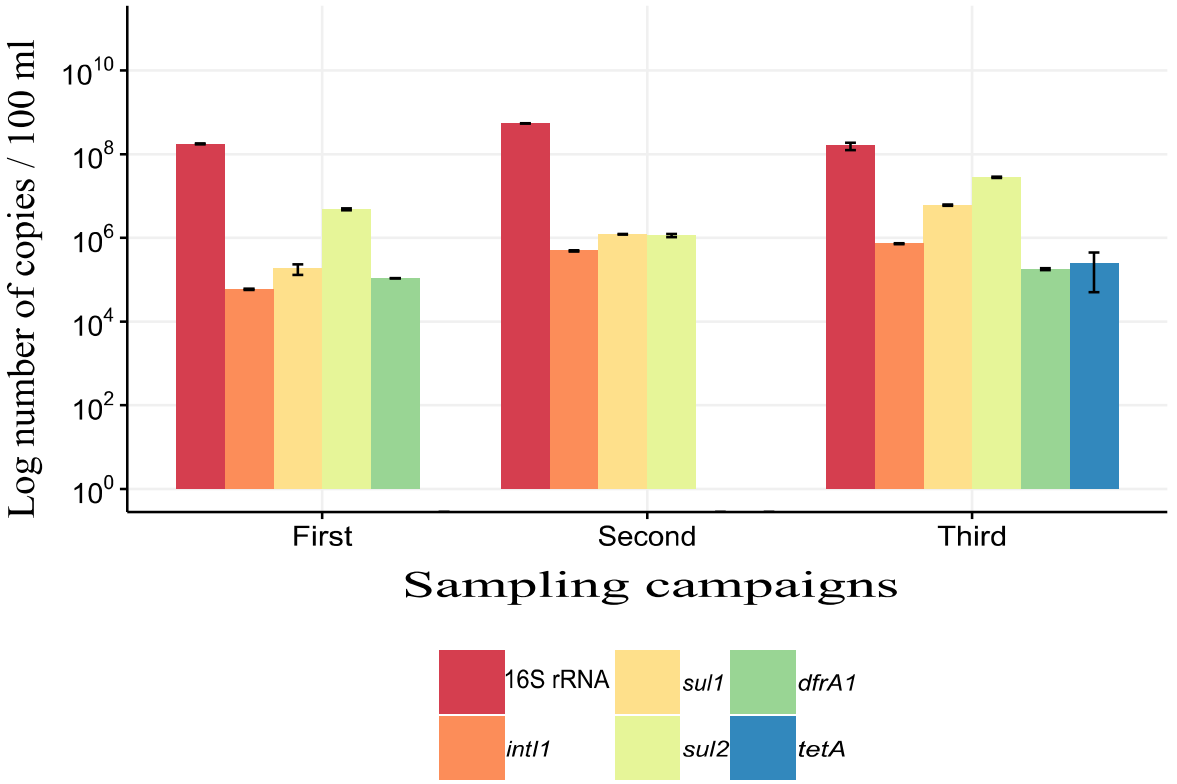

Fig. 4.25. Mean absolute abundance (copy number/ 100 ml) of 16S, *intI1*, *sul1*, *sul2*, *dfrA1* and*tetA* in the metagenomic DNA of water sample AW2.

4.19 Absolute abundance of ARGs and *intI1* in samples from Ikeja computer village E-waste dumpsite

4.19.1 Absolute gene abundances in sample IKJ

In sample IKJ, 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA* genes were quantified in samples from the three sampling periods, however, $bla_{\text{CTX-M-1}}$ was below level of quantification in all the samples. The log transformed mean gene copy number of 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA* per gram of sample IKJ are presented in Fig. 4.26. The number of copies of *sul1* and *sul2* were similar across the sampling periods. The absolute mean copy numbers ± standard deviation of *sul1 and sul2* at all the sampling periods were $2.53 \times 10^7 \pm 1.21 \times 10^7$ and $3.06 \times 10^7 \pm 1.39 \times 10^7$ respectively. The mean of gene copies ± standard deviation of *intI1*, *dfrA1* and *tetA* ranged from $4.29 \times 10^7 \pm 5.15 \times 10^6$ to $7.39 \times 10^6 \pm 3.57 \times 10^5$, $1.98 \times 10^6 \pm 6.01 \times 10^5$ to $4.75 \times 10^5 \pm 7.27 \times 10^3$ and $3.36 \times 10^5 \pm 3.08 \times 10^4$ to $2.57 \times 10^5 \pm 9.16 \times 10^3$ respectively.

4.19.2 Absolute gene abundances in sample IKB

In sample IKB, *tetA* obtained from the first and third sampling periods was below level of quantification, whereas $bla_{\text{CTX-M-1}}$ was below level of quantification in samples from the third sampling period. Other quantified genes including 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* were present in all samples from IKB. Further, the log transformed mean number of copies of ARG and *intI1* per 100 ml of IKB are presented in Fig. 4.27. The mean gene copy numbers ± standard deviation were $2.80 \times 10^5 \pm 4.61 \times 10^3$ to $4.84 \times 10^4 \pm 6.22 \times 10^3$(*intI1*), $1.69 \times 10^6 \pm 3.08 \times 10^4$ to $7.18 \times 10^5 \pm 4.48 \times 10^3$ (*sul1*), $7.10 \times 10^5 \pm 7.47 \times 10^4$ to $3.05 \times 10^5 \pm 4.33 \times 10^3$ (*sul2*), $9.59 \times 10^5 \pm 1.18 \times 10^5to2.58 \times 10^5 \pm 9.15 \times 10^3$ (*dfrA1*) and $7.10 \times 10^5 \pm 1.06 \times 10^5$ to $4.76 \times 10^4 \pm 1.41 \times 10^4$ ($bla_{\text{CTX-M-1}}$), whereas it was $1.93 \times 10^5 \pm 9.84 \times 103$ for *tetA*.

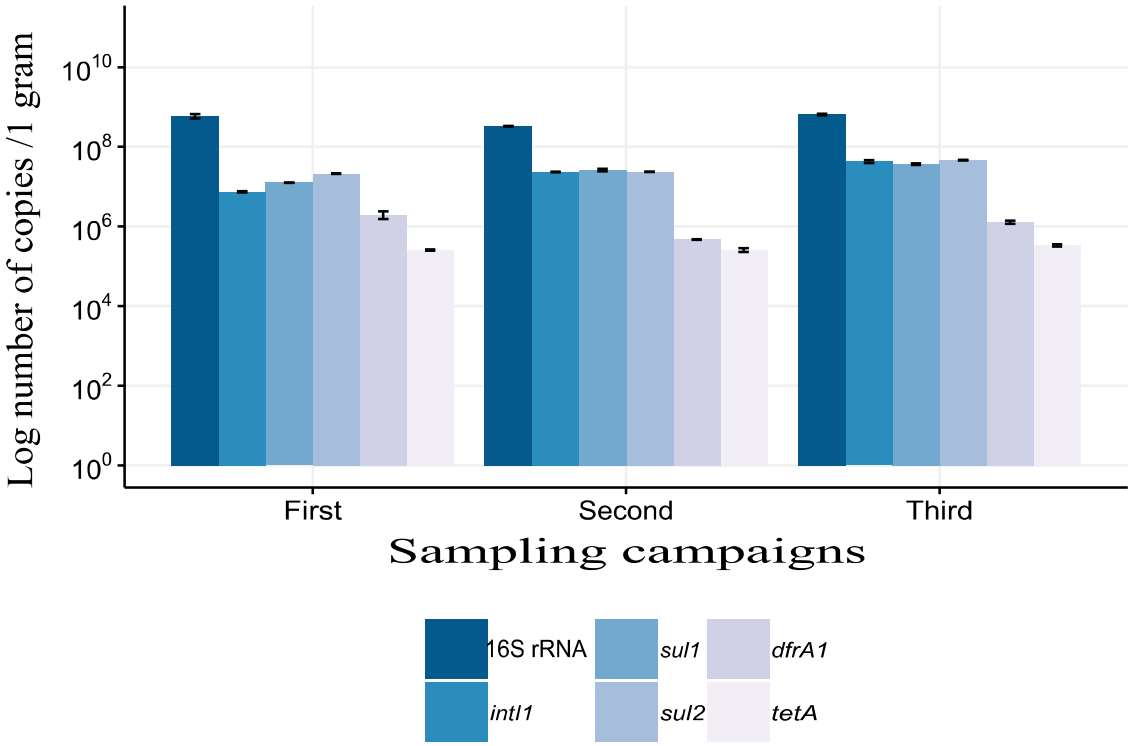

Fig. 4.26. Mean absolute abundance (number of copies/ gram) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1* and *tetA* in the metagenomic DNA of soil sample IKJ.

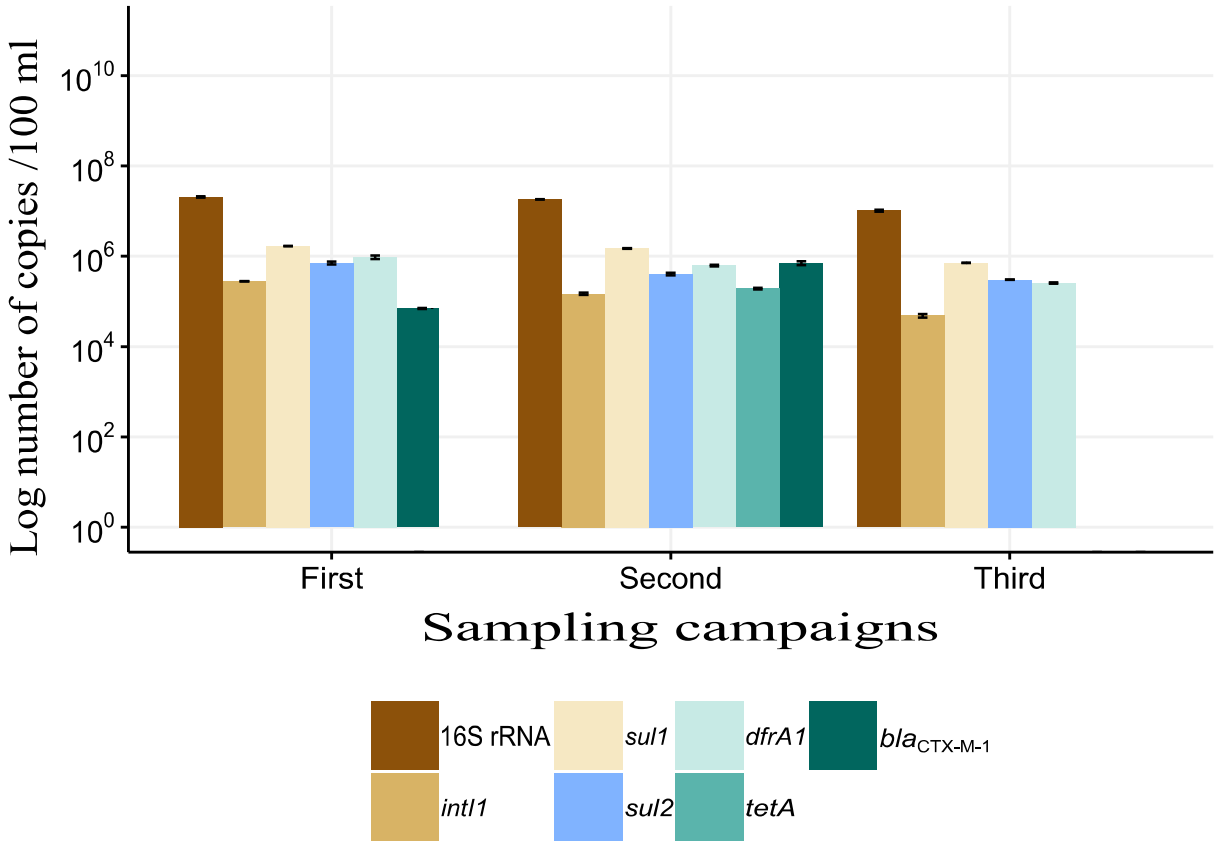

Fig. 4.27. Mean absolute abundance (copy number/ 1000ml) of 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1*, *tetA* and $bla_{CTX-M-1}$ in the metagenomic DNA of water sample IKB.

4.20 Absolute abundance of ARGs and, *intI1* in the samples from Aswani market E-waste dumpsite

4.20.1 Absolute gene abundances in sample AS

Genes quantified in sample AS obtained during the 3 sampling periods were 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA*. $bla_{\text{CTX-M-1}}$ was below level of quantification in all AS samples. The log transformed mean copies of 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA* per gram of sampleAS are presented in Fig.4.28. The number of copies of *intI1, sul1, sul2, dfrA1 and tetA* were similaracross the three sampling periods, as it deviated slightly away from the mean. The absolute mean copy numbers ± standard deviation of *intI1, sul1, sul2, dfrA1 and tetA* during the three sampling periods were $4.15 \times 10^7 \pm 1.95 \times 10^7$, $6.04 \times 10^7 \pm 2.89 \times 10^7$, $5.63 \times 10^7 \pm 2.53 \times 10^7$, $3.62 \times 10^6 \pm 3.48 \times 10^6$ and $2.82 \times 10^5 \pm 3.93 \times 10^4$ respectively.

4.20.2 Absolute gene abundances in sample ASB

In sample ASB, 16S rRNA, *intI1*, *sul1, sul2* and $bla_{\text{CTX-M-1}}$ were quantified in all the ASB samples from the different sampling period. However, *dfrA1* was below level of quantification in ASB samples from the second and third sampling periods, whereas *tetA* was below level of quantification in ASB sample from the second sampling period. The log transformed mean copy numbers of ARG and *intI1* per 100 ml of ASB are presented in Fig. 4.29. Copy number of *sul1* in the third sampling period (4.07×10^7) was $>10^2$ times more than what was observed in the first and second sampling period (3.62×10^5 and 1.07×10^5 respectively).The mean copy numbers ± standard deviation of *intI1*, *sul2,* and $bla_{\text{CTX-M-1}}$ranged from $4.77 \times 10^5 \pm 8.52 \times 10^4$ to $1.05 \times 10^4 \pm 4.95 \times 10^2$, $2.20 \times 10^6 \pm 1.68 \times 10^5$ to $4.75 \times 10^4 \pm 2.58 \times 10^3$ and $4.65 \times 10^5 \pm 3.87 \times 10^4$ to $5.01 \times 10^4 \pm 1.69 \times 10^4$ respectively, whereas it was $1.54 \times 10^5 \pm 5.74 \times 10^3$ to $1.58 \times 10^5 \pm 6.68 \times 10^3$ for *tetA*.

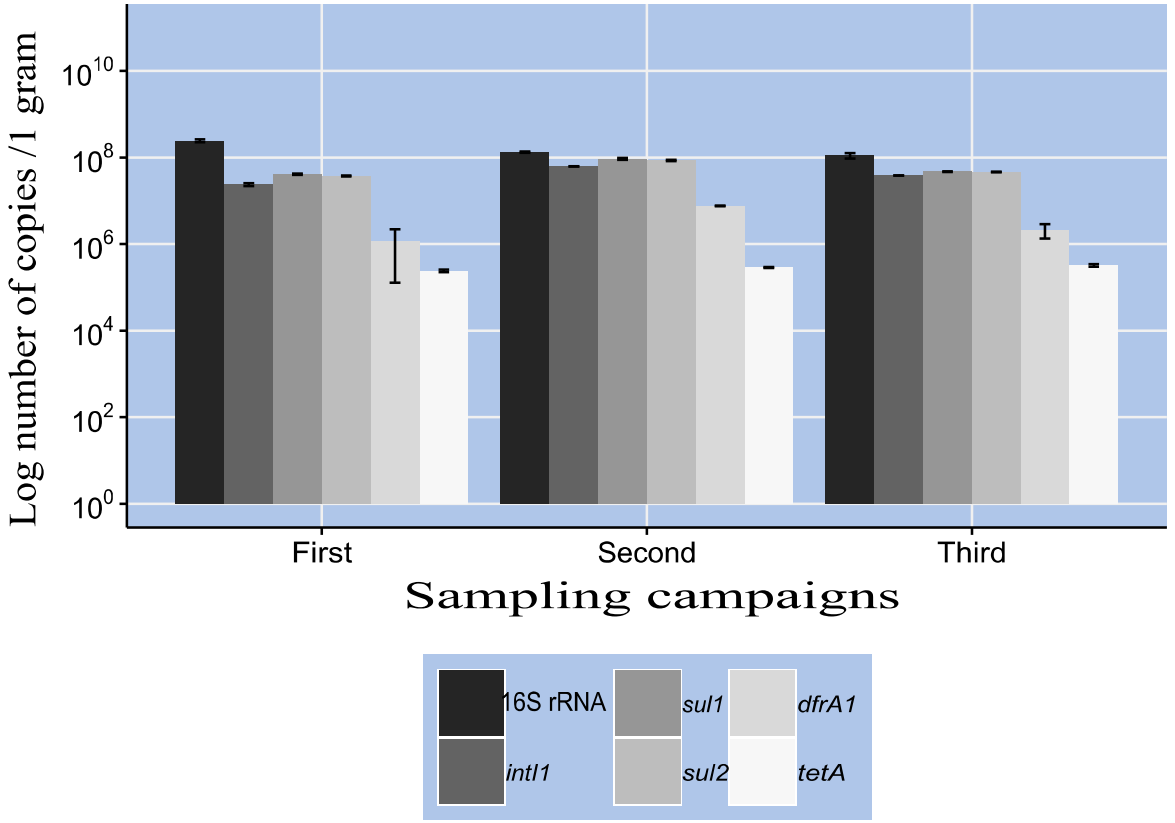

Fig. 4.28. Mean absolute abundance (copy number/ gram) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1* and *tetA* in the metagenomic DNA of soil sample AS.

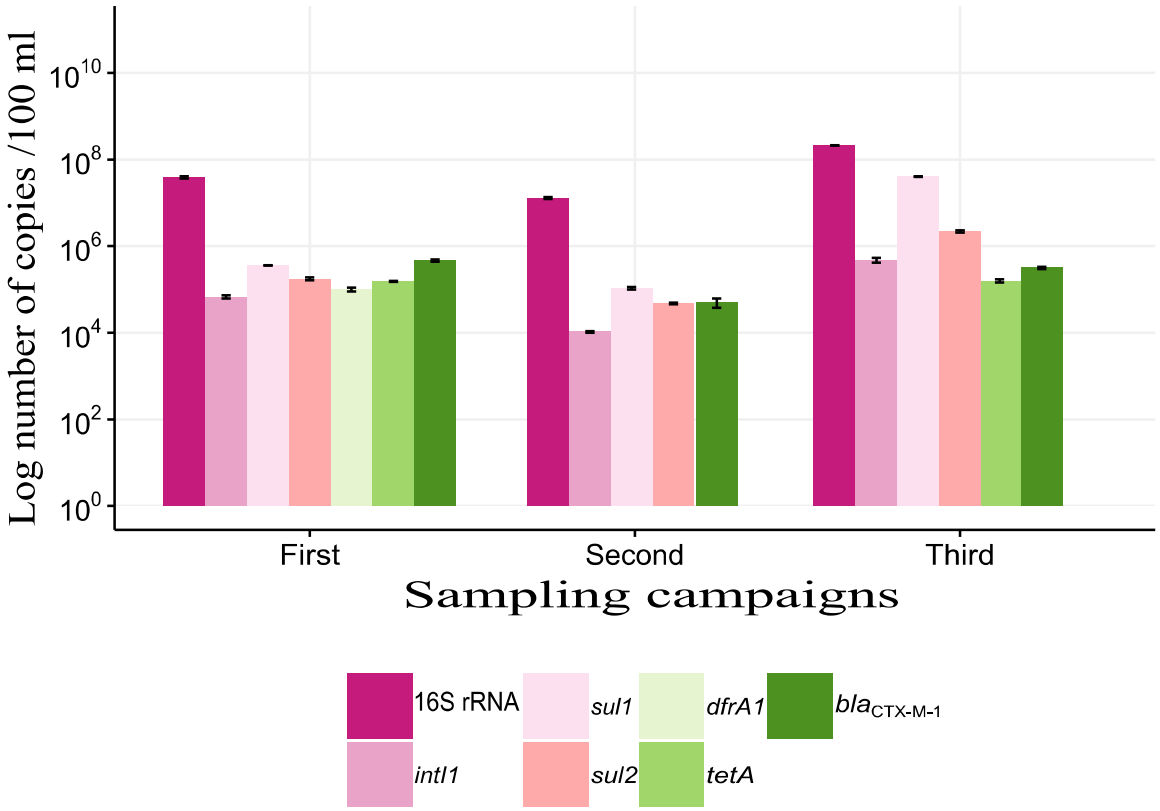

Fig. 4.29. Mean absolute abundance (copy number/ 100ml) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1*, *tetA* and $bla_{CTX-M-1}$ in the metagenomic DNA of water sample ASB.

4.21 Absolute abundance of ARGs and *intI1* in the samples from Ogunpa market E-waste dumpsite

4.21.1 Absolute gene abundances in sample OS

Genes detected and quantified in sample OS were 16S rRNA, *intI1*, *sul1, sul2*, and *dfrA1*. $bla_{\text{CTX-M-1}}$ and *tetA* were below level of quantification in OR samples from the first sampling. The log transformed mean copies of 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1*, *tetA* and $bla_{\text{CTX-M-1}}$ per gram of sample AS are presented in Fig. 4.30. The mean copy numbers ± standard deviation during the three sampling periods ranged from $1.63 \times 10^7 \pm 1.18\times 10^6$ to $6.73 \times 10^6 \pm 3.62\times 10^5$for *intI1*, $1.28 \times 10^7 \pm 1.57\times 10^5$to $7.19 \times 10^6 \pm 1.53\times 10^6$for *sul1*, $2.55 \times 10^7 \pm 5.82\times 10^6$to $1.45 \times 10^7 \pm 1.09\times 10^6$ for *sul2*, $2.13 \times 10^7 \pm 1.45\times 10^7$ to $5.24 \times 10^5 \pm 5.03\times 10^5$ for *dfrA1* and $2.55 \times 10^5 \pm 9.84\times 10^3$ to $1.77 \times 10^5 \pm 2.67\times 10^3$ for *tetA*, whereas it was 2.08×10^5 for $bla_{\text{CTX-M-1}}$.

4.21.2 Absolute gene abundances in sample OR

All genes (16S rRNA, *intI1*, *sul1, sul2*, *dfrA1tetA* and $bla_{\text{CTX-M-1}}$) were quantified in OR samples from the different sampling periods. The log transformed mean copy numbers of ARG and *intI1* per 100 ml of OR are presented in Fig. 4.31. The average copy number of $bla_{\text{CTX-M-1}}$ from the three sampling periods was 2.88×10^6. Copy number of $bla_{\text{CTX-M-1}}$ in sample OR was higher than all the other samples analysed in this study. The mean copy numbers ± standard deviation ranged from $5.41 \times 10^6 \pm 3.74\times 10^5$ to $1.17 \times 10^6 \pm 1.14\times 10^5$for *intI1,* $2.32 \times 10^7 \pm 1.32\times 10^6$ to $4.47 \times 10^6 \pm 7.43\times 10^4$ for *sul1,* $2.72 \times 10^7 \pm 3.86\times 10^6$ to $2.02 \times 10^7 \pm 1.43\times 10^7$ for *sul2,* $1.40 \times 10^6 \pm 7.03\times 10^4$ to $9.02 \times 10^5 \pm 5.30\times 10^4$ for *dfrA1* and$3.08 \times 10^5 \pm 1.14\times 10^4$ to $2.54 \times 10^5 \pm 3.54\times 10^3$for *tetA*.

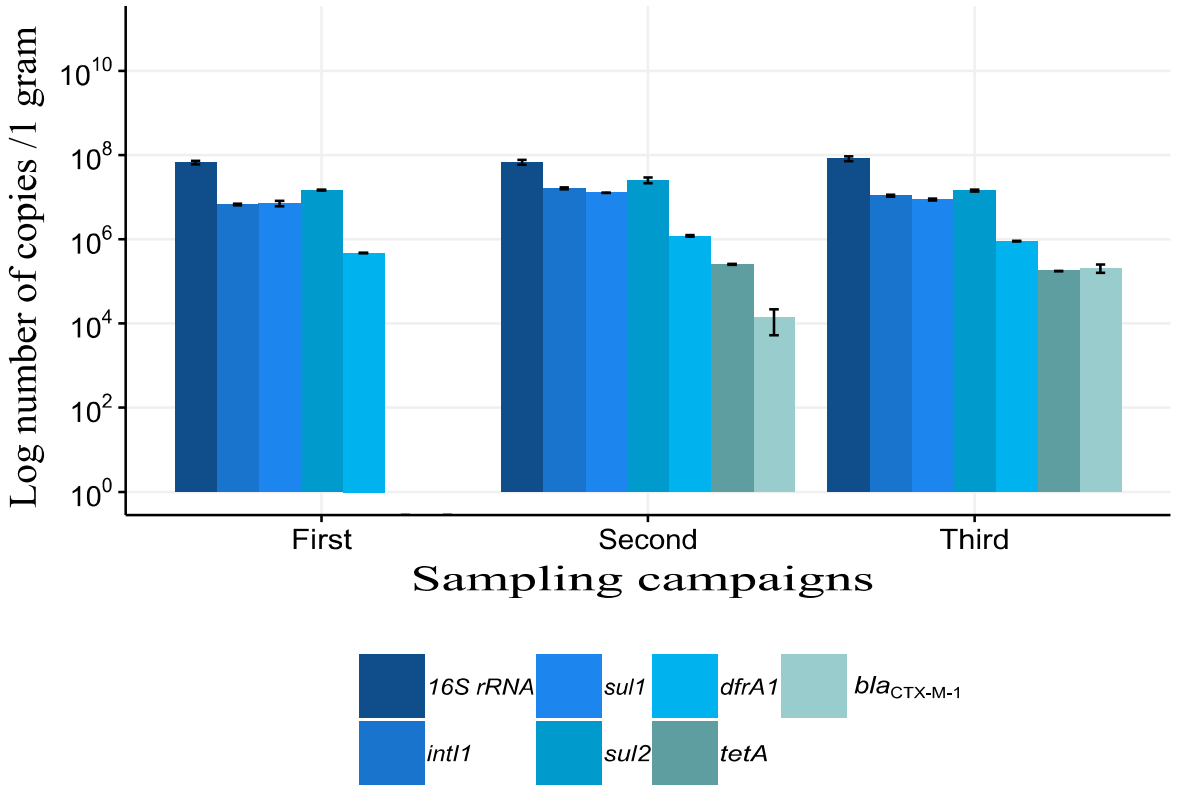

Fig. 4.30. Mean absolute abundance (copy number/ gram) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1*, *tetA* and $bla_{CTX-M-1}$ in the metagenomic DNA of soil sample OS.

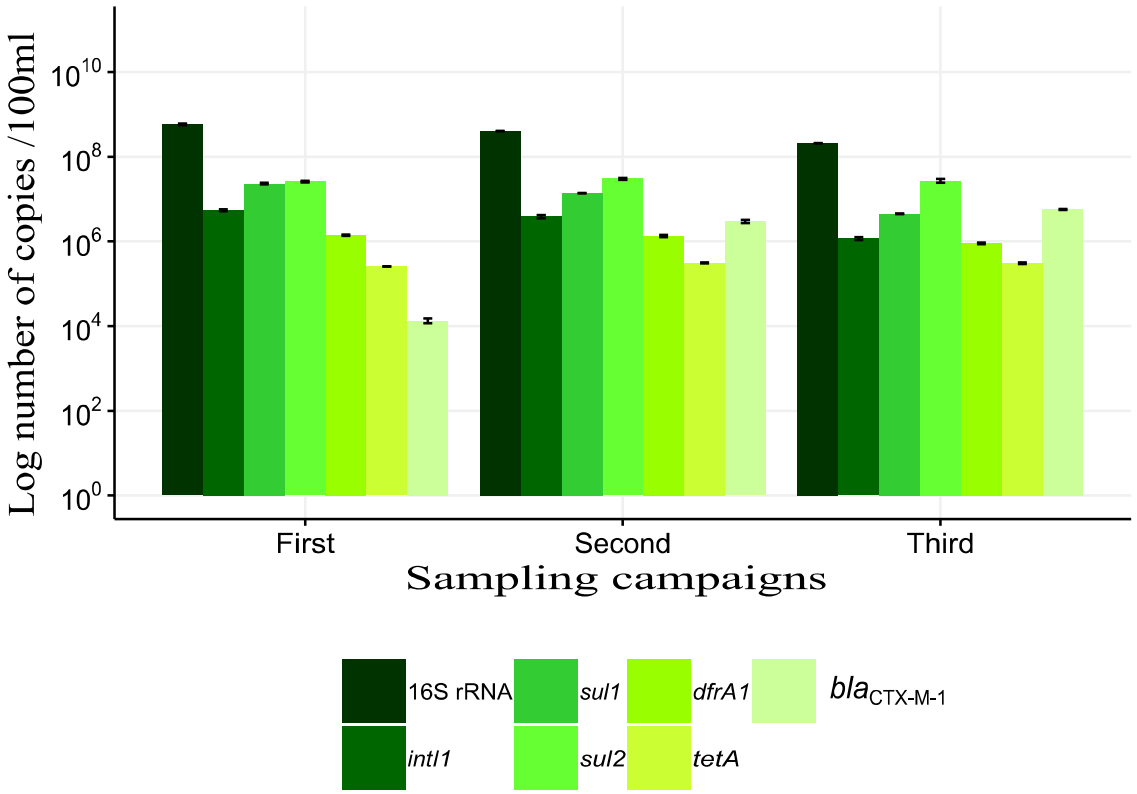

Fig. 4.31. Mean absolute abundance (copy number/ 100ml) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1*, *tetA* and $bla_{CTX-M-1}$ in the metagenomic DNA of water sample OR.

4.22 Absolute abundance of ARGs and *intI1* in soil and water samples from Arulogun E-waste dumpsite

4.22.1 Absolute gene abundances in sample ARU

16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and *tetA* were quantified in ARU in the 3 sampling periods. $bla_{\text{CTX-M-1}}$ was below level of quantification in ARU samples from the first and second sampling. The log transformed mean copies of 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1*, *tetA* and $bla_{\text{CTX-M-1}}$ per gram of sample ARU are presented in Fig. 4.32. The mean copy numbers ± standard deviation for the three sampling periods ranged from $8.76 \times 10^7 \pm 3.89\times 10^6$ to $6.03 \times 10^5 \pm 1.05\times 10^5$ for *intI1*, $1.23 \times 10^8 \pm 6.06\times 10^6$ to $1.27 \times 10^6 \pm 1.19\times 10^5$ for *sul1*, $9.53 \times 10^7 \pm 3.20\times 10^6$ to $1.48 \times 10^6 \pm 1.04\times 10^5$ for *sul2*, $1.32 \times 10^7 \pm 1.37\times 10^7$ to $9.23 \times 10^5 \pm 2.32\times 10^4$ for *dfrA1* and$1.10 \times 10^6 \pm 3.75\times 10^4$ to $1.29 \times 10^5 \pm 1.59\times 10^4$ for *tetA*, whereas it was $9.90 \times 10^4 \pm 3.99\times 10^4$ for $bla_{\text{CTX-M-1}}$.

4.22.2 Absolute gene abundances in sample UW1

In hand-dug well water sample UW1, *tetA* was below level of quantification in the samples from UW1, whereas *dfrA1* was only quantified in UW1 samples from second sampling and $bla_{\text{CTX-M-1}}$ in the first and third sampling periods. 16S rRNA and ARGs *intI1*, *sul1, sul2*, *dfrA1* were quantified in samples from UW1. Furthermore, the log transformed mean number f copies of ARGs and *intI1* per 100 ml of UW1 are presented in Fig. 4.33. The mean copy numbers ± standard deviation ranged from $2.64 \times 10^5 \pm 1.19\times 10^4$ to $5.82 \times 10^4 \pm 4.98\times 10^3$ for *intI1,* $4.84 \times 10^6 \pm 1.20\times 10^5$ to $2.36 \times 10^5 \pm 1.32\times 10^4$ for *sul1,* $9.05 \times 10^7 \pm 1.89\times 10^6$ to $7.85 \times 10^5 \pm 9.94\times 10^4$ for *sul2,* and $5.58 \times 10^4 \pm 1.19\times 10^4$ to $4.72 \times 10^4 \pm 1.27\times 10^4$ for $bla_{\text{CTX-M1}}$ whereas, *dfrA1* was $1.25 \times 10^6 \pm 5.87\times 10^4$.

4.22.3 Absolute gene abundances in sample UW2

Genes detected and quantified in sample UW2 obtained during the 3 sampling periods were 16S rRNA, *intI1*, *sul1, sul2*, *dfrA1* and $bla_{\text{CTX-M-1}}$. *tetA* was below level of quantification in the samples from UW2, whereas *dfrA1* was quantified in UW1 samples from second and third sampling. The log transformed mean copy numbers of

ARG and *intI1* per 100 ml of UW2 are presented in Fig. 4.34. The mean copy numbers ± standard

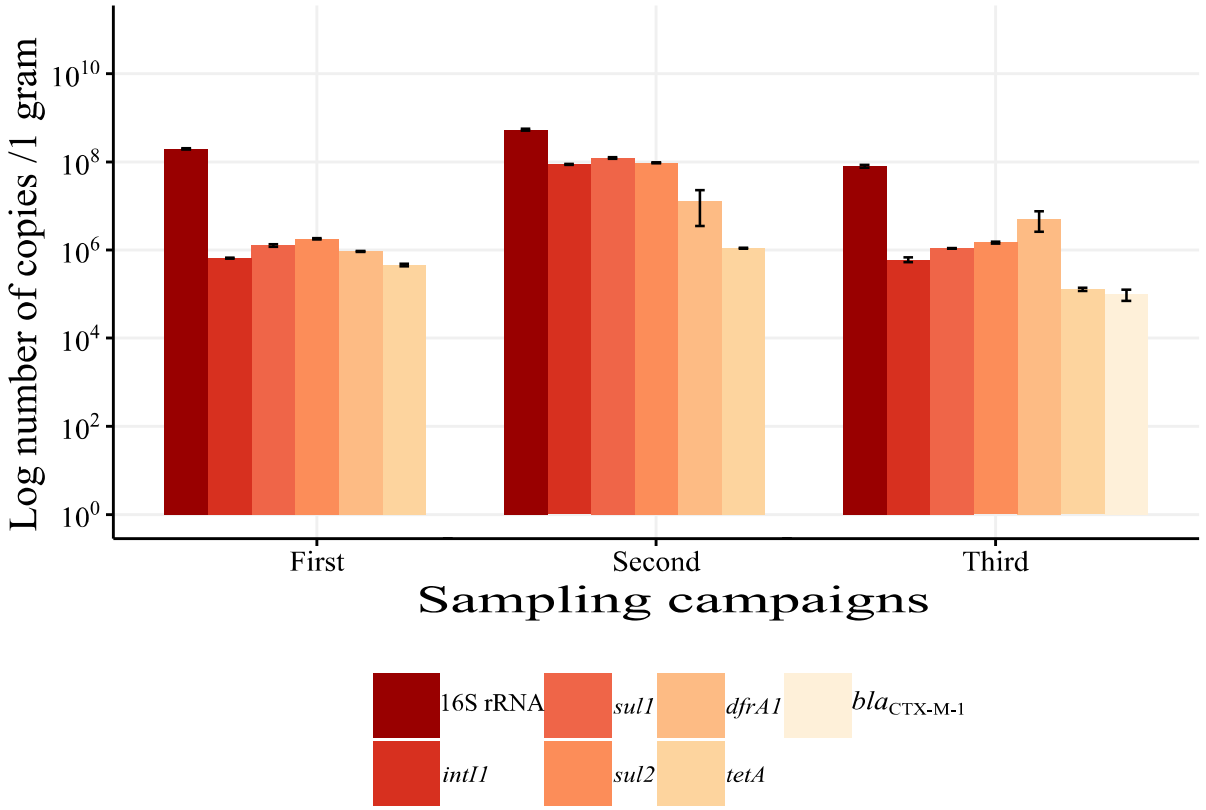

Fig. 4.32. Mean absolute abundance (copy number/ gram) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1*, *tetA* and $bla_{CTX-M-1}$ in the metagenomic DNA of soil sample ARU.

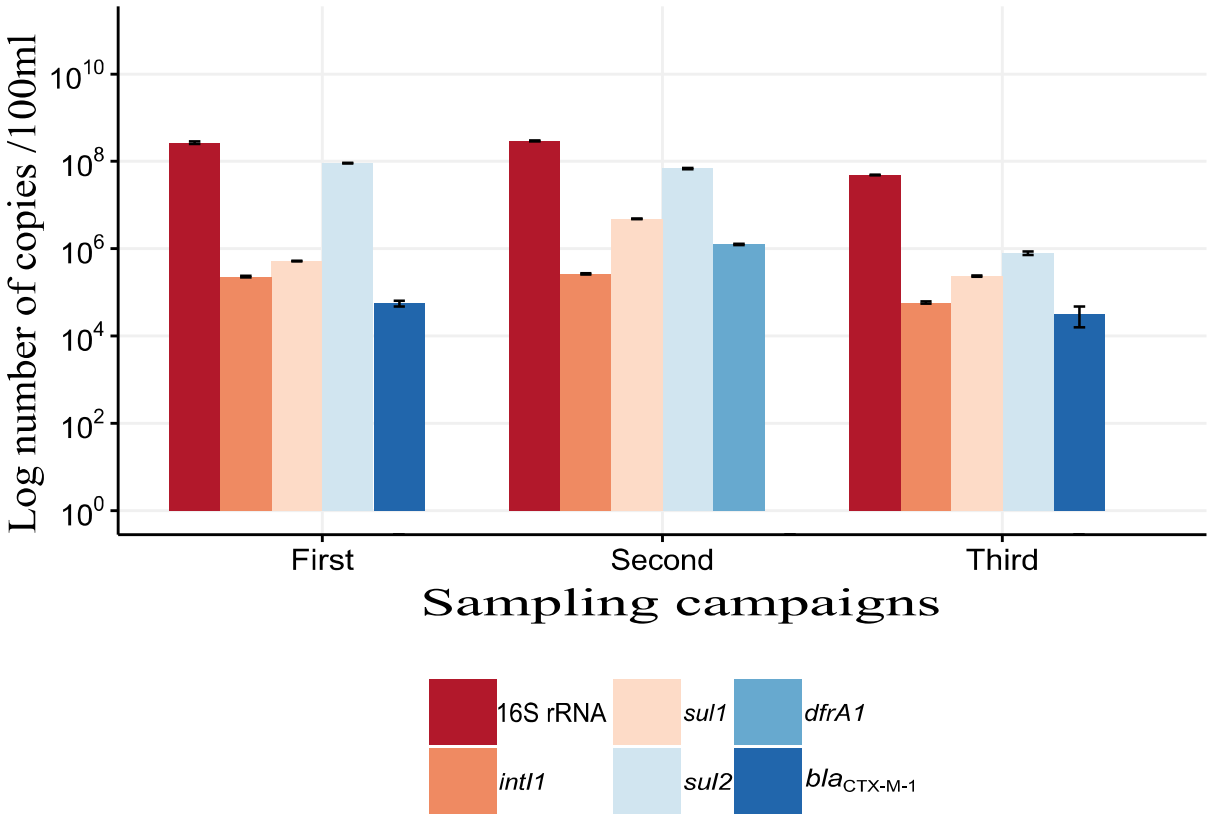

Fig. 4.33. Mean absolute abundance (copy number/ 100ml) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1* and $bla_{CTX\text{-}M\text{-}1}$ in the metagenomic DNA of water sample UW1.

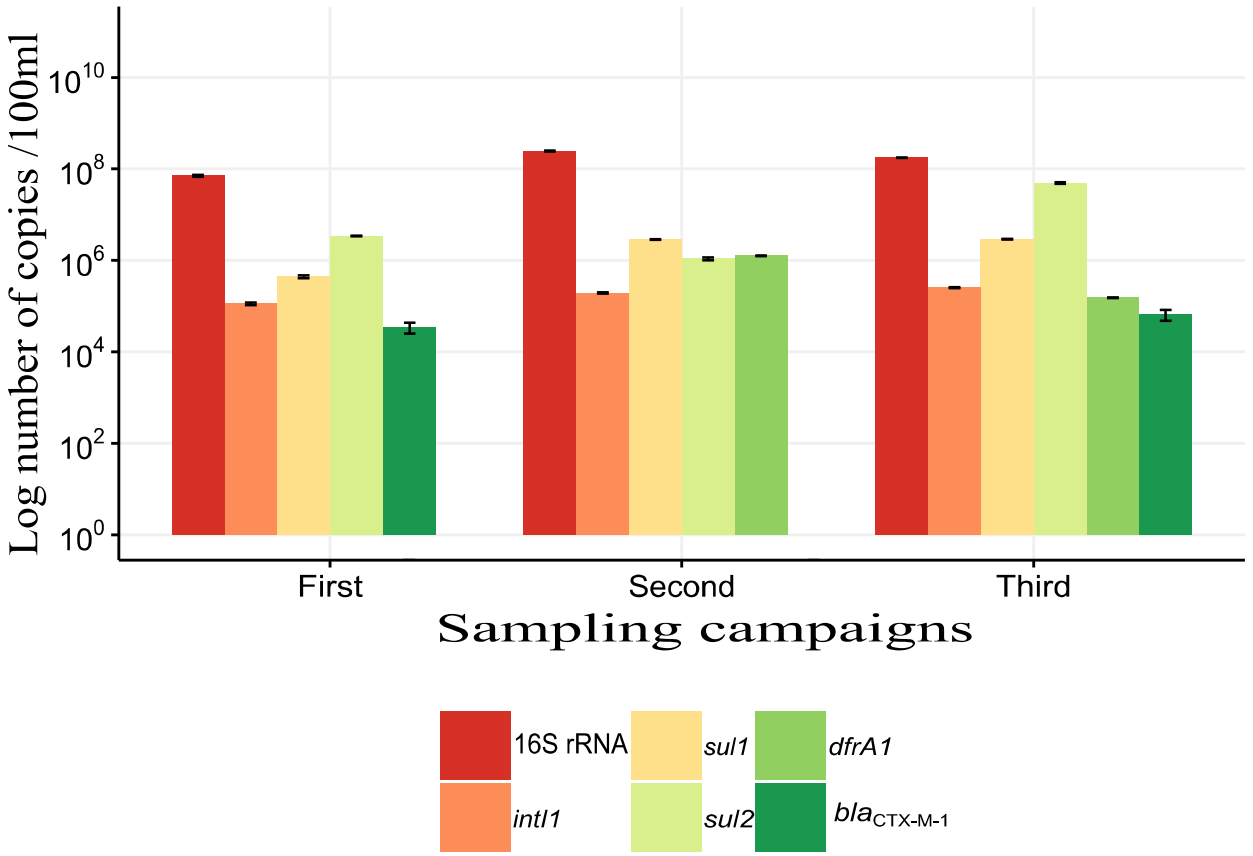

Fig. 4.34. Mean absolute abundance (copy number/ 100 ml) of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1* and bla_{CTX-M1} in the metagenomic DNA of water sample UW2.

deviation ranged from $2.54 \times 10^5 \pm 8.19 \times 10^3$ to $1.13 \times 10^5 \pm 9.80 \times 10^3$ for *intI1,* $2.93 \times 10^6 \pm 6.43 \times 10^4$ to $4.40 \times 10^5 \pm 3.23 \times 10^4$ for *sul1,* $4.91 \times 10^7 \pm 2.77 \times 10^6$to $3.43 \times 10^6 \pm 7.57 \times 10^4$ for *sul2,* $1.26 \times 10^6 \pm 3.84 \times 10^4$ to $1.52 \times 10^5 \pm 2.49 \times 10^3$ for *dfrA1*, and $1.25 \times 10^5 \pm 1.55 \times 10^4$to $6.52 \times 10^4 \pm 2.44 \times 10^4$for $bla_{\text{CTX-M-1}}$.

4.23 Relative abundance of ARGs, *intI1* from samplings sites in Lagos and Ibadan

The relative or normalized abundance of the quantified ARGs or MGE *intI1*, were determined by using the formular:

$$\frac{Total\ gene\ copy\ number\ of\ ARGs\ or\ MGE\ per\ sample}{Total\ gene\ copy\ number\ of\ 16S\ rRNA\ per\ sample}$$

In samples from Lagos, the normalized abundance of ARGs and *intI1* contamination varied from 1.12×10^{-1} (AL3) to 7.82×10^{-2} (IKB3) for *sul1*, 4.06×10^{-1} (AS3) to 6.19×10^{-3} (ASB3) for *sul2*, 1.33×10^{-2} (AR3) to 6.25×10^{-4} (AW2III) for *dfrA1*, 1.06×10^{-2} (IKB3) to 6.19×10^{-4} (IKJ3) for *tetA*, 2.11×10^{-2} (IKB3) to 4.90×10^{-5} (AR3) for $bla_{\text{CTX-M-1}}$ and 3.06×10^{-1} (AS3) to 8.80×10^{-3} (IKB3) for *intI1* (Fig. 4.35). Whereas in samples from Ibadan the normalized abundance of ARG and *intI1* contamination varied from 1.34×10^{-1} (OS3) to 6.44×10^{-3} (UW1III) for *sul1*, 1.22×10^{-1} (UW2III) to 7.47×10^{-2} (OR3) for *sul2*, 3.07×10^{-2} (ARU3) to 3.34×10^{-3} (OR3) for *dfrA1*, 1.99×10^{-3} (ARU3) to 8.85×10^{-4} (OR3) for *tetA*, 1.72×10^{-2} (OR3) to 2.07×10^{-4} (UW1III) for $bla_{\text{CTX-M-1}}$ and 1.57×10^{-1} (OS3) to 6.58×10^{-4} (UW1III) for *intI1* (Fig. 36).

The relative calculated abundances were compared with literature values from study sites with similar ARGs or MGE contamination. Results showed that the ARGs and MGE contamination status of E-waste dumpsites in this study were similar or above values reported in literature (Fig. 35 and 36).

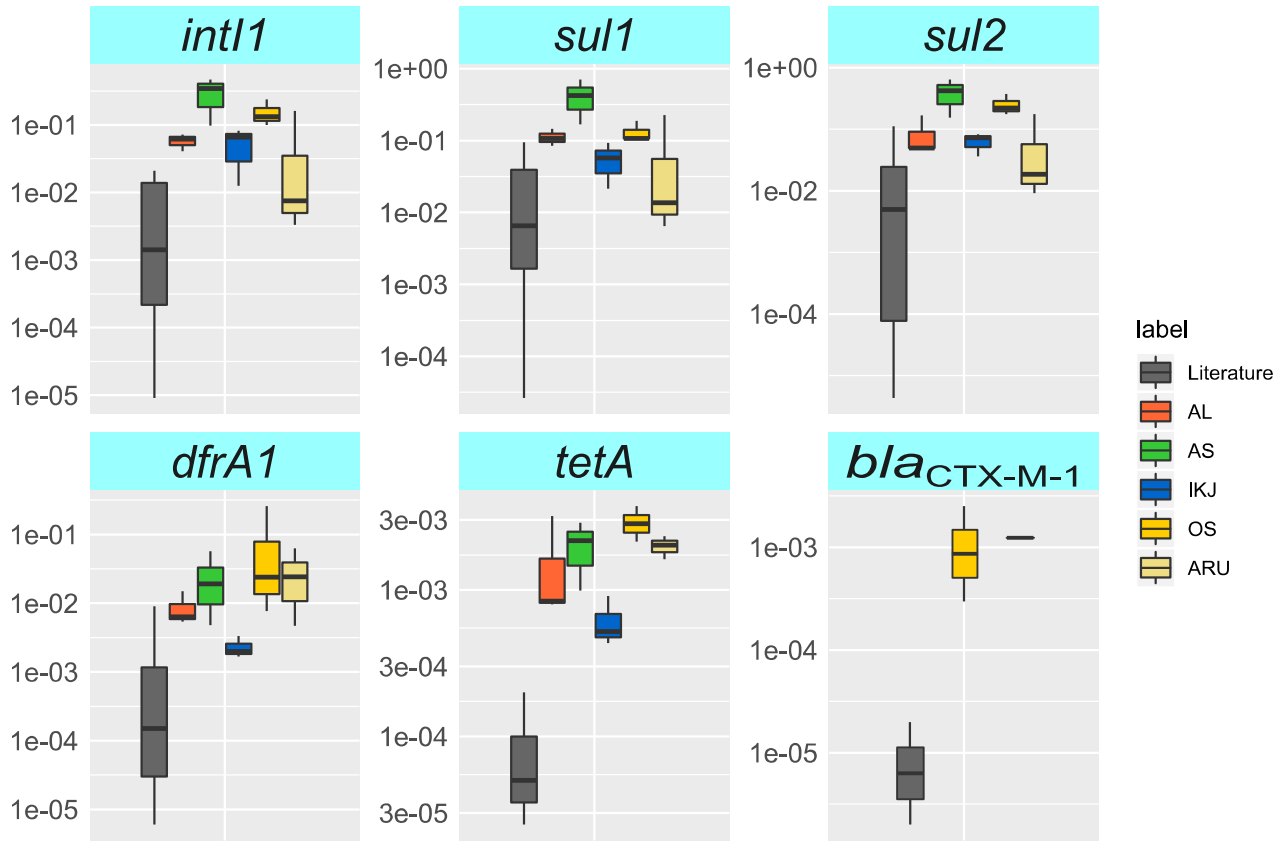

Fig. 4.35. Relative abundance (ARGs copy number/16S rRNA) of ARGs and *intI1* from metagenomic DNA samples of soil samples from the Lagos and Ibadan electronic waste dumpsites. Boxplots shows the mean (25th, 50th and 75th percentile) of three replicates from samples obtained during the sampling campaigns.

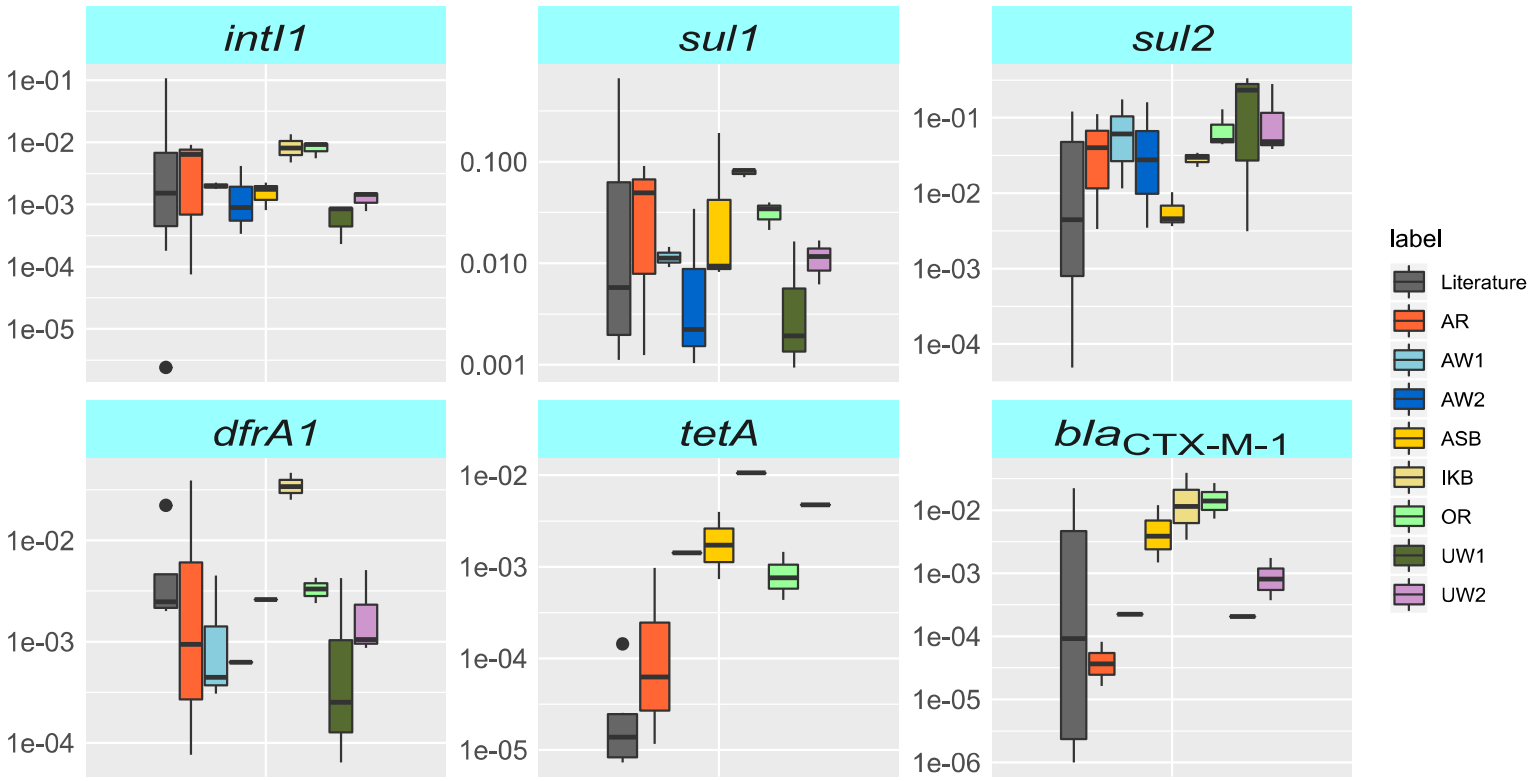

Fig. 4.36. Relative abundance (ARGs copy number/16S rRNA) of ARGs and *intI1* from metagenomic DNA of water samples from the Lagos and Ibadan electronic waste dumpsites. Boxplots shows the mean (25th, 50th and 75th percentile) of three replicates from samples obtained during the sampling campaigns.

4.24 Correlations between ARGs, *intI1* and heavy metals

Bivariate correlations ($p<0.05$) between the absolute abundance of the ARGs and *intI1* in the soil and water samples from the sampling sites are shown in Table 4.8. Results revealed that the absolute abundance of *intI1* had strongest relationship with *sul1* (correlation coefficient, r = 0.9). Further results from correlations analysis of the absolute abundance of *intI1* and other ARGs were 0.76 (*dfrA1*), 0.7 (*sul2*), 0.66 (*tetA*). However, weak and insignificant correlations were observed between *intI1* and $bla_{\text{CTX-M-1}}$ (r = -0.02).Overall, the relative abundance of $bla_{\text{CTX-M-1}}$ had weak and insignificant correlations with the absolute abundance of all the ARGs quantified in this study (Table 4.8)

Bivariate correlations between the absolute abundance of ARGs or *intI1*and the measured concentrations of metals present in soil and water samples from the E-waste dumpsites sampling locations are provided in Table 4.9 (*intI1* and metals), Table 4.10 (*sul1* and metals), Table 4.11 (*sul2* and metals), Table 4.12 (*dfrA1* and metals), Table 4.13 (*tetA* and metals) and Table 4.13 ($bla_{\text{CTX-M-1}}$ and metals). Correlations between *intI1* and heavy metals were strongest with Cu (0.73) and least with Se (0.29) for *sul1* it was Pb (0.51) and Se (0.24) respectively. Weak correlations were observed with the absolute abundance of *sul2* and heavy metals, occurring within the range of 0.35 (Cr) and 0.19 (Cd). Correlations between *dfrA1* and heavy metals were strongest with Pb (0.48) and least with Se (0.13) whereas it was Al (0.52) and Co (0.17) respectively for *tetA*. On the other hand, $bla_{\text{CTX-M-1}}$ had weak and negative correlations with all the heavy metals.

PCA multivariate correlation analysis of ARGs, *intI1* and the heavy metals explained 82.1% of the variability in the dataset (Fig. 4.37). As earlier observed with bivariate correlation analysis, *intI1* had strongest relationship between the ARGs and with the heavy metals in the PCA biplot (Fig. 37). Further PCA analysis of the measured concentrations of metals and ARGs occurring in samples during the three sampling times (Fig. 4.38) and in the sampling areas (Fig. 4.39) showed that seasonal changes and geographic location had minimal or no effect towards the proliferation of ARGs, *intI1* and heavy metals.

Table 4.8. Bivariate correlations between log transformed absolute abundance of ARGs from E-waste dumpsites. Correlation was performed at 95% confidence level.

ARG	ARG	r	p<0.05
intI1	*sul1*	0.900578	5.77×10^{-15}
intI1	*sul2*	0.702507	6.19×10^{-7}
intI1	*dfrA1*	0.760551	1.93×10^{-8}
intI1	*tetA*	0.699747	7.16×10^{-7}
intI1	$bla_{\text{CTX-M-1}}$	-0.02344	0.887381
sul1	*sul2*	0.714641	3.22×10^{-7}
sul1	*dfrA1*	0.765314	1.39×10^{-8}
sul1	*tetA*	0.695344	8.98×10^{-7}
sul1	$bla_{\text{CTX-M-1}}$	-0.00709	0.965843
sul2	*dfrA1*	0.512451	0.000852
sul2	*tetA*	0.526895	0.000569
sul2	$bla_{\text{CTX-M-1}}$	0.043748	0.79144
dfrA1	*tetA*	0.493943	0.001395
dfrA1	$bla_{\text{CTX-M-1}}$	0.044574	0.787589
tetA	$bla_{\text{CTX-M-1}}$	0.174577	0.287803

where;

r = correlation coefficient

p-value = significance level of t-test

Table 4.9. Bivariate correlation of log transformed absolute *intI1* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Bivariate correlation between *intI1* and Se were weak and insignificant.

Gene	Metals	r	p<0.05	t	df
intI1	Cu	0.7369014	8.84×10^{-5}	6.6308	37
intI1	Zn	0.7049182	5.45×10^{-4}	6.0453	37
intI1	Pb	0.7446666	5.47×10^{-5}	6.7866	37
intI1	Mn	0.720228	2.35×10^{-4}	6.315	37
intI1	Fe	0.6471014	8.52×10^{-6}	5.1628	37
intI1	Al	0.7301748	1.32×10^{-4}	6.5004	37
intI1	Co	0.3180467	0.04848	2.0406	37
intI1	Cr	0.7229644	2.01×10^{-4}	6.3652	37
intI1	Ni	0.5951639	6.43×10^{-2}	4505	37
intI1	Cd	0.5310284	0.000505	3812	37
intI1	Se	0.2980599	0.06533	18994	37

where;

r = Correlation coefficient

df = degrees of freedom

t = t-test statistic

p-value = significance level of t-test

Table 4.10. Bivariate correlation of log transformed absolute *sul1* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Bivariate correlation between *sul1* and metals, Co and Se were weak and insignificant.

Gene	Metals	r	p<0.05	t	df
sul1	Cu	0.508956	0.0009298	3.5993	37
sul1	Zn	0.459598	0.003246	3.1478	37
sul1	Pb	0.5193566	0.000704	3.6968	37
sul1	Mn	0.4711378	0.002467	3.249	37
sul1	Fe	0.4397168	0.005095	2.978	37
sul1	Al	0.4832832	0.00183	3.3579	37
sul1	Co	0.2428593	0.1363	1.5228	37
sul1	Cr	0.4708418	0.002485	3.2464	37
sul1	Ni	0.4342723	0.005739	2.9325	37
sul1	Cd	0.3270121	0.04215	2.1049	37
sul1	Se	0.2486423	0.1269	1.5615	37

where;

r = Correlation coefficient

df = degrees of freedom

t = t-test statistic

p-value = significance level of t-test

Table 4.11. Bivariate correlation of log transformed absolute *sul2* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Bivariate correlation between *sul2* and metals, Cd, Co, Zn, Fe, Ni and Se were weak and insignificant.

Gene	Metals	r	p<0.05	t	df
sul2	Cu	0.2803761	0.08384	1.7767	37
sul2	Zn	0.2363213	0.1475	1.479	37
sul2	Pb	0.3400879	0.03415	2.1998	37
sul2	Mn	0.2903466	0.07296	1.8456	37
sul2	Fe	0.2112666	0.1967	1.3148	37
sul2	Al	0.3026746	0.06108	1.9317	37
sul2	Co	0.1761914	0.2833	1.0888	37
sul2	Cr	0.3506149	0.02864	2.2773	37
sul2	Ni	0.2635187	0.105	1.6617	37
sul2	Cd	0.1904217	0.2456	1.1799	37
sul2	Se	0.3388639	0.03484	2.1908	37

where;

r = Correlation coefficient

df = degrees of freedom

t = t-test statistic

p-value = significance level of t-test

Table 4.12. Bivariate correlation of log transformed absolute *sul2* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Bivariate correlation between *dfrA1* and metals Cd, Co and Se were weak and insignificant.

Gene	Metals	r	p<0.05	t	df
dfrA1	Cu	0.4508193	0.003974	3.0721	37
dfrA1	Zn	0.4631551	0.002986	3.1788	37
dfrA1	Pb	0.482538	0.001864	3.3511	37
dfrA1	Mn	0.4370909	0.005398	2.956	37
dfrA1	Fe	0.4033486	0.01089	2.6813	37
dfrA1	Al	0.4565565	0.003484	3.1214	37
dfrA1	Co	0.1878463	0.2521	1.1633	37
dfrA1	Cr	0.4005984	0.0115	2.6595	37
dfrA1	Ni	0.3620247	0.02353	2.3624	37
dfrA1	Cd	0.2885467	0.07484	1.8331	37
dfrA1	Se	0.137095	0.4053	0.84187	37

where;

r = Correlation coefficient

df = degrees of freedom

t = t-test statistic

p-value = significance level of t-test

Table 4.13. Bivariate correlation of log transformed absolute *tetA* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Bivariate correlation between *tetA* and metals, Cd, Co and Se were weak and insignificant.

Gene	Metals	r	p<0.05	t	df
tetA	Cu	0.4857254	0.001721	3.3801	37
tetA	Zn	0.4774107	0.002117	3.3049	37
tetA	Pb	0.5121647	0.0008589	3.6272	37
tetA	Mn	0.4875437	0.001643	3.3967	37
tetA	Fe	0.436494	0.005468	2.951	37
tetA	Al	0.5273939	0.0005607	3.7758	37
tetA	Co	0.1735066	0.2908	1.0717	37
tetA	Cr	0.5477341	0.0003072	3.9822	37
tetA	Ni	0.4411659	0.004935	2.9902	37
tetA	Cd	0.3205468	0.04664	2.0584	37
tetA	Se	0.1999985	0.2222	1.2416	37

where;

r = Correlation coefficient

df = degrees of freedom

t = t-test statistic

p-value = significance level of t-test

Table 4. 14. Bivariate correlation of log transformed absolute *bla*$_{CTX-M-1}$ gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. There were no significant correlations between *bla*$_{CTX-M-1}$ and heavy metals.

Gene	Metals	r	p<0.05	t	df
bla$_{CTX-M-1}$	Cu	-0.1564031	0.3417	-0.96322	37
bla$_{CTX-M-1}$	Zn	-0.1523605	0.3545	-0.93772	37
bla$_{CTX-M-1}$	Pb	-0.1757999	0.2844	-1.0863	37
bla$_{CTX-M-1}$	Mn	-0.2232288	0.1719	-1.393	37
bla$_{CTX-M-1}$	Fe	-0.1064737	0.5188	-0.65136	37
bla$_{CTX-M-1}$	Al	-0.2058068	0.2088	-1.2793	37
bla$_{CTX-M-1}$	Co	-0.2214135	0.1755	-1.3811	37
bla$_{CTX-M-1}$	Cr	-0.2184401	0.1816	-1.3616	37
bla$_{CTX-M-1}$	Ni	-0.19813	0.2266	-1.2296	37
bla$_{CTX-M-1}$	Cd	-0.1397605	0.3961	-0.85856	37
bla$_{CTX-M-1}$	Se	-0.08959653	0.5875	-0.5472	37

where;

r = Correlation coefficient

df = degrees of freedom

t = t-test statistic

p-value = significance level of t-test

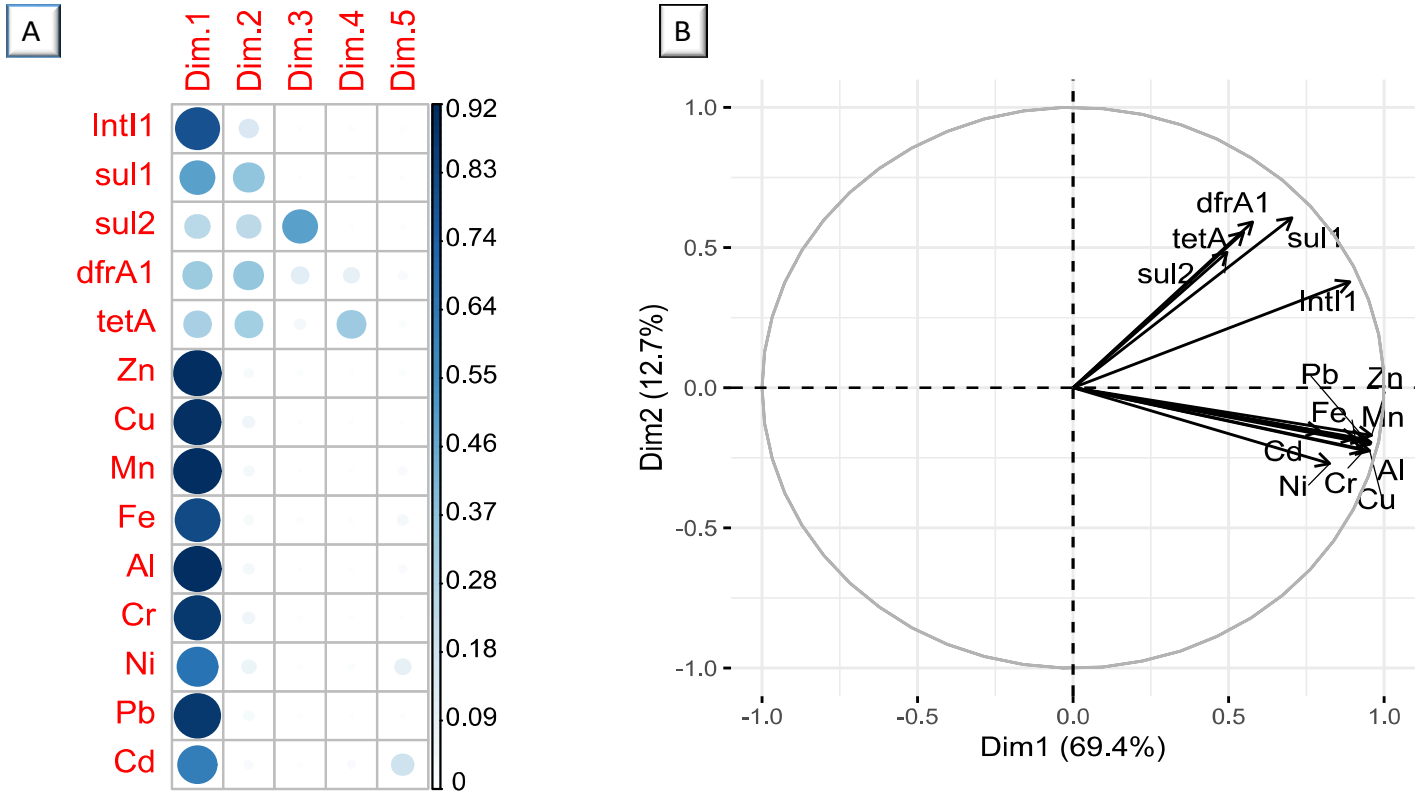

Fig. 4.37. Multivariate Principal Component Analysis (PCA) showing relationships occurring between ARGs, MGE and HMs present in samples from E-waste dumpsites. Plate A shows the percentage distribution (represented by intensity of white to blue colouration) of thevariables into principal components (Dimensions – Dim). PCA explains 82.1% of the variability in the dataset, and shows positive correlations within and between ARGs, *intI1*, and HMs. Correlation between *intI1* and HMs is strongest.

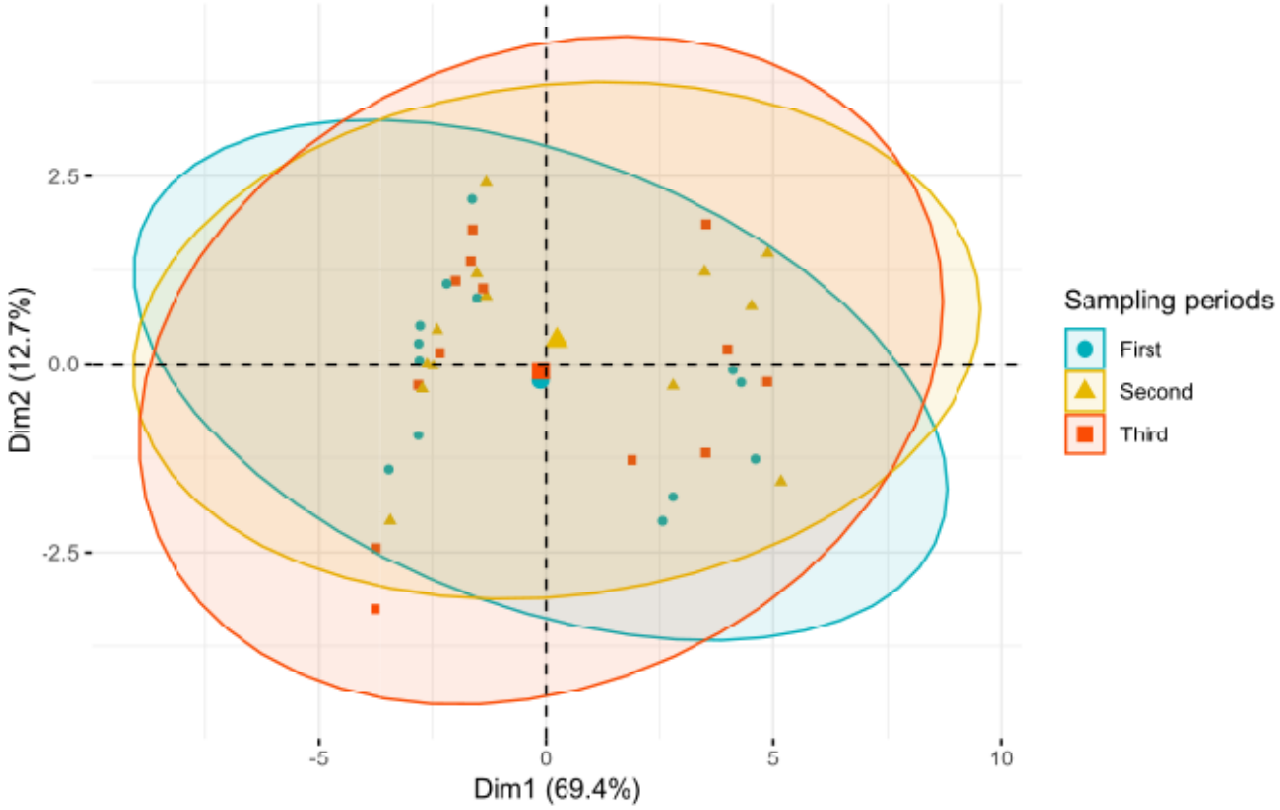

Fig. 4.38. PCA showing ellipses clustering of variables (ARGs, *intI1* and HMs) from E-waste dumpsites according to the different sampling periods. Results shows seasonal changes occuring during the sampling campaigns in Lagos and Ibadan to have minimal or no variation on the proliferation of HMs, *intI1* and ARGs in the E-waste dumpsites.

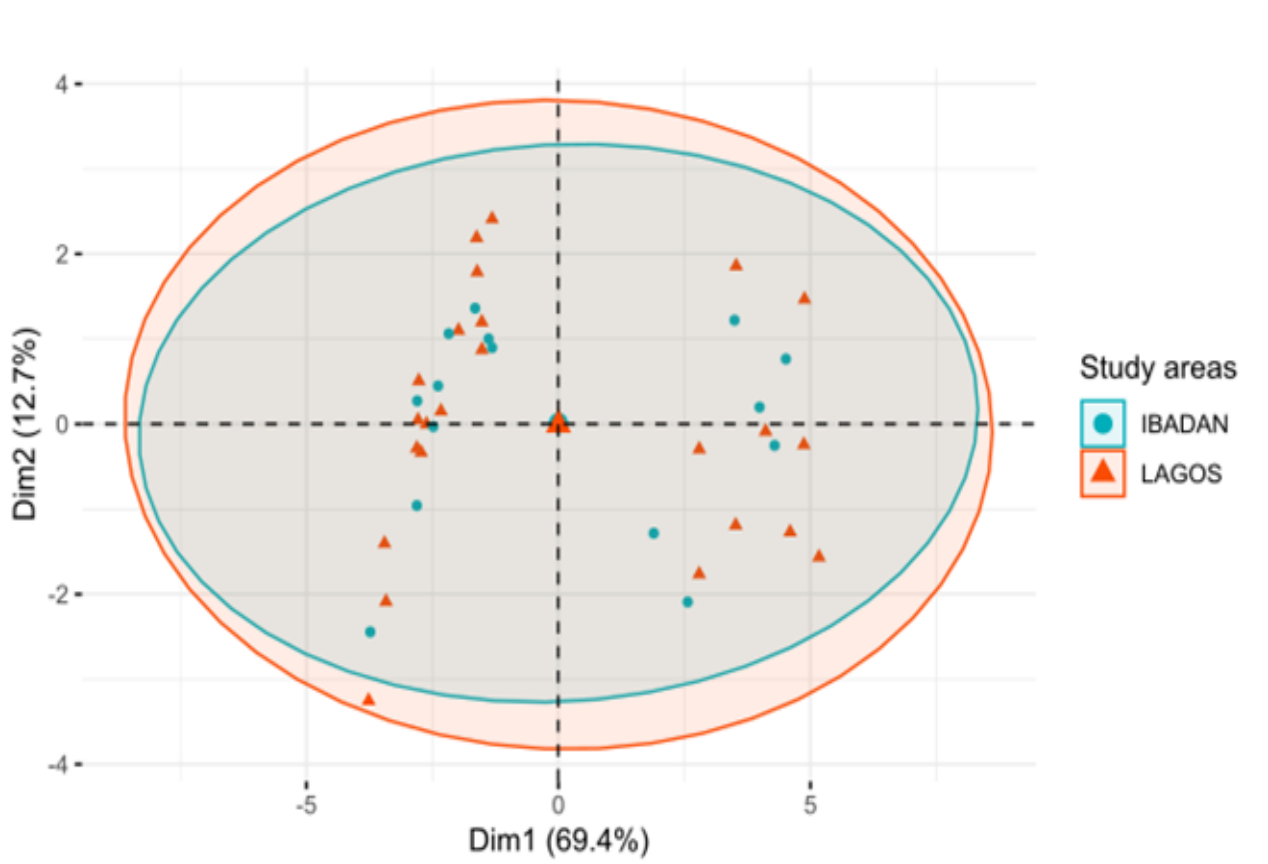

Fig. 4.39. PCA showing ellipses clustering of variables (ARGs, *intI1* and HMs) from E-waste dumpsites, according to the different sampling areas. Results shows electronic waste dumpsites in Lagos and Ibadan to be comparable in the proliferation of *intI1*, ARGs and HMs

CHAPTER FIVE

DISCUSSION

5.1 Physicochemical properties of soil and water samples from E-waste dumpsites

The physicochemical propertiesof the soil and water samples from the dumpsites in this study varied greatly in comparison to the control samples and permissible limits by regulatory bodies respectively. This variance is largely influenced by activities occurring within the respective sampling sites (Obianefo *et al.*,2017).

According to the EPA (2001) and SON (2015) permissible limits for water quality, the TDS and EC of water should not exceed 500 mg/l and 1000 µS/cm respectively. This was however not the case with water samples from rivers and hand-dug wells in this study which had much higher TDS and EC values. Similarly, these findings contradicted reports from previous studies in Nigeria that found low levels of TDS and electrical conductivity in underground water when compared to permissible limits by regulatory bodies(Badmus*et al.*, 2014; Mbaka*et al.*, 2017). This is however not surprising as the rivers and hand-dug wells sampled around the E-waste dumpsites were mostly shallow and might support the input of debris and other organic material from around the dumpsites, especially through runoffs during the rainy season. Also, rock weathering may also contribute to the increasing levels of TDS in the hand-dug wells (Anning, 2011; Badmus *et al.*, 2014). However, the opposite was observed in borehole samples (ASB, IKB), where TDS and Electrical conductivity were below EPA recommended limits. Usually, elevated TDS raises water densities and consequentlyreduces the solubility of oxygen, making such water unsuitable for consumption (Mbaka *et al.*, 2017). Furthermore, electrical conductivity had a perfect and significant linear correlation with TDS (correlation coefficient of 0.9996) (Figure 4.1), indicating that the measure of electrical conductivity was congruent with the TDS in all the water samples from E-waste dumpsites.

E-waste dumpsites are composed mainly of non-biodegradable waste and may therefore have poor organic matter composition (Nnorom *et al.*, 2013; Adesokan*et al*., 2016). In the present study, the percentage organic carbon and organic nitrogen in soils from the E-waste dumpsites were much lower than what was observed with soil from the control site (IITA Garden soil). This contrasted with observations in MSW dumpsites in Nigeria where high level of organic matter was attributed to wastes coming from households with high organic matter content(Ideriah *et al.*, 2006; Obianefo *et al.*, 2017). Observed percentagesof organic carbon and nitrogen in this present study were similar with what was observed by Sanusi (2015) and Adesokan *et al.* (2016) in electronic waste dumps in Lagos and Ibadan respectively. Low organic matter contentfrom E-waste dumps may suggest the presence of fewer humic materials for adsorption of pollutants and could thus enhance the leaching of heavy metals to lower soil horizons and ultimately to ground water (Adesokan *et al.*, 2016).

Also, the leachability of pollutants like metals is intensified in soils with a sandy texture, particularly during the rainy season (Brady and Weil, 2008). This is because sandy soils support higher cation mobility in contrast to clayey soils (Adesokan *et al.*, 2016). As earlier determined, all the soil samples from E-waste dumpsite in this study were characterized as sandy. This has equally been corroborated in reports from other E-waste and MSW dumpsites in Nigeria (Ideriah *et al.,* 2006; Azeez *et al.*, 2011; Badmus *et al.* 2014; Adesokan*et al.*, 2016). Generally, soil types in the south-western Nigeria have been classified to have mostly sandy texture(Fasina *et al*., 2015). Soil texture correspondingly influences several physical properties in the soil such as water holding capacity, cultivation, permeability and general soil productivity in terms of nutrient availability (Brady and Weil, 2008). Nutrient availability is strongly tied to the soil pH (McKenzie, 2003; Miller, 2016), a parameter that will influence microbial activity and plant growth.

Generally, a majority of plants and microbes grow well at pH range of 6.5 and 7.5 (Wang *et al.*, 2014). This pH range was observed in 51.28% of the soil and water samples tested in this study. Two hand-dug wells in Ibadan (ARW1 and ARW2) and two boreholes from Lagos (ASB and IKB) had pH values below permissible limits for drinking water set by the EPA (2001) and (SON, 2015) (Table 4.3). The acidic pH of underground waters has similarly been reported in other studies in Nigeria (Aina and Oshunrinade, 2016; Ukpaka and Ukpaka, 2016), Ghana (Tay *et al.*, 2018) and

Australia (Appleyard *et al.*, 2004). Several reasons have been proffered to explain the characteristic low pH observed in underground water. For example, rain water has been implicated to carry with it dissolved gases that contribute to the formation of various kinds of acids (Ukpaka and Ukpaka, 2016). Also, high concentrations of Fe which was observed in underground water in this study especially during the rainy season (39.22 – 59.18 mg/l), have earlier been described to play a part in reducing pH of these waters(Tay*et al*., 2018; Ukpaka and Ukpaka, 2016). Over a period of time as borehole casings become corroded, more Fe is introduced into the water. Fe present as soluble ferrous state in these waters is oxidized to insoluble ferric state upon exposure to air and may further hydrolyse to form insoluble hydrated ferric oxide that could undergo biochemical oxidations reaction within sedimentary rock formations and cause a drop in the water pH(Tay*et al*., 2018; Ukpaka and Ukpaka, 2016). In addition, soil formations contain Fe in the form of FeS (pyrite), the oxidation of pyrite in water leads to formation of iron-sulphate and sulphuric acid, thus further increasing pH of ground water. Water pH values lower than 6.5 as observed in 50% of the water samples in this study, are considered to be too acidic for human consumption and could lead to several health-related issues including acidosis (Nkansah *et al.*, 2010).

The pH of soil samples observed in this study were within range of values observed by Sanusi (2015) in an electronic waste dumpsite in Lagos. However, this was lower than that recorded in the study by Lenart and Wolny-Koładka (2013) in metal contaminated sites in Poland steelworks. The variation in pH values could be largely influenced by differences in the physical, chemical and microbiological qualities of the samples due to heavy metal contamination (Kazlauskaite-Jadzevičė*et al.*, 2014). In tandem, the slightly higher pH observed with the soil samples obtained durinsg the third sampling period (rainy season) may be as a result of dilution by rainwater (Ideriah *et al.*, 2006). Generally, increase in the soil pH increases binding efficiencies of divalent cations, making it increasingly non-bioavailable (Hakim *et al.*, 2019). In this study, 73.33% and 100% of the soil samples had higher concentration of Ca^{2+} and Na^{+} when compared to the control garden soils. Mccauley *et al.* (2017) explains that soil pH within range of 6.5 to 8.0 is responsible for the availability of Ca^{2+} and Na^{+} in soils which corroborated with the data presented in this present study (Table 4.2). Also, the total phosphorus in soil is most available at pH 5.5 to 7.5, which may explain why higher phosphorus content were determined in 53.3% of the samples when compared

to garden soil (Mccauley *et al.*, 2017). Similar results for Ca^{2+}, Na^{+} and available phosphorus were observed by Azeez *et al.* (2011) in metal contaminated MSW dumpsites in Abeokuta, Nigeria. Also, Mg^{2+}, and K^{+} which were higher in the control garden soil may be attributed to the higher cation exchange capacity that is characteristic of its high clay/organic matter content.

Both Ca^{2+} and Mg^{2+} are essential minerals in human health, although, excess of these minerals in the form of $CaCO_3$ and $MgCO_3$ can cause water to be hard. Traditionally, water hardness is used to refer to the ability of water to react in the presence of soap, and may be a representation of the presence of a variety of polyvalent metallic ions including aluminium, barium, iron, manganese, strontium and zinc in water (WHO, 2011). Although $CaCO_3$ and $MgCO_3$ were generally higher in the hand-dug well water samples, samples AR3, AW1III and AW2III obtained during the rainy season exceeded the acceptable limit (150mg/ml of $CaCO_3$) fixed by the Nigerian Standard for drinking water NIS 554 (SON, 2015). On the other hand, surface waters are prone to excessive enrichment with nutrients from anthropogenic sources(Singh, 2013; Adesuyi *et al.*, 2015). In this present study, river samples OR and AR, had concentrations of PO_4 and NH_4 above EPA permissible limits. Although, phosphate and nitrate containing compounds are important nutrients needed in microbial metabolism, high concentrations can significantly change the aquatic ecosystem and ultimately lead to eutrophication. Various anthropogenic sources ranging from fecal and urine contamination, manures and chemical fertilizers, pharmaceutical and industrial effluents have been implicated in the input of nutrients into water bodies(Singh, 2013). The anthropogenic input of these nutrients deteriorates water quality and may also favour the growth of algae which are starters of eutrophication(Singh, 2013; Adesuyi *et al.*, 2015). Algae, bacteria and fungi are capable of converting nitrate ions to form ammonia using nitrate and nitrite reductases (Singh, 2013). This, in addition to the anthropogenic sources described above may explain the high ammonia content in AR and OR.

5.2 Heavy metal pollution in E-waste dumpsites

All the heavy metals analysed in this study are importantparts of many electronic machines and were hence expected to be present in E-waste and subsequently as

contaminants in the dumpsites(Basel Action Network, 2011; Omole *et al.*, 2015). The HMsanalysed were present at concentrations in several magnitudes above their concentration in the garden soil (control) and permissible limits set by regulatory bodies for the soil and water samples respectively. The heavy metals contained in E-waste enter into the soil environment following substandard metal extraction methods(Baldé *et al.,* 2017). The determination of spatial extent and mobility of heavy metals contaminations in soils has become a critical point of consideration in metal pollution studies (Shaheen and Iqbal, 2018). Soil properties including texture, electrical conductivity, pH, etc., have been reported to aid the mobility, proliferation and leaching of heavy metals cations to other ecosystems (Azeez *et al.*, 2011; Kazlauskaite-Jadzeviče *et al.*, 2014; Mccauley *et al.*, 2017; Shaheen and Iqbal, 2018). Hence, this phenomenon may likely explain the occurrence of the analysedHMs in the water sources from this study.

Reports on metal contamination in the soil, sediment, water, and air of E-waste dumpsites resulting from various non-sustainable E-waste metal extraction processes has been reported in several regions of the world, including Nigeria (Olafisoye *et al.*, 2013; Omole *et al.*, 2015; Sanusi, 2015; Adesokan *et al.*, 2016), Ghana(Tokumaru, 2015), China(Xu*et al.*, 2015), India (Ha *et al.*, 2009; Gangwar *et al.*, 2019), Thailand(Pookkasorn and Sharp, 2016), Philippines (Celestial, 2018), and Russia (Labunska *et al.*, 2010).

Due to their toxicological effects, pollution arising from HMs has become a serious concern to public health(Shaheen and Iqbal, 2018). Heavy metals from E-waste dumpsites pose significant threat to water, the food chain and humans which could serve as sinks for their bio-accumulation and cause further detrimental consequences from long term exposure(Gangwar *et al.*, 2019). For instance, exposures to metal pollution from E-waste dumpsites have been reported to be associated with prevalence of cardiovascular morbidity in workers and nearby inhabitants (Gangwar *et al.*, 2019). Exposure to heavy metals is linked to prostatic proliferative lesions, cancer, nephropathy, blood poisoning, breakdown of central nervous system, plumbism, anaemia, bone fractures, kidney dysfunction and other vital organ failures (Jaishankar*et al.*, 2014; Omole *et al.*, 2015; Shaheen and Iqbal, 2018).

5.3 Cultivable metal-tolerant bacteria community in samples from E-waste dumpsites

In this present study, results from the determination of total cultivable metal (Cu, Pb and Zn) tolerant bacteria (Appendix III to V) during the three sampling periods revealed that there was a constant selection of metal tolerant strains in all the sampling sites. This, in addition to the results of the HMs analysis in the samples, indicates that the metal pollutants which are continuously added to the samples might be responsible for the proliferation of metal tolerant bacteria in the sampling sites (Singh *et al.*, 2004). Except in the borehole samples (IKB and ASB), the proportion of metal resistant bacteria in the water samples had higher occurrence in the 3rd sampling campaign (rainy season) in comparison to results from the other two sampling campaigns. In contrast soil samples largely had higher percentages of metal tolerant strains occurring during the dry season (First and second sampling periods). Effect of seasonal changes on the proliferation of heavy metal resistant bacterial populations was similarly investigated by Odokuma and Ijeomah (2004) from river water and sediment impacted by industrial effluent discharges, where results showed that during the summer a greater proportion of HM resistant bacteria were enumerated in contrast to the wet season. Similarly, results from this present study were in line with what was obtained by Ansari and Malik (2010) who observed seasonal variations in the proliferation of Ni and Cd tolerant coliform bacteria from heavy metal impacted industrial wastewaters and agricultural soils in India where total coliform count were maximum in spring and summer and then in the winter and post-monsoon seasons. The observed higher percentage of HM tolerant bacteria in the water samples (except in boreholes) during the rainy season could be attributed higher heavy metal levels in these waters during this season and the likely enrichment of the water bodies with metal-tolerant bacteria from sub-soil of the E-waste dumpsites due to runoff from rainfall.

Some HMs, such as Cu and Zn play vital roles in life processes of microbes where they serve as essential nutrients, catalyst for biochemical reactions and other processes involved in microbial metabolism (Bruins *et al.*, 2000). These metals, including Pb (which have no biological roles), when available at high concentrations could be toxic to microorganisms and may cause changes in bacterial populations in favour of the spreading of metal tolerant species in the natural environment(Bruins *et al.*, 2000). It is possible that the proliferation of metal tolerance in these E-waste sampling sites could be a consequence of the inculcation of various metal-stress adaptation mechanisms, ranging from mineralization, metal sorption, extracellular precipitation, enzymatic

oxidation, uptake and accumulation and metal efflux by the indigenous microbes (Bruins*et al.*, 2000; Nies, 2000).

5.4 Heavy metal tolerance of the isolated enterobacterial isolates

Similar pattern of metal tolerance (Pb^{2+}>Zn^{2+}>Cu^{2+}) observed among the Enterobacteriaceae isolates in this study werealso reported by Kabeer *et al.* (2018), where *Chromobacterium, Vibrio* and *Pseudomonas* from plant rhizospehere receiving non-point metal contamination source showed metal resistance in the order of Pb^{2+}>Zn^{2+}>Cu^{2+}>Cd^{2+}. However, the level of metal resistance of the Enterobacteriaceae strains in this present study were much greater than what was reported by Eghomwanre *et al.* (2016), where heavy metal minimum inhibitory concentrations of Enterobacteriaceae strains such as *E. coli, Klebsiella mobilis, Enterobacter* sp. from heavy metal contaminated mechanic workshops in Warri, Nigeria were between 5-20mg/l for Pb^{2+}, Cd^{2+}, Fe^{2+} and Zn^{2+}.

In the present study, Pb^{2+} had no inhibitory effect on the isolated enterobacterial strains at 1100 µg/ml. The high Pb^{2+} tolerance exhibited by the Gram negative bacteria strains were close to what was observed byNeethu *et al.* (2015), where Gram negative bacteria (n = 130) isolated from Kongsfjord in the Arctic tolerated up to 1000 µg/l of Pb^{2+}, with the total Pb -tolerant Gram negative bacteria reducing to 49% only at 2000 µg/l(Neethu *et al.*, 2015). Brunis *et al.* (2000) described Pb^{2+} as toxic to bacterial growth even at low concentrations, however, the resistance levels observed in this study suggests that these strains had developed reliable mechanisms to detoxify the lethal effects of Pb^{2+} on their cellular metabolism. The prevalence of Pb^{2+} tolerance by Gram negative bacteria and their corresponding resistance mechanisms has been widely reported(Jarosławiecka and Piotrowska-Seget, 2014; Neethu *et al.*, 2015). A key bacterial mechanism against the toxicity of Pb is the limitation of its (Pb) movement across the cell envelope (Bruins *et al.*, 2000), a role performed by lipopolysaccharides in Gram negative bacterial species (Jarosławiecka and Piotrowska-Seget, 2014). Other Pb resistance mechanisms in bacteria include extra-and intracellular precipitation of Pb^{2+}, biosorption of Pb on extracellular polymeric substances, binding of Pb^{2+} by siderophores and other specific proteins,

biotransformation of lead compounds, metallo-regulatory proteins and Pb^{2+} sensing, and efflux systems(Jarosławiecka and Piotrowska-Seget, 2014). In this study, Pb associated resistance gene *zntA* was observed to be present in all the sequenced strains, this may explain the elevated Pb tolerance by the enterobacterial isolates.

In contrast to results of the Pb^{2+} tolerance assay, Cu^{2+} and Zn^{2+} were toxic to the enterobacterial strains in the present study. Furthermore, investigations on the toxicity of Cu and Zn on Enterobacteriaceae have been determined in previous studies (Resende *et al.*, 2012; Neethu *et al.*, 2015). These elements are reported to disturb normal redox reactions involved in cellular metabolism to further lead to production of toxic hydroxyl ions(Porcheron *et al.*, 2013). However, Cu and Zn tolerance levels in enterobacterial strains from this study were much less than what was reported by Resende *et al.* (2012) where enterobacterial isolates (n=195) from the Nile tilapia aquaculture farm in Leopoldina, Brazil, had Cu and Zn $MIC_{90\%}$ at >1,024 µg/ml respectively.

Defence mechanisms against copper toxicity are conserved in many Gram negative bacteria and can be achieved by P-type ATPases that effectively pump Cu(II) out of the cell. Also, periplasmic Cu can be effectively effluxed out of the cell by large multi-component protein complexes, such as *cusCBA* of *E. coli*(Dupont*et al.*, 2011). In the present study, the *cusABCRS* gene cluster werepresent in 74.36% of the enterobacterial strains. In addition, these strains harboured a variety of other copper resistance genes including *pcoABDRS*, *cutCEF*, *cueO* and *copA* which were detected in 32.05%, 74.36%, 97.44% and 94.87% of the isolates respectively.

Tolerance to HMs such as Cu and Pb are usually connected with Zn tolerance. Zinc has however been described as the most occurring divalent positively charged ion in the environment and also the second most significant transition element in living things after Fe(Nies, 2000; Porcheron *et al.*, 2013; Neethu *et al.*, 2015). Resulting from their ubiquity and bioavailability (within pH of 5 to 7) (Mccauley *et al.*, 2017), various microorganisms have successfully adapted vital zinc homeostasis mechanisms which could be either chromosomal plasmid or transposon-encoded(Bruins*et al.*, 2000; Nies, 2000). In Enterobacteriaceae, homeostasis of Zn is largely facilitated via a coordination of specialized influx and efflux pumps(Porcheron *et al.* 2013). Bacterial tolerance to Zn usually results from mechanisms involving the sequestration, bioaccumulation and detoxification supported by various Zn binding proteins (Nies,

2000), however, in Enterobacteriaceae, detoxification is largely carried-out by the $P_{IB-type}$ ATPase *zntA* and several other trans-membrane proteins including *zitB* and *YiiP*(Porcheron*et al.*, 2013). In this study, zinc resistance genes such as *zntA*, *zitB*, *znuABC*, *zraSR*, *zinT* and *zupT* were detected in 100%,96.15%, 100%, 75.64%, 92.30% and 92.30% of the strains respectively. The observed phenotypic tolerance to Cu, Pb and Zn and the correlating genotypic resistance determinants in the enterobacterial strains of this study explains the elevated metal tolerance profiles of the isolates when compared to other literature reports described above.

While bacterial exposure to heavy metals has existed for a long time, predating human history(Koditschek and Guyre, 1974; Sütterlin *et al*., 2018), anthropogenic sources have exacerbated their input in the environment and now characterises a major source of metal contamination and concern worldwide (Jaishankar *et al.*, 2014). Detrimental effects of anthropogenic derived metals on exposed bacterial populations, such as what is obtained in E-waste dumpsites, have been particularly linked with the development and proliferation bacterial resistance to antibiotics (Pal*et al.*, 2015; Poole, 2017). The HMs selected for bacterial tolerance analysis in this study (Cu, Pb and Zn) have been reported in several studies to aid the co-selection of antibiotic resistance(Di Cesare*et al.*, 2016; Poole, 2017). In tandem, several of the metal resistance genes detected in this study have been well reported to aid co-selection of antibiotic resistance (Table 2.1 and Table 2.2).

5.5 Phenotypic antibiotic resistance profile of metal tolerant Enterobacteriaceae

Most of the heavy metal tolerant Enterobacteriaceae in this present study (89.29%) were observed to be resistant to more than one class of antibiotics. The development and proliferation of multidrug resistant Enterobacteriaceae species has severely complicated the treatment and control of infections and diseases in humans. From the present study, a larger percentage of the Enterobacteriaceaedisplayed phenotypic resistance to members of the third-generation cephalosporin antibiotics when compared to the carbapenem antibiotics used in susceptibility testing. This was in tandem with observations from other studies that have reported a greater selection of cephalosporin resistance in Enterobacteriaceae(Rohde *et al.*, 2018; Amador*et al.*, 2019; Rizzo*et al.*, 2019). The observed prevalence of resistance to the third generation cephalosporins

and carbapenems is concerning as infections resulting from carbapenems and cephalosporins resistant Enterobacteriaceae are a rising health care problem worldwide (Paterson, 2006; Rizzo *et al.*, 2019), and their occurrence have been described in various environmental matrices including livestock (Amador *et al.*, 2019), clinic (Kpoda *et al.*, 2018; Rohde *et al.*, 2018), wastewater(Caltagirone*et al.*, 2017), drinking water (Tanner *et al.*, 2019) and food (Ye *et al.*, 2018). The global increase in bacterial resistance to cephalosporinshas largely been credited to the proliferation of extended-spectrum β-lactamases (ESBLs).ESBLs conferring resistance to beta-lactam drugs are commonly carried on MGEs and are reported to often which often co-occur withothergenesconferring resistance to other antibiotic classes such as sulphonamides, fluoroquinolones and aminoglycosides(Paterson, 2006; Caltagirone *et al.*, 2017). Also, in this study, the aminoglycoside, gentamicin, was the most active against the Enterobacteriaceae (11.90 % resistant strains), whereas kanamycin had higher number of resistant strains (45.24%). These group of antibiotic are known to be particularly potent against Enterobacteriaceae(Krause *et al.*, 2016). In tandem with the findings of this study, surveillance studies have shown gentamicin to show good inhibition against Gram negative pathogens (Sader *et al.*, 2014) and Enterobacteriaceae(Sader *et al.*, 2015).

Furthermore, resistance displayed by the enterobacterial isolates from this this study to florfenicol (76.20%), sulphamethoxazole/trimethoprim and tetracycline (53.57% respectively) and ciprofloxacin (17.86%), were above what was reported from *E. coli* isolates in Tibetan pigs where resistance to tetracycline, florfenicol, sulphamethoxazole/trimethoprim, and ciprofloxacin were 40%, 27.9%, 19.4% and 7.8% respectively (Li *et al.* 2014). Similarly, the resistance profiles of this study were higher than those reported from Enterobacteriaceae species isolated from wastewaters around the Choupal wastewater treatment plant in Portugal which showed resistance to sulphamethoxazole/trimethoprim, tetracycline and ciprofloxacin of 21.1%, 18.2% and 14.1% respectively (Amador *et al.*, 2015). Based on extensive search to available published literatures, this study is very likely the first report of antimicrobial susceptibility of Enterobacteriaceaeisolates from E-waste dumpsites. Importantly, the observed high frequency of antibiotic resistance phenotypes by the metal tolerant Enterobacteriaceae in this study might strongly be a consequence of anthropogenic activities occurring in the metal contaminated E-waste sampling sites.

Correlation studies on bacterial resistance to heavy metal and antibiotic has been on the increase (Knapp *et al.*, 2017; Nguyen*et al.*, 2019) as soil and water ecosystems are known to have the ability to provide important platforms for selection and proliferation of resistance to multiple antibiotics and HMs (Hg^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+})(Nguyen *et al.*, 2019). Associationsoccurring between microbial resistance to antibiotics and tolerance to HMs from soil and water ecosystem has been widely reported(Narasimhulu*et al.*, 2010; Oyetibo *et al*., 2010; Romero *et al*., 2017). In tandem with the study of Oyetibo *et al.* (2010), where twenty two (n=22) bacteria isolates from heavy metals polluted soil and water samples within industrial estates in Lagos, Nigeria showed dual tolerance to heavy metals (Cd^{2+}, Co^{2+}, Ni^{2+}, Cr^{6+} and Hg^{2+}) and 18 clinically relevant antibiotics, this study for the first time in E-waste dumpsites also obtained strong evidence of co-resistance to HMs (Cu^{2+}, Pb^{2+}, Zn^{2+}) and antibiotics by Enterobacteriacea isolates.

The anthropogenic contamination of heavy metals such as what is observed in E-waste dumpsites in this study has been described to aid the proliferation of bacterial resistance to several antibiotics, including those analysed in this study, such as β-lactams(Hu *et al*., 2017), aminoglycosides(Li*et al.*, 2017), sulphamethoxazole/trimethoprim(Oyetibo *et al.*, 2010; Martins *et al.*, 2014), and tetracycline resistance(Lin *et al.*, 2016; Zhou *et al*., 2017). Further research into likely bacterial mechanism of metal and antibiotic co-resistance by the examination of the occurrence of resistance determinants to both antimicrobials provided insight to the crucial roles of heavy metals inselecting for antibiotic resistance in bacterial strains (Pal *et al.*, 2015).

5.6 The antibiotic resistome of the enterobacterial isolates

The enterobacterial isolates carried a plethora of ARGs which were widely distributed amongst the strains and were also observed to belong to different STs. Here, enterobacterial strains belonging to *E. coli* ST10 were the most common (n=10), coming from samples AR (n=5), AW1 (n=2), OR (n=2) and OS (n=1). All the *E. coli* ST-10 strains in this study contained at least one or more ARGs specifying resistance to antibiotics including the aminoglycosides [*aph (3")-Ib*, *aph(6)-Id*, *aadA17*, *aadA1*], sulphonamides (*sul1*, *sul2*, *sul3*), β-lactams ($bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$), trimethoprim (*dfrA7*, *dfrA12*, *dfrA14*), tetracyclines (*tetA*) and macrolides [*mdf(A)*]. The other ST-10 isolates

contained phenicol resistance genes *floR* (EC7) and *catA1* (EC16, EC25, EC28, EC65) and the plasmid mediated quinolone resistance gene, *qnrS1* (EC81 and EC82). The dominance and widespread occurrence of the endemic antibiotic resistant *E. coli* ST-10 in different environments has been described in other regions of the world(Aibinu*et al.*, 2012; Sonda *et al.*, 2018; Falgenhauer *et al.*, 2019). As a result of the wide range of ARGs possessed by members of *E. coli* ST-10, ST-38, ST-131 and ST-648, they have been described as emerging versatile clones of multidrug resistant bacteria with enhanced virulence in human and animals hosts(dos Anjos *et al.* 2019), and thus represents key actors in the AMR crisisworldwide. Consistent with this, two members of the *E. coli* ST-38 (EC37 and EC39) carrying *aadA5*, *sul2*, *qnrB7*, *dfrA17*, *tetA* and *mdf(A)* in their genomes were isolated in this study. However, unlike what was observed in *E. coli* ST-38 members in this study, members of the this ST have received increased public health attention for harbouring the carbapenem hydrolysing β-lactamase $bla_{\text{OXA-48}}$ (Izdebski *et al.*, 2018).

In this presentresearch, *E. coli* ST-215 (n=8) were the most common after ST-10. Majority of the members were isolated from samples AR (EC6, EC18, EC23, EC24, EC26 and EC32), AW1 (EC17) and OS (EC41). With the exception of *E. coli* EC8 which had an additional *tetA* gene, all the *E. coli* ST-215 carried the macrolide resistance gene *mdf(A)* as the only ARG in their resistome. More so, the *mdf(A)* gene were detected in all the *E. coli*isolates in this study. They are proton motive force driven efflux pumps which confers resistance to a broad spectrum of cationic or zwitterionic lipophilic compounds and various antibiotics including erythromycin, chloramphenicol, rifampin, puromycin, tetracycline, certain aminoglycosides and fluoroquinolones(Lewinson*et al.*, 2003). Cells expressing *mdf(A)* from multi-copy plasmids are regarded to be considerably more resistant, and has been observed in multi-drug resistant bacteria from clinical isolates(Wang *et al.*, 2013). Like the *mdf(A)*, the tetracycline resistance genes *tetA*, *tetC* and *tetD* observed among theEnterobacteriaceaeisolates from this study confer resistance by coding for energy-dependent efflux pumps. The high prevalence of *tetA* in the enterobacterial strains in this study (75%) has similarly been reported in Enterobacteriaceae from recent research studies (Sheykhsaran *et al.*, 2018; Amador *et al.*, 2019). However, *tetD* was found only in *C. freundii* EC11 ST-116 which interestingly shared the antibiotic

resistance phenotype CAZ, TET, SXT and FFC with a similar multidrug *C. freundii* ST116 isolated from diarrheal patients in a clinic in China (Liu *et al.*, 2018).

The *Citrobacter* strains in this study (except *Citrobacter* sp. EC71 which has *qnrB69* as the only ARG in its resistome) harboured resistance to at least six classes of antibiotics, with each strain containing a variant of the *ampC* β-lactamsase bla_{CMY} ($bla_{\text{CMY-100}}$, $bla_{\text{CMY-129}}$ and $bla_{\text{CMY-135}}$). In-line with results obtained in this study, the presence of $bla_{\text{CMY-100}}$ and $bla_{\text{CMY-135}}$ in *C. freundii* has been previously reported(Antonelli*et al.*, 2015), however, there is little or no report on the detection of $bla_{\text{CMY-129}}$ in *C. portucalensis*. Previous studies on *C. portucalensis* have reported $bla_{\text{CMY-37}}$ and $bla_{\text{CMY-13}}$ from companion dogs in Japan (Harada *et al.*, 2019), $bla_{\text{CMY-127}}$ from leafy vegetable in Nigeria (Igbinosa *et al.*, 2018) and $bla_{\text{CMY-39}}$ from poultry in Bangladesh (Hasan *et al.*, 2019). The ability of *C. portucalensis* to harbour a plethora of resistance genes against several antibiotic classes and their presence in diverse environments, including for the first time in E-waste dumpsites, might suggest *C. portucalensis* are fast emerging superbugs and deserve active surveillance to determine the extent of current risk to global antimicrobial resistance.

Similar to what was observed with the *Citrobacter* strains, the *ampC* β -lactamase gene bla_{MIR} or bla_{ACT} were detected in all the *Enterobacter* strains except in *Enterobacter kobei* EC53 which carried *fosA* as the only ARG in its genome. All the *Enterobacter* strains in this study (*En. cloacae*, *En. kobei*, *En. roggenkampii* and *En. hormaechei*) are members of the *E. cloacae* complex (Hoffmann and Roggenkamp, 2003; Sutton *et al*,. 2018). bla_{MIR} amino acid sequences have been reported to be common with the *En. cloacae* complex (Wu *et al.*, 2018), however, reports of the detection of bla_{MIR} in *En. roggenkampii* is scarce. In this study, $bla_{\text{MIR-1}}$, $bla_{\text{MIR-3}}$, $bla_{\text{MIR-5}}$ and $bla_{\text{MIR-6}}$ were detected in *En. roggenkampii* strains EC43, EC60, EC61 and EC52 respectively. Recently, the $bla_{\text{MIR-6}}$ detected in *En.cloacae* in a medical centre in Taiwan was reported to be carried on plasmids (Ku *et al.*, 2019). Similarly, members of the bla_{ACT}*ampC* β-lactamases are carried on plasmids and have been reported to undergo successful conjugative transfer to other bacterial species (Ku *et al.*, 2019). In this study, one isolate *En. hormaechei* EC63 carried a $bla_{\text{ACT-7}}$. Martins *et al.* (2019) recently reported the isolation of two $bla_{\text{ACT-7}}$ carrying *En. hormaechei* strains from urinary tract infections in Brazil. Generally, bacterial strains belonging to the *Enterobacter cloacae* complex are widely recognised as nosocomial pathogens with

the ability of causing an assortment of infections including pneumonia, bladder ailments, and septicaemia (Annavajhala *et al.*, 2019; Martins *et al.*, 2019) and are an emerging threat globally (Annavajhala*et al.*, 2019). The occurrence of *ampC* beta-lactamases carrying *Enterobacter* strains in water and soil samples within E-waste dumpsites from this study highlights the public health significance of these dumpsites as likely sources of public health important antibiotic resistant bacteria. Amongst the *Enterobacter* strains in this study, *Enterobacter* sp. EC64 carried the largest number of ARGs (n=8) in their resistome specifying resistance across six (6) antibiotic classes, however, the observed genotypic resistance did not correlate with antibiotic resistance phenotype.

5.7 Discrepancies between antibiotic phenotypes and genotype

In this study, there were several cases of antibiotic resistance phenotypes occurring without a corresponding genotype. A distinct example is with the *E. coli* EC4 ST-167 and *E. coli* EC6 ST-9428 which displayed phenotypic resistance to all the test antibiotics. Consistent with the antibiotic resistance phenotype, *E. coli* EC4 ST-167 harboured at least one ARG specifying resistance to each antibiotics it was resistant to. However, *E. coli* EC6 ST-9428 harboured no ARG in its genome. Studies have similarly reported discrepancies in correlating antibiotic resistance genotypes and phenotypes in resistant bacteria strains(Davis *et al.*, 2011; Ruppé *et al.*, 2017). Several reasons have been proffered for this phenomenon in bacterial strains. For example, the increased phenotypic resistance to third generation cephalosporins by *Enterobactericeae* as observed in this study has been linked to mutational events that cause the over-expression of chromosomally encoded *ampC* β-lactamase (Davis *et al.*, 2011; Ruppé *et al.*, 2017), indicating that ceftazidime, cefpodozime, cefoxitin, ceftriaxone may not constitute good markers to differentiate between phenotypic and genotypic resistance mechanisms in *E. coli*(Mammeri *et al.*, 2008). Additionally, resistance to carbapenems inEnterobacteriacea such as in*E. coli* and *K. pneumoniae* has been linked to production of ESBLs coupled with a shortfallin the function of outer membrane porins (Reuter *et al.*, 2013). Mutations occurring in *ampC* for instance, in combination with enhanced efflux leads to increase in clinical levels of resistance in fluoroquinolones(Fernandes*et al.*, 2003). In addition, several chromosomally occurring

efflux pump mechanisms are abundant in Gram negative bacteria and make significant contributions in raising the level of antimicrobial resistance in the bacteria(Li *et al.*, 2015b), for example, homologues of the RND, *Acr* and *Mex* efflux systems mediate intrinsic and acquired multidrug resistance in many Gram negative bacteria (Baucheron *et al.*, 2004; Piddock, 2006; Li *et al.,* 2015b). Furthermore, the over-expression of an efflux pump contribute to antibiotic resistance phenotypes and impacts greatly on therapeutics (Piddock, 2006).

5.8 SNP analysis and spread of antibiotic resistant clones

SNP analysis amongst different bacterial clonal complexes provides vital information of epidemiological relevance about the spreading and dissemination of resistant bacteria clones globally (Singh *et al.*, 2018). In this present study, SNP analysis showed theoccurence of multiple potential clones in different sample sites, for example, the zero (0) SNP count observed between multidrug resistant *E. coli* strains EC25 and EC16 obtained from river AR and hand-dug well AW1 samples respectively at different sampling periods within the same sampling area indicate possible transmission between two sampling sites. Several direct and indirect means of transmission mediated by human, animals and other activities could be responsible for the transmission of this bacterial clone from either location. Similarly, interspecies transmission of bacteria clones have been reported in literature. For example, the study of Schaufler *et al.* (2016), reported ten (10) *E. coli* ST-410 strains isolated from wild birds, environmental dog feaces and human clinical environment to show almost identical macrorestriction and pulsed-field gel electrophoresispatterns. In addition, further SNP investigation of the whole genomes revealed very low numbers of single nucleotide polymorphisms among the strains, thus providing important information on interspecies transmission of the clones.

Although this study inadvertently provided evidence of bacterial transmission between two water bodies within a smaller space (river and –dug well water within the Alaba International Market), the results however underscores the vital nature of the "One-health" approach in tackling antimicrobial resistance. In tandem with bacterial transmissions, antibiotic resistant clones (especially the high risk clones) have contributed greatly to the proliferation of multi-drug resistance worldwide via a varied number of mobile genetic elements, including the acquisition and dissemination of

plasmids harbouring antibiotic resistance genes in Gram negative bacteria(Woodford*et al.*, 2011).

5.9 Plasmids on the enterobacterial isolates

In this study, 93.59% of the Enterobacteriaceae contained diverse plasmid replicon types including multiple hybrid replicon such as IncFIB(K), IncFIA(HI1), IncFII(pRSB107), IncFIB(pB171), IncFII(Yp),IncFII(29), IncFIB(AP001918),IncFII(pECLA), IncFIB(pECLA),IncFII(pSE11), IncHI1B(R27), IncHI1B(CIT). All the plasmid groups detected in the Enterobacteriaceae strains in this present study have earlier been associated with harbouring a variety of ARGs (Rozwandowicz *et al.*, 2018). Similarly, most of the ARGs detected in this study have been previously described to be present on plasmids. However, *in silico* plasmid analysis to confirm their presence on plasmids and their subsequent transmission to other bacteria remains unknown. The high plasmid diversity observed within the enterobacterial strains in this study may be indicative of a well-established evolutionary mechanism for adaptation to environmental stresses imposed by toxins from the metal polluted E-waste dumpsites.

Furthermore, the inherent ability of plasmids to acquire and integrate novel ARGs, initiate their transfer and be stably replicated in a wide variety of host microorganisms makes them the very efficient vectors in the spread and evolution of antimicrobial resistance (Rozwandowicz *et al.* 2018). This probable feature further underscores the public health importance of E-waste dumpsites in the spread of AMR. Additionally, most of the metal resistance genes detected in bacterial isolates from the current study including *cop*, *cus*, *mer*, *pco*, *rcn*, *sil*, *ars* and *ter* gene clusters have been reported to be carried on mobile plasmids in Enterobacteriaceae(Kariuki *et al.*, 2015;Fang *et al.*, 2016; Falgenhauer *et al.*, 2017;Wu *et al.*, 2018). Furthermore, the probable co-localization of metal resistance genes and ARGs on detected plasmids may further facilitate their persistence and dissemination of resistance between bacteria species or genera within the E-waste dumpsites (Poole, 2017; Wu *et al.*, 2018).

5.10 ARG pollution in E-waste dumpsites

In this study, qPCR measurements showed that the absolute abundance of the ARGs which included*sul1*, *sul2*, *tetA*, *dfrA1*, *bla*$_{\text{CTX-M-1}}$ and MGE*intI1* were mostly persistent in the soil and water samples in the E-waste sampling sites during the three sampling campaigns. The overall normalized abundance (ARGs or *intI1*/ 16S rRNA) of the ARGs and *intI1* in soil samples were either similar or higher than values presented from severalregions of the world including China(Luo *et al.*, 2010; Xiong*et al.*, 2014; Chen *et al.*, 2015a), Finland (Tamminen *et al.*, 2011; Muziasari *et al.*, 2014), Nigeria (Adelowo *et al.*, 2018b), Pakistan (Khan *et al.*, 2013), Poland (Koczura*et al.*, 2016), Sweden (Berglund *et al.*, 2015), Switzerland(Devarajan *et al*., 2015) and USA(Pruden *et al*., 2006). Similar to the measured absolute abundance, the 16S normalized abundance of the ARGs and *intI1* in water samples were either similar or higher than values presented from severalregions of the world including China (Luo *et al.*, 2010; Xiong *et al.*, 2014; Chen *et al.*, 2015a; Lu *et al.*, 2018), Singapore (Le *et al.*, 2016), Poland (Koczura *et al.*, 2016) and pan Europe (Cacace *et al.*, 2019).

The *intI1*, *sul1* and *sul2* were the most abundant and most frequently occurring (in 100% of the samples) ARGs in samples from this study. The sulfonamides were the first drugs with a selective effect on bacteria (Sköld, 2000). Microorganism have since developed efficient mechanisms for combating the effects of the drug by encoding dihydropteorate synthase enzymes that do not bind to the drug (Sánchez-Osuna *et al.*, 2018). Such mechanisms are encoded by sulphonamide resistance genes (*sul1*, *sul2*, *sul3* and more recently *sul4*) (Sköld, 2000; Razavi *et al.*, 2017). These genes are predominantly plasmid and integron borne and thus supporting their widespread dissemination (Sánchez-Osuna *et al.*, 2018). High abundances of the sulphonamide resistance genes have been reported in many human-impacted environments (Chen *et al.*, 2015a; Koczura *et al.*, 2016; Adelowo *et al.* 2018b). In the present study, there were strong positive correlation between the absolute gene abundance of *intI1* with *sul1* ($r = 0.90$) and *sul2* ($r = 0.70$) at $P<0.05$. The correlation between *intI1* and *sul1* were higher than what was reported in wetlands in Nigeria (Adelowo *et al.*, 2018b) and aquaculture farms in the Baltic Sea (Muziasari *et al.*, 2014). This strong positive correlation is however expected as *sul1* forms one of the backbone genes of the 3' conserved segments of the class 1 integrons(Gillings, 2014; Romero *et al.*, 2017; Amos *et al.*, 2018; Murray *et al.*, 2019). Also, *sul2* have been reported to be found on non-conjugative small plasmids or on large multi-drug resistance plasmids in

bacteria(Hamidian*et al.*, 2016). Similar correlations between *intI1* and s*ul* genes have been described in other anthropogenic contaminated environments (Luo *et al.*, 2010; Chen *et al.*, 2015a).

Although some studies have reported *sul1* to be more prevalent in many bacterial strains than *sul2*(Muziasari et al. 2014; Domínguez *et al*., 2019), the mean absolute number of copies of *sul2* (5.91×10^7) were slightly higher than *sul1* (4.39×10^7) in this study. This trend was similar in polluted wetland in Nigeria (Adelowo *et al.*, 2018b), but different from results obtained by Koczura *et al.* (2016) which reported much lower abundance of *sul2* (from 0.051 to 0.083%) compared to *sul1* in water and sediment samples from an industrial waste water treatment facility in Poland. The mean copy numbers of the sulphonamide resistance genes determined in this present study were only one order of magnitude greater than *dfrA1*, suggesting that the prevalence of these ARGs in the natural environment deserves equal attention as sulphonamides and trimethoprim antibiotics are usually administered in combination (Muziasari *et al.*, 2014; CLSI, 2017). The absolute abundance of *dfrA1* measured in two hospital wastewaters in Singapore were higher (10^1 to 10^3) than 83.33% (n=20) of the water samples analysed in this study (Le *et al.*, 2016). The high abundance may have resulted from selection pressure owing to direct antibiotics pollution from hospital operations. For instance, Le *et al.* (2016) reported trimethoprim concentrations of 6.61 – 71.8 µg/l and 0.78 – 11.87 µg/l in the wastewaters from the study hospitals. *dfrA1* have been frequently described to occur as gene cassettes associated with Class 1 and 2 integrons (Odetoyin *et al*., 2017). This implies that they may be transferred when integrons are present on conjugative plasmids. In this study, the absolute gene abundance of *dfrA1* correlated positively with*intI1* (r = 0.76), *sul1* (r = 0.76) and *sul2* (r = 0.51). Strong correlations with *intI1* and *sul1* might indicate the possibility of them being associated with the class 1 integrons.

Like *dfrA1* and *sul* genes, *tetA* genes have also been reported to be carried by integrons(Asgharpour *et al.*, 2018). Thus, the absolute gene abundance of *tetA* significantly correlated with *intI1* (r = 0.69). Also, correlations (r) with *tetA* were 0.69 for *sul1*, 0.52 for *sul2* and 0.49 for *dfrA1*. Studies have reported the co-occurrence of *tetA*, *sul1*, *sul2*, *dfrA* in class 1 integrons found on *Enterobactericeae* strains (Dessie *et al.*, 2013; Drugdová and Kmeť, 2013). More so, several of the sequenced strains in this study including EC2, EC5, EC20, EC21, EC34, carried all 4 genes in their genomes

and these strains harboured diverse plasmid replicon types including IncB/O/K/Z, IncFII (29), IncFIB (AP001918), TrfA IncFIA(pRSB107) and IncQ1. Although the probability is high, it is still unclear if the four genes are genetically linked on these plasmids. Also, the absolute copy number of *tetA* in the present study were several magnitudes above (about 10^2 to 10^4) what was reported in drinking water treatment plant in China (Lu *et al.*, 2018). A similar pattern (about 10^1 to 10^3) was observed in soil samples in this study when compared with sediments from aquaculture farms located in Turku Achipelago, Finland (Tamminen *et al.*, 2011).

In contrast to other ARGs quantified in this present study, the absolute gene abundance of $bla_{\text{CTX-M-1}}$ had weak and insignificant correlations with the absolute abundance of *intI1* (-0.023), *sul1* (-0.007), *sul2* (0.04), *dfrA1* (0.044) and *tetA* (0.174). In contrast, the 16S normalized abundance of the gene were significant for *intI1* (0.54), *sul1* (0.32) and *tetA* (0.34). Discrepancies in bivariate correlations of ARG and metals using absolute and normalized gene abundances were similarly observed by Knapp *et al.* (2017) and was attributed to lower metal concentrations occurring in the soils. Similarly, Props *et al.* (2017) observed discrepancies in interpreting absolute and relative abundances in the quantification of microbial taxon abundances. These studies highlight the importance of considering both relative and absolute gene abundances for the interpretation of gene abundance and correlation data (Knapp *et al.*, 2017; Props *et al.*, 2017).

In soil samples where $bla_{\text{CTX-M-1}}$ were above level of quantification, their absolute abundance occurred in the upper limits of 10^1 – 10^2 above what was reported from sediment samples in Vidy Bay (Devarajan *et al.*, 2015). On the other hand, the quantified abundance of $bla_{\text{CTX-M-1}}$ in 75% of water samples from this study were below those reported from hospital wastewaters in Singapore (Le *et al.*, 2016). This is however not surprising as the prevalence and even outbreaks of $bla_{\text{CTX-M-1}}$ in hospital environment have been well recorded in several counties of the world, such as in Chile (Pavez *et al.*, 2019), France (Carrër *et al.*, 2009), Italy (Giani *et al.*, 2017), Netherlands (Dautzenberg *et al.*, 2014), Scotland (Younes *et al.*, 2011) and Canada (Boyd *et al.*, 2004), making hospitals a well-established reservoir of $bla_{\text{CTX-M-1}}$. The observed low frequency of occurrence of $bla_{\text{CTX-M-1}}$ in this study (43.5%) is in slight contrast with observation by Adelowo *et al.* (2018b) that pollution of the Nigerian environment with clinically relevant ARGs might still be at its early stages. There is currently little or no

information of the gene abundances of $bla_{\text{CTX-M-1}}$, *dfrA1* and *tetA* in the Nigerian environment, and hence the reason for poor comparison locally. Nonetheless, the high abundance of these genes compared with corresponding ARG contaminated sites is sufficient in characterizing the E-waste dumpsites as reservoirs for proliferation of AMR in microorganisms. Furthermore, *E. coli* (*uidA*) revealed moderate (0.40) [Co] to strong (0.61) [Al] correlations with all the heavy metals (except Se and Cd) in this study. The relationship between *E. coli* and other members of the Enterobacteriacea with heavy metals in proliferation of AMR in metal contaminated environments has been investigated in several studies (Porcheron *et al*., 2013; Fang *et al*., 2016).

5.11 Role of heavy metals in the proliferation of resistance genes in E-waste dumpsites

In the present study, the absolute abundances of *intI1* showed strongest correlations with the heavy metals (except Se) in the samples ($0.53>r<0.73$). Heavy metal contamination in the environment has been associated with increased prevalence of *intI1*(Rosewarne *et al.*, 2010). Heavy metals contaminants which were abundant in samples from E-waste dumpsites in this study, can cause perturbations in the bacterial communities which can trigger bacterial stress response systems (Baharoglu and Mazel, 2014). Such bacterial stress response systems causes the up-regulation of integron activity, dynamically rearranging the genes cassettes within the variable regions of the integrons(Baharoglu and Mazel, 2014; Gillings, 2018). The *intI1* and metal correlation seen in the present study were in line with observation by Su *et al.* (2014) for Cu and Zn but contrary to reports by Zhang *et al.* (2018a) where weak and insignificant correlations between *intI1* and heavy metals, Pb and Cd were reported in metal contaminated agricultural soils. The class 1 integrons arewell-described to be a good proxy in determining anthropogenic pollution activitiesoccurring in the natural environment owing to; the presence of connected resistance determinants conferring resistance to antibiotics, metals and biocides; its widespread diversity in bacteria strains; rapid response and dissemination between bacterial species owing to environmental changes (Gillings *et al.*, 2015). As earlier determined, evidence from correlation analysis and literature reports suggests that quantified genes in this study may be present on class 1 integrons. Thus, the possible selection of these genes within mobile elements by metals may also significantly impact on the promotion of AMR in the natural environment (Poole, 2017).

Various correlation patterns were observed between ARGs and HMs concentration in the samples from the E-waste sampling sites in this study. Correlation observed for *sul2* and *tetA* with Cu and Zn (Table 4.11 and 413) were much greater than the correlation figures reported by Zhang *et al.* (2018b) for Cu and Zn at r = 0.204 and 0.071 for *tetA* and *sul2* respectively. The absolute gene abundance of $bla_{\text{CTX-M-1}}$ showed negative and insignificant correlations ($-0.08 \geq r \leq -0.22$) with all the heavy metals, however, the reverse was observed (except for Co) when analysed with the 16S normalized abundance of the gene, $r > 0.36 < 0.56$ (Appendix XXV). This may be as a result of the discrepancy earlier described. Furthermore, a study by Laffite *et al.* (2016) on hospital effluents in Kinshasa, Democratic Republic of Congo reported significant correlation between$bla_{\text{CTX-M}}$ and the heavy metals Cd (r = 0.44), (r = 0.54), Cr and Cu(r = 0.54), Zn (r= 0.51), Pb (r= 0.52) and Hg (r = 0.37). In general Cd, Se and Co had weak correlations with the ARG measured in this study. Similar observations were observed with different ARG/ heavy metal correlated pair in the study of Knapp *et al.* (2017). Since bacterial response to heavy metals, like antibiotics, is concentration dependent, the phenomenon may be credited to the relatively lower concentration of the metals (Cd, Se and Co) in this study, (Bernier and Surette, 2013).

The results of the correlation between the quantified ARGs and HMs in the E-waste dumpsites in this study underscores the complex and vital relationships that exists between HM pollution and the proliferation of AMR in environmental reservoirs. However, to date, despite the presence of metals in E-waste dumpsites, very little has been done to investigate this phenomenon in E-waste dumpsite. This study will probably be the first to provide empirical evidence of the link between metals and AMR in E-waste dumpsites. Further, multivariate PCA analysis gave strong indications that the HM concentrations and ARG abundances in both soil and water samples obtained from the sampling sites during the three sampling campaigns showed no significant difference (observed from ellipses clusters from the PCA biplots) in the proliferation of AMR in these dumpsites. The PCA results consolidates on previous studies that have recognised the role for heavy metals, even at sub-lethal concentrations, in the evolution,propagation and spread of antimicrobial resistance in bacterial strains and bacterial communities (Chen *et al.* 2015b; Xu *et al.*, 2017).

CHAPTER SIX

SUMMARY AND CONCLUSIONS

6.1 Summary

For a long time, the practice of indiscriminate dumping, open burning and other non-sustainable metal extraction activities in E-wastes dumpsites have been a common activity in many parts of Nigeria and other developing nations. E-waste dumpsites are usually located in ecologically sensitive areas in close proximity to public water sources, agricultural farms and other human activities. The results of the present study which linked metal pollution to proliferation of ARGs within E-waste dumpsite is new and for the first time highlights the important contribution of metals found in E-waste dumpsites to the global AMR crisis. This study used classical microbiology techniques as well as culture dependent and culture independent molecular analysis to show that the selected E-waste sampling sites located in Lagos and Ibadan are reservoirs of AMR and that heavy metals contamination plays significant roles in the proliferation of AMR in the selected dumpsites, thus, establishing a role for E-waste dumpsites as a contributor to the global AMR crisis and a viable threat to public health.

6.2 Conclusion

This research was directed at investigating metal and antibiotic co-resistance in enterobacterial isolates from selected E-waste dumpsites in Lagos and Ibadan and further examine the roles of metal selection pressure to providing an important platform for the evolution and dissemination of AMR in the E-waste dumpsites. Hence, at the end of this study, the following conclusions were made:

1. The measured physicochemical properties of soil and water samples from the dumpsites showed that values are higher than that measured in control garden soil and permissible limits set by various regulatory bodies, suggesting that the soil health of the dumpsites is poor and the water unsuitable for human consumption.

2. Heavy metals analysis confirmed HMs contamination of the analysed samples obtained within the E-waste dumpsites. Thus giving significance on the input of toxic HMs into the natural environment due to informal and sub-standard heavy metals recycling practices on the E-waste dumpsites. Also, the presence of HMs in the water samples elucidatesfurther on the importance of soil-water contamination of heavy metals to public health.
3. This study confirmed the proliferation of metal-tolerant strains in all the samples from the E-waste dumpsites during the three sampling periods. Thus indicating that there is a constant selection of these strains resulting from heavy metal contamination in these sites.
4. The enterobacterial isolatesexhibited phenotypic co-resistance to selected HMs (Cu^{2+}, Pb^{2+} and Zn^{2+}) and ten clinically relevant antibiotics. Further investigation of the genotypic mechanisms of the observed HMs and antibiotic phenotypic resistance in the enterobacterial isolates revealed a large diversity of HMs and ARGs The detected metal resistance genes may enhance cross-resistance to antibiotics. The occurrence of genes specifying resistance to diverse antibiotics in the enterobacterial strains from the E-waste dumpsites is of serious concern to public health especially if they are located on mobilizable plasmids.
5. The study also detected a diverse range of plasmid types, indicating these strains may have a well-established means of receiving and disseminating novel resistance genes to cope with the toxins such as those resulting from heavy metal pollution in the E-waste dumpsites.
6. The microbial community of E-waste dumpsites have been largely unexplored, hence, further phylogenetic analysis of the enterobacterial strains revealed these isolates were spread across several clonal complexes and sequence types, including the discovery of five novel *E. coli* sequence types.
7. Real time quantitative PCR quantification of *intI1*, *sul1*, *sul2*, *dfrA1*, *tetA* and $bla_{\text{CTX-M-1}}$ provided important insights into the ARG contamination of samples from the E-waste dumpsites when compared to similar ARG contaminated sites around the world.
8. Heavy metal correlation analysis using bivariate and multivariate correlation confirmed that anthropogenic pollution of HMs in the E-waste dumpsites played significant roles in the proliferation of AMR in the E-waste dumpsites.

6.3 Recommendations

In line with the findings described above, the following major recommendation are proffered;

Results from the metal tolerance, drug resistance profiles and ARG content described in the enterobacterial isolates in addition to the high abundance of ARGs quantified in the soil and water samples underscore the need for active surveillance to determine extent of proliferation of resistance in the environment and their contribution to the burden of MR in the clinic or vice versa. An active AMR surveillance system would provide vital information on the extent of ARG pollution in the environment and would help in proffering efficient mitigation strategies. Similarly, such surveillance systems should be carried out strongly taking the "one-health" approach into account. The "one-health" approach is particularly important as toxins such as heavy metals from E-waste leach into surrounding soil, water and other ecosystems, thus further driving the indigenous microbial flora towards the evolution of AMR to cope with the toxicity of the toxins.

This study also strongly recommends the institution of effective clean-up management plans on existing E-waste dumpsites. Also, more efficient disposal and metal extraction processes of E-waste which does not any pose ecological risks should be employed. Furthermore, partnership at regional, national and continental levels including the engagement of relevant stakeholders characterized by adequate legislation, policies, strategies, and provision of appropriate resources and instruments to check and prevent the trans-boundary movement of end-of-life electrical electronics into Nigeria and other developing countries. This is necessary to reduce the health hazards related with E-wastes.

6.4 Contributions to knowledge

1. This study established for the first time that the selected E-waste dumpsites in Lagos and Ibadan are reservoirs of AMR. This information from these sites has extensive implications to similar E-waste dumpsites in developing countries.
2. The role of heavy metal pollutants in the E-waste dumpsites to the proliferation of AMR was also established in this study.
3. This study provided the first reports on the quantification of ARGs contaminants at E-waste dumpsites and *tetA* and *dfrA1* contamination in the Nigerian environment.

4. This research led to the discovery and curation of 5 novel *E. coli* sequence types (ST- 9428, ST-9815, ST-9816, ST-9817 and ST-9897).

REFERENCES

Abraham, E. P. and Chain, E. 1940. An Enzyme from Bacteria able to Destroy Penicillin. *Nature*, *146.* 3713: 837–837. https://doi.org/10.1038/146837a0

Achi, H. A., Adeofun, C. O., Gbadebo, A. M., Ufoegbune, G. C., Oyedepo, J. A., Amori, A. A. and Uwadiegwu, B. O. 2013. Solid Waste Management in Nigeria. *Waste Management for Everyone1*.1:1–7. https://doi.org/10.5901/jesr.2013.v3n4p45

Adelowo, O. O., Vollmers, J., Mäusezahl, I., Kaster, A. K. and Müller, J. A. 2018a. Detection of the carbapenemase gene bla VIM-5 in members of the *Pseudomonas putida* group isolated from polluted Nigerian wetlands. *Scientific Reports8*.1:. https://doi.org/10.1038/s41598-018-33535-3

Adelowo, O. O., Helbig, T., Knecht, C., Reincke, F., Mäusezahl, I. and Müller, J. A. 2018b. High abundances of class 1 integrase and sulfonamide resistance genes, and characterisation of class 1 integron gene cassettes in four urban wetlands in Nigeria. *PLoS ONE13*.11:. https://doi.org/10.1371/journal.pone.0208269

Adeniji, A. O., Okoh, O. O. and Okoh, A. I. 2018. Distribution pattern and health risk assessment of polycyclic aromatic hydrocarbons in the water and sediment of Algoa Bay, South Africa. *Environmental Geochemistry and Health* 1–18. https://doi.org/10.1007/s10653-018-0213-x

Adesokan, M. D., Adie, G. U. and Osibanjo, O. 2016. Soil Pollution by Toxic Metals near E-waste Recycling Operations in Ibadan, Nigeria. *Journal of Health and Pollution6*.11:26–33. https://doi.org/10.5696/2156-9614-6-11.26

Adesuyi, A. A., Nnodu, V. C., Njoku, K. L. and Anuoluwapo, J. 2015. Nitrate and Phosphate Pollution in Surface Water of. *International Journal of Geology, Agriculture and Environmental Sciences14*.20:14–20.

Aditi, S., Maitreyi, M., Parul, T. and Shweta, S. 2015. Resistance of heavy metals on some pathogenic bacterial species. *African Journal of Microbiology Research*, *9*.16: 1162–1164. https://doi.org/10.5897/ajmr2014.7344

Aendekerk, S., Ghysels, B., Cornelis, P. and Baysse, C. 2002. Characterization of a new efflux pump*, MexGHI-OpmD,* from *Pseudomonas aeruginosa* that confers resistance to vanadium. *Microbiology* (Vol. 148). https://doi.org/10.1099/00221287-148-8-2371

Aibinu, I., Odugbemi, T., Koenig, W. and Ghebremedhin, B. 2012. Sequence Type ST131 and ST10 Complex (ST617) predominant among CTX-M-15-producing *Escherichia coli* isolates from Nigeria. *Clinical Microbiology and Infection18*.3:2011–2013. https://doi.org/10.1111/j.1469-0691.2011.03730.x

Aina, A. T. and Oshunrinade, O. O. 2016. Comparison of water quality from boreholes and handdug wells around and within the University of Lagos, Lagos, Nigeria. *International Journal of Research in Environmental Studies3*.July:93–100.

Allen, H. K., Donato, J., Wang, H. H., Cloud-hansen, K. A., Davies, J. and

Handelsman, J. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.8*.4:251–259. https://doi.org/10.1038/nrmicro2312

Amador, P., Fernandes, R., Prudêncio, C. and Duarte, I. 2019. Prevalence of antibiotic resistance genes in multidrug-resistant Enterobacteriaceae on portuguese livestock manure. *Antibiotics8*.1:. https://doi.org/10.3390/antibiotics8010023

Amador, P. P., Fernandes, R. M., Prudêncio, M. C., Barreto, M. P. and Duarte, I. M. 2015. Antibiotic resistance in wastewater: Occurrence and fate of Enterobacteriaceae producers of Class A and Class C β-lactamases. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering50*.1:26–39. https://doi.org/10.1080/10934529.2015.964602

Amos, G. C. A., Ploumakis, S., Zhang, L., Hawkey, P. M., Gaze, W. H. and Wellington, E. M. H. 2018. The widespread dissemination of integrons throughout bacterial communities in a riverine system. *The ISME Journal12*.3:681–691. https://doi.org/10.1038/s41396-017-0030-8

Andersson, D. I. and Hughes, D. 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature Reviews Microbiology 2010 8:48*.4:260. https://doi.org/10.1038/nrmicro2319

Annavajhala, M. K., Gomez-Simmonds, A. and Uhlemann, A. C. 2019. Multidrug-resistant *Enterobacter cloacae* complex emerging as a global, diversifying threat. *Frontiers in Microbiology10*.JAN:44. https://doi.org/10.3389/fmicb.2019.00044

Anning, D. W. 2011. Modeled Sources, Transport, and Accumulation of Dissolved Solids in Water Resources of the Southwestern United States1. *JAWRA Journal of the American Water Resources Association47*.5:1087–1109. https://doi.org/10.1111/j.1752-1688.2011.00579.x

Ansari, M. I. and Malik, A. 2010. Seasonal variation of different microorganisms with nickel and cadmium in the industrial wastewater and agricultural soils. *Environmental Monitoring and Assessment167*.1–4:151–163. https://doi.org/10.1007/s10661-009-1038-y

Antonelli, A., D'Andrea, M. M., Vaggelli, G., Docquier, J. D. and Rossolini, G. M. 2015. OXA-372, a novel carbapenem-hydrolysing class D β-lactamase from a *Citrobacter freundii* isolated from a hospital wastewater plant. *Journal of Antimicrobial Chemotherapy70*.10:2749–2756. https://doi.org/10.1093/jac/dkv181

APHA and AWWA. 2012. *Standard methods for the examination of water and wastewater*. (E. W. Rice, Ed.) (22nd ed.). Washington, D.C. : American Water Works Association, 2012.

Appleyard, S., Wong, S., Willis-Jones, B., Angeloni, J. and Watkins, R. 2004. Groundwater acidification caused by urban development in Perth, Western Australia: Source, distribution, and implications for management. *Australian Journal of Soil Research42*.5–6:579–585.

Armalytė, J., Skerniškytė, J., Bakienė, E., Krasauskas, R., Šiugždinienė, R., Kareivienė, V. and Ružauskas, M. 2019. Microbial diversity and antimicrobial resistance profile in microbiota from soils of conventional and organic farming systems. *Frontiers in Microbiology*, *10.* APR: 892. https://doi.org/10.3389/fmicb.2019.00892

Asgharpour, F., Mahmoud, S., Marashi, A., and Moulana, Z. 2018. Molecular detection of class 1, 2 and 3 integrons and some antimicrobial resistance genes in Salmonella Infantis isolates. *Iranian Journal of Microbiology10*.2:104–110. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/29997750

Atieno, N., Owuor, O., and Omwoyo, O. 2013. Heavy metal and associated antibiotic resistance of fecal coliforms, fecal streptococci and pathogens isolated from wastewaters of abattoirs in Nairobi, Kenya. *Journal of Applied Biosciences64*.1:4858. https://doi.org/10.4314/jab.v64i1.88476

Asiimwe, E. N. and Åke, G. 2012. E-Waste Management in East African Community. *Handbook of Research on E-Government in Emerging Economies* 307–327. https://doi.org/10.4018/978-1-4666-0324-0.ch015

Awoniyi, M. A. 2016. The Emergence of Common Market in West Africa: An Examination of Cross Culture and Ethnographic Marketing System of Alaba International Market, Lagos-Nigeria. *American Journal of Industrial and Business Management*, *06.*02: 136–154. https://doi.org/10.4236/ajibm.2016.62014

Azeez, J. O., Hassan, O. A. and Egunjobi, P. O. 2011. Soil contamination at dumpsites: Implication of soil heavy metals distribution in municipal solid waste disposal system: A case study of Abeokuta, southwestern Nigeria. *Soil and Sediment Contamination20*.4:370–386. https://doi.org/10.1080/15320383.2011.571312

Badmus, B. S., Ozebo, V. C., Idowu, O. A., Ganiyu, S. A. and Olurin, O. T. 2014. Physico-chemical properties of soil samples and dumpsite environmental impact on groundwater quality in South Western Nigeria. *African Review of Physics9*.July:103–114.

Baharoglu, Z. and Mazel, D. 2014. SOS, the formidable strategy of bacteria against aggressions. *FEMS Microbiology Reviews38*.6:1126–1145. https://doi.org/10.1111/1574-6976.12077

Baker-Austin, C., Wright, M. S., Stepanauskas, R. and McArthur, J. V. 2006. Co-selection of antibiotic and metal resistance. *Trends in Microbiology14*.4:176–182. https://doi.org/10.1016/j.tim.2006.02.006

Baldé, C. P., Forti, V., Gray, V., Kuehr, R. and Stegmann, P. 2017. *The Global E-waste monter 2017*. United Nations University (UNU), International Telecommunication Union (ITU) & International Solid Waste Association (ISWA), Bonn/Geneva/Vienna. Retrieved from www.unu.edu

Balfour, E. 1943. THE LIVING SOIL evidence of the importance to human health of soil vitality, with special reference to national planning. *The Living Soil: Evidence of the Importance to Human Health of Soil Vitality, with Special Reference to*

Post-War Planning. Retrieved from http://biologia.ucr.ac.cr/profesores/Garcia Jaime/SUELO/THE LIVING SOIL-LIBRO COMPLETO.pdf

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S. and Pevzner, P. A. 2012. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology19*.5:455–477. https://doi.org/10.1089/cmb.2012.0021

Basel Action Network. 2011. Where are WEEE in Africa. E-waste Africa Programme WEEE UNEP-CHW-EWASTE-PUB-WeeAfricaReport.English.

Baucheron, S., Tyler, S., Boyd, D., Mulvey, M. R., Chaslus-Dancla, E. and Cloeckaert, A. 2004. AcrAB-TolC directs efflux-mediated multidrug resistance in Salmonella enterica serovar typhimurium DT104. *Antimicrobial Agents and Chemotherapy48*.10:3729–3735. https://doi.org/10.1128/AAC.48.10.3729-3735.2004

Bedenić, B., Slade, M., Starčević, L. Ž., Sardelić, S., Vranić-Ladavac, M., Benčić, A. and Tambić-Andraševic, A. 2018. Epidemic spread of OXA-48 beta-lactamase in Croatia. *Journal of Medical Microbiology67*.8:1031–1041. https://doi.org/10.1099/jmm.0.000777

Bengtsson-Palme, J., Kristiansson, E. and Larsson, D. G. J. 2018. Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Reviews42*.1:68–80. https://doi.org/10.1093/femsre/fux053

Benveniste, R. and Davies, J. 1973. Aminoglycoside antibiotic inactivating enzymes in actinomycetes similar to those present in clinical isolates of antibiotic resistant bacteria. *Proceedings of the National Academy of Sciences of the United States of America70*.8:2276–2280. https://doi.org/10.1073/pnas.70.8.2276

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F. and Martinez, J. L. 2015. Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology13*.5:310–317. https://doi.org/10.1038/nrmicro3439

Berglund, B., Fick, J. and Lindgren, P. E. 2015. Urban wastewater effluent increases antibiotic resistance gene concentrations in a receiving northern European river. *Environmental Toxicology and Chemistry34*.1:192–196. https://doi.org/10.1002/etc.2784

Bernier, S. P. and Surette, M. G. 2013. Concentration-dependent activity of antibiotics in natural environments. *Frontiers in Microbiology4*.FEB:20. https://doi.org/10.3389/fmicb.2013.00020

Blanco, P., Hernando-Amado, S., Reales-Calderon, J., Corona, F., Lira, F., Alcalde-Rico, M. and Martinez, J. 2016. Bacterial Multidrug Efflux Pumps: Much More Than Antibiotic Resistance Determinants. *Microorganisms4*.1:14. https://doi.org/10.3390/microorganisms4010014

Bolger, A. M., Lohse, M. and Usadel, B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics30*.15:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bosi, E., Donati, B., Galardini, M., Brunetti, S., Sagot, M.-F., Lió, P. and Fondi, M. 2015. MEDUSA: a multi-draft based scaffolder. *Bioinformatics* .15:2443–2451. https://doi.org/10.1093/bioinformatics/btv171

Boyd, D. A., Tyler, S., Christianson, S., McGeer, A., Muller, M. P., Willey, B. M. and Mulvey, M. R. 2004. Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. *Antimicrobial Agents and Chemotherapy48*.10:3758–3764. https://doi.org/10.1128/AAC.48.10.3758-3764.2004

Brady, N. C. and Weil, R. R. 2008. *The Nature and Properties of Soils* (Fourteenth). Retrieved from https://1642598126.rsc.cdn77.org/sites/tbbooks/pdf/Solutions-Manual-Nature-Properties-of-Soils-14th-Edition-Brady.pdf

Bruins, M. R., Kapil, S. and Oehme, F. W. 2000. Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety45*.3:198–207. https://doi.org/10.1006/eesa.1999.1860

Bush, K. 2018. Past and present perspectives on β-lactamases. *Antimicrobial Agents and Chemotherapy*, *62.* 10, e01076-18. https://doi.org/10.1128/AAC.01076-18

Cacace, D., Fatta-Kassinos, D., Manaia, C. M., Cytryn, E., Kreuzinger, N., Rizzo, L. and Berendonk, T. U. 2019. Antibiotic resistance genes in treated wastewater and in the receiving water bodies: A pan-European survey of urban settings. *Water Research162*:320–330. https://doi.org/10.1016/j.watres.2019.06.039

Caltagirone, M., Nucleo, E., Spalla, M., Zara, F., Novazzi, F., Marchetti, V. M. and Pagani, L. 2017. Occurrence of extended spectrum β-lactamases, KPC-Type, and MCR-1.2-producing enterobacteriaceae from wells, river water, and wastewater treatment plants in Oltrepò Pavese area, Northern Italy. *Frontiers in Microbiology8*.NOV:2232. https://doi.org/10.3389/fmicb.2017.02232

Carattoli, A., Zankari, E., Garciá-Fernández, A., Larsen, M. V., Lund, O., Villa, L. and Hasman, H. 2014. In Silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. *Antimicrobial Agents and Chemotherapy58*.7:3895–3903. https://doi.org/10.1128/AAC.02412-14

Carrër, A., Lassel, L., Fortineau, N., Mansouri, M., Anguel, N., Richard, C. and Nordmann, P. 2009. Outbreak of CTX-M-15-Producing *Klebsiella pneumoniae* in the Intensive Care Unit of a French Hospital. *Microbial Drug Resistance15*.1:47–54. https://doi.org/10.1089/mdr.2009.0868

Cavaco, L. M., Hasman, H., Stegger, M., Andersen, P. S., Skov, R., Fluit, A. C. and Aarestrup, F. M. 2010. Cloning and occurrence of czrC, a gene conferring cadmium and zinc resistance in methicillin-resistant *Staphylococcus aureus* CC398 isolates. *Antimicrobial Agents and Chemotherapy54*.9:3605–3608. https://doi.org/10.1128/AAC.00058-10

Celestial, R. G. 2018. "E-Waste Management in the Philippines." *E-Waste Management in the Philippines* .July: https://doi.org/10.13140/RG.2.2.17965.74728

Chait, R., Vetsigian, K. and Kishony, R. (2012). What counters antibiotic resistance in nature? *Nature Chemical Biology*, *8*.1: 2–5. https://doi.org/10.1038/nchembio.745

Chapin, K. C. and Lauderdale, T. 2003. Reagents, stains and media: bacteriology. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, & R. H. Yolken (Eds.), *Manual of Clinical Microbiology* (8th ed., p. 358). ASM Press, Washington D.C.

Chapman, J. S. (2003). Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *International Biodeterioration and Biodegradation*, *51*.4: 271–276. https://doi.org/10.1016/S0964-8305(03)00044-1

Chen, B., Liang, X., Nie, X., Huang, X., Zou, S. and Li, X. 2015a. The role of class I integrons in the dissemination of sulfonamide resistance genes in the Pearl River and Pearl River Estuary, South China. *Journal of Hazardous Materials282*:61–67. https://doi.org/10.1016/j.jhazmat.2014.06.010

Chen, S., Li, X., Sun, G., Zhang, Y., Su, J. and Ye, J. 2015b. Heavy metal induced antibiotic resistance in bacterium LSJC7. *International Journal of Molecular Sciences16*.10:23390–23404. https://doi.org/10.3390/ijms161023390

Clardy, J., Fischbach, M. A. and Currie, C. R. 2007. The natural history of antibiotics. *Current Biology*, *19*.11: 1–5. Retrieved from https://www.cell.com/current-biology/pdf/S0960-9822(09)00918-X.pdf

CLSI - Clinical and Laboratory Standards Institute. 2017. *M100-S24 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement An informational supplement for global application developed through the Clinical and Laboratory Standards Institute consensus process*. (P. Wayne, Ed.), *Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement M100-S15.\r* (27th Editi). Retrieved from www.clsi.org.

Cobbing, M. 2008. Toxic Tech: Not in Our Backyard. Uncovering the Hidden Flows of e-waste. Report from Greenpeace International. Retrieved June 18, 2019, from http://www.greenpeace.org/raw/content/ belgium/fr/press/reports/toxic-tech.pdf, Amsterdam, 2008.

Colclough, A., Corander, J., Sheppard, S. K., Bayliss, S. C. and Vos, M. 2019. Patterns of cross-resistance and collateral sensitivity between clinical antibiotics and natural antimicrobials. *Evolutionary Applications12*.5:878–887. https://doi.org/10.1111/eva.12762

Collignon, P. and Beggs, J. J. 2019. Socioeconomic Enablers for Contagion: Factors Impelling the Antimicrobial Resistance Epidemic. *Antibiotics8*.3:86. https://doi.org/10.3390/antibiotics8030086

Collignon, P., Beggs, J. J. and Walsh, T. R., Gandra, S., and Laxminarayan, R. 2018. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *The Lancet Planetary Health2*.9:e398–e405. https://doi.org/10.1016/S2542-5196(18)30186-4

Colomer-Lluch, M., Jofre, J. and Muniesa, M. 2011. Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. *PLoS ONE6*.3:.

https://doi.org/10.1371/journal.pone.0017549

Curutiu, C., Lazar, V. and Chifiriuc, M. C. 2017. *Pesticides and antimicrobial resistance: from environmental compartments to animal and human infections*. *New Pesticides and Soil Sensors*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-804299-1.00011-4

Cuzon, G., Ouanich, J., Gondret, R., Naas, T. and Nordmann, P. 2011. Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in France. *Antimicrobial Agents and Chemotherapy55*.5:2420–2423. https://doi.org/10.1128/AAC.01452-10

Danner, M. C., Robertson, A., Behrends, V. and Reiss, J. 2019. Antibiotic pollution in surface fresh waters: Occurrence and effects. *Science of the Total Environment664*:793–804. https://doi.org/10.1016/j.scitotenv.2019.01.406

Dautzenberg, M. J., Ossewaarde, J. M., de Kraker, M. E., van der Zee, A., van Burgh, S., de Greeff, S. C. and Bonten, M. J. 2014. Successful control of a hospital-wide outbreak of OXA-48 producing enterobacteriaceae in the Netherlands, 2009 to 2011. *Eurosurveillance19*.9:20723. https://doi.org/10.2807/1560-7917.ES2014.19.9.20723

Davies, J., and Davies, D. 2010. Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews74*.3:417–433. https://doi.org/10.1128/mmbr.00016-10

Davis, M. A., Besser, T. E., Orfe, L. H., Baker, K. N. K., Lanier, A. S., Broschat, S. L. and Call, D. R. 2011. Genotypic-phenotypic discrepancies between antibiotic resistance characteristics of *Escherichia coli* isolates from calves in management settings with high and low antibiotic use. *Applied and Environmental Microbiology77*.10:3293–3299. https://doi.org/10.1128/AEM.02588-10

De Rore, H., Top, E., Houwen, F., Mergeay, M. and Verstraete, W. 1994. Evolution of heavy metal resistant transconjugants in a soil environment with a concomitant selective pressure. *FEMS Microbiology Ecology14*.3:263–273. https://doi.org/10.1016/0168-6496(94)90008-6

Dessie, H. K., Bae, D. H. and Lee, Y. J. 2013. Characterization of integrons and their cassettes in Escherichia coli and Salmonella isolates from poultry in Korea. *Poultry Science92*.11:3036–3043. https://doi.org/10.3382/ps.2013-03312

Devarajan, N., Laffite, A., Graham, N. D., Meijer, M., Prabakar, K., Mubedi, J. I. and Poté, J. 2015. Accumulation of clinically relevant antibiotic-resistance genes, bacterial load, and metals in freshwater lake sediments in central europe. *Environmental Science and Technology49*.11:6528–6537. https://doi.org/10.1021/acs.est.5b01031

Di Cesare, A., Eckert, E. M. and Corno, G. 2016. Co-selection of antibiotic and heavy metal resistance in freshwater bacteria. *Journal of Limnology75*.2S:59–66. https://doi.org/10.4081/jlimnol.2016.1198

Domínguez, M., Miranda, C. D., Fuentes, O., de la Fuente, M., Godoy, F. A., Bello-Toledo, H. and González-Rocha, G. 2019. Occurrence of Transferable Integrons

and sul and dfr Genes Among Sulfonamide-and/or Trimethoprim-Resistant Bacteria Isolated From Chilean Salmonid Farms. *Frontiers in Microbiology10*:748. https://doi.org/10.3389/fmicb.2019.00748

dos Anjos, C., Sabino, C. P., Bueris, V., Fernandes, M. R., Pogliani, F. C., Lincopan, N. and Sellera, F. P. 2019. Antimicrobial blue light inactivation of international clones of multidrug-resistant *Escherichia coli* ST10, ST131 and ST648. *Photodiagnosis and Photodynamic Therapy27*.May:51–53. https://doi.org/10.1016/j.pdpdt.2019.05.014

Drugdová, Z. and Kmeť, V. 2013. Prevalence of β-lactam and fluoroquinolone resistance, and virulence factors in *Escherichia coli* isolated from chickens in Slovakia. *Biologia (Poland)68*.1:11–17. https://doi.org/10.2478/s11756-012-0142-6

Duan, M., Gu, J., Wang, X., Li, Y., Li, P. and Yin, Y. 2018. Combined effects of compost containing Sulfamethazine and zinc on pakchoi (Brassica chinensis L.) growth, soil sulfonamide resistance genes, and microbial communities. *Archives of Agronomy and Soil Science64*.2:231–243. https://doi.org/10.1080/03650340.2017.1342033

Dupont, C. L., Grass, G. and Rensing, C. 2011. Copper toxicity and the origin of bacterial resistance - New insights and applications. *Metallomics3*.11:1109–1118. https://doi.org/10.1039/c1mt00107h

Eghomwanre, A. F., Obayagbona, N. O., Osarenotor, O. and Enagbonma, B. J. 2016. Evaluation of antibiotic resistance patterns and heavy metals tolerance of some bacteria isolated from contaminated soils and sediments from Warri, Delta State, Nigeria. *Journal of Applied Sciences and Environmental Management20*.2:287-291–291. Retrieved from https://www.ajol.info/index.php/jasem/article/view/140398

El-Sayed, M. H. 2016. Multiple Heavy Metal and Antibiotic Resistance of *Acinetobacter baumannii* Strain HAF – 13 Isolated from Industrial Effluents. *American Journal of Microbiological Research, Vol. 4, 2016, Pages 26-364*.1:26–36. https://doi.org/10.12691/AJMR-4-1-3

EPA - Environmental Protection Agency. 2001. Parameters of water quality Interpretation and Standards. *Environmental Protection AGENCY, Ireland.* 1–133. https://doi.org/10.1017/CBO9781107415324.004

EPA. 2014. *Soil sampling - U.S environmental protection Agency (EPA)*.

Falgenhauer, L., Ghosh, H., Guerra, B., Yao, Y., Fritzenwanker, M., Fischer, J. and Chakraborty, T. 2017. Comparative genome analysis of IncHI2 VIM-1 carbapenemase-encoding plasmids of *Escherichia coli* and *Salmonella enterica* isolated from a livestock farm in Germany. *Veterinary Microbiology200*:114–117. https://doi.org/10.1016/j.vetmic.2015.09.001

Falgenhauer, L., Imirzalioglu, C., Oppong, K., Akenten, C. W., Hogan, B., Krumkamp, R. and Eibach, D. 2019. Detection and characterization of ESBL-producing *Escherichia coli* from humans and poultry in Ghana. *Frontiers in Microbiology10*.JAN:3358. https://doi.org/10.3389/fmicb.2018.03358

Fang, L., Li, X., Li, L., Li, S., Liao, X., Sun, J. and Liu, Y. 2016. Co-spread of metal and antibiotic resistance within ST3-IncHI2 plasmids from *E. coli* isolates of food-producing animals. *Scientific Reports6*.1:25312. https://doi.org/10.1038/srep25312

Fasina, A. S., Raji, A., Oluwatosin, G. A., Omoju, O. J. and Oluwadare, D. A. 2015. Properties, Genesis, Classification, Capability and Sustainable Management of Soils from South-Western Nigeria. *International Journal of Soil Science10*.3:142–152. https://doi.org/10.3923/ijss.2015.142.152

Ferguson, J. E. 1990. The heavy elements: chemistry, environmental impact and health effects. Retrieved from http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=UACHBC.xis&method=post&formato=2&cantidad=1&expresion=mfn=074673

Fernandes, P., Ferreira, S. B. and Joaquim, M. S. C. 2003. Solvent tolerance in bacteria: Role of efflux pumps and cross-resistance with antibiotics. *International Journal of Antimicrobial Agents22*.3:211–216. https://doi.org/10.1016/S0924-8579(03)00209-7

Feßler, A. T., Zhao, Q., Schoenfelder, S., Kadlec, K., Brenner Michael, G., Wang, Y. and Schwarz, S. 2017. Complete sequence of a plasmid from a bovine methicillin-resistant *Staphylococcus aureus* harbouring a novel ica-like gene cluster in addition to antimicrobial and heavy metal resistance genes. *Veterinary Microbiology200*:95–100. https://doi.org/10.1016/j.vetmic.2016.07.010

Flach, C. F., Johnning, A., Nilsson, I., Smalla, K., Kristiansson, E. and Larsson, D. G. J. 2015. Isolation of novel IncA/C and IncN fluoroquinolone resistance plasmids from an antibiotic-polluted lake. *Journal of Antimicrobial Chemotherapy70*.10:2709–2717. https://doi.org/10.1093/jac/dkv167

Fonseca, E. L. and Vicente, A. C. P. 2013. Epidemiology of qnrVC alleles and emergence out of the Vibrionaceae family. *Journal of Medical Microbiology*, *62.* PART10, 1628–1630. https://doi.org/10.1099/jmm.0.062661-0

Gangwar, C., Choudhari, R., Chauhan, A., Kumar, A., Singh, A. and Tripathi, A. 2019. Assessment of air pollution caused by illegal E-waste burning to evaluate the human health risk. *Environment International125*:191–199. https://doi.org/10.1016/J.ENVINT.2018.11.051

Ghosh, A., Singh, A., Ramteke, P. W. and Singh, V. P. 2000. Characterization of large plasmids encoding resistance to toxic heavy metals in Salmonella abortus equi. *Biochemical and Biophysical Research Communications272*.1:6–11. https://doi.org/10.1006/bbrc.2000.2727

Giani, T., Antonelli, A., Caltagirone, M., Mauri, C., Nicchi, J., Arena, F. and Rossolini, G. M. 2017. Evolving beta-lactamase epidemiology in Enterobacteriaceae from Italian nationwide surveillance, October 2013: KPC-carbapenemase spreading among outpatients. *Eurosurveillance22*.31:1–11. https://doi.org/10.2807/1560-7917.ES.2017.22.31.30583

Gillings, M. R. 2014. Integrons: Past, Present, and Future. *Microbiology and Molecular Biology Reviews78*.2:257–277. https://doi.org/10.1128/mmbr.00056-

Gillings, M. R. 2018. DNA as a Pollutant: the Clinical Class 1 Integron. *Current Pollution Reports4*.1:49–55. https://doi.org/10.1007/s40726-018-0076-x

Gillings, M. R., Gaze, W. H., Pruden, A., Smalla, K., Tiedje, J. M. and Zhu, Y. G. 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME Journal9*.6:1269–1279. https://doi.org/10.1038/ismej.2014.226

Gochfeld, M. 2003. Cases of mercury exposure, bioavailability, and absorption. *Ecotoxicology and Environmental Safety56*.1:174–179. https://doi.org/10.1016/S0147-6513(03)00060-5

Gómez-Sanz, E., Kadlec, K., Feßler, A. T., Zarazaga, M., Torres, C. and Schwarz, S. 2013. Novel erm(T)-carrying multiresistance plasmids from porcine and human isolates of methicillin-resistant Staphylococcus aureus ST398 that also harbor cadmium and copper resistance determinants. *Antimicrobial Agents and Chemotherapy57*.7:3275–3282. https://doi.org/10.1128/AAC.00171-13

Gorovtsov, A. V., Sazykin, I. S. and Sazykina, M. A. 2018. The influence of heavy metals, polyaromatic hydrocarbons, and polychlorinated biphenyls pollution on the development of antibiotic resistance in soils. *Environmental Science and Pollution Research25*.10:9283–9292. https://doi.org/10.1007/s11356-018-1465-9

Gren, I. 2012. science Microbial transformation of xenobiotics. *Chemik66*.8:839–842.

Griffiths, P. A., Babb, J. R., Bradley, C. R. and Fraise, A. P. 1997. Glutaraldehyde-resistant mycobacterium chelonae from endoscope washer disinfectors. *Journal of Applied Microbiology*, *82.* 4: 519–526. https://doi.org/10.1046/j.1365-2672.1997.00171.x

Guan, X., Xue, X., Liu, Y., Wang, J., Wang, Y., Wang, J. and Pan, L. 2013. Plasmid-mediated quinolone resistance - Current knowledge and future perspectives. *Journal of International Medical Research41*.1:20–30. https://doi.org/10.1177/0300060513475965

Guo, L., An, J., Ma, Y., Ye, L., Luo, Y., Tao, C. and Yang, J. 2016. Nosocomial outbreak of oxa-48-producing klebsiella pneumoniae in a Chinese hospital: Clonal transmission of st147 and st383. *PLoS ONE11*.8:e0160754. https://doi.org/10.1371/journal.pone.0160754

Gweme, F., Maringe, H., Ngoyi, L. and van Stam, G. 2016. E-Waste in Zimbabwe and Zambia. *1st Institute of Lifelong Learning and Development Studies International Research Conference, Chinhoyi University of Technology, 2-5 August 2016, Chinhoyi, Zimbabwe* .August:

Ha, N. N., Agusa, T., Ramu, K., Tu, N. P. C., Murata, S., Bulbule, K. A. and Tanabe, S. 2009. Contamination by trace elements at E-waste recycling sites in Bangalore, India. *Chemosphere76*.1:9–15. https://doi.org/10.1016/j.chemosphere.2009.02.056

Hakim, A., Suzuki, T. and Kobayashi, M. 2019. Strength of Humic Acid Aggregates: Effects of Divalent Cations and Solution pH. *ACS Omega4*.5:8559–8567. research-article. https://doi.org/10.1021/acsomega.9b00124

Hall, A. R. and Corno, G. 2014. Tetracycline modifies competitive interactions in experimental microcosms containing bacteria isolated from freshwater. *FEMS Microbiology Ecology90*.1:168–174. https://doi.org/10.1111/1574-6941.12388

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* .Series No. 41:95–98. Retrieved from http://jwbrown.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf

Hamidian, M., Ambrose, S. J. and Hall, R. M. 2016. A large conjugative Acinetobacter baumannii plasmid carrying the sul2 sulphonamide and strAB streptomycin resistance genes. *Plasmid87–88*:43–50. https://doi.org/10.1016/j.plasmid.2016.09.001

Händel, N., Hoeksema, M., Mata, M. F., Brul, S. and Ter Kuile, B. H. 2016. Effects of stress, reactive oxygen species, and the SOS response on de novo acquisition of antibiotic resistance in Escherichia coli. *Antimicrobial Agents and Chemotherapy60*.3:1319–1327. https://doi.org/10.1128/AAC.02684-15

Harada, K. and Asai, T. 2010. Role of antimicrobial selective pressure and secondary factors on antimicrobial resistance prevalence in *Escherichia coli* from food-producing animals in Japan. *Journal of Biomedicine and Biotechnology2010*:180682. https://doi.org/10.1155/2010/180682

Harada, K., Shimizu, T., Ozaki, H., Kimura, Y., Miyamoto, T. and Tsuyuki, Y. 2019. Characterization of Antimicrobial Resistance in *Serratia* spp. and *Citrobacter* spp. Isolates from Companion Animals in Japan: Nosocomial Dissemination of Extended-Spectrum Cephalosporin-Resistant *Citrobacter freundii*. *Microorganisms7*.3:64. https://doi.org/10.3390/microorganisms7030064

Harrison, J. J., Ceri, H. and Turner, R. J. 2007. Multimetal resistance and tolerance in microbial biofilms. *Nature Reviews Microbiology5*.12:928–938. https://doi.org/10.1038/nrmicro1774

Hasan, M. S., Sultana, M. and Hossain, M. A. 2019. Complete genome arrangement revealed the emergence of a poultry origin superbug *Citrobacter portucalensis* strain NR-12. *Journal of Global Antimicrobial Resistance18*:126–129. https://doi.org/10.1016/j.jgar.2019.05.031

Hearn, E. M., Dennis, J. J., Gray, M. R. and Foght, J. M. 2003. Identification and Characterization of the emhABC Efflux System for Polycyclic Aromatic Hydrocarbons in Pseudomonas fluorescens cLP6a. *Journal of Bacteriology185*.21:6233–6240. https://doi.org/10.1128/JB.185.21.6233-6240.2003

Hernando, M. D., Mezcua, M., Fernández-Alba, A. R. and Barceló, D. 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta69*.2:334–342. https://doi.org/10.1016/J.TALANTA.2005.09.037

Hilty, L. M. 2005. Electronic waste—an emerging risk? *Environmental Impact Assessment Review* 431–435. Retrieved from https://www.infona.pl/resource/bwmeta1.element.elsevier-a75e7a1b-d155-321d-

b6bc-2ae34eacf50a

Hoffmann, H. and Roggenkamp, A. 2003. Population genetics of the nomenspecies Enterobacter cloacae. *Applied and Environmental Microbiology69*.9:5306–5318. https://doi.org/10.1128/AEM.69.9.5306-5318.2003

Holmes, A. H., Moore, L. S. P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A. and Piddock, L. J. V. 2016. Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet387*.10014:176–187. https://doi.org/10.1016/S0140-6736(15)00473-0

Hoskisson, P. A. (2016). Streptomyces in Nature and Medicine: the Antibiotic Makers. In *Microbe Magazine* (Vol. 3). https://doi.org/10.1128/microbe.3.151.1

Hu, H. W., Wang, J. T., Li, J., Shi, X. Z., Ma, Y. B., Chen, D. and He, J. Z. 2017. Long-term nickel contamination increases the occurrence of antibiotic resistance genes in agricultural soils. *Environmental Science and Technology51*.2:790–800. https://doi.org/10.1021/acs.est.6b03383

Ideriah, T. J. K., Omuaru, V. O. T. and Adiukwu, P. U. 2006. Soil quality around a solid waste dumpsite in Port Harcourt, Nigeria. *African Journal of Ecology44*.3:388–394. https://doi.org/10.1111/j.1365-2028.2006.00632.x

Igbinosa, E. O., Rathje, J., Habermann, D., Brinks, E., Cho, G. S. and Franz, C. M. A. P. 2018. Draft genome sequence of multidrug-resistant strain *Citrobacter portucalensis* MBTC-1222, isolated from uziza (Piper guineense) leaves in Nigeria. *Genome Announcements6*.9:e00123-18. https://doi.org/10.1128/genomeA.00123-18

Ikhimiukor, O. O. and Adelowo, O. O. 2018. Selective metal accumulation by metal-resistant bacteria growing on spent engine oil in single and ternary metal mixtures. *International Journal of Environmental Science and Technology*. https://doi.org/10.1007/s13762-018-2137-5

IWRG. 2009. Industrial Waste Resource Guideline for Sampling Contaminated Soil .June:1–12.

Izdebski, R., Baraniak, A., Zabicka, D., Machulska, M., Urbanowicz, P., Fiett, J. and Wcisło-Wach, B. 2018. Enterobacteriaceae producing OXA-48-like carbapenemases in Poland, 2013-January 2017. *Journal of Antimicrobial Chemotherapy73*.3:620–625. https://doi.org/10.1093/jac/dkx457

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B. and Beeregowda, K. N. 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology7*.2:60–72. https://doi.org/10.2478/intox-2014-0009

Jarosławiecka, A. and Piotrowska-Seget, Z. 2014. Lead resistance in micro-organisms. *Microbiology (United Kingdom)160*.PART 1:12–25. https://doi.org/10.1099/mic.0.070284-0

Jia, B., Raphenya, A. R., Alcock, B., Waglechner, N., Guo, P., Tsang, K. K. and McArthur, A. G. 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Research45*.D1:D566–D573. https://doi.org/10.1093/nar/gkw1004

Kaas, R. S., Leekitcharoenphon, P., Aarestrup, F. M. and Lund, O. 2014. Solving the problem of comparing whole bacterial genomes across different sequencing platforms. *PLoS ONE9*.8:e104984. https://doi.org/10.1371/journal.pone.0104984

Kabeer, R., Rinoy, V., and Sylas, V. P. 2018. Metal tolerant and antibiotic resistant bacteria from the rhizosphere of water hyacinth: a study from a wetland receiving non point source of contamination. *International Research Journal of Environmental Sciences7*.6:34–45.

Kariuki, S., Okoro, C., Kiiru, J., Njoroge, S., Omuse, G., Langridge, G. and Revathi, G. 2015. Ceftriaxone-Resistant *Salmonella enterica* Serotype Typhimurium Sequence Type 313 from Kenyan Patients Is Associated with the $bla_{CTX-M-15}$ Gene on a Novel IncHI2 Plasmid. *Antimicrobial Agents and Chemotherapy59*.6:3133–3139. https://doi.org/10.1128/AAC.00078-15

Kaushik, R., Balasubramanian, R. and de la Cruz, A. A. 2012. Influence of air quality on the composition of microbial pathogens in fresh rainwater. *Applied and Environmental Microbiology78*.8:2813–2818. https://doi.org/10.1128/AEM.07695-11

Kazlauskaite-Jadzeviče, A., Volungevičius, J., Gregorauskiene, V. and Marcinkonis, S. 2014. The role of pH in heavy metal contamination of urban soil. *Journal of Environmental Engineering and Landscape Management22*.4:311–318. https://doi.org/10.3846/16486897.2013.872117

Kerrn, M. B., Klemmensen, T., Frimodt-Møller, N. and Espersen, F. 2002. Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. *Journal of Antimicrobial Chemotherapy50*.4:513–516. https://doi.org/10.1093/jac/dkf164

Khakabimamaghani, S., Najafi, A., Ranjbar, R. and Raam, M. 2013. GelClust: A software tool for gel electrophoresis images analysis and dendrogram generation. *Computer Methods and Programs in Biomedicine111*.2:512–518. https://doi.org/10.1016/j.cmpb.2013.04.013

Khan, F. A., Söderquist, B. and Jass, J. 2019. Prevalence and Diversity of Antibiotic Resistance Genes in Swedish Aquatic Environments Impacted by Household and Hospital Wastewater. *Frontiers in Microbiology10*:688. https://doi.org/10.3389/fmicb.2019.00688

Khan, G. A., Berglund, B., Khan, K. M., Lindgren, P. E. and Fick, J. 2013. Occurrence and Abundance of Antibiotics and Resistance Genes in Rivers, Canal and near Drug Formulation Facilities - A Study in Pakistan. *PLoS ONE8*.6:e62712. https://doi.org/10.1371/journal.pone.0062712

Kimiran-Erdem, A., Arslan-Aydoğdu, E. Ö., Gürün, S. and Altun, Ö. 2015. Determination of multiple antibiotic and heavy metal resistance of the bacteria isolated from the Küçükçekmece Lagoon, Turkey. *Polish Journal of Environmental Studies*, *24*.3: 1077–1084. https://doi.org/10.15244/pjoes/29202

Kleiner, D. K., Katz, S. E. and Ward, P. M. L. 2007. Development of in vitro antimicrobial resistance in bacteria exposed to residue level exposures of

antimicrobial drugs, pesticides and veterinary drugs. *Chemotherapy53*.2:132–136. https://doi.org/10.1159/000100012

Knapp, C. W., Callan, A. C., Aitken, B., Shearn, R., Koenders, A. and Hinwood, A. 2017. Relationship between antibiotic resistance genes and metals in residential soil samples from Western Australia. *Environmental Science and Pollution Research24*.3:2484–2494. https://doi.org/10.1007/s11356-016-7997-y

Koczura, R., Mokracka, J., Taraszewska, A. and Łopacinska, N. 2016. Abundance of Class 1 Integron-Integrase and Sulfonamide Resistance Genes in River Water and Sediment Is Affected by Anthropogenic Pressure and Environmental Factors. *Microbial Ecology72*.4:909–916. https://doi.org/10.1007/s00248-016-0843-4

Koditschek, L. K. and Guyre, P. 1974. Resistance transfer fecal coliforms isolated from the whippany river. *Water Research8*.10:747–752. https://doi.org/10.1016/0043-1354(74)90019-0

Kola, A., Piening, B., Pape, U. F., Veltzke-Schlieker, W., Kaase, M., Geffers, C. and Gastmeier, P. 2015. An outbreak of carbapenem-resistant OXA-48 - producing *Klebsiella pneumonia* associated to duodenoscopy. *Antimicrobial Resistance and Infection Control4*.1:8. https://doi.org/10.1186/s13756-015-0049-4

Kpoda, D. S., Ajayi, A., Somda, M., Traore, O., Guessennd, N., Ouattara, A. S. and Dosso, M. 2018. Distribution of resistance genes encoding ESBLs in Enterobacteriaceae isolated from biological samples in health centers in Ouagadougou, Burkina Faso. *BMC Research Notes11*.1:471. https://doi.org/10.1186/s13104-018-3581-5

Krause, K. M., Serio, A. W., Kane, T. R. and Connolly, L. E. 2016. Aminoglycosides: An overview. *Cold Spring Harbor Perspectives in Medicine6*.6:. https://doi.org/10.1101/cshperspect.a027029

Ku, Y.-H., Lee, M.-F., Chuang, Y.-C. and Yu, W.-L. 2019. Detection of Plasmid-Mediated β-Lactamase Genes and Emergence of a Novel AmpC (CMH-1) in *Enterobacter cloacae* at a Medical Center in Southern Taiwan. *Journal of Clinical Medicine8*.1:8. https://doi.org/10.3390/jcm8010008

Kücken, D., Feucht, H. H. and Kaulfers, P. M. 2000. Association of qacE and qacEΔ1 with multiple resistance to antibiotics and antiseptics in clinical isolates of Gram-negative bacteria. *FEMS Microbiology Letters183*.1:95–98. https://doi.org/10.1016/S0378-1097(99)00636-9

Kurenbach, B., Hill, A. M., Godsoe, W., Van Hamelsveld, S. and Heinemann, J. A. 2018. Agrichemicals and antibiotics in combination increase antibiotic resistance evolution. *PeerJ2018*.10:e5801. https://doi.org/10.7717/peerj.5801

Kurenbach, B., Marjoshi, D., Amábile-Cuevas, C. F., Ferguson, G. C., Godsoe, W., Gibson, P. and Heinemann, J. A. 2015. Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and Glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium. *MBio6*.2:e00009-15. https://doi.org/10.1128/mBio.00009-15

Labunska, I., Brigden, K., Santillo, D., Kiselev, A. and Paul, J. 2010. *Russian R fuse 2 : Russian R fuse 2 : an update on PBDEs and other contaminants detected in St-Petersburg area, Russia*. *Greenpeace Research Laboratories Technical Note 04/2010*.

Laffite, A., Kilunga, P. I., Kayembe, J. M., Devarajan, N., Mulaji, C. K., Giuliani, G. and Poté, J. 2016. Hospital Effluents Are One of Several Sources of Metal, Antibiotic Resistance Genes, and Bacterial Markers Disseminated in Sub-Saharan Urban Rivers. *Frontiers in Microbiology7*:1128. https://doi.org/10.3389/fmicb.2016.01128

Larsen, M. V., Cosentino, S., Rasmussen, S., Friis, C., Hasman, H., Marvig, R. L. and Lund, O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. *Journal of Clinical Microbiology50*.4:1355–1361. https://doi.org/10.1128/JCM.06094-11

Lawal, S. 2019. Nigeria has become an e-waste dumpsite for Europe, US and Asia. *TRTWORLD*. Retrieved from https://www.trtworld.com/magazine/nigeria-has-become-an-e-waste-dumpsite-for-europe-us-and-asia-24197. Accessed February 15 2019.

Le, T. H., Ng, C., Chen, H., Yi, X. Z., Koh, T. H., Barkham, T. M. S. and Gin, K. Y. H. 2016. Occurrences and characterization of antibiotic-resistant bacteria and genetic determinants of hospital wastewater in a tropical country. *Antimicrobial Agents and Chemotherapy60*.12:7449–7456. https://doi.org/10.1128/AAC.01556-16

Lenart, A. and Wolny-Koładka, K. 2013. The effect of heavy metal concentration and soil pH on the abundance of selected microbial groups within ArcelorMittal Poland steelworks in Cracow. *Bulletin of Environmental Contamination and Toxicology90*.1:85–90. https://doi.org/10.1007/s00128-012-0869-3

Levy, S. B. 1997. Antibiotic resistance: an ecological imbalance. *JOHN WILEY & SONS*. https://doi.org/10.1002/9780470515358

Lewinson, O., Adler, J., Poelarends, G. J., Mazurkiewicz, P., Driessen, A. J. M. and Bibi, E. 2003. The Escherichia coli multidrug transporter MdfA catalyzes both electrogenic and electroneutral transport reactions. *Proceedings of the National Academy of Sciences of the United States of America100*.4:1667–1672. https://doi.org/10.1073/pnas.0435544100

Lewis, A. 2010. *Europe breaking electronic waste export ban*. *Bbc* (Vol. 2011). Retrieved from http://www.bbc.co.uk/news/world-europe-10846395

Li, A. D., Li, L. G. and Zhang, T. 2015b. Exploring antibiotic resistance genes and metal resistance genes in plasmid metagenomes from wastewater treatment plants. *Frontiers in Microbiology6*.SEP: https://doi.org/10.3389/fmicb.2015.01025

Li, L. G., Xia, Y. and Zhang, T. 2017. Co-occurrence of antibiotic and metal resistance genes revealed in complete genome collection. *ISME Journal11*.3:651–662. https://doi.org/10.1038/ismej.2016.155

Li, P., Wu, D., Liu, K., Suolang, S., He, T., Liu, X. and Lin, D. 2014. Investigation of antimicrobial resistance in *Escherichia coli* and enterococci isolated from Tibetan pigs. *PLoS ONE9*.4:. https://doi.org/10.1371/journal.pone.0095623

Li, X. Z., Plésiat, P. and Nikaido, H. 2015a. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clinical Microbiology Reviews28*.2:337–418. https://doi.org/10.1128/CMR.00117-14

Lin, W. H. and Kussell, E. 2016. Complex Interplay of Physiology and Selection in the Emergence of Antibiotic Resistance. *Current Biology26*.11:1486–1493. https://doi.org/10.1016/j.cub.2016.04.015

Liu, L., Chen, D., Liu, L., Lan, R., Hao, S., Jin, W. and Xu, J. 2018. Genetic diversity, multidrug resistance, and virulence of *Citrobacter freundii* from diarrheal patients and healthy individuals. *Frontiers in Cellular and Infection Microbiology8*.JUL:233. https://doi.org/10.3389/fcimb.2018.00233

Livermore, D. M., Canton, R., Gniadkowski, M., Nordmann, P., Rossolini, G. M., Arlet, G. and Woodford, N. 2007. CTX-M: Changing the face of ESBLs in Europe. *Journal of Antimicrobial Chemotherapy59*.2:165–174. https://doi.org/10.1093/jac/dkl483

Looft, T., Johnson, T. A., Allen, H. K., Bayles, D. O., Alt, D. P., Stedtfeld, R. D. and Stanton, T. B. 2012. In-feed antibiotic effects on the swine intestinal microbiome. *Proceedings of the National Academy of Sciences109*.5:1691–1696. https://doi.org/10.1073/pnas.1120238109

Loucif, L., Kassah-Laouar, A., Saidi, M., Messala, A., Chelaghma, W. and Rolain, J. M. 2016. Outbreak of OXA-48-producing *Klebsiella pneumoniae* involving a sequence type 101 clone in Batna University Hospital, Algeria. *Antimicrobial Agents and Chemotherapy60*.12:7494–7497. https://doi.org/10.1128/AAC.00525-16

Lu, J., Tian, Z., Yu, J., Yang, M. and Zhang, Y. 2018. Distribution and abundance of antibiotic resistance genes in sand settling reservoirs and drinking water treatment plants across the Yellow River, China. *Water (Switzerland)10*.3:7–10. https://doi.org/10.3390/w10030246

Luo, Y., Mao, D., Rysz, M., Zhou, Q., Zhang, H., Xu, L. and Alvarez, P. J. J. 2010. Trends in antibiotic resistance genes occurrence in the Haihe River, China. *Environmental Science and Technology44*.19:7220–7225. https://doi.org/10.1021/es100233w

Lutgring, J. D., Zhu, W., De Man, T. J. B., Avillan, J. J., Anderson, K. F., Lonsway, D. R. and Limbago, B. M. 2018. Phenotypic and genotypic characterization of enterobacteriaceae producing oxacillinase-48-like carbapenemases, United States. *Emerging Infectious Diseases24*.4:700–709. https://doi.org/10.3201/eid2404.171377

Mammeri, H., Eb, F., Berkani, A. and Nordmann, P. 2008. Molecular characterization of AmpC-producing Escherichia coli clinical isolates recovered in a French hospital. *Journal of Antimicrobial Chemotherapy61*.3:498–503. https://doi.org/10.1093/jac/dkm538

Martínez-Martínez, L., Pascual, A. and Jacoby, G. A. 1998. Quinolone resistance from a transferable plasmid. *Lancet351*.9105:797–799. https://doi.org/10.1016/S0140-6736(97)07322-4

Martinez, J. L. 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution157*.11:2893–2902. https://doi.org/10.1016/j.envpol.2009.05.051

Martinez, J. L., Sánchez, M. B., Martínez-Solano, L., Hernandez, A., Garmendia, L., Fajardo, A. and Alvarez-Ortega, C. 2009. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. *FEMS Microbiology Reviews33*.2:430–449. https://doi.org/10.1111/j.1574-6976.2008.00157.x

Martins, E. R., Bueno, M. F. C., Francisco, G. R., Casella, T., de Oliveira Garcia, D., Cerdeira, L. T. and Nogueira, M. C. L. 2019. Genome and plasmid context of two rmtG-carrying *Enterobacter hormaechei* isolated from urinary tract infections in Brazil. *Journal of Global Antimicrobial Resistance*. https://doi.org/10.1016/j.jgar.2019.06.020

Martins, V. V., Zanetti, M. O. B., Pitondo-Silva, A. and Stehling, E. G. 2014. Aquatic environments polluted with antibiotics and heavy metals: a human health hazard. *Environmental Science and Pollution Research21*.9:5873–5878. https://doi.org/10.1007/s11356-014-2509-4

Máthé, I., Benedek, T., Táncsics, A., Palatinszky, M., Lányi, S. and Márialigeti, K. 2012. Diversity, activity, antibiotic and heavy metal resistance of bacteria from petroleum hydrocarbon contaminated soils located in Harghita County (Romania). *International Biodeterioration and Biodegradation73*:41–49. https://doi.org/10.1016/j.ibiod.2012.05.018

Mathers, A. J., Hazen, K. C., Carroll, J., Yeh, A. J., Cox, H. L., Bonomo, R. A. and Sifri, C. D. 2013. First clinical cases of OXA-48-producing carbapenem-resistant klebsiella pneumoniae in the united states: The "menace" arrives in the new world. *Journal of Clinical Microbiology51*.2:680–683. https://doi.org/10.1128/JCM.02580-12

Mbaka, P. K., Mwangi, J. K. and Kiptum, C. K. 2017. Assessment of water quality in selected shallow wells of Keiyo Highlands, Kenya. *African Journal of Science, Technology, Innovation and Development9*.3:329–338. https://doi.org/10.1080/20421338.2017.1327476

McEwen, S. A. and Collignon, P. J. 2018. Antimicrobial Resistance: a One Health Perspective. In *Antimicrobial Resistance in Bacteria from Livestock and Companion Animals* (Vol. 6, pp. 521–547). American Society of Microbiology. https://doi.org/10.1128/microbiolspec.arba-0009-2017

Mccauley, A., Jones, C. and Olson-Rutz, K. 2017. Soil pH and Organic Matter. *Nutrient ManagementModule No.*4449–8:16 p.

McKenzie, H. R. 2003. Soil pH and Plant Nutrients. *Ag-Info Centre Fact Sheet* .May:1–2.

Miller, J. 2016. Soil pH Affects Nutrient Availability. *University of Maryland*

Extension .July:1–5. https://doi.org/10.13140/RG.2.1.2423.5768

Mohammed, A. S., Kapri, A. and Goel, R. 2011. Heavy Metal Pollution: Source, Impact, and Remedies (pp. 1–28). Springer, Dordrecht. https://doi.org/10.1007/978-94-007-1914-9_1

Mosolygó, T., Kincses, A., Csonka, A., Tönki, Á. S., Witek, K., Sanmartín, C. and Spengler, G. 2019. Selenocompounds as novel antibacterial agents and bacterial efflux pump inhibitors. *Molecules*. https://doi.org/10.3390/molecules24081487

Mourão, J., Novais, C., Machado, J., Peixe, L. and Antunes, P. 2015. Metal tolerance in emerging clinically relevant multidrug-resistant *Salmonella enterica* serotype 4,[5],12:i:- clones circulating in Europe. *International Journal of Antimicrobial Agents45*.6:610–616. https://doi.org/10.1016/j.ijantimicag.2015.01.013

Murray, A. K., Zhang, L., Snape, J. and Gaze, W. H. 2019. Comparing the selective and co-selective effects of different antimicrobials in bacterial communities. *International Journal of Antimicrobial Agents53*.6:767–773. https://doi.org/10.1016/j.ijantimicag.2019.03.001

Muziasari, W. I., Managaki, S., Pärnänen, K., Karkman, A., Lyra, C., Tamminen, M. and Virta, M. 2014. Sulphonamide and trimethoprim resistance genes persist in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. *PLoS ONE9*.3:e92702. https://doi.org/10.1371/journal.pone.0092702

Naphade, S. R., Durve, A. A., Bhot, M., Varghese, J., Chandra, N. and Thane, D. 2012. Isolation , characterization and identification of pesticide tolerating bacteria from garden soil. *Europian Journal of Experimental Biology2*.5:1943–1951.

Narasimhulu, K., Rao, S. and Venu Vinod, A. 2010. Isolation and Identifi cation of Bacterial Strains and Study of their Resistance to Heavy Metals and Antibiotics. *Journal of Microbial & Biochemical Technology02*.03:074–076. https://doi.org/10.4172/1948-5948.1000027

Ndihokubwayo, J., Yahaya, A., Desta, A., Ki-Zerbo, G., Odei, E., Keita, B. and Nkhoma, W. 2013. *Antimicrobial resistance in the African Region: issues, challenges and actions proposed. Key Determinants for the African Region.WHO Press* (Vol. 16). Retrieved from http://www.who.int/bulletin/%0Ahttps://www.aho.afro.who.int/en/ahm/issue/16/reports/antimicrobial-resistance-african-region-issues-challenges-and-actions-proposed

Neethu, C. S., Rahiman, K. M. M., Saramma, A. V. and Hatha, A. A. M. 2015. Heavy-metal resistance in gram-negative bacteria isolated from kongsfjord, Arctic. *Canadian Journal of Microbiology61*.6:429–435. https://doi.org/10.1139/cjm-2014-0803

Ng, L. K., Martin, I., Alfa, M. and Mulvey, M. 2001. Multiplex PCR for the detection of tetracycline resistant genes. *Molecular and Cellular Probes15*.4:209–215. https://doi.org/10.1006/mcpr.2001.0363

Nguyen, C. C., Hugie, C. N., Kile, M. L. and Navab-Daneshmand, T. 2019.

Association between heavy metals and antibiotic-resistant human pathogens in environmental reservoirs: A review. *Frontiers of Environmental Science and Engineering13*.3:46. https://doi.org/10.1007/s11783-019-1129-0

Nies, D. H. 1999. Microbial heavy-metal resistance. *Applied Microbiology and Biotechnology51*.6:730–750. https://doi.org/10.1007/s002530051457

Nies, D. H. 2000. Heavy metal-resistant bacteria as extremophiles: Molecular physiology and biotechnological use of *Ralstonia* sp. CH34. *Extremophiles4*.2:77–82. https://doi.org/10.1007/s007920050140

Nies, D. H. 2003. Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiology Reviews27*.2–3:313–339. https://doi.org/10.1016/S0168-6445(03)00048-2

Nies, D. H., and Silver, S. 1995. Ion efflux systems involved in bacterial metal resistances. *Journal of Industrial Microbiology14*.2:186–199. https://doi.org/10.1007/BF01569902

Nkansah, M. A., Boadi, N. O. and Badu, M. 2010. Assessment of the Quality of Water from Hand-Dug Wells in Ghana. *Environmental Health Insights4*:EHI.S3149. https://doi.org/10.4137/ehi.s3149

Nnorom, I. C., Osibanjo, O., Okechukwu, K., Nkwachukwu, O. and R.C, C. 2013. Evaluation of Heavy Metal Release from the Disposal of Waste Computer Monitors at an Open Dump. *International Journal of Environmental Science and Development1*.3:227–233. https://doi.org/10.7763/ijesd.2010.v1.44

Nolivos, S., Cayron, J., Dedieu, A., Page, A., Delolme, F. and Lesterlin, C. 2019. Role of AcrAB-TolC multidrug efflux pump in drug-resistance acquisition by plasmid transfer. *Science (New York, N.Y.)364*.6442:778–782. https://doi.org/10.1126/science.aav6390

Obianefo, F., Agbagwa, I. and Tanee, F. B. . 2017. Physicochemical Characteristics of Soil from Selected Solid Waste Dump Sites in Port. *J. Appl. Sci. Environ. Manage21*.6:1153–1156.

Odetoyin, B. W., Labar, A. S., Lamikanra, A., Aboderin, A. O. and Okeke, I. N. 2017. Classes 1 and 2 integrons in faecal *Escherichia coli* strains isolated from mother-child pairs in Nigeria. *PLoS ONE12*.8:e0183383. https://doi.org/10.1371/journal.pone.0183383

Odokuma, L. O., and Ijeomah, S. O. 2004. Seasonal changes in the heavy metal resistant bacterial population of the New Calabar River, Nigeria. *Global Journal of Pure and Applied Sciences9*.4:425–434. https://doi.org/10.4314/gjpas.v9i4.16048

Odunaike, R. K., Alausa, S. K., Oyebanjo, O. A., Ijeoma, G. C. and Alo, A. O. 2008a. Measurement of Radiation Level in Refuse Dumps across Lagos metropolis, Southwestern Part of Nigeria. *Environmental Research Journal2*.4:174–176.

Odunaike, R. K., Laoye, J. A., Alausa, S. K., Ijeoma, G. C. and Adelaja, A. D. 2008b. Radiation Emission Characterization of Waste Dumpsites in the City of Ibadan in Oyo State of Nigeria. *Research Journal of Environmental Toxicology2*.2:100–

103. https://doi.org/10.3923/rjet.2008.100.103

OECD. 2013. OECD environmental outlook to 2030. https://doi.org/10.5860/choice.46-1270

Ogungbuyi, O., Osibanjo, O. and Schluep, M. 2012. e-Waste Country Assessment Nigeria e-Waste Africa project of the Secretariat of the Basel Convention e-Waste Country Assessment Nigeria .May: Retrieved from http://www.basel.int/Portals/4/Basel Convention/docs/eWaste/EwasteAfrica_Nigeria-Assessment.pdf

Ohajinwa, C., Peijnenburg, W. and Osibanjo, O. 2016. 383 Incidence and injury patterns among electronic waste workers in informal sector in Ibadan, Nigeria. *Injury Prevention22*.Suppl 2:A140.2-A140. https://doi.org/10.1136/injuryprev-2016-042156.383

Okorhi, O. J., Okereka, E. E., Akhimie, C. O. and Enekwenchi, K. K. 2018. Frontiers and prospects for recycling Waste Electrical and Electronic Equipment (WEEE) in Nigeria. *Journal of Applied Sciences and Environmental Management21*.7:1382. https://doi.org/10.4314/jasem.v21i7.30

Olafisoye, O. B., Adefioye, T. and Osibote, O. A. 2013. Heavy metals contamination of water, soil, and plants around an electronic waste dumpsite. *Polish Journal of Environmental Studies22*.5:1431–1439.

Omole, D. O., Tenebe, I. T., Emenike, C. P., Umoh, A. S. and Badejo, A. A. 2015. Causes, impact and management of electronic wastes: Case study of some nigerian communities. *ARPN Journal of Engineering and Applied Sciences10*.18:7876–7874. Retrieved from http://eprints.covenantuniversity.edu.ng/5632/

Oriomah, C., Adelowo, O. O. and Adekanmbi, A. O. 2015. Bacteria from spent engine-oil-contaminated soils possess dual tolerance to hydrocarbon and heavy metals, and degrade spent oil in the presence of copper, lead, zinc and combinations thereof. *Annals of Microbiology65*.1:207–215. https://doi.org/10.1007/s13213-014-0851-x

Oyetibo, G. O., Ilori, M. O., Adebusoye, S. A., Obayori, O. S. and Amund, O. O. 2010. Bacteria with dual resistance to elevated concentrations of heavy metals and antibiotics in Nigerian contaminated systems. *Environmental Monitoring and Assessment168*.1–4:305–314. https://doi.org/10.1007/s10661-009-1114-3

Pal, C., Asiani, K., Arya, S., Rensing, C., Stekel, D. J., Larsson, D. G. J. and Hobman, J. L. 2017. *Metal Resistance and Its Association With Antibiotic Resistance*. *Advances in Microbial Physiology* (1st ed., Vol. 70). Elsevier Ltd. https://doi.org/10.1016/bs.ampbs.2017.02.001

Pal, C., Bengtsson-Palme, J., Kristiansson, E. and Larsson, D. G. J. 2015. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. *BMC Genomics16*.1:1–14. https://doi.org/10.1186/s12864-015-2153-5

Pal, C., Bengtsson-Palme, J., Rensing, C., Kristiansson, E. and Larsson, D. G. J. 2014.

BacMet: antibacterial biocide and metal resistance genes database. *Nucleic Acids Research42*.D1:D737–D743. https://doi.org/10.1093/nar/gkt1252

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P. and Tyson, G. W. 2015. CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research25*.7:1043–1055. https://doi.org/10.1101/gr.186072.114

Paterson, D. L. 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. *American Journal of Infection Control34*.5 SUPPL.:20–28. https://doi.org/10.1016/j.ajic.2006.05.238

Pavez, M., Troncoso, C., Osses, I., Salazar, R., Illesca, V., Reydet, P. and Barrientos, L. 2019. High prevalence of CTX-M-1 group in ESBL-producing enterobacteriaceae infection in intensive care units in southern Chile. *The Brazilian Journal of Infectious Diseases23*.2:102–110. https://doi.org/10.1016/J.BJID.2019.03.002

Peltier, E., Vincent, J., Finn, C. and Graham, D. W. 2010. Zinc-induced antibiotic resistance in activated sludge bioreactors. *Water Research44*.13:3829–3836. https://doi.org/10.1016/j.watres.2010.04.041

Peralta, G. L. and Fontanos, P. M. 2006. E-waste issues and measures in the Philippines. *Journal of Material Cycles and Waste Management8*.1:34–39. https://doi.org/10.1007/s10163-005-0142-5

Piddock, L. J. V. 2006. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clinical Microbiology Reviews19*.2:382–402. https://doi.org/10.1128/CMR.19.2.382-402.2006

Pirš, M., Cerar Kišek, T., Križan Hergouth, V., Seme, K., Mueller Premru, M., Jeverica, S. and Lejko Z., T. 2019. Successful control of the first OXA-48 and/or NDM carbapenemase-producing Klebsiella pneumoniae outbreak in Slovenia 2014–2016. *Journal of Hospital Infection101*.2:142–149. https://doi.org/10.1016/j.jhin.2018.10.022

Poirel, L., Potron, A. and Nordmann, P. 2012. OXA-48-like carbapenemases: The phantom menace. *Journal of Antimicrobial Chemotherapy67*.7:1597–1606. https://doi.org/10.1093/jac/dks121

Poirel, L., Rodriguez-Martinez, J. M., Mammeri, H., Liard, A. and Nordmann, P. 2005. Origin of plasmid-mediated quinolone resistance determinant *qnrA*. *Antimicrobial Agents and Chemotherapy49*.8:3523–3525. https://doi.org/10.1128/AAC.49.8.3523-3525.2005

Pookkasorn, S. and Sharp, A. 2016. The Management of Waste from Electrical and Electronic Equipment (WEEE) in Bangkok, Thailand 6–9. https://doi.org/10.15242/iicbe.c0816218

Poole, K. 2017. At the Nexus of Antibiotics and Metals: The Impact of Cu and Zn on Antibiotic Activity and Resistance. *Trends in Microbiology25*.10:820–832. https://doi.org/10.1016/j.tim.2017.04.010

Porcheron, G., Garénaux, A., Proulx, J., Sabri, M. and Dozois, C. M. 2013. Iron,

copper, zinc, and manganese transport and regulation in pathogenic Enterobacteria: Correlations between strains, site of infection and the relative importance of the different metal transport systems for virulence. *Frontiers in Cellular and Infection Microbiology3*.DEC:90. https://doi.org/10.3389/fcimb.2013.00090

Props, R., Kerckhof, F.-M., Rubbens, P., De Vrieze, J., Hernandez Sanabria, E., Waegeman, W. and Boon, N. 2017. Absolute quantification of microbial taxon abundances. *The ISME Journal11*.2:584–587. https://doi.org/10.1038/ismej.2016.117

Pruden, A., Pei, R., Storteboom, H. and Carlson, K. H. 2006. Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado. *Environmental Science and Technology40*.23:7445–7450. https://doi.org/10.1021/es0604131

Ramadan, A. A., Abdelaziz, N. A., Amin, M. A. and Aziz, R. K. 2019. Novel *bla*CTX-M variants and genotype-phenotype correlations among clinical isolates of extended spectrum beta lactamase-producing *Escherichia coli*. *Scientific Reports*, *9.* 1: 4224. https://doi.org/10.1038/s41598-019-39730-0

Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N. and Megharaj, M. 2019. Local applications but global implications: Can pesticides drive microorganisms to develop antimicrobial resistance? *Science of the Total Environment654*:177–189. https://doi.org/10.1016/j.scitotenv.2018.11.041

Razavi, M., Marathe, N. P., Gillings, M. R., Flach, C.-F., Kristiansson, E., and Joakim Larsson, D. G. 2017. Discovery of the fourth mobile sulfonamide resistance gene. *Microbiome5*.1:160. https://doi.org/10.1186/s40168-017-0379-y

Resende, J. A., Silva, V. L., Fontes, C. O., Souza-Filho, J. A., De Oliveira, T. L. R., Coelho, C. M. and Diniz, C. G. 2012. Multidrug-resistance and toxic metal tolerance of medically important bacteria isolated from an aquaculture system. *Microbes and Environments27*.4:449–455. https://doi.org/10.1264/jsme2.ME12049

Reuter, S., Ellington, M. J., Cartwright, E. J. P., Köser, C. U., Török, M. E., Gouliouris, T. and Peacock, S. J. 2013. Rapid bacterial whole-genome sequencing to enhance diagnostic and public health microbiology. *JAMA Internal Medicine173*.15:1397–1404. https://doi.org/10.1001/jamainternmed.2013.7734

Review on Antimicrobial Resistance. 2014. *Antimicrobial Resistance : Tackling a crisis for the health and wealth of nations*. *Review on Antimicrobial Resistance*. London: Wellcome Trust & HM Government.

Rizzo, K., Horwich-Scholefield, S. and Epson, E. 2019. Carbapenem and Cephalosporin Resistance among Enterobacteriaceae in Healthcare-Associated Infections, California, USA1. *Emerging Infectious Diseases25*.7:1389–1393. https://doi.org/10.3201/eid2507.181938

Robicsek, A., Jacoby, G. A. and Hooper, D. C. 2006. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infectious Diseases*, *6.*10: 629–640. https://doi.org/10.1016/S1473-3099(06)70599-0

Robustillo-Rodela, A., Pérez-Blanco, V., Espinel Ruiz, M. A., Ruiz Carrascoso, G., Figueira Iglesias, J. C. and Abad Martín, D. 2017. Successful control of 2 simultaneous outbreaks of OXA-48 carbapenemase-producing Enterobacteriaceae and multidrug-resistant *Acinetobacter baumannii* in an intensive care unit. *American Journal of Infection Control45*.12:1356–1362. https://doi.org/10.1016/j.ajic.2017.07.018

Roemhild, R. and Schulenburg, H. 2019. Evolutionary ecology meets the antibiotic crisis. *Evolution, Medicine and Public Health2019*.1:37–45. https://doi.org/10.1093/emph/eoz008

Rohde, A. M., Zweigner, J., Wiese-Posselt, M., Schwab, F., Behnke, M., Kola, A. and Wolke, S. 2018. Incidence of infections due to third generation cephalosporin-resistant Enterobacteriaceae - A prospective multicentre cohort study in six German university hospitals. *Antimicrobial Resistance and Infection Control7*.1:159. https://doi.org/10.1186/s13756-018-0452-8

Romero, J. L., Grande Burgos, M. J., Pérez-Pulido, R., Gálvez, A. and Lucas, R. 2017. Resistance to Antibiotics, Biocides, Preservatives and Metals in Bacteria Isolated from Seafoods: Co-Selection of Strains Resistant or Tolerant to Different Classes of Compounds. *Frontiers in Microbiology8*:1650. https://doi.org/10.3389/fmicb.2017.01650

Rosewarne, C. P., Pettigrove, V., Stokes, H. W. and Parsons, Y. M. 2010. Class 1 integrons in benthic bacterial communities: abundance, association with Tn *402* -like transposition modules and evidence for coselection with heavy-metal resistance. *FEMS Microbiology Ecology72*.1:35–46. https://doi.org/10.1111/j.1574-6941.2009.00823.x

Ross, J. S. and Katz, M. H. 2015. No time to wait. *JAMA Internal Medicine175*.12:1986. https://doi.org/10.1001/jamainternmed.2015.5393

Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, B. and Hordijk, J. 2018. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy73*.5:1121–1137. https://doi.org/10.1093/jac/dkx488

Ruppé, E., Cherkaoui, A., Lazarevic, V., Emonet, S. and Schrenzel, J. 2017, November 29. Establishing genotype-to-phenotype relationships in bacteria causing hospital-acquired pneumonia: A prelude to the application of clinicalmetagenomics. *Antibiotics*. Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/antibiotics6040030

Sader, H. S., Farrell, D. J., Flamm, R. K. and Jones, R. N. 2014. Antimicrobial susceptibility of Gram-negative organisms isolated from patients hospitalised with pneumonia in US and European hospitals: Results from the SENTRY Antimicrobial Surveillance Program, 2009-2012. *International Journal of Antimicrobial Agents43*.4:328–334. https://doi.org/10.1016/j.ijantimicag.2014.01.007

Sader, H. S., Rhomberg, P. R., Farrell, D. J. and Jones, R. N. 2015. Arbekacin activity against contemporary clinical bacteria isolated from patients hospitalized with pneumonia. *Antimicrobial Agents and Chemotherapy59*.6:3263–70.

https://doi.org/10.1128/AAC.04839-14

Sánchez-Osuna, M., Cortés, P., Barbé, J. and Erill, I. 2018. Origin of the mobile di-hydro-pteroate synthase gene determining sulfonamide resistance in clinical isolates. *BioRxiv* 472472. https://doi.org/10.1101/472472

Sandegren, L., Linkevicius, M., Lytsy, B., Melhus, Å. and Andersson, D. I. 2012. Transfer of an *Escherichia coli* ST131 multiresistance cassette has created a *Klebsiella pneumoniae*-specific plasmid associated with a major nosocomial outbreak. *Journal of Antimicrobial Chemotherapy67*.1:74–83. https://doi.org/10.1093/jac/dkr405

Sanusi, A. 2015. Impact of Burning E-waste on Soil Physicochemical Properties and Soil Microorganisms. *British Microbiology Research Journal8*.2:434–442. https://doi.org/10.9734/bmrj/2015/16874

Schaufler, K., Semmler, T., Wieler, L. H., Wöhrmann, M., Baddam, R., Ahmed, N. and Guenther, S. 2016. Clonal spread and interspecies transmission of clinically relevant ESBL-producing *Escherichia coli* of ST410-another successful pandemic clone? *FEMS Microbiology Ecology92*.1:fiv155. https://doi.org/10.1093/femsec/fiv155

SCENIHR. 2009. *Assessment of the Antibiotic Resistance Effects of Biocides Antibiotic Resistance Effects of Biocides*. Retrieved from http://ec.europa.eu/health/ph_risk/risk_en.htm

Schluep, M., Müller, E., Hilty, L. M., Ott, D., Widmer, R. and Böni, H. 2013. Insights from a decade of development cooperation in e-waste management. In *First International Conference on Information and Communication Technologies for Sustainability* (pp. 45–51). Zurich, Switzerland. https://doi.org/10.5167/uzh-84886

Seiler, C. and Berendonk, T. U. 2012. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology3*.DEC:1–10. https://doi.org/10.3389/fmicb.2012.00399

Shafiani, S. and Malik, A. 2003. Tolerance of pesticides and antibiotic resistance in bacteria isolated from wastewater-irrigated soil. *World Journal of Microbiology and Biotechnology19*.9:897–901. https://doi.org/10.1023/B:WIBI.0000007290.94694.4f

Shaheen, A. and Iqbal, J. 2018. Spatial distribution and mobility assessment of carcinogenic heavy metals in soil profiles using geostatistics and Random Forest, Boruta Algorithm. *Sustainability (Switzerland)10*.3:. https://doi.org/10.3390/su10030799

Sheykhsaran, E., Bannazadeh Baghi, H., Soroush Barhaghi, M. H., Alizadeh, N., Memar, M. Y., Etemadi, S. and Ghotaslou, R. 2018. The rate of resistance to tetracyclines and distribution of tetA, tetB, tetC, tetD, tetE, tetG, tetJ and tetY genes in enterobacteriaceae isolated from Azerbaijan, Iran during 2017. *Physiology and Pharmacology (Iran)22*.3:205–212. Retrieved from http://1463.iranjournals.net/article_381016.html

Shistar, T. and Curle, C. 2014. Agricultural Uses of Antibiotics Escalate Bacterial Resistance. *Pesticide and YouWinter 201*:9–17. Retrieved from https://beyondpesticides.org/assets/media/documents/journal/bp-36.4-w17-Antibiotics-Cited2.pdf

Sinegani, S. A. A. and Younessi, N. 2017. Antibiotic resistance of bacteria isolated from heavy metal-polluted soils with different land uses. *Journal of Global Antimicrobial Resistance10*:247–255. https://doi.org/10.1016/j.jgar.2017.05.012

Singh, A. 2013. Nitrate and phosphate contamination in water and possible remedial measures. In N. Dwivedi (Ed.), *Environmenta Problems and Plant* (pp. 44–56).

Singh, K. P., Mohan, D., Sinha, S. and Dalwani, R. 2004. Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. *Chemosphere55*.2:227–255. https://doi.org/10.1016/j.chemosphere.2003.10.050

Singh, N. K., Bezdan, D., Checinska Sielaff, A., Wheeler, K., Mason, C. E. and Venkateswaran, K. 2018. Multi-drug resistant Enterobacter bugandensis species isolated from the International Space Station and comparative genomic analyses with human pathogenic strains. *BMC Microbiology18*.1:175. https://doi.org/10.1186/s12866-018-1325-2

Singh, Y., Ramteke, P. W. and Shukla, P. K. 2014. *Characterization z of Pseudomonas spp. isolated from sewage irrigated soils and their plant growth promoting traits in vitro*. *Int J Microbial Resour Technol* (Vol. 2). Retrieved from http://ijmrt.inpressco.com

Sköld, O. 2000. Sulfonamide resistance: Mechanisms and trends. *Drug Resistance Updates3*.3:155–160. https://doi.org/10.1054/drup.2000.0146

SON - Standards Organization of Nigeria. 2015. Nigerian Standard for Drinking Water Quality .52:19–24.

Sonda, T., Kumburu, H., van Zwetselaar, M., Alifrangis, M., Mmbaga, B. T., Aarestrup, F. M. and Lund, O. 2018. Whole genome sequencing reveals high clonal diversity of Escherichia coli isolated from patients in a tertiary care hospital in Moshi, Tanzania. *Antimicrobial Resistance and Infection Control7*.1:72. https://doi.org/10.1186/s13756-018-0361-x

Sparks, T. C. and Nauen, R. 2015. IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology121*:122–128. https://doi.org/10.1016/j.pestbp.2014.11.014

Spellberg, B. and Doi, Y. 2015. The rise of fluoroquinolone-resistant Escherichia coli in the community: Scarier than we thought. *Journal of Infectious Diseases212*.12:1853–1855. https://doi.org/10.1093/infdis/jiv279

Stepanauskas, R., Glenn, T. C., Jagoe, C. H., Tuckfield, R. C., Lindell, A. H., King, C. J. and McArthur, J. V. 2006. Coselection for microbial resistance to metals and antibiotics in freshwater microcosms. *Environmental Microbiology8*.9:1510–1514. https://doi.org/10.1111/j.1462-2920.2006.01091.x

Sthiannopkao, S. and Wong, M. H. 2013. Handling e-waste in developed and

developing countries: Initiatives, practices, and consequences. *Science of the Total Environment463–464*:1147–1153. https://doi.org/10.1016/j.scitotenv.2012.06.088

Stickler, D. J. and Thomas, B. 1980. Antiseptic and antibiotic resistance in Gram-negative bacteria causing urinary tract infection. *Journal of Clinical Pathology*, *33*.3: 288–296. https://doi.org/10.1136/JCP.33.3.288

Su, H. C., Pan, C. G., Ying, G. G., Zhao, J. L., Zhou, L. J., Liu, Y. S. and He, L. Y. 2014. Contamination profiles of antibiotic resistance genes in the sediments at a catchment scale. *Science of the Total Environment490*:708–714. https://doi.org/10.1016/j.scitotenv.2014.05.060

Sun, M., Ye, M., Wu, J., Feng, Y., Wan, J., Tian, D. and Kengara, F. O. 2015. Positive relationship detected between soil bioaccessible organic pollutants and antibiotic resistance genes at dairy farms in Nanjing, Eastern China. *Environmental Pollution206*:421–428. https://doi.org/10.1016/j.envpol.2015.07.022

Sun, F., Zhou, D., Sun, Q., Luo, W., Tong, Y., Zhang, D. and Xia, P. 2016. Genetic characterization of two fully sequenced multi-drug resistant plasmids pP10164-2 and pP10164-3 from Leclercia adecarboxylata. *Scientific Reports6*.1:33982. https://doi.org/10.1038/srep33982

Sütterlin, S., Téllez-Castillo, C. J., Anselem, L., Yin, H., Bray, J. E. and Maiden, M. C. J. 2018. Heavy metal susceptibility of *Escherichia coli* isolated from urine samples from Sweden, Germany, and Spain. *Antimicrobial Agents and Chemotherapy62*.5:e00209-18. https://doi.org/10.1128/AAC.00209-18

Sutton, G. G., Brinkac, L. M., Clarke, T. H. and Fouts, D. E. 2018. Enterobacter hormaechei subsp. hoffmannii subsp. nov., *Enterobacter hormaechei* subsp. *xiangfangensis* comb. nov., *Enterobacter roggenkampii* sp. nov., and *Enterobacter muelleri* is a later heterotypic synonym of *Enterobacter asburiae* based on computational a. *F1000Research7*:521. https://doi.org/10.12688/f1000research.14566.2

Tacão, M., Araújo, S., Vendas, M., Alves, A. and Henriques, I. 2018. Shewanella species as the origin of blaOXA-48 genes: insights into gene diversity, associated phenotypes and possible transfer mechanisms. *International Journal of Antimicrobial Agents51*.3:340–348. https://doi.org/10.1016/j.ijantimicag.2017.05.014

Tadesse, B. T., Ashley, E. A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I. J. and Dittrich, S. 2017. Antimicrobial resistance in Africa: a systematic review. *BMC Infectious Diseases17*.1:616. https://doi.org/10.1186/s12879-017-2713-1

Tamminen, M., Karkman, A., Lõhmus, A., Muziasari, W. I., Takasu, H., Wada, S. and Virta, M. 2011. Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. *Environmental Science and Technology45*.2:386–391. https://doi.org/10.1021/es102725n

Tanner, W. D., VanDerslice, J. A., Goel, R. K., Leecaster, M. K., Fisher, M. A.,

Olstadt, J. and Gundlapalli, A. V. 2019. Multi-state study of Enterobacteriaceae harboring extended-spectrum beta-lactamase and carbapenemase genes in U.S. drinking water. *Scientific Reports9*.1:3938. https://doi.org/10.1038/s41598-019-40420-0

Tatusova, T., Dicuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E. P., Zaslavsky, L. and Ostell, J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Research44*.14:6614–6624. https://doi.org/10.1093/nar/gkw569

Tay, K. C., Dorleku, M. and Koranteng, S. S. 2018. Hydrochemical evolution of ground and surface water within the Amansie and Adansi districts of the Ashanti region, Ghana. *West African Journal of Applied Ecology26*.1:108–133.

Thomsen, M. C. F., Ahrenfeldt, J., Cisneros, J. L. B., Jurtz, V., Larsen, M. V., Hasman, H. and Lund, O. 2016. A bacterial analysis platform: An integrated system for analysing bacterial whole genome sequencing data for clinical diagnostics and surveillance. *PLoS ONE11*.6:e0157718. https://doi.org/10.1371/journal.pone.0157718

Tokumaru, T. 2015. *Determination of the Extent of Pollution in Soils, Water Bodies, and human hair at the e-waste recycline sites in Ghana*. Ghana.

Ukpaka, C. P. and Ukpaka, C. 2016. Characteristics of Groundwater in Port-Harcourt Local Government Area Characteristics of Groundwater in Port-Harcourt Local Government Area Abstract : .December:136–144.

UN Environement. 2019. UN report: Time to seize opportunity, tackle challenge of e-waste. Retrieved July 2, 2019, from https://www.unenvironment.org/news-and-stories/press-release/un-report-time-seize-opportunity-tackle-challenge-e-waste

UNEP. 2006. UNEP in 2006. *UN Documentation* 7–14. Retrieved from www.unep.org/governingbodies.

UNEP. 2019. Nigeria turns the tide on electronic waste | UN Environment. Retrieved June 21, 2019, from https://www.unenvironment.org/ar/node/25118

United Nations University. 2017. E-waste Rises 8% by Weight in 2 Years as Incomes Rise, Prices Fall - United Nations University. Retrieved June 21, 2019, from https://unu.edu/media-relations/releases/ewaste-rises-8-percent-by-weight-in-2-years.html

USEPA. 1996. *6010B METHOD - INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY*. Retrieved from https://www.epa.gov/sites/production/files/documents/6010b.pdf

Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A. and Laxminarayan, R. 2014. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases14*.8:742–750. https://doi.org/10.1016/S1473-3099(14)70780-7

Versalovic, J., Koeuth, T. and Lupski, R. 1991. Distribution of repetitive DNA sequences in eubacteria and application to finerpriting of bacterial enomes. *Nucleic Acids Research19*.24:6823–6831. https://doi.org/10.1093/nar/19.24.6823

Vieweger, A. and Döring, T. F. 2014. Assessing health in agriculture - towards a common research framework for soils, plants, animals, humans and ecosystems. *Journal of the Science of Food and Agriculture95*.3:438–446. https://doi.org/10.1002/jsfa.6708

Wales, A. and Davies, R. 2015. Co-Selection of Resistance to Antibiotics, Biocides and Heavy Metals, and Its Relevance to Foodborne Pathogens. *Antibiotics4*.4:567–604. https://doi.org/10.3390/antibiotics4040567

Wang, C., Zhou, S., Song, J. and Wu, S. 2018. Human health risks of polycyclic aromatic hydrocarbons in the urban soils of Nanjing, China. *Science of the Total Environment612*:750–757. https://doi.org/10.1016/j.scitotenv.2017.08.269

Wang, D., Hu, E., Chen, J., Tao, X., Gutierrez, K. and Qi, Y. 2013. Characterization of novel ybjG and dacC variants in *Escherichia coli*. *Journal of Medical Microbiology62*.PART 11:1728–1734. https://doi.org/10.1099/jmm.0.062893-0

Wang, J.-P. and Guo, X.-K. 2006. *Impact of electronic wastes recycling on environmental quality.Biomedical and environmental sciences : BES* (Vol. 19). Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16827186

Wang, L., Chen, Z., Shang, H., Wang, J. and Zhang, P. Y. 2014. Impact of simulated acid rain on soil microbial community function in Masson pine seedlings. *Electronic Journal of Biotechnology17*.5:199–203. https://doi.org/10.1016/j.ejbt.2014.07.008

Wang, Y. and Qian, P. Y. 2009. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS ONE4*.10:e7401. https://doi.org/10.1371/journal.pone.0007401

Webber, M. A. and Piddock, L. J. V. 2003. The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy51*.1:9–11. https://doi.org/10.1093/jac/dkg050

WHO. 2011. *Hardness in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality (GDWQ)*. *World Health Organization*. Retrieved from http://www.who.int/water_sanitation_health/dwq/chemicals/hardness.pdf

Wilson, L. A. and Sharp, P. M. 2006. Enterobacterial repetitive intergenic consensus (ERIC) sequences in *Escherichia coli*: Evolution and implications for ERIC-PCR. *Molecular Biology and Evolution23*.6:1156–1168. https://doi.org/10.1093/molbev/msj125

Woodford, N., Turton, J. F. and Livermore, D. M. 2011. Multiresistant Gram-negative bacteria: The role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiology Reviews35*.5:736–755. https://doi.org/10.1111/j.1574-6976.2011.00268.x

WHO - World Health Organization. 2019. New report calls for urgent action to avert antimicrobial resistance crisis. Retrieved June 25, 2019, from https://www.who.int/news-room/detail/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis

World Population Review. 2019a. Lagos Population 2019 (Demographics, Maps, Graphs). Retrieved July 25, 2019, from http://worldpopulationreview.com/world-cities/lagos-population/

World Population Review. 2019b. Population of Cities in Nigeria (2019). Retrieved July 25, 2019, from http://worldpopulationreview.com/countries/nigeria-population/cities/

Wu, C., Lin, C., Zhu, X., Liu, H., Zhou, W., Lu, J. and Hu, Y. 2018. The β-lactamase gene profile and a plasmid-carrying multiple heavy metal resistance genes of *Enterobacter cloacae*. *International Journal of Genomics2018*:1–12. https://doi.org/10.1155/2018/4989602

Wuertz, S., and Mergeay, M. 1997. The impact of heavy metals on soil microbial communities and their activities. *Mordern Soil Microbiology.* En: Modern soil microbiology. -- New York, US : Marcel Dekker, 1997. Retrieved from http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=earth.xis&method=post&formato=2&cantidad=1&expresion=mfn=025674

Xiong, W., Sun, Y., Ding, X., Zhang, Y. and Zeng, Z. 2014. Antibiotic resistance genes occurrence and bacterial community composition in the Liuxi River. *Frontiers in Environmental Science2*.DEC:61. https://doi.org/10.3389/fenvs.2014.00061

Xu, X., Zeng, X., Boezen, H. M. and Huo, X. 2015. E-waste environmental contamination and harm to public health in China. *Frontiers of Medicine9*.2:220–228. https://doi.org/10.1007/s11684-015-0391-1

Xu, Y., Xu, J., Mao, D. and Luo, Y. 2017. Effect of the selective pressure of sub-lethal level of heavy metals on the fate and distribution of ARGs in the catchment scale. *Environmental Pollution220*:900–908. https://doi.org/10.1016/j.envpol.2016.10.074

Ye, M., Sun, M., Wan, J., Feng, Y., Zhao, Y., Tian, D. and Jiang, X. 2016. Feasibility of lettuce cultivation in sophoroliplid-enhanced washed soil originally polluted with Cd, antibiotics, and antibiotic-resistant genes. *Ecotoxicology and Environmental Safety124*:344–350. https://doi.org/10.1016/j.ecoenv.2015.11.013

Ye, Q., Wu, Q., Zhang, S., Zhang, J., Yang, G., Wang, J. and Chen, M. 2018. Characterization of extended-spectrum β-lactamase-producing Enterobacteriaceae from retail food in China. *Frontiers in Microbiology9*.AUG:1709. https://doi.org/10.3389/fmicb.2018.01709

Younes, A., Hamouda, A., Dave, J. and Amyes, S. G. B. 2011. Prevalence of transferable $bla_{CTX\text{-}M\text{-}15}$ from hospital- and community-acquired *Klebsiella pneumoniae* isolates in Scotland. *Journal of Antimicrobial Chemotherapy66*.2:313–318. https://doi.org/10.1093/jac/dkq453

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O. and Larsen, M. V. 2012. Identification of acquired antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy67*.11:2640–2644. https://doi.org/10.1093/jac/dks261

Zhai, Y., He, Z., Kang, Y., Yu, H., Wang, J., Du, P. and Gao, Z. 2016. Complete nucleotide sequence of pH11, an IncHI2 plasmid conferring multi-antibiotic resistance and multi-heavy metal resistance genes in a clinical Klebsiella pneumoniae isolate. *Plasmid86*:26–31. https://doi.org/10.1016/j.plasmid.2016.04.001

Zhang, M., Chen, L., Ye, C. and Yu, X. 2018a. Co-selection of antibiotic resistance via copper shock loading on bacteria from a drinking water bio-filter. *Environmental Pollution233*:132–141. https://doi.org/10.1016/j.envpol.2017.09.084

Zhang, F., Zhao, X., Li, Q., Liu, J., Ding, J., Wu, H. and Zhu, J. 2018b. Bacterial community structure and abundances of antibiotic resistance genes in heavy metals contaminated agricultural soil. *Environmental Science and Pollution Research25*.10:9547–9555. https://doi.org/10.1007/s11356-018-1251-8

Zhou, B., Wang, C., Zhao, Q., Wang, Y., Huo, M., Wang, J. and Wang, S. 2016. Prevalence and dissemination of antibiotic resistance genes and coselection of heavy metals in Chinese dairy farms. *Journal of Hazardous Materials320*:10–17. https://doi.org/10.1016/j.jhazmat.2016.08.007

Zhou, Y., Niu, L., Zhu, S., Lu, H. and Liu, W. 2017. Occurrence, abundance, and distribution of sulfonamide and tetracycline resistance genes in agricultural soils across China. *Science of the Total Environment599–600*:1977–1983. https://doi.org/10.1016/j.scitotenv.2017.05.152

Zhou, Z., Alikhan, N.-F., Mohamed, K., Group, the A. S. and Achtman, M. 2019. The user's guide to comparative genomics with EnteroBase. Three case studies: micro-clades within Salmonella enterica serovar Agama, ancient and modern populations of Yersinia pestis, and core genomic diversity of all Escherichia. *BioRxiv* 613554. https://doi.org/10.1101/613554

Zhu, Y.-G., Johnson, T. A., Su, J.-Q., Qiao, M., Guo, G.-X., Stedtfeld, R. D. and Tiedje, J. M. 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proceedings of the National Academy of Sciences110*.9:3435–3440. https://doi.org/10.1073/pnas.1222743110

APPENDICES

APPENDIX I: Measured heavy metals concentration in the soil samples (mg/kg)

	Zn	Cu	Mn	Fe	Al	Co	Cr	Ni	Pb	Se	Cd
AL1	825.870^{g}	190.210^{b}	136.520^{e}	2539.510^{d}	6370.230^{j}	0.000^{gh}	229.810^{b}	353.600^{b}	173.900^{h}	43. 400^{a}	12. 100^{c}
AS1	287.900^{i}	11.320^{i}	85.950^{j}	2697.170^{b}	6822.000^{h}	10.950^{d}	59.700^{i}	257.600^{c}	1372.000^{b}	0.O00^{d}	0.O00^{d}
IKJ1	916.700^{d}	16.730^{g}	102.720^{h}	2093.800^{h}	4787.000^{i}	27.100^{a}	34.300^{k}	47.100^{g}	818.800^{d}	0.O00^{d}	0.O00^{d}
ẠRU1	446.040^{k}	3.150^{k}	77.600^{i}	2336.360^{f}	7122.000^{g}	3.900^{e}	107.500^{c}	122.100^{e}	267.300^{f}	0.O00^{d}	0.O00^{d}
ỌS1	1108.530^{b}	25.220^{e}	119.570^{f}	2436.930^{e}	5426.000^{k}	1.000^{gh}	76.300^{d}	39.100^{i}	192.000^{g}	0.O00^{d}	13.600^{c}
AL2	945.100^{c}	296.660^{a}	284.030^{a}	2539.510^{d}	8194.010^{c}	2.000^{fg}	246.210^{a}	387.100^{a}	1554.800^{a}	1.170^{d}	23. 500^{b}
AS2	908.260^{e}	28.690^{d}	147.860^{d}	1863.850^{i}	7827.000^{d}	15.900^{b}	61.400^{hi}	83.300^{f}	788.100^{e}	0.O00^{d}	0. 000^{d}
IKJ2	858.390^{f}	13.230^{h}	63.730^{n}	1775.410^{k}	2909.000^{m}	1.000^{gh}	71.600^{f}	10.200^{k}	23.500^{k}	0.O00^{d}	0. 000^{d}
ẠRU2	282.850^{m}	4.080^{k}	202.260^{c}	2859.370^{a}	6583.000^{i}	2.000^{fg}	62.300^{h}	7.800^{l}	18.400^{m}	0.O00^{d}	0. 000^{d}
ỌS2	789.360^{h}	2.720^{k}	93.740^{i}	2543.670^{c}	7590.000^{e}	1.350^{gh}	81.000^{c}	40.800h	153.000^{i}	40.O00^{b}	12.100^{c}
AL3	1164.680^{a}	102.470^{c}	94.320^{i}	1402.410^{l}	1847.000^{n}	0.000^{gh}	60.700^{hi}	258.600c	1360.200^{b}	0. 000^{d}	25.700^{a}
AS3	825.870^{g}	20.260^{f}	83.160^{k}	2141.300^{g}	1493.000^{o}	12.500^{cd}	29.800^{i}	0.000^{m}	173.900^{h}	0.O00^{d}	0. 000^{d}
IKJ3	143.220^{n}	12.590^{h}	108.330^{g}	1775.410^{k}	8794.000^{a}	13.700^{c}	81.500^{c}	170.800^{d}	1234.000^{c}	0. 000^{d}	0. 000^{d}
ẠRU3	462.430^{j}	6.280^{j}	269.290^{b}	1819.380^{j}	7545.000^{f}	3.300^{ef}	64.500^{g}	1.100^{m}	21.500^{l}	0. 000^{d}	0. 000^{d}
ỌS3	513.160^{i}	2.840^{k}	74.820^{m}	2539.510^{d}	8311.000^{b}	2.000^{fg}	46.700^{j}	20.000^{j}	80.400^{j}	20.000^{c}	22.200^{b}
Garden soil IITA	10.700^{o}	0.410^{i}	38.120^{o}	124.200^{m}	50.000^{p}	0.000^{h}	0.000^{m}	0.800^{m}	0.000^{n}	0.100^{d}	0.000^{d}
SEM	49.62	11.73	9.97	93.79	385.53	1.11	9.27	18.69	81.13	2.07	1.38

Mean of the variables occurring on the same column with dissimilar superscripts are significantly different ($p < 0.05$) using one-way ANOVA

SEM = Standard Error Mean

APPENDIX II: Measured metals concentration in the water samples from the sampling sites (mg/l)

Sample ID	Cd	Co	Cr	Ni	Pb	Al	Se	Mn	Fe	Cu	Zn
ĄR1	0.540^{a}	2.820^{a}	16.260^{a}	8.580^{a}	2.480^{bc}	43.280^{d}	4.880^{a}	0.290^{a}	0.780^{r}	0.270	0.600
AW1I	0.460^{a}	2.440^{ab}	3.340^{c}	2.520^{b}	1.020^{cdf}	26.340^{h}	1.200^{bc}	0.200^{a}	2.040^{qr}	0.180	0.300
AW2I	0.500^{a}	2.380^{ab}	2.740^{cd}	2.100^{b}	1.020^{cdef}	23.100^{ijk}	4.780^{a}	0.290^{a}	3.460^{pq}	0.140	0.800
ASB1	0.420^{a}	2.380^{ab}	1.260^{de}	2.360^{b}	0.380^{def}	26.580^{h}	0.900^{bc}	0.110^{a}	4.490^{p}	0.260	0.800
IKB1	0.440^{a}	2.120^{ab}	1.360^{de}	1.320^{bc}	1.720^{bcd}	35.480^{f}	0.420^{c}	0.200^{a}	6.160^{o}	0.150	1.000
UW1I	0.440^{a}	2.180^{ab}	5.480^{b}	1.960^{b}	2.100^{bcd}	39.760^{e}	4.980^{a}	0.290^{a}	6.750^{no}	0.150	0.800
UW2I	0.460^{a}	2.220^{ab}	1.160^{de}	1.200^{bc}	1.420^{cdef}	23.380^{ijk}	0.420^{c}	0.110^{a}	7.980^{mn}	0.130	1.000
OR1	0.480^{a}	2.280^{ab}	1.300^{de}	1.260^{bc}	2.100^{bcd}	21.340^{el}	1.120^{bc}	0.110^{a}	9.280^{m}	0.160	1.300
ĄR2	0.540^{a}	2.340^{ab}	1.680^{cde}	1.480^{bc}	5.520^{a}	69.680^{c}	4.420^{a}	0.200^{a}	11.350^{l}	0.230	1.500
AW1II	0.460^{a}	2.340^{ab}	1.220^{de}	1.420^{bc}	1.400^{cdef}	22.480^{kl}	0.620^{c}	0.200^{a}	16.780^{k}	0.150	2.000
AW2II	0.440^{a}	2.360^{ab}	1.160^{de}	1.560^{bc}	1.140^{cdef}	24.360^{ij}	2.660^{b}	0.390^{a}	17.630^{k}	0.390	2.300
ASB2	0.460^{a}	2.400^{ab}	1.120^{de}	1.720^{bc}	0.960^{cdef}	31.060^{g}	0.540^{c}	0.290^{a}	21.170^{j}	0.130	3.100
IKB2	0.440^{a}	2.380^{ab}	1.420^{de}	2.160^{b}	1.540^{bcd}	24.420^{ij}	0.680^{c}	0.110^{a}	24.000^{i}	0.170	3.600
UW2II	0.440^{a}	2.360^{ab}	1.120^{de}	2.100^{b}	0.900^{cdef}	22.420^{kl}	0.700^{bc}	0.390^{a}	28.010^{h}	0.220	2.800
UW1II	0.420^{a}	2.320^{ab}	1.060^{de}	1.980^{b}	0.600^{cdef}	25.420^{hi}	0.840^{bc}	0.290^{a}	33.400^{g}	0.150	3.300
OR2	0.500^{a}	0.520^{bc}	1.530^{cde}	1.590^{bc}	2.070^{bcd}	32.120^{g}	1.100^{bc}	0.540^{a}	32.200^{g}	0.140	1.240
ĄR3	0.420^{a}	2.300^{ab}	1.540^{cde}	2.300^{b}	1.020^{cdf}	75.520^{b}	5.240^{a}	0.200^{a}	40.430^{ef}	0.330	3.800
AW1III	0.420^{a}	2.320^{ab}	1.240^{de}	2.380^{b}	0.000^{f}	25.740^{hi}	1.240^{bc}	0.110^{a}	39.220^{f}	0.160	0.300
AW2III	0.400^{a}	2.280^{ab}	1.200^{de}	2.440^{b}	0.000^{f}	23.480^{ijk}	1.600^{bc}	0.290^{a}	45.450^{d}	0.190	0.600
ASBIII	0.400^{a}	2.260^{ab}	1.100^{de}	2.500^{b}	0.000^{f}	22.86^{jkl}	1.100^{bc}	0.200^{a}	49.390^{c}	0.260	1.000
IKB3	0.380^{a}	2.260^{ab}	1.040^{de}	2.560^{b}	0.000^{f}	25.900^{hi}	0.980^{bc}	0.200^{a}	41.660^{e}	0.170	2.800
UW1III	0.440^{a}	2.080^{ab}	1.300^{de}	1.260^{bc}	2.020^{bcde}	19.780^{m}	0.280^{c}	0.110^{a}	46.750^{d}	0.150	1.900
UW2III	0.480^{a}	2.160^{ab}	2.980^{cd}	2.240^{b}	3.180^{b}	95.340^{a}	1.600^{bc}	0.390^{a}	54.880^{b}	0.150	2.200
OR3	0.460^{a}	2.180^{ab}	1.460^{de}	1.400^{bc}	1.800^{bcde}	31.260^{g}	0.980^{bc}	0.110^{a}	59.180^{a}	0.250	2.400
EPA	0.005^{b}	0.000^{c}	0.050^{e}	0.000^{c}	0.500^{ef}	0.200^{n}	0.010^{c}	0.050^{b}	1.000^{r}	0.050	0.300
SON	0.01	0.000	0.500	0.100	0.100	0.200^{n}	0.000^{d}	0.200	0.500	0.010	0.200
SEM	0.1	0.12	0.37	0.19	0.17	2.28	0.21	0.1	2.15	0.100	1.000

Mean of the variables occurring on the same column with dissimilar superscripts are significantly different ($p < 0.05$) using ANOVA

APPENDIX III: Total Heterotrophic Bacteria Count and Metal tolerant Bacteria Count (Cu^{2+}, Pb^{2+} and Zn^{2+}) from samples obtained during the first sampling campaign

Samples	Total Heterotrophic Bacteria Count (cfu/ml)	Metal treatments (50 ug/ml)		
		Total copper tolerant strains	Total lead tolerant strains	Total zinc tolerant strains
AL	2.40Ex+06 ± 1.27Ex+01	1.15Ex+06 ± 2.12Ex+00	1.45Ex+06 ± 7.07E-01	1.15Ex+06 ± 7.07E-01
AW1	1.80Ex+06 ± 4.24Ex+00	5.00Ex+05 ± 4.24Ex+00	1.15Ex+06 ± 7.07E-01	8.50Ex+05 ± 7.07E-01
AW2	4.05Ex+06 ± 2.12Ex+00	2.35Ex+06 ± 2.12Ex+00	3.25Ex+06 ± 7.07E-01	1.50Ex+06 ± 4.24Ex+00
ẠR	2.51Ex+07 ± 4.24Ex+00	1.17Ex+07 ± 2.83Ex+00	1.78Ex+07 ± 8.49Ex+00	1.69Ex+07 ± 1.48Ex+01
AS	7.00Ex+05 ± 4.24Ex+00	5.50Ex+05 ± 3.54Ex+00	6.50Ex+06 ± 7.07E-01	1.50Ex+05 ± 7.07E-01
ASB	9.35Ex+06 ± 2.47Ex+01	8.25Ex+06 ± 1.77Ex+01	6.90Ex+06 ± 2.26Ex+01	7.00Ex+05 ± 2.83Ex+00
IKJ	3.02Ex+07 ± 4.95Ex+00	1.20Ex+07 ± 2.12Ex+00	2.03Ex+07 ± 1.06Ex+01	1.60Ex+07 ± 1.06Ex+01
IKB	1.00Ex+04 ± 1.41Ex+00	1.50Ex+03 ± 7.07E-01	3.50Ex+03 ± 7.07E-01	6.50Ex+03 ± 2.12Ex+00
ẠRU	6.65Ex+06 ± 1.20Ex+01	1.60Ex+06 ± 7.07Ex+00	1.55Ex+06 ± 2.12Ex+00	1.65Ex+06 ± 7.07E-01
UW1	6.00Ex+05 ± 2.83Ex+00	1.00Ex+05 ± 1.00Ex+00	2.00Ex+05 ± 2.83Ex+00	4.00Ex+05 ± 1.41Ex+00
UW2	5.00Ex+03 ± 2.83Ex+00	5.00Ex+02 ± 7.07E-01	3.50Ex+03 ± 2.12Ex+00	5.00Ex+02 ± 7.07E-01
ỌS	9.05Ex+06 ± 9.19Ex+00	2.70Ex+06 ± 7.07Ex+00	5.20Ex+06 ± 9.90Ex+00	3.65Ex+06 ± 4.95Ex+00
OR	6.00Ex+05 ± 2.83Ex+00	2.50Ex+05 ± 7.07E-01	5.00Ex+05 ± 1.41Ex+00	3.50Ex+05 ± 2.12Ex+00

Data represents mean of three replicate plate counts ± Standard deviation.

Ex+ = Exponential

APPENDIX IV: Total Heterotrophic Bacteria Count and Metal tolerant Bacteria Count (Cu^{2+}, Pb^{2+} and Zn^{2+}) [cfu/ml] from samples obtained during the second sampling campaign

Samples	Total Heterotrophic Bacteria Count (cfu/ml)	Metal treatments (50 ug/ml)		
		Total copper tolerant strains	Total Lead tolerant strains	Total zinc tolerant strains
AL	2.60Ex+06 ± 2.83Ex+00	1.00Ex+06 ± 2.83Ex+00	2.10Ex+06 ± 2.83Ex+00	1.55Ex+06 ± 3.54Ex+00
AW1	2.05Ex+06 ± 2.12Ex+00	7.50Ex+05 ± 3.54Ex+00	1.40Ex+06 ± 1.41Ex+00	5.50Ex+05 ± 2.12Ex+00
AW2	6.50Ex+05 ± 3.54Ex+00	3.00Ex+05 ± 1.41Ex+00	5.50Ex+05 ± 2.12Ex+00	1.50Ex+05 ± 7.07E-01
ẠR	1.38Ex+07 ± 6.36Ex+00	5.50Ex+06 ± 1.70Ex+01	8.90Ex+06 ± 4.24Ex+00	5.80Ex+06 ± 2.83Ex+00
AS	1.55Ex+06 ± 4.95Ex+00	5.50Ex+05 ± 2.12Ex+00	4.50Ex+05 ± 2.12Ex+00	5.00Ex+05 ± 4.24Ex+00
ASB	3.50Ex+06 ± 2.83Ex+00	2.75Ex+06 ± 7.07E-01	2.25Ex+06 ± 2.12Ex+00	4.00Ex+05 ± 5.66Ex+00
IKJ	7.75Ex+06 ± 4.95Ex+00	6.40Ex+06 ± 1.70Ex+01	6.95Ex+06 ± 1.20Ex+01	4.90Ex+06 ± 7.07Ex+00
IKB	3.50Ex+05 ± 7.07E-01	1.50Ex+05 ± 7.07E-01	1.00Ex+05 ± 1.41Ex+00	2.00Ex+05 ± 1.41Ex+00
ẠRU	2.10Ex+06 ± 4.24Ex+00	7.00Ex+05 ± 1.41Ex+00	2.05Ex+06 ± 2.12Ex+00	1.05Ex+06 ± 2.12Ex+00
UW1	4.50Ex+05 ± 7.07E-01	1.00Ex+05 ± 1.41Ex+00	2.00Ex+05 ± 0.00Ex+00	5.00Ex+04 ± 7.07E-01
UW2	4.00Ex+03 ± 1.41Ex+00	5.00Ex+02 ± 7.07E-01	1.50Ex+03 ± 7.07E-01	1.00Ex+03 ± 0.00Ex+00
ỌS	6.50Ex+06 ± 1.56Ex+01	3.70Ex+06 ± 5.66Ex+00	5.70Ex+06 ± 7.07Ex+00	3.55Ex+06 ± 6.36Ex+00
OR	8.00Ex+05 ± 1.41Ex+00	4.50Ex+05 ± 2.12Ex+00	6.50Ex+05 ± 7.07E-01	4.00Ex+05 ± 1.41Ex+00

Data represents mean of three replicate plate counts ± Standard deviation.

Ex+ = Exponential

APPENDIX V: Total Heterotrophic Bacteria Count and Metal tolerant Bacteria Count (Cu2+, Pb2+ and Zn2+) from samples obtained during the second sampling campaign

Samples	Total Heterotrophic Bacteria Count (cfu/ml)	Metal treatments (50 ug/ml)		
		Total copper tolerant strains	Total Lead tolerant strains	Total zinc tolerant strains
AL	2.00Ex+06 ± 1.41Ex+00	1.25Ex+06 ± 2.12Ex+00	9.00Ex+05 ± 1.41Ex+00	1.20Ex+06 ± 5.66Ex+00
AW1	4.30Ex+06 ± 5.66Ex+00	1.70Ex+06 ± 5.66Ex+00	3.05Ex+06 ± 2.12Ex+00	1.40Ex+06 ± 2.83Ex+00
AW2	2.45Ex+06 ± 7.78Ex+00	2.20Ex+06 ± 2.83Ex+00	1.80Ex+06 ± 2.83Ex+00	1.05Ex+06 ± 3.54Ex+00
ẠR	3.75Ex+06 ± 7.78Ex+00	1.85Ex+06 ± 4.95Ex+00	2.90Ex+06 ± 9.90Ex+00	2.40Ex+06 ± 2.83Ex+00
AS	5.65Ex+06 ± 1.34Ex+01	3.30Ex+06 ± 7.07Ex+00	4.75Ex+06 ± 6.36Ex+00	4.25Ex+06 ± 6.36Ex+00
ASB	3.90Ex+06 ± 4.24Ex+00	1.70Ex+06 ± 7.07Ex+00	3.05Ex+06 ± 2.12Ex+00	1.65Ex+06 ± 9.19Ex+00
IKJ	1.94Ex+07 ± 2.26Ex+01	1.28Ex+07 ± 6.36Ex+00	1.76Ex+07 ± 1.56Ex+01	8.85Ex+06 ± 1.48Ex+01
IKB	1.20Ex+06 ± 1.41Ex+00	6.00Ex+05 ± 1.41Ex+00	1.05Ex+06 ± 7.07E-01	5.50Ex+05 ± 2.12Ex+00
ẠRU	5.90Ex+06 ± 5.66Ex+00	4.00Ex+06 ± 5.66Ex+00	4.90Ex+06 ± 9.90Ex+00	1.25Ex+06 ± 7.07E-01
UW1	1.50Ex+06 ± 5.66Ex+00	1.50Ex+05 ± 7.07E-01	3.00Ex+05 ± 1.41Ex+00	2.00Ex+05 ± 1.41Ex+00
UW2	9.50Ex+05 ± 7.07E-01	1.00Ex+05 ± 1.41Ex+00	8.00Ex+05 ± 4.24Ex+00	2.50Ex+05 ± 7.07E-01
ỌS	7.25Ex+06 ± 2.33Ex+01	3.65Ex+06 ± 1.06Ex+01	5.50Ex+06 ± 1.41Ex+00	3.35Ex+06 ± 3.54Ex+00
OR	1.10Ex+06 ± 1.41Ex+00	8.50Ex+05 ± 2.12Ex+00	1.05Ex+06 ± 3.54Ex+00	2.83Ex+00 ± 7.00Ex+05

Data represents mean of three replicate plate counts ± Standard deviation.

Ex+ = Exponential

APPENDIX VI: Measured zones of inhibition from antibiotic susceptibility test on copper tolerant bacteria isolates

Isolate code	Antibiotics										
	CN	K	CIP	TET	FFC	SXT	CPD	CAZ	IPM	MEM	ETP
C1	25	18	24	22	28	25	13	21	14	13	14
C2	22	20	25	24	27	24	24	26	32	22	22
C3	16	15	30	20	19	20	13	14	25	20	21
C4	22	24	25	25	25	29	24	22	27	26	25
C5	18	13	10	12	13	-	10	-	12	15	16
C6	20	18	20	21	28	22	26	24	ה	20	16
C7	18	-	26	15	21	20	-	20	-	-	-
C8	19	16	15	20	18	-	8	16	19	19	14
C9	19	15	26	14	8	-	-	15	19	15	12
C10	24	16	20	16	21	-	-	20	27	25	17
C11	19	15	19	17	21	-	-	10	18	15	10
C12	17	14	21	15	8	17	-	-	24	23	12
C13	16	21	22	21	26	19	16	18	20	19	22
C14	20	18	25	24	28	-	16	18	29	25	18
C15	18	17	26	15	8	-	-	16	21	17	13
C16	17	12	23	18	21	28	20	21	16	-	-
C17	16	11	22	20	25	24	14	15	22	20	20
C18	30	21	32	22	33	32	10	24	38	30	34
C19	20	15	24	17	11	21	11	16	27	23	15
C20	19	11	18	16	21	-	-	8	25	21	23
C21	21	18	16	18	22	13	24	22	25	27	22

C22	22	18	21	19	24	26	25	25	30	28	25
C23	24	20	24	20	23	25	23	22	28	29	24
C24	20	19	19	26	23	27	20	21	36	30	28
C25	21	24	16	18	11	-	-	10	27	22	22
C26	25	21	33	25	29	40	-	-	34	30	24
C27	25	23	34	29	30	40	-	-	39	36	30
C28	29	19	35	21	25	28	-	12	11	14	11
C29	25	22	30	21	29	31	20	21	38	32	20
C30	29	27	30	22	30	38	26	25	28	27	30
C31	24	23	30	30	27	27	22	14	37	32	26
C32	16	12	15	10	19	-	12	12	25	22	18
C33	16	15	23	20	25	19	10	14	9	11	10
C34	29	20	28	15	27	32	12	11	37	32	27
C35	25	21	25	17	26	25	17	10	34	31	28
C36	31	24	28	17	30	34	15	18	ה	37	33
C37	26	20	27	26	30	27	18	11	34	29	28
C38	20	12	14	15	22	8	-	-	20	13	14
C39	24	24	24	25	27	22	22	21	ה	24	23
C40	21	11	18	15	23	19	8	10	ה	20	22
C41	21	17	20	20	24	22	15	19	24	31	28
C42	22	17	26	24	20	29	28	29	37	33	38
C43	15	17	16	8	21	-	19	20	22	25	21
C44	26	21	31	15	26	32	-	12	43	35	30
C45	20	15	19	22	28	20	16	18	17	19	10
C46	18	16	21	16	18	19	12	15	16	19	24

C47	14	13	25	24	21	-	8	15	15	18	19
C48	19	15	19	20	24	-	-	-	30	25	26
C49	27	23	30	22	30	32	21	24	42	32	32
C50	19	17	23	20	26	15	-	11	30	26	25
C51	20	15	21	18	25	-	25	29	38	33	29
C52	25	22	30	25	26	23	25	13	38	33	29
C53	23	16	26	20	16	29	-	-	32	27	26
C54	21	16	23	23	24	22	23	22	26	26	22
C55	22	20	24	22	24	27	29	27	35	31	28
C56	24	24	23	24	29	27	28	27	36	34	27
C57	25	20	27	18	28	32	22	19	38	31	28
C58	15	11	16	14	21	-	-	-	30	26	25
C59	22	20	24	20	24	26	22	20	28	23	21
C60	18	14	19	17	16	21	19	25	20	24	21
C61	32	20	27	13	26	27	18	17	44	31	34
C62	16	11	18	15	20	-	-	-	26	22	23
C63	21	13	22	18	7	-	8	8	26	24	20
C64	19	14	18	11	8	-	-	14	20	15	12
C65	18	14	19	11	8	-	-	14	20	15	12
C66	17	-	28	25	34	28	31	29	ה	26	30
C67	16	13	22	13	16	16	8	13	23	15	-
C68	26	27	27	24	29	28	26	26	ה	29	24
C69	30	30	38	31	32	22	31	22	ה	39	32
C70	19	18	20	18	23	22	22	22	24	26	22
C71	28	22	30	18	30	29	13	15	38	31	34

C72	25	24	21	21	26	28	26	27	32	30	26
C73	22	10	19	11	23	-	-	8	26	24	21
C74	33	29	37	31	34	34	-	-	[illegible]	36	34
C75	29	28	32	29	33	26	34	27	[illegible]	39	34
C76	30	32	31	31	36	32	25	21	[illegible]	35	34
C77	25	24	32	30	32	34	10	8	[illegible]	34	27
C78	30	26	30	29	28	32	35	34	[illegible]	37	38
C79	25	24	26	28	32	36	14	12	[illegible]	31	29
C80	20	17	22	18	26	-	9	10	[illegible]	26	24
C81	23	21	23	15	20	10	16	21	[illegible]	22	20
C82	31	29	37	31	34	41	22	16	[illegible]	37	32
C83	22	21	22	25	30	23	23	21	[illegible]	30	24
C84	34	30	33	21	32	38	20	18	[illegible]	36	34
C85	19	18	20	22	23	18	19	23	[illegible]	30	30
C86	16	11	14	17	20	-	-	10	[illegible]	13	-
C87	30	25	44	24	32	26	-	21	[illegible]	19	13
C88	19	19	18	15	20	8	10	10	[illegible]	16	12
C89	20	20	16	20	11	15	9	14	[illegible]	18	18
C90	32	31	36	34	30	35	24	16	[illegible]	33	30
C91	25	21	28	18	30	-	10	13	[illegible]	28	28
C92	15	-	14	15	18	-	8	11	[illegible]	22	23
C93	22	16	23	20	23	-	-	-	[illegible]	14	-
C94	20	21	21	14	13	14	13	16	[illegible]	22	16
C95	22	19	20	18	31	25	26	30	[illegible]	15	11
C96	25	23	23	19	22	-	14	14	[illegible]	25	19

C97	23	29	21	19	33	23	29	30	ה	26	21
C98	18	-	21	12	8	18	-	28	ה	-	-
C99	19	17	21	14	10	17	13	15	ה	21	15
C100	21	14	24	14	22	18	16	19	ה	12	10
C101	19	16	26	16	14	12	8	23	ה	21	17
C102	22	14	21	24	29	-	25	27	ה	-	21
C103	17	-	22	15	20	21	15	18	ה	23	24
C104	15	-	21	16	21	19	14	19	ה	20	24
C105	23	18	23	20	23	18	-	11	ה	25	24
C106	26	22	25	25	34	34	23	23	ה	29	25
C107	28	32	33	26	25	22	27	29	ה	32	36
C108	19	18	19	12	10	14	10	13	ה	13	11
C109	21	16	21	14	13	-	8	11	ה	22	23
C110	19	14	20	14	10	13	9	12	ה	19	12
C111	16	9	17	14	10	18	11	15	ה	21	12
C112	18	13	18	14	11	11	10	12	ה	16	12
C113	19	16	24	18	30	24	-	-	ה	25	25
C114	20	17	22	16	11	17	12	15	ה	18	16
C115	22	18	18	12	17	-	14	16	ה	16	11
C116	19	18	21	21	23	16	20	21	ה	25	25
C117	27	26	28	18	20	19	10	24	ה	28	30
C118	20	21	22	15	11	-	-	18	ה	16	17
C119	20	20	29	17	13	-	-	19	ה	18	16
C120	18	18	23	13	14	-	-	25	ה	24	13

Where - = no zone of inhibition; ה = antibiotic not available

APPENDIX VII: Measured zones of inhibition from antibiotic susceptibility test on lead tolerant bacteria isolates

Isolate code	Antibiotics										
	CN	K	CIP	TET	FFC	SXT	CPD	CAZ	IPM	MEM	ETP
Po1											
Po2	16	11	11	21	-	15	8	22	14	16	10
Po3	18	14	15	22	22	17	-	20	16	16	18
Po4	16	13	20	19	24	20	15	18	20	19	18
Po5	18	14	14	18	21	-	11	20	20	21	14
Po6	18	16	24	15	8	-	-	16	20	13	11
Po7	25	20	24	34	22	-	20	34	36	35	24
Po8	24	20	35	29	29	38	8	9	35	37	38
Po9	21	15	24	14	19	16	16	17	31	27	20
Po10	15	-	26	14	24	15	-	23	-	-	-
Po11	15	-	26	14	24	15	-	23	-	-	-
Po12	21	17	19	15	10	15	10	13	28	18	18
Po13	18	18	27	16	10	-	-	22	25	18	18
Po14	30	22	30	22	30	32	15	16	40	35	34
Po15	26	23	30	21	30	30	11	13	38	32	30
Po16	26	23	30	21	30	30	11	13	38	32	30
Po17	24	23	30	26	28	32	28	27	34	31	20
Po18	19	15	20	20	19	-	20	-	23	27	21
Po19	19	16	19	18	19	20	15	16	22	21	16
Po20	25	20	27	14	26	22	11	12	48	35	36
Po21	21	15	14	13	22	19	21	9	25	20	19

P°22	22	19	23	17	23	26	22	20	19	21	19
P°23	14	11	7	19	26	23	20	16	32	17	11
P°24	22	18	23	25	29	28	27	15	35	32	30
P°25	16	17	16	18	20	16	19	19	20	21	19
P°26	31	21	34	37	34	22	20	19	34	34	31
P°27	24	20	34	24	16	24	10	25	26	27	20
P°28	16	14	20	15	20	18	-	21	-	8	8
P°29	22	17	21	19	13	14	10	28	18	19	15
P°30	23	17	29	17	19	18	9	9	13	14	12
P°31	26	24	25	26	30	27	30	27	34	30	26
P°32	21	19	25	25	26	28	27	25	21	25	24
P°33	23	22	25	24	21	22	26	25	33	29	26
P°34	27	28	27	20	30	30	29	28	ה	24	28
P°35	17	14	24	11	20	14	-	-	25	18	22
P°36	29	25	35	30	34	30	25	28	38	39	49
P°37	23	20	25	24	26	26	25	26	31	28	25
P°38	25	25	34	25	25	32	24	15	42	33	32
P°39	28	25	31	30	30	32	28	14	42	36	42
P°40	32	26	38	36	38	39	40	42	40	39	40
P°41	15	-	15	8	19	-	8	8	16	23	13
P°42	22	19	29	25	30	36	-	-	24	32	21
P°43	21	14	16	20	24	-	19	22	22	24	26
P°44	31	21	32	19	33	34	24	16	40	34	32
P°45	24	19	26	11	20	24	14	23	35	34	30
P°46	25	19	23	21	21	25	16	18	38	29	27
P°47	28	23	34	17	24	32	21	20	34	29	30

P°48	22	19	23	18	23	25	20	20	24	22	19
P°49	21	-	30	11	12	12	-	25	23	31	29
P°50	21	-	30	11	12	13	-	24	21	32	29
P°51	10	8	15	15	16	-	19	17	25	19	18
P°52	17	11	20	16	23	18	25	26	-	-	-
P°53	15	14	16	14	26	-	13	18	18	21	19
P°54	10	11	10	9	19	-	15	15	12	15	15
P°55	35	26	32	18	34	32	14	15	ה	38	34
P°56	30	12	34	30	32	32	28	26	ה	27	24
P°57	24	24	25	22	26	25	24	25	ה	29	24
P°58	24	24	25	22	26	25	24	25	ה	29	25
P°59	27	28	30	25	30	29	27	28	ה	31	25
P°60	32	26	33	16	31	34	20	18	ה	32	30
P°61	22	18	20	16	28	-	8	11	ה	23	24
P°62	22	22	25	23	28	22	24	21	ה	26	22
P°63	30	26	31	14	30	32	20	16	ה	34	29
P°64	27	26	25	28	29	25	23	21	ה	24	25
P°65	30	29	35	31	32	34	32	24	ה	39	35
P°66	30	28	34	18	34	38	12	16	ה	34	32
P°67	24	23	26	20	28	8	8	10	ה	25	25
P°68	24	23	25	19	30	-	8	10	ה	23	21
P°69	32	30	38	18	35	40	14	17	ה	36	34
P°70	24	21	26	26	30	-	-	-	ה	26	27
P°71	28	25	28	19	28	34	-	-	ה	-	-
P°72	30	24	24	21	32	31	23	24	ה	27	24
P°73	32	29	34	32	32	34	14	16	ה	38	32

P°74	31	26	21	16	30	38	24	24	ה	39	32
P°75	28	25	30	24	29	36	12	-	ה	35	32
P°76	31	23	25	24	26	36	8	-	ה	28	24
P°77	23	18	26	16	30	-	8	9	ה	22	22
P°78	27	20	26	36	38	24	9	-	ה	-	-
P°79	19	18	19	17	23	12	16	17	ה	25	16
P°80	20	17	27	18	27	11	8	10	ה	21	22
P°81	28	17	27	18	27	11	8	10	ה	21	22
P°82	32	27	36	20	30	36	16	15	ה	35	32
P°83	33	34	39	28	32	38	25	30	ה	39	42
P°84	34	31	28	26	29	39	28	28	ה	40	42
P°85	17	10	20	-	22	28	18	18	ה	29	-
P°86	29	27	30	29	32	34	21	14	ה	34	-
P°87	21	22	25	25	24	26	12	13	ה	28	26
P°88	28	26	28	23	27	28	32	25	ה	36	31
P°89	24	22	25	12	18	18	18	22	ה	25	20
P°90	18	19	19	14	10	-	11	12	ה	16	15
P°91	19	19	21	11	15	10	-	-	ה	-	-
P°92	24	24	24	25	28	30	29	26	ה	30	9
P°93	20	20	22	9	11	19	14	18	ה	20	16
P°94	17	16	20	21	30	24	23	20	ה	19	17
P°95	22	23	23	25	21	26	24	24	ה	26	24
P°96	18	19	20	14	10	11	12	15	ה	16	14
P°97	22	24	21	20	25	28	28	25	ה	30	25
P°98	19	11	20	11	23	21	-	28	ה	-	-
P°99	25	22	27	22	25	27	20	24	ה	21	19

Pº100	23	22	28	27	30	-	16	21	ה	35	-
Pº101	23	20	28	21	26	21	7	8	ה	30	21
Pº102	26	19	26	23	30	18	-	-	ה	23	25
Pº103	31	29	34	31	32	30	29	24	ה	34	30
Pº104	23	23	26	27	29	28	18	18	ה	32	28
Pº105	27	16	16	15	22	9	-	8	ה	21	21
Pº106	20	18	20	14	10	12	-	11	ה	16	11
Pº107	19	17	17	18	23	-	-	-	ה	14	-
Pº108	18	18	21	16	23	-	-	10	ה	23	23
Pº109	22	24	22	22	25	15	25	24	ה	26	22
Pº110	18	8	20	14	11	12	11	19	ה	16	14
Pº111	21	-	22	22	32	28	22	24	ה	26	22
Pº112	22	21	26	14	17	14	-	-	ה	-	-
Pº113	22	20	9	-	12	12	16	16	ה	19	13
Pº114	18	17	-	-	-	-	-	19	ה	25	18
Pº115	18	19	20	11	15	-	-	12	ה	14	10
Pº116	20	18	20	14	10	12	-	11	ה	16	11

Where - = no zone of inhibition

ה = antibiotic not available

APPENDIX VIII: Measured zones of inhibition from antibiotic susceptibility test on zinc tolerant bacteria isolates

Isolate code	Antibiotics										
	CN	K	CIP	TET	FFC	SXT	CPD	CAZ	IPM	MEM	ETP
Zų1	17	12	16	15	8	17	8	12	14	-	9
Zų2	21	17	25	20	24	19	21	20	33	28	19
Zų3	19	-	24	11	10	-	8	12	22	18	12
Zų4	16	12	20	15	9	12	10	15	19	19	15
Zų5	15	12	20	14	10	15	8	-	21	18	13
Zų6	15	12	20	14	10	13	10	14	26	19	14
Zų7	18	14	22	15	10	15	10	12	22	18	14
Zų8	10	15	19	14	10	-	-	19	20	13	14
Zų9	16	14	20	15	10	15	8	12	19	20	13
Zų10											
Zų11	16	11	19	15	16	26	14	16	13	-	-
Zų12	19	16	17	17	10	22	12	20	28	22	17
Zų13	17	12	21	15	10	14	10	11	25	20	24
Zų14	21	18	22	19	25	26	25	24	31	27	24
Zų15	19	16	17	14	21	-	-	-	26	24	24
Zų16	23	15	19	19	26	10	-	8	23	25	26
Zų17	22	18	23	21	20	12	-	-	21	33	24
Zų18	25	15	26	19	24	10	-	-	20	27	24
Zų19	23	17	25	27	28	22	11	10	37	32	28
Zų20	18	15	22	18	25	10	8	11	ה	24	-
Zų21	20	15	11	10	23	-	15	18	25	24	26

Zų22	23	16	25	21	27	21	12	20	11	15	14
Zų23	21	17	25	22	23	18	17	23	8	12	11
Zų24	15	12	14	8	22	-	-	7	21	21	22
Zų25	27	28	29	20	32	25	27	24	ה	29	25
Zų26	21	14	20	19	23	15	9	10	33	25	25
Zų27	21	20	23	22	25	26	22	22	25	29	22
Zų28	21	18	17	20	22	23	25	24	29	25	22
Zų29	20	14	18	26	22	-	-	9	26	22	22
Zų30	15	8	21	11	14	-	16	19	30	25	16
Zų31	22	13	28	20	26	10	-	-	24	18	24
Zų32	21	15	26	26	29	18	-	8	28	28	28
Zų33	15	8	10	11	20	-	20	15	23	27	22
Zų34	16	17	9	18	21	-	14	20	20	23	23
Zų35	20	20	27	23	26	27	21	25	29	26	25
Zų36	16	17	9	18	21	-	14	20	20	23	23
Zų37	27	18	21	16	25	18	-	-	32	26	27
Zų38	32	21	35	30	34	32	27	24	40	40	40
Zų39	19	11	25	18	22	8	-	11	35	24	28
Zų40	29	22	29	25	27	34	-	-	ה	32	24
Zų41	23	21	29	25	27	34	-	-	ה	32	24
Zų42	25	20	25	14	29	13	8	10	ה	20	20
Zų43	21	10	29	10	17	10	-	18	ה	29	23
Zų44	19	8	26	9	15	10	-	20	ה	30	22
Zų45	16	19	21	20	28	20	16	19	19	18	23
Zų46	20	18	24	26	25	24	17	21	36	30	26
Zų47	34	28	34	20	32	36	22	18	ה	34	30

Zų48	29	26	21	26	24	32	-	-	ה	32	24
Zų49	23	18	20	15	26	15	8	10	ה	24	22
Zų50	20	15	23	17	24	33	29	29	ה	32	33
Zų51	33	24	32	24	28	32	-	-	ה	34	27
Zų52	23	18	25	18	26	-	8	10	ה	25	24
Zų53	29	28	31	18	31	34	25	26	ה	31	30
Zų54	23	18	22	20	27	10	10	12	ה	25	24
Zų55	21	21	19	22	25	21	23	21	ה	28	24
Zų56	21	21	19	22	25	21	23	21	ה	28	24
Zų57	23	17	25	15	25	12	8	10	ה	18	22
Zų58	20	21	21	19	25	12	8	10	ה	18	22
Zų59	24	24	25	24	27	25	25	23	ה	27	23
Zų60	18	13	20	12	26	22	9	17	ה	8	10
Zų61	26	28	34	31	36	34	25	-	ה	12	10
Zų62	25	18	26	16	20	-	8	9	ה	22	21
Zų63	22	19	23	18	12	-	8	8	ה	26	27
Zų64	25	18	30	20	20	10	8	12	ה	26	25
Zų65	29	24	28	11	28	34	-	-	ה	-	-
Zų66	34	26	29	25	32	36	-	-	ה	31	24
Zų67	23	20	28	23	29	22	9	12	ה	29	29
Zų68	17	16	16	16	20	10	8	10	ה	20	20
Zų69	19	13	24	14	28	26	-	21	ה	8	11
Zų70	34	34	39	35	32	36	29	30	ה	38	39
Zų71	25	24	22	21	24	15	19	17	ה	28	16
Zų72	22	21	22	16	21	12	17	19	ה	25	17
Zų73	25	25	25	28	31	26	31	27	ה	30	28

Zų74	21	20	20	14	19	14	15	16	ה	25	18
Zų75	18	16	21	16	21	16	17	20	ה	21	25
Zų76	21	19	16	12	16	-	15	18	ה	24	19
Zų77	22	22	23	11	33	20	28	29	ה	11	8
Zų78	20	19	30	14	19	25	18	20	ה	22	24
Zų79	23	20	31	14	18	24	19	22	ה	25	22
Zų80	24	23	-	8	22	11	16	16	ה	20	26
Zų81	16	14	11	15	14	-	-	-	ה	-	-
Zų82	29	29	22	10	15	12	-	-	ה	11	-
Zų83	17	20	23	13	11	22	14	18	ה	16	22
Zų84	17	18	29	18	14	-	16	22	ה	20	27
Zų85	16	15	21	-	11	-	13	15	ה	15	20
Zų86	20	22	23	16	9	23	-	-	ה	-	12
Zų87	20	21	19	14	9	18	-	-	ה	-	15
Zų88	16	19	30	18	25	26	8	-	ה	-	20
Zų89	19	11	25	15	25	18	-	-	ה	-	-
Zų90	18	18	20	29	34	25	23	22	ה	25	22
Zų91	20	18	20	29	34	25	23	22	ה	25	22
Zų92	22	19	26	28	27	28	20	17	ה	30	28
Zų93	27	24	28	25	34	24	-	-	ה	26	26
Zų94	34	36	36	25	32	36	34	32	ה	39	42
Zų95	20	23	22	24	26	24	25	24	ה	27	23
Zų96	18	16	17	15	11	17	11	14	ה	12	15
Zų97	32	34	36	28	33	38	37	38	ה	40	42
Zų98	20	19	19	14	20	10	13	15	ה	20	16
Zų99	23	24	25	20	24	18	8	14	ה	28	25

Zų100	21	20	20	15	21	14	18	20	ה	25	18
Zų101	19	16	20	15	10	14	12	14	ה	20	16
Zų102	18	16	19	14	10	14	10	15	ה	20	14
Zų103	30	25	30	16	24	8	-	18	ה	20	14
Zų104	17	18	21	15	11	19	10	16	ה	14	14
Zų105	25	26	25	22	23	27	24	24	ה	30	26
Zų106	21	20	22	14	26	-	20	23	ה	16	19
Zų107	25	25	26	27	30	27	30	29	ה	31	27
Zų108	22	22	25	17	12	11	-	17	ה	14	14
Zų109	18	19	21	13	10	8	-	28	ה	29	22
Zų110	-	-	-	10	-	-	-	12	ה	-	8
Zų111	19	20	25	12	12	10	-	27	ה	28	21
Zų112	17	18	29	18	14	-	16	22	ה	20	27
Zų113	16	15	21	-	11	-	13	15	ה	15	20
Zų114	20	22	23	16	9	23	-	-	ה	-	12

Where - = no zone of inhibition

ה = antibiotic not available

APPENDIX IX: Measured zones of inhibition from antibiotic susceptibility test on Enterobacteriaceae strains

Isolate code	CN	K	Cip	Tet	FFC	SXT	CPD	CAZ	IPM	MEM	ETP
EC 1	15	12	16	17	18	12	12	12	22	21	24
EC 2	14	12	-	-	18	-	15	18	18	20	20
EC 3	13	11	17	8	20	-	11	18	21	20	21
EC 4	10	9	11	-	18	-	8	13	16	19	18
EC 5	12	12	19	-	10	-	13	13	16	24	20
EC 6	10	12	13	-	12	-	-	13	17	18	18
EC 7	12	10	-	-	-	-	-	12	19	18	23
EC 8	16	12	16	8	17	14	8	14	23	21	25
EC 9	14	14	28	11	20	-	13	17	22	23	26
EC 10	10	12	20	12	18	15	11	16	20	20	26
EC 11	12	10	-	-	16	-	10	16	17	22	22
EC 12	14	10	-	8	16	-	15	16	21	20	20
EC 13	13	12	-	-	15	-	10	16	22	19	24
EC 14	19	14	18	-	18	-	11	12	20	20	24
EC 15	14	12	20	12	23	16	13	20	15	20	20
EC 16	15	14	20	12	23	16	13	20	15	20	20
EC 17	15	12	22	19	26	18	15	18	15	21	19
EC 18	18	12	26	15	17	20	12	22	24	20	21
EC 19	18	13	14	10	20	20	14	18	19	20	24
EC 20	18	12	23	10	18	-	13	16	18	22	23
EC 21	12	12	18	10	20	-	14	17	16	24	24
EC 22	-	-	-	10	13	-	-	14	20	19	22
EC 23	17	12	24	16	18	17	10	14	20	18	22
EC 24	18	11	23	16	18	14	-	14	19	16	20
EC 25	20	14	8	10	21	-	10	13	19	18	22

EC 26	20	15	29	19	24	22	10	13	19	18	22
EC 27	14	12	17	13	14	1	8	14	21	18	23
EC 28	20	17	9	8	21	-	12	14	21	22	22
EC 29	14	12	-	16	20	18	15	21	23	24	28
EC 30	16	14	18	19	24	18	16	18	25	24	28
EC 31	16	15	25	9	18	-	19	20	22	21	25
EC 32	20	15	29	19	19	20	12	15	21	20	22
EC 33	20	15	20	8	22	-	20	22	23	24	26
EC 34	18	15	30	9	23	-	12	15	22	23	26
EC 35	17	12	20	16	17	-	10	15	14	20	20
EC 36	15	15	20	16	16	19	16	18	19	18	25
EC 37	16	14	25	8	20	-	8	18	23	22	26
EC 38	17	14	22	10	22	-	16	15	22	22	25
EC 39	16	15	27	10	25	-	15	13	20	22	25
EC 40	18	18	20	8	24	-	17	22	23	25	24
EC 41	20	15	35	20	21	25	16	18	23	15	15
EC 42	17	15	18	8	20	-	14	17	24	20	24
EC 43	14	10	19	10	18	12	-	11	13	16	18
EC 44	19	15	27	20	9	-	12	17	25	24	24
EC 45	21	17	34	19	24	26	13	17	13	21	23
EC 46	19	14	28	-	18	-	14	16	24	24	26
EC 47	16	14	24	11	22	-	14	18	16	23	24
EC 48	18	19	30	20	19	25	-	14	11	15	18
EC 49	16	14	28	-	24	-	10	14	14	17	28
EC 50	18	14	23	21	22	21	13	12	12	16	16
EC 51	16	13	25	18	22	20	18	21	20	20	24
EC 52	16	14	22	18	20	19	19	20	22	20	22

EC 53	18	13	21	22	16	18	18	19	14	20	22
EC 54	18	14	27	9	22	24	17	23	27	25	25
EC 55	19	13	26	18	21	-	16	16	15	20	20
EC 56	10	12	-	-	15	-	-	12	18	16	20
EC 57	15	13	24	18	26	18	13	18	26	24	24
EC 58	13	11	18	15	21	12	8	12	20	18	20
EC 59	14	10	20	26	18	18	11	15	24	21	25
EC 60	18	12	18	18	23	-	17	18	14	20	24
EC 61	16	15	34	21	20	16	18	22	16	20	22
EC 62	17	15	33	20	26	24	8	15	16	21	22
EC 63	17	12	28	20	23	20	14	15	17	21	20
EC6 4	16	14	30	12	21	-	10	13	14	17	18
EC 65	20	18	22	15	-	-	-	10	27	18	9
EC 66	166	15	17	-	24	-	12	16	[illegible]	23	22
EC 67	10	13	-	-	17	-	13	17	[illegible]	22	21
EC 68	11	12	-	-	23	-	-	11	[illegible]	19	19
EC 69	16	13	20	-	22	16	13	16	[illegible]	20	21
EC 70	15	15	16	-	20	-	10	16	[illegible]	20	22
EC 71	17	15	18	14	16	15	11	15	[illegible]	20	21
EC 72	17	15	21	-	22	-	16	16	[illegible]	22	24
EC 73	19	18	28	9	25	-	16	18	[illegible]	23	24
EC 74	20	20	23	-	21	18	16	19	[illegible]	23	23
EC 75	18	14	21	8	22	16	16	19	[illegible]	22	24
EC 76	19	19	25	-	24	-	14	14	[illegible]	22	21
EC 77	15	16	28	16	22	16	15	20	[illegible]	21	22
EC 78	20	18	26	8	22	-	16	19	[illegible]	25	25

EC 79	17	17	24	-	19	-	16	16	ה	25	25
EC 80	19	19	21	16	25	20	19	21	ה	25	26
EC 81	18	18	22	10	25	-	12	12	ה	23	23
EC 82	16	15	20	15	20	18	12	17	ה	22	22
EC 83	18	18	26	15	20	17	10	15	ה	21	22
EC 84	15	10	20	14	16	9	10	14	10	14	15

Where; - = no zone of inhibition; ה = antibiotic not available

APPENDIX X: CLSI breakpoints for Enterobacteriaceae strains (CLSI, 2017)

Antibiotic	Susceptible	Intermediate	Resistant	Disc content
Ceftazidime	≥21	18 – 20	≤17	30µg
Cefpodoxime	≥21	18 – 2-	≤17	30µg
Ertapenem	≥23	20-22	≤19	10µg
Imipenem	≥23	20-22	≤19	10µg
Meropenem	≥23	20-22	≤19	10µg
Gentamicin	≥15	13-14	≤12	10µg
Kanamycin	≥18	14-17	≤13	30µg
Tetracycline	≥15	12-Dec	≤11	30µg
Ciprofloxacin	≥21	16-20	≤15	5µg
Trimethoprim/Sulfamethoxazole	≥16	11-15	≤10	1.25/23.75 g

APPENDIX XI: Metadata for Dendograms generated using GelClust following ERIC PCR

Dendogram code	Isolate name	Location of isolation	Plate	Dendogram code	Isolate name	Location of isolation	Plate
1	*E. coli*EC1	Alaba River	A	13	*E. coli*EC78	Alaba River	C
2	*E. coli*EC2	Alaba River	A	14	*E. coli*EC79	Alaba River	C
3	*E. coli*EC3	Alaba River	A	15	*E. coli*EC80	Alaba River	C
4	*E. coli*EC4	Alaba River	A	16	*E. coli*EC81	Alaba River	C
5	*E. coli*EC5	Alaba River	A				
6	*E. coli*EC6	Alaba River	A	1	*E. coli*E15	Aaba well1	D
7	*E. coli*EC7	Alaba River	A	2	*E. coli*EC16	Aaba well1	D
8	*E. coli*EC8	Alaba River	A	3	*E. coli*EC17	Aaba well1	D
9	*E. coli*EC9	Alaba River	A	4	*E. coli*EC84	Aaba well1	D
10	*E. coli*EC10	Alaba River	A				
11	*C. freundii* EC11	Alaba River	A				
12	*E. coli*EC12	Alaba River	A	1	*E. coli*EC36	Ogunpa soil	A
13	*E. coli*EC13	Alaba River	A	2	*E. coli*EC37	Ogunpa soil	A
14	*E. coli*EC14	Alaba River	A	3	*E. coli*EC38	Ogunpa soil	A
15	*E. coli*EC18	Alaba River	A	4	*E. coli*EC39	Ogunpa soil	A
16	*E. coli*EC19	Alaba River	A	5	*E. coli*EC40	Ogunpa soil	A
1	*E. coli*EC20	Alaba River	B	6	*E. coli*EC41	Ogunpa soil	A
2	*C. freundii* E21	Alaba River	B	7	*E. cloacae* EC64	Ogunpa soil	A
3	*E. coli*EC22	Alaba River	B	8	*E. coli*EC65	Ogunpa soil	A
4	*E. coli*EC23	Alaba River	B				
5	*E. coli*EC24	Alaba River	B	1	*E. coli*EC42	Ogunpa River	A
6	*E. coli*EC25	Alaba River	B	2	*E. roggenkampii* EC43	Ogunpa River	A
7	*E. coli*EC26	Alaba River	B	3	*E. coli*EC44	Ogunpa River	A
8	*E. coli*EC27	Alaba River	B	4	*E. coli*EC45	Ogunpa River	A
9	*E. coli*EC28	Alaba River	B	5	*E. coli*EC46	Ogunpa River	A
10	*E. coli*EC29	Alaba River	B	6	*C. portucalensis* EC47	Ogunpa River	A

11	*E. coli*EC30	Alaba River	B	7	*E. coli*EC48	Ogunpa River	A
12	*E. coli*EC31	Alaba River	B	8	*C. portucalensis* EC49	Ogunpa River	A
13	*E. coli*EC32	Alaba River	B	9	*E. cloacea* EC50	Ogunpa River	A
14	*E. coli*EC33	Alaba River	B	10	*Kluyvera sp.* EC51	Ogunpa River	A
15	*E. coli*EC34	Alaba River	B	11	*E. roggenkampii* EC52	Ogunpa River	A
16	*C. freundii* EC35	Alaba River	B	12	*E. cloacea* EC53	Ogunpa River	A
1	*E. coli*EC66	Alaba River	C	13	*E. coli*EC54	Ogunpa River	A
2	*E. coli*EC67	Alaba River	C	14	*E. coli*EC55	Ogunpa River	A
3	*E. coli*EC68	Alaba River	C	15	*E. coli*EC56	Ogunpa River	A
4	*E. coli*EC69	Alaba River	C	16	*E. cloacea* EC57	Ogunpa River	A
5	*E. coli*EC70	Alaba River	C	17	*Leclercia sp.* EC58	Ogunpa River	A
6	*Citrobacter* sp EC71	Alaba River	C	18	*E. coli* EC59	Ogunpa River	A
7	*E. coli*EC72	Alaba River	C	19	*E. roggenkampii* EC60	Ogunpa River	A
8	*E. coli*EC73	Alaba River	C	20	*E. roggenkampii* EC61	Ogunpa River	A
9	*E. coli*EC74	Alaba River	C	21	*E. cloacea* EC62	Ogunpa River	A
10	*E. coli*EC75	Alaba River	C	22	*E. hormaeche* EC63	Ogunpa River	A
11	*E. coli*EC76	Alaba River	C	23	*E. coli*EC82	Ogunpa River	A
12	*E. coli*EC77	Alaba River	C	24	*E. coli*EC83	Ogunpa River	A

APPENDIX XII: Metadata obtained from MLST profiling of the *E.coli*strains on Enterobase

Strain codes	ST	ST complex	Lineage	*adk*	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*
EC 2	424		AxB1	6	30	32	16	11	8	7
EC 3	1721	ST10 Cmplx	A	222	11	4	8	8	8	2
EC 4	167	ST10 Cmplx	A	10	11	4	8	8	13	2
EC 5	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 6	9428		A	6	153	163	91	7	8	6
EC 7	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 8	215	ST10 Cmplx	A	10	11	4	8	8	18	2
EC 9	207	ST10 Cmplx	A	61	11	4	8	8	8	2
EC 10	216		ABD	10	11	57	8	7	18	6
EC 12	1721	ST10 Cmplx	A	222	11	4	8	8	8	2
EC 13	424		AxB1	6	30	32	16	11	8	7
EC 14	167	ST10 Cmplx	A	10	11	4	8	8	13	2
EC 15	450		A	6	11	95	104	8	7	2
EC 16	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 17	215	ST10 Cmplx	A	10	11	4	8	8	18	2
EC 18	182		ABD	6	58	54	54	1	2	47
EC 19	215	ST10 Cmplx	A	10	11	4	8	8	18	2
EC 20	450		A	6	11	95	104	8	7	2
EC 22	206	ST206 Cmplx	AxB1	6	7	5	1	8	18	2
EC 23	215	ST10 Cmplx	A	10	11	4	8	8	18	2
EC 24	215	ST10 Cmplx	A	10	11	4	8	8	18	2
EC 25	10	ST10 Cmplx	A	10	11	4	8	8	8	2

EC 26	215	ST10 Cmplx	A	10	11	4	8	8	18	2
EC 27	5700			503	4	15	102	9	73	6
EC 28	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 29	398	ST398 Cmplx		64	7	1	1	8	8	6
EC 30	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 31	9815		A	10	27	4	10	8	1	2
EC 32	215	ST10 Cmplx	A	10	11	4	8	8	18	2
EC 33	398	ST398 Cmplx		64	7	1	1	8	8	6
EC 34	450		A	6	11	95	104	8	7	2
EC 36	226	ST226 Cmplx	A	10	27	5	8	8	7	2
EC 37	38	ST38 Cmplx	D	4	26	2	25	5	5	19
EC 39	38	ST38 Cmplx	D	4	26	2	25	5	5	19
EC 40	219		ABD	58	53	53	58	24	1	42
EC 41	215	ST10 Cmplx	A	10	11	4	8	8	18	2
EC42	9816	ST206 Cmplx	AxB1	6	7	856	1	8	18	2
EC 44	540		AxB1	6	7	57	1	8	8	2
EC 45	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 46	541	ST522 Cmplx		111	23	109	8	8	8	2
EC 54	218	ST10 Cmplx	A	10	11	4	12	8	8	2
EC 56	156	ST156 Cmplx	AxB1	6	29	32	16	11	8	44
EC 59	409		A	10	11	1	1	71	8	6
EC65	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 66	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 67	1196		AxB1	6	6	33	26	11	8	2
EC 68	361		A	10	99	5	91	8	7	2

EC 69	9817			10	7	5	8	8	737	6
EC72	2456		A	6	374	4	10	8	8	2
EC 73	9817			10	7	5	8	8	737	6
EC 74	165	ST165 Cmplx	A	10	27	5	10	12	8	2
EC 75	9817			10	7	5	8	8	737	6
EC 77	394	ST394 Cmplx		21	35	61	52	5	5	4
EC78	165	ST165 Cmplx	A	10	27	5	10	12	8	2
EC 79	609	ST46 Cmplx		8	7	1	8	8	7	6
EC 80	8763			6	11	57	140	7	175	6
EC 81	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 82	10	ST10 Cmplx	A	10	11	4	8	8	8	2
EC 83	8677	ST10 Cmplx		10	11	292	8	8	18	2

APPENDIX XIII: Antibiotic resistance genes detected in the sequenced enterobacterial (n=78) strains

Strain	Aminoglycoside	Sulphonamide	Beta-lactam	Quinolone	Trimethoprim	Tetracycline	Fosfomycin	Phenicol	Macrolide
*E. coli*EC2	*aadA2, strA, strB*	*sul1,sul2*	$bla_{\text{TEM-1B}}$	*qepA-like*	*dfrA12*	*tetA*			*mphA, mdfA*
*E. coli*EC3	*strA-like, strB*	*sul2*	$bla_{\text{TEM-1B}}$	*qnrS1*	*dfrA14-like*	*tetA*			*mdfA*
*E. coli*EC4	*aadA1-like, strA, strB*	*sul2*	$bla_{\text{OXA-1}}$, $bla_{\text{TEM-1B}}$	*qnrS1*	*dfrA14-like*	*tetA*		*catA1*	*mdfA*
*E. coli*EC5	*strA, strB*	*sul1,sul2*	$bla_{\text{TEM-1B}}$		*dfrA7*	*tetA*			*mdfA*
*E. coli*EC6									*mdfA*
*E. coli*EC7	*aadA2-like*	*sul1,sul3*	$bla_{\text{TEM-1B}}$		*dfrA12*	*tetA-like*		*floR*	*mphA, mdfA*
*E. coli*EC8						*tetA*			*mdfA*
*E.coli*EC9									*mdfA*
*E. coli*EC10									*mdfA*
C. freundii EC11	*aac(3)-IId-like, aadA2*	*sul1, sul2*	$bla_{\text{CMY-135}}$		*dfrA12*	*tetD*			*mphA*
*E. coli*EC12	*strA-like, strB*	*sul2*	$bla_{\text{TEM-1B}}$	*qnrS1*	*dfrA14-like*	*tetA*			*mdfA*
*E. coli*EC13	*aadA2, strA, strB*	*sul1,sul2*	$bla_{\text{TEM-1B}}$	*qepA4*	*dfrA12*	*tetA-like*			*mphA, mdfA*
*E. coli*EC14		*sul2*	$bla_{\text{TEM-1B}}$	*qnrS1*		*tetA*			*mdfA*
*E. coli*EC15	*aadA5, strA, strB-like*	*sul1,sul2*	$bla_{\text{TEM-1B}}$		*dfrA17*	*tetA*			*mphA, mdfA*
*E. coli*EC16	*aadA1-like,aadA2*	*sul1,sul3*	$bla_{\text{OXA-1}}$		*dfrA12*	*tetA*		*catA1-like*	*mphA, mdfA*
*E. coli*EC17									*mdfA*
*E. coli*EC18									*mdfA*
*E. coli*EC19									*mdfA*
*E. coli*EC20	*aadA5,strA,strB-like*	*sul1,sul2*	$bla_{\text{TEM-1B}}$		*dfrA17*	*tetA*			*mphA, mdfA*
C. freundii sp. EC21	*aadA1,aadA24-like*	*sul1,sul2*	$bla_{\text{TEM-1C}}$, $bla_{\text{CMY-100}}$	*qnrB12-like*	*dfrA1,dfrA15*	*tetA*		*catA2-like*	

*E. coli*EC22	*strA, strB*	*sul2*	*bla*$_{\text{TEM-1B}}$	*qnrS1*	*dfrA14-like*	*tetA-like*			*mdfA*
*E. coli*EC23									*mdfA*
*E.coli*EC24									*mdfA*
E. coli EC25	*aadA1-like,aadA2-like*	*sul1,sul3*	*bla*$_{\text{OXA-1}}$		*dfrA12*	*tetA*		*catA1-like*	*mphA, mdfA*
E.coli EC26									*mdfA*
E.coli EC27									*mdfA*
E.coli EC28	*aadA1-like,aadA2-like*	*sul1,sul3*	*bla*$_{\text{OXA-1}}$		*dfrA12*	*tetA*		*catA1-like*	*mphA, mdfA*
E.coli EC29									*mdfA*
E.coli EC30									*mdfA*
E.coli EC31	*strA-like,strB*	*sul2*	*bla*$_{\text{TEM-1B}}$		*dfrA14*	*tetA*			*mdfA*
E.coli EC32									*mdfA*
E.coli EC33	*strA,strB-like*	*sul2*	*bla*$_{\text{TEM-1B}}$	*qnrS1*	*dfrA14*	*tetA*			*mdfA*
E.coli EC33	*aadA5,strA,strB-like*	*sul1,sul2*	*bla*$_{\text{TEM-1B}}$		*dfrA17*	*tetA*			*mphA, mdfA*
C. freundii EC35	*aadA24-like*	*sul1,sul2*	*bla*$_{\text{TEM-1B}}$, *bla*$_{\text{CMY-100}}$	*qnrB12-like*	*dfrA15*			*catA2-like*	
E.coli EC36									*mdfA*
E.coli EC37	*aadA5*	*sul2*		*qnrB7*	*dfrA17*	*tetA*			*mdfA*
E.coli EC39	*aadA5*	*sul2*		*qnrB7*	*dfrA17*	*tetA*			*mdfA*
E.coli EC40		*sul2*	*bla*$_{\text{TEM-1B}}$	*qnrS1*	*dfrA14-like*	*tetA*			*mdfA*
E.coli EC41									*mdfA*
E.coli EC42	*strB-like*	*sul3*	*bla*$_{\text{TEM-1B}}$	*qnrS1*	*dfrA14*	*tetA-like*			*mdfA*
En. roggenkampii EC43			*bla*$_{\text{MIR-1}}$			*tetA*	*fosA-like*		
E.coli EC44	*strA-like,strB*	*sul2*	*bla*$_{\text{TEM-1B}}$		*dfrA14*	*tetA*			*mdfA*
E.coli EC45									*mdfA*

E.coli EC46									*mdfA*
C. portucalensis EC47	*aadA1*	*sul1*	$bla_{\text{CMY-129}}$, $bla_{\text{TEM-1C}}$	*qnrB17*	*dfrA1*	*tetA*			
C. portucalensis EC49	*aadA1*	*sul1*	$bla_{\text{CMY-129}}$, $bla_{\text{TEM-1C}}$	*qnrB17*	*dfrA1*	*tetA*			
Enterobacter sp EC50			$bla_{\text{MIR-1}}$				*fosA-like*		
Kluyvera sp. EC51									
En. roggenkampii EC52			$bla_{\text{MIR-6}}$						
E. kobei EC53							*fosA-like*		
E.coli EC54			$bla_{\text{TEM-1B}}$			*tetA*			*mdfA*
E.coli EC56	*aac(3)-IId-like,strA,strB*	*sul2*	$bla_{\text{TEM-1B}}$	*qepA-like*	*dfrA17*	*tet(B)*			*mdfA*
Enterobacter sp. EC57			$bla_{\text{HERA-8}}$					*catA1-like*	
Leclercia sp EC58						*tet(C)*			
E.coli EC59									*mdfA*
En. roggenkampii EC60			$bla_{\text{MIR-3}}$	*qnrS1*	*dfrA14*				
En. roggenkampii EC61		*sul2*	$bla_{\text{MIR-5}}$				*fosA-like*	*catA1-like*	
Enterobacter sp EC62			$bla_{\text{MIR-1}}$				*fosA-like*		
En. hormaeche EC63			$bla_{\text{ACT-7}}$				*fosA-like*		
Enterobacter sp	*strA-like,strB*	*sul2*	$bla_{\text{MIR-5}}$,$bla_{\text{TEM-1B}}$		*dfrA14-like*	*tetA*	*fosA-like*		

EC64									
*E. coli*EC65	*aadA1-like,aadA2-like*	*sul1,sul3*	bla_{OXA-1}		*dfrA12*	*tetA*		*catA1-like*	*mphA, mdfA*
*E. coli*EC66	*aadA8b-like,strA,strB*	*sul2*	bla_{TEM-1B}	*qnrS1*	*dfrA14-like*	*tetA,tet(B)*	*fosA-like*		*mdfA*
*E. coli*EC67	*aac(3)-IIa-like,aadA1,aadA2*	*sul3-like*	bla_{OXA-1},bla_{TEM-1B}		*dfrA12*	*tetA*		*catB3-like,cmlA1-like*	
*E. coli*EC68	*aac(3)-IIa-like, aac(6')Ib-cr strA, strB, aac(6')Ib-cr*	*sul2*	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1B}		*dfrA14*	*tetA*		*catB3-like*	*mphA, mdfA*
*E. coli*EC69				*qnrS1*		*tetA-like*			*mdfA*
*E. coli*EC70	*strA-like,strB*	*sul2*	bla_{TEM-1B}		*dfrA14*	*tetA*			*mdfA*
Citrobacter sp EC71				*qnrB69-like*					
*E. coli*EC72	*strB-like*	*sul3-like*	bla_{TEM-1B}	*qnrS1*	*dfrA14*	*tetA-like*			*mdfA*
*E. coli*EC73				*qnrS1*		*tetA-like*			*mdfA*
*E. coli*EC74	*strA-like,strB*	*sul2*	bla_{TEM-1B}		*dfrA14*	*tetA-like*			*mdfA*
*E. coli*EC75				*qnrS1*		*tetA-like*			*mdfA*
*E. coli*EC77									*mdfA*
E.coli EC78	*strA,strB*	*sul2*	bla_{TEM-1B}	*qnrS1*	*dfrA14*	*tetA*			*mdfA*
E.coli EC79	*aadA1-like,strA,strB*	*sul2*	bla_{OXA-1}, bla_{TEM-1B}		*dfrA5*	*tet(B)*		*catA1-like*	*mdfA*
E.coli EC80									*mdfA*
E.coli EC81	*strA,strB*	*sul2*	bla_{TEM-1B}	*qnrS1*	*dfrA14*	*tetA-like*			*mdfA*
E.coli EC82	*strA,strB*	*sul2*	bla_{TEM-1B}	*qnrS1*	*dfrA14*	*tetA-like*			*mdfA*
E.coli EC83									*mdfA*

Where En = Enterobacter

APPENDIX XIV: Plasmid replicon types in Enterobacteriaceae strains

Strain names	Plasmids replicon types
*E. coli*EC2	ColRNAI,IncB/O/K/Z
*E. coli*EC3	IncFIB(K),IncY,ColRNAI
*E. coli*EC4	IncFIA(HI1),IncR,ColRNAI
*E. coli*EC5	IncFII(pRSB107),Col(MG828),IncQ1,Col156,ColpVC,ColRNAI
*E. coli*EC6	IncY,IncR
*E. coli*EC7	IncY
*E. coli*EC8	Col(MG828),ColRNAI
*E. coli*EC9	NA
*E. coli*EC10	IncFIB(K),p0111
C. freundii EC11	IncFIB(pB171),IncFII(Yp),IncR,ColRNAI
*E. coli*EC12	IncFIB(K),IncY,ColRNAI
*E. coli*EC13	IncX1,ColRNAI
*E. coli*EC14	IncY,ColRNAI
*E. coli*EC15	IncFII(29),IncFIB(AP001918),Col156
*E. coli*EC16	IncY,ColRNAI
*E. coli*EC17	IncFIA(HI1),IncFIB(pQil),IncFIB(AP001918),IncFIB(K)
*E. coli*EC18	NA
*E. coli*EC19	IncFIA(HI1),IncFIB(pQil),IncFIB(AP001918),IncFIB(K)
*E. coli*EC20	IncFII(29),IncFIB(AP001918),Col156
Citrobacter sp. EC21	TrfA,ColRNAI
*E. coli*EC22	IncHI1B(CIT),IncFIB(K),p0111
*E. coli*EC23	IncFIA(HI1),IncFIB(pQil),IncFIB(AP001918), IncFIB(K)
*E. coli*EC24	IncFIA(HI1),IncFIB(pQil),IncFIB(AP001918),IncFIB(K)
*E. coli*EC25	IncY,ColRNAI
*E. coli*EC26	IncFIA(HI1),IncFIB(AP001918),IncFIB(pQil),IncFIB(K)
*E. coli*EC27	IncR,ColRNAI
*E. coli*EC28	IncY,ColRNAI
*E. coli*EC29	NA
*E. coli*EC30	IncY,IncR
*E. coli*EC31	IncFII,IncI1,IncFIA,Col(BS512),ColRNAI
*E. coli*EC32	IncFIA(HI1),IncFIB(pQil),IncFIB(AP001918),IncFIB(K)
*E. coli*EC33	IncFIB(K)
*E. coli*EC33	IncFII(29),IncFIB(AP001918),Col156
C. freundii EC35	IncFIB(pHCM2),TrfA,ColRNAI
*E. coli*EC36	ColRNAI
*E. coli*EC37	IncI1
*E. coli*EC39	IncI1
*E. coli*EC40	IncY
*E. coli*EC41	IncFIA(HI1),IncR,ColRNAI
*E. coli*EC42	IncX1,ColRNAI
En. roggenkampii EC43	IncFII(pECLA),IncFIB(pECLA),ColRNAI
*E. coli*EC44	IncR,ColRNAI

*E. coli*EC45	NA
*E. coli*EC46	IncHI1B(R27),IncHI1A,IncY,IncR,ColRNAI
C. portucalensis EC47	IncFIB(K),IncR,ColRNAI
C. portucalensis EC49	IncFIB(K),IncR,ColRNAI
Enterobacter sp EC50	IncFIB(K),IncY,ColRNAI
Kluyvera sp. EC51	IncFIB(K),ColRNAI
En. roggenkampii EC52	IncFIB(K),ColRNAI
En. kobei EC53	IncR,ColRNAI
*En. coli*EC54	IncFIC(FII),IncFII(pSE11),IncI1,IncFIB(AP001918),ColRNAI
*En. coli*EC56	IncFIA,IncFIB(pB171),IncQ1,ColRNAI
Enterobacter sp. EC57	ColRNAI
Leclercia sp EC58	IncFIB(pCTU3),ColRNAI
*E. coli*EC59	ColRNAI
En. roggenkampii EC60	IncFIA(HI1),IncFIB(K),IncFIB(pQil),IncR,ColRNAI
En. roggenkampii EC61	IncFIB(K),ColRNAI
Enterobacter sp EC62	IncFIA(HI1),IncFIB(K),IncN,IncR,ColRNAI
En. hormaeche EC63	ColRNAI
Enterobacter sp EC64	IncY,ColRNAI
*E. coli*EC65	IncY,ColRNAI
*E. coli*EC66	IncFIA(HI1),IncFIB(K),IncY,IncR,ColRNAI
*E. coli*EC67	NA
*E. coli*EC68	IncY,IncQ1
*E. coli*EC69	IncY
*E. coli*EC70	IncR,ColRNAI
Citrobacter sp EC71	TrfA,ColRNAI
*E. coli*EC72	IncX1,ColRNAI
*E. coli*EC73	IncY
*E. coli*EC74	IncFIA(HI1),IncR
*E. coli*EC75	IncY
*E. coli*EC77	NA
*E. coli*EC78	IncFIB(K),IncFIB(AP001918)
*E. coli*EC79	IncFIA(HI1),IncHI1A,IncHI1B(R27),IncQ1,ColpVC,ColRNAI
*E. coli*EC80	IncFIA(HI1),IncFIB(K),IncFII(Y),IncY,IncR,ColRNAI
*E. coli*EC81	IncY
*E. coli*EC82	IncY
*E. coli*EC83	IncFII,IncFIB(K)

Where; NA = Not present

APPENDIX XV: Heavy metal resistance genes present on Enterobacterial isolates from E-waste dumpsites in this study

Enterobacterial isolates	Heavy metal resistance genes present
E. coli EC2	*arsB, mntH, rcnA, copA, pcoABDRS, cusABCRS, cueO, cutCEF, silABCPRS, nikABCDE,zntA, zitB, zinT, znuABC, zupT, zraR, tehAB, mobABCE, fecDE, fieF*
E. coli EC3	*arsB, mntH, rcnA, copA, pcoABDRS, cusABCRS, cueO, cutCEF, silABCPRS, nikABCDE,zntA, zitB, zinT, znuABC, zupT, zraR, tehAB, mobABCE, fecDE, fief*
E. coli EC4	*arsB, mntH, rcnA, copA, cusABCRS, cueO, cutCEF, nikABCDE,zntA, zitB, zinT, znuABC, zupT, zraSR, , tehAB, mobABCE, fecDE, fief*
E. coli EC5	*arsB, mntH, rcnA, copA, cusABCRS, cueO, cutCEF, nikABCDE, ,zntA, zitB, zinT, znuABC, zupT, zraSR, , tehAB, mobABCE, fief*
E. coli EC6	*arsAB, mntH, rcnA, copA, cusABCR, cueO, cutCEF, nikABCDE, ,zntA, zitB, zinT, znuABC, zupT, zraR, tehAB, mobABCE, fief*
E. coli EC7	*arsB, mntH, rcnA, copA, cusABCSR, cueO, cutCEF, nikABCDE, ,zntA, zitB, zinT, znuABC, zupT, zraSR, tehAB, mobABCE, fief*
E. coli EC8	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*

E. coli EC9	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC10	*arsA, arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
Citrobacter freundii EC11	*arsH, mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC12	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, tehAB, modABCE, fecD, fecE, fief*
E. coli EC13	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB,modABCE, fecD, fecE, fief*
E. coli EC14	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecD, fecE, fief*
E. coli EC15	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecD, fecE, fief*
E. coli EC16	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*

E. coli EC17	*arsA, arsB, mntH, rcnA, copA, pcoB, pcoD, pcoR, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC18	*arsB, mntH, rcnA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC19	*arsA, arsB, mntH, rcnA, copA, pcoD, pcoR, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC20	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecD, fecE, fief*
Citrobacter werkmanii EC21	*arsH, mntH, copA, pcoA, pcoB , pcoD, pcoR, pcoS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC22	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC23	*arsA, arsB, mntH, rcnA, copA, pcoD, pcoR, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC24	*arsA, arsB, mntH, rcnA, copA, pcoD, pcoR, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*

E. coli EC25	*arsA , arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC26	*arsA, arsB, mntH, rcnA, pcoD, pcoR, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC27	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC28	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC29	*arsB, mntH, rcnA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC30	*arsB, mntH, rcnA, copA, pcoA, pcoB, cusA, cusB, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC31	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC32	*arsA, arsB, mntH, rcnA, copA, pcoD, pcoR, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE*

E. coli EC33	*arsB, mntH, rcnA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC34	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecD, fecE, fief*
Citrobacter werkmanii EC35	*arsH, mntH, copA, cusA, cusR, zntA, zitB,zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE,fief*
E. coli EC36	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC37	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC39	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
E. coli EC40	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zraS, zraR, tehAB, modABCE, fief*
E. coli EC41	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*

E. coli EC42	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
En roggenkampii EC43	*mntH, copA, cutF, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, modABCE, fief*
E. coli EC44	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, tehAB, modABCE, fecE, fecD, fief*
E. coli EC45	*arsA, arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC46	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, tehAB, modABCE, fief*
C. portucalensis EC47	*arsA, arsB, mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, silA, silB, silC, silP, silR, silS, zntA, znuABC, modABCE, fief*
C. portucalensis EC49	*arsA, arsB, mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, silA, silB, silC, silP, silR, silS, zntA, znuABC, modABCE, fief*
Enterobacter sp EC50	*mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, cutF, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, modABCE, fief, merA*
Kluyvera sp EC51	*mntH, copA, cueO, cutC, cutF, zntA, zitB, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*

En roggenkampii EC52	*mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, cutF, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, zupT, modABCE, fief*
Enterobacter kobei EC53	*arsA, arsB, mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, tehAB, modABCE, fief*
E. coli EC54	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC56	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC57	*mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, tehAB, modABCE, fief*
Leclercia sp EC58	*arsB, arsH, mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, cutC, cutF, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, zupT, tehAB, modABCE, fief*
E. coli EC59	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
En. roggenkampii EC60	*mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, cutF, silA, silB, silC, silP, silR, silS, zntA, zitB, znuABC, zupT, modABCE, fief*

En. roggenkampii EC61	*arsA, arsB, mntH, rcnA, copA, cueO, cutF, silA, zntA, zitB, zinT, znuABC, zupT, tehAB, modABCE, fecE, fecD, fief*
Enterobacter sp EC62	*arsA, arsB, mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, cutF, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, zupT, tehAB, modABCE, fief*
En. hormaechei EC63	*mntH, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cueO, silA, silB, silC, silP, silR, silS, zntA, zitB, zinT, znuABC, zupT, tehAB, modABCE, fief*
Enterobacter sp EC64	*mntH, copA, cueO, cutF, silA, zntA, zitB, zinT, znuABC, zupT, modABCE, fief, merA*
E. coli EC65	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC66	*arsA, arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
E. coli EC67	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief, terCDZ*
E. coli EC68	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
E. coli EC69	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT,*

	zraS, zraR, tehAB, modABCE, fief
E. coli EC70	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
Citrobacter sp EC71	*arsA, arsB, mntH, copA, cusA, cusR, cueO, cutF, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC72	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC73	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC74	*arsB, mntH, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
E. coli EC75	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC77	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
E. coli EC78	*arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT,*

	zraS, zraR, tehAB, modABCE, fief
E. coli EC79	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
E. coli EC80	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fief*
E. coli EC81	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
E. coli EC82	*arsB, mntH, rcnA, copA, pcoA, pcoB, pcoD, pcoR, pcoS, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, silA, silB, silC, silP, silR, silS, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*
E. coli EC83	*arsA, arsB, mntH, rcnA, copA, cusA, cusB, cusC, cusR, cusS, cueO, cutC, cutE, cutF, nikABCDE, zntA, zitB, zinT, znuABC, zupT, zraS, zraR, tehAB, modABCE, fecE, fecD, fief*

APPENDIX XVI: DNA concentration of Enterobacteriaceae strains used for Whole Genome Sequencing

Strain code	ng/ µl	260/280nm	Strain code	ng/ µl	260/280
EC1	150	1.80	EC43	52	1.88
EC2	52	1.98	EC44	29	1.70
EC3	29	1.90	EC45	36	1.71
EC4	55	1.92	EC46	56	1.87
EC5	44	1.88	EC47	55	1.98
EC6	70	1.95	EC48	48	1.87
EC7	42	1.94	EC49	37	1.98
EC8	13	1.89	EC50	55	1.86
EC9	74	1.89	EC51	56	1.72
EC10	46	1.87	EC52	53	1.78
EC11	46	1.71	EC53	48	1.87
EC12	42	1.83	EC54	21	1.92
EC13	33	1.79	EC55	15	1.98
EC14	11	1.78	EC56	40	1.92
EC15	23	2.09	EC57	9.6	1.94
EC16	30	1.93	EC58	39	1.85
EC17	82	1.64	EC59	35	1.69
EC18	37	1.88	EC60	83	1.86
EC19	15	1.94	EC 61	76	1.70
EC20	30	1.79	EC62	71	1.87
EC21	59	1.80	EC63	30	1.88
EC22	34	2.02	EC64	59	1.78
EC23	21	2.11	EC65	23	1.98
EC24	49	1.86	EC 66	7.4	1.81
EC25	57	1.92	EC67	54	1.84
EC26	26	1.73	EC68	56	1.86
EC27	38	1.83	EC69	52	1.71
EC28	33	1.71	EC70	59	1.73
EC29	10.2	1.88	EC71	65	1.85
EC30	37	1.96	EC72	31	1.76
EC31	56	1.79	EC73	70	1.70
EC32	25	1.74	EC74	73	1.76
EC33	40	1.83	EC75	84	1.75
EC34	78	1.88	EC76	45	1.73
EC35	39	1.89	EC77	102	1.78
EC36	31	1.89	EC78	83	1.80
EC37	26	1.75	EC79	57	1.82
EC38	22	1.73	EC80	76	1.82
EC39	48	1.72	EC81	28	1.91
EC40	21	1.76	EC82	79	1.72
EC41	38	2.08	EC83	93	1.72
EC42	28	1.74			

APPENDIX XVII: Measured copy numbers of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1*, *tetA* and $bla_{\text{CTX-M-1}}$ per 100ml of water samples obtained within selected E-waste dumpsites

Sample	16SrRNA	*intI1*	*sul1*	*sul2*	*dfrA1*	*tetA*	$bla_{\text{CTX-M-1}}$
ĄR1	1.32Ex+09 ± 1.03Ex+08	8.39Ex+06 ± 6.61Ex+05	6.53Ex+07 ± 1.74Ex+06	1.47Ex+08 ± 8.60Ex+06	1.24Ex+06 ± 1.62Ex+05	1.28Ex+06 ± 8.66Ex+04	1.08Ex+05 ± 8.01Ex+04
ĄR2	2.88Ex+09 ± 1.03Ex+08	2.61Ex+07 ± 5.32Ex+05	2.61Ex+08 ± 3.05Ex+06	1.16Ex+08 ± 1.62Ex+07	1.12Ex+08 ± 5.06Ex+06	1.80Ex+05 ± 3.98Ex+03	4.69Ex+04 ± 2.06Ex+04
ĄR3	8.26Ex+09 ± 5.08Ex+08	6.20Ex+05 ± 3.42Ex+04	1.04Ex+07 ± 1.87Ex+05	2.77Ex+07 ± 8.06Ex+05	6.30Ex+05 ± 4.16Ex+04	9.62Ex+04 ± 1.42Ex+04	0.00Ex+00
AW1I	2.33Ex+08 ± 2.70Ex+06	4.17Ex+05 ± 1.33Ex+04	2.14Ex+06 ± 5.10Ex+04	4.10Ex+07 ± 9.12Ex+06	7.17Ex+04 ± 7.59Ex+03	4.68Ex+04 ± 1.17Ex+04	0.00Ex+00
AW1II	1.97Ex+08 ± 6.87Ex+06	4.42Ex+05 ± 1.19Ex+05	2.85Ex+06 ± 1.07Ex+05	2.29Ex+06 ± 1.54Ex+04	8.87Ex+05 ± 2.26Ex+04	2.23Ex+04 ± 3.25Ex+03	0.00Ex+00
AW1III	2.50Ex+08 ± 4.66Ex+06	4.97Ex+05 ± 2.88Ex+04	2.79Ex+06 ± 9.19Ex+04	1.53Ex+07 ± 4.17Ex+05	1.11Ex+05 ± 1.71Ex+04	2.30Ex+04 ± 4.13Ex+02	0.00Ex+00
AW2I	1.75Ex+08 ± 6.06Ex+06	5.93Ex+04 ± 2.45Ex+03	1.82Ex+05 ± 7.28Ex+04	4.84Ex+06 ± 3.48Ex+05	1.08Ex+05 ± 1.14Ex+03	0.00Ex+00	0.00Ex+00
AW2II	5.45Ex+08 ± 5.93Ex+06	4.86Ex+05 ± 2.76Ex+04	1.22Ex+06 ± 3.40Ex+04	1.90Ex+06 ± 1.08Ex+06	0.00Ex+00	0.00Ex+00	0.00Ex+00

AW2III	1.75Ex+08 ± 4.49Ex+07	7.27Ex+05 ± 2.85Ex+04	6.04Ex+06 ± 3.20Ex+05	2.81Ex+07 ± 1.48Ex+06	1.79Ex+05 ± 1.02Ex+04	2.49Ex+05 ± 2.80Ex+05	0.00Ex+00
ASB1	3.86Ex+07 ± 3.11Ex+06	6.76Ex+04 ± 7.76Ex+03	3.62Ex+05 ± 2.47Ex+03	1.77Ex+05 ± 1.99Ex+04	1.00Ex+05 ± 1.02Ex+04	1.54Ex+05± 5.74Ex+03	4.65Ex+05 ± 3.87Ex+04
ASB2	1.29Ex+07 ± 9.46Ex+05	1.05Ex+04 ± 4.95Ex+02	1.07Ex+05 ± 9.09Ex+03	4.75Ex+04 ± 2.58Ex+03	0.00Ex+00	0.00Ex+00	5.01Ex+04 ± 1.69Ex+04
ASB3	2.13Ex+08 ± 2.31Ex+06	4.77Ex+05 ± 8.52Ex+04	4.07Ex+07 ± 6.95Ex+05	2.20Ex+06 ± 1.68Ex+05	0.00Ex+00	1.58Ex+05 ± 6.68Ex+03	3.15Ex+05 ± 2.41Ex+04
IKB1	2.06Ex+07 ± 8.08Ex+05	2.80Ex+05 ± 4.61Ex+03	1.69Ex+06 ± 3.08Ex+04	7.10Ex+05 ± 7.47Ex+04	9.59Ex+05 ± 1.18Ex+05	0.00Ex+00	6.99Ex+04 ± 2.18Ex+03
IKB2	1.83Ex+07 ± 5.06Ex+05	1.49Ex+05 ± 1.21Ex+04	1.50Ex+06 ± 3.47Ex+04	4.07Ex+05 ± 4.11Ex+04	6.25Ex+05 ± 3.83Ex+04	1.93Ex+05 ± 9.84Ex+03	7.10Ex+05 ± 1.06Ex+05
IKB3	1.02Ex+07 ± 7.29Ex+05	4.84Ex+04 ± 6.22Ex+03	7.18Ex+05 ± 4.48Ex+03	3.05Ex+05 ± 4.33Ex+03	2.58Ex+05 ± 9.15Ex+03	0.00Ex+00	4.76Ex+04 ± 1.41Ex+04
OR1	5.83Ex+08 ± 3.30Ex+07	5.41Ex+06 ± 3.74Ex+05	2.32Ex+07 ± 1.32Ex+06	2.59Ex+07 ± 1.71Ex+06	1.40Ex+06 ± 7.03Ex+04	2.54Ex+05 ± 3.54Ex+03	0.00Ex+00
OR2	4.05Ex+08 ± 5.75Ex+06	3.87Ex+06 ± 4.47Ex+05	1.39Ex+07 ± 1.03Ex+05	2.02Ex+07 ± 1.43Ex+07	1.34Ex+06 ± 1.23Ex+05	3.08Ex+05 ± 1.14Ex+04	2.97Ex+06 ± 3.53Ex+05
OR3	2.10Ex+08 ± 4.41Ex+06	1.17Ex+06 ± 1.14Ex+05	4.47Ex+06 ± 7.43Ex+04	2.72Ex+07 ± 3.86Ex+06	9.02Ex+05 ± 5.30Ex+04	3.07Ex+05 ± 1.77Ex+04	5.67Ex+06 ± 1.55Ex+05

UW1I	2.69Ex+08 ± 2.24Ex+07	2.28Ex+05 ± 1.25Ex+04	5.20Ex+05 ± 1.39Ex+04	9.05Ex+07 ± 1.89Ex+06	0.00Ex+00	0.00Ex+00	5.58Ex+04 ± 1.19Ex+04
UW1II	2.95Ex+08 ± 9.99Ex+06	2.64Ex+05 ± 1.19Ex+04	4.84Ex+06 ± 1.20Ex+05	6.83Ex+07 ± 3.48Ex+06	1.25Ex+06 ± 5.87Ex+04	0.00Ex+00	0.00Ex+00
UW1III	2.50Ex+08 ± 3.57Ex+05	5.82Ex+04 ± 4.98Ex+03	2.36Ex+05 ± 1.32Ex+04	7.85Ex+05 ± 9.94Ex+04	0.00Ex+00	0.00Ex+00	4.72Ex+04 ± 1.27Ex+04
UW2I	7.13Ex+07 ± 4.60Ex+06	1.13Ex+05 ± 9.80Ex+03	4.40Ex+05 ± 3.23Ex+04	3.43Ex+06 ± 7.57Ex+04	0.00Ex+00	0.00Ex+00	1.25Ex+05 ± 1.55Ex+04
UW2II	2.47Ex+08 ± 8.04Ex+06	1.93Ex+05 ± 8.90Ex+03	2.87Ex+06 ± 5.20Ex+04	9.49Ex+06 ± 1.19Ex+07	1.26Ex+06 ± 3.84Ex+04	0.00Ex+00	0.00Ex+00
UW2III	1.75Ex+08 ± 2.10Ex+06	2.54Ex+05 ± 8.19Ex+03	2.93Ex+06 ± 6.43Ex+04	4.91Ex+07 ± 2.77Ex+06	1.52Ex+05 ± 2.49Ex+03	8.31Ex+05 ± 4.51Ex+04	6.52Ex+04 ± 2.44Ex+04

Each measurements represents the average of three replicate samples

Ex+ = Exponential

APPENDIX XVIII: Measured copy number of 16S rRNA, *intI1*, *sul1*, *sul2*, *dfrA1*, *tetA* and $bla_{\text{CTX-M-1}}$ per gram of soil samples obtained within selected E-waste dumpsites

Sample	16SrRNA	*intI1*	*sul1*	*sul2*	*dfrA1*	*tetA*	$bla_{\text{CTX-M-1}}$
AL1	1.84Ex+08 ± 3.59Ex+07	7.52Ex+06 ± 2.29Ex+05	1.98Ex+07 ± 5.36Ex+05	9.17Ex+06 ± 5.48Ex+05	2.77Ex+06 ± 9.65Ex+05	1.47Ex+05 ± 2.04Ex+04	0.00Ex+00
AL2	2.29Ex+08 ± 2.85Ex+07	1.65Ex+07 ± 7.29Ex+05	1.94Ex+07 ± 1.65Ex+05	1.14Ex+07 ± 1.18Ex+06	1.24Ex+06 ± 1.35Ex+05	1.93Ex+05 ± 2.44Ex+03	0.00Ex+00
AL3	1.90Ex+08 ± 9.32Ex+06	1.17Ex+07 ± 1.04Ex+06	2.75Ex+07 ± 8.02Ex+05	3.17Ex+07 ± 4.20Ex+05	1.20Ex+06 ± 6.91Ex+05	6.07Ex+05 ± 5.40Ex+04	0.00Ex+00
ỌS1	6.74Ex+07 ± 8.91Ex+06	6.73Ex+06 ± 3.62Ex+05	7.19Ex+06 ± 1.53Ex+06	1.49Ex+07 ± 4.84Ex+05	1.61Ex+06 ± 4.05Ex+04	0.00Ex+00	0.00Ex+00
ỌS2	6.83Ex+07 ± 1.29Ex+07	1.63Ex+07 ± 1.18Ex+06	1.28Ex+07 ± 1.57Ex+05	2.55Ex+07 ± 5.82Ex+06	5.24Ex+05 ± 5.03Ex+05	2.55Ex+05 ± 9.84Ex+03	2.03Ex+04 ± 8.22Ex+03
ỌS3	8.28Ex+07 ± 1.41Ex+07	1.10Ex+07 ± 8.11Ex+05	8.86Ex+06 ± 6.23Ex+05	1.45Ex+07 ± 1.09Ex+06	2.13Ex+07 ± 1.45Ex+07	1.77Ex+05 ± 2.67Ex+03	2.08Ex+05 ± 6.54Ex+04
AS1	2.42Ex+08 ± 2.38Ex+07	2.37Ex+07 ± 2.59Ex+06	4.09Ex+07 ± 1.55Ex+06	3.74Ex+07 ± 1.73Ex+06	1.16Ex+06 ± 1.46Ex+06	2.40Ex+05 ± 2.07Ex+04	0.00Ex+00
AS2	1.33Ex+08 ± 6.75Ex+06	6.24Ex+07 ± 1.22Ex+06	9.36Ex+07 ± 6.97Ex+06	8.51Ex+07 ± 3.89Ex+06	7.61Ex+06 ± 1.71Ex+05	2.88Ex+05 ± 1.03Ex+04	0.00Ex+00
AS3	1.10Ex+08 ±	3.83Ex+07 ±	4.67Ex+07 ±	4.64Ex+07 ±	2.10Ex+06 ±	3.18Ex+05 ±	0.00Ex+00

	2.12Ex+07	3.26Ex+05	1.30Ex+06	1.30Ex+06	1.07Ex+06	2.12Ex+04	
IKJ1	5.92Ex+08 ± 1.02Ex+08	7.39Ex+06 ± 3.57Ex+05	1.27Ex+07 ± 1.43Ex+05	2.15Ex+07 ± 6.09Ex+05	1.98Ex+06 ± 6.01Ex+05	2.57Ex+05 ± 9.16Ex+03	0.00Ex+00
IKJ2	2.85Ex+08 ± 6.38Ex+07	2.33Ex+07 ± 7.51Ex+05	2.64Ex+07 ± 2.64Ex+06	2.37Ex+07 ± 1.99Ex+05	4.75Ex+05 ± 7.27Ex+03	2.58Ex+05 ± 2.73Ex+04	0.00Ex+00
IKJ3	6.47Ex+08 ± 4.58Ex+07	4.29Ex+07 ± 5.15Ex+06	3.69Ex+07 ± 2.55Ex+06	4.66Ex+07 ± 1.19Ex+06	1.29Ex+06 ± 1.54Ex+05	3.36Ex+05 ± 3.08Ex+04	0.00Ex+00
ẠRU1	1.97Ex+08 ± 9.66Ex+06	6.54Ex+05 ± 2.77Ex+04	1.27Ex+06 ± 1.19Ex+05	1.80Ex+06 ± 8.25Ex+04	9.23Ex+05 ± 2.32Ex+04	4.59Ex+05 ± 4.25Ex+04	0.00Ex+00
ẠRU2	5.44Ex+08 ± 3.98Ex+07	8.76Ex+07 ± 3.89Ex+06	1.23Ex+08 ± 6.06Ex+06	9.53Ex+07 ± 3.20Ex+06	1.32Ex+07 ± 1.37Ex+07	1.10Ex+06 ± 3.75Ex+04	0.00Ex+00
ẠRU3	7.98Ex+07 ± 7.93Ex+06	6.03Ex+05 ± 1.05Ex+05	1.09Ex+06 ± 1.37Ex+04	1.48Ex+06 ± 1.04Ex+05	5.04Ex+06 ± 3.47Ex+06	1.29Ex+05 ± 1.59Ex+04	9.90Ex+04 ± 3.99Ex+04

Each measurements represents the average of three replicate samples, Ex = Exponential

APPENDIX XIX: Bivariate correlations between log transformed relative abundance of ARGs and *intI1* from E-waste dumpsites. Correlation was performed at 95% confidence interval.

ARG	ARG	r	p<0.05
intI1	*sul1*	0.8838152	9.10E-14
intI1	*sul2*	0.6197304	2.59E-05
intI1	*dfrA1*	0.719063	2.51E-07
intI1	*tetA*	0.6287299	1.82E-05
intI1	$bla_{\text{CTX-M-1}}$	0.5465225	3.19E-04
sul1	*sul2*	0.5669777	1.68E-04
sul1	*dfrA1*	0.7316231	1.22E-07
sul1	*tetA*	0.6454047	9.16E-06
sul1	$bla_{\text{CTX-M-1}}$	0.3225171	4.52E-02
sul2	*dfrA1*	0.3656837	2.21E-02
sul2	*tetA*	0.3901173	1.41E-02
sul2	$bla_{\text{CTX-M-1}}$	0.2125364	1.94E-01
dfrA1	*tetA*	0.5353199	4.46E-04
dfrA1	$bla_{\text{CTX-M-1}}$	0.2532894	1.20E-01
tetA	$bla_{\text{CTX-M-1}}$	0.3475343	3.02E-02

where;

r = Correlation coefficient

p-value = significance level of t-test

APPENDIX XX: Bivariate correlation of log transformed relative *intI1* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Bivariate correlation between *intI1* and metals, Cd, Co and Se were weak and insignificant.

Gene	Metals	r	p<0.05	tt	dff
intI1	Cu	0.7746844	7.15E-06	7.4519	37
intI1	Zn	0.7746844	7.15E-06	7.4519	37
intI1	Pb	0.7710557	9.29E-06	7.3656	37
intI1	Mn	0.7806129	4.61E-06	7.5971	37
intI1	Fe	0.7260858	1.68E-04	6.4232	37
intI1	Al	0.7735092	7.78E-06	7.4238	37
intI1	Co	0.2799538	0.08432	1.7738	37
intI1	Cr	0.7493837	4.05E-05	6.8843	37
intI1	Ni	0.6041118	4.66E-02	4.6112	37
intI1	Cd	0.17718	0.2806	1.095	37
intI1	Se	0.17718	0.2806	1.0951	37

where;

r = Correlation coefficient

dff = degree of freedom

tt = t-test statistics

p-value = significance level of t-test

APPENDIX XXI: Bivariate correlation of log transformed relative *sul1* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Correlations between *sul1* and metals, Co and Se were weak and insignificant

Gene	Metals	r	p<0.05	tt	dff
sul1	Cu	0.55363	0.0002562	4.0439	37
sul1	Zn	0.5567217	0.0002326	4.0766	37
sul1	Pb	0.5489336	0.0002962	3.9947	37
sul1	Mn	0.5410218	0.0003763	3.913	37
sul1	Fe	0.536748	0.0004272	3.8696	37
sul1	Al	0.5311075	0.0005038	3.8128	37
sul1	Co	0.1947613	0.2348	1.2078	37
sul1	Cr	0.4957967	0.001329	3.4727	37
sul1	Ni	0.4457818	0.004453	3.0292	37
sul1	Cd	0.4542664	0.003673	3.1017	37
sul1	Se	0.09410195	0.5688	0.57495	37

where;

r = Correlation coefficient

dff = degree of freedom

tt = t-test statistics

p-value = significance level of t-test

APPENDIX XXII: Bivariate correlation of log transformed relative *sul2* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Correlations between *sul2* and metals, Co and Se were weak and insignificant

Gene	Metals	r	p<0.05	tt	dff
sul2	Cu	0.335703	0.03668	2.1678	37
sul2	Zn	0.345223	0.03136	2.2375	37
sul2	Pb	0.3974643	0.01222	2.6347	37
sul2	Mn	0.3843158	0.01571	2.5322	37
sul2	Fe	0.3155311	0.05038	2.0226	37
sul2	Al	0.3743653	0.01888	2.4558	37
sul2	Co	0.1376451	0.4034	0.84531	37
sul2	Cr	0.4145666	0.008691	2.771	37
sul2	Ni	0.2892599	0.07409	1.838	37
sul2	Cd	0.3432641	0.0324	2.223	37
sul2	Se	0.2359316	0.1482	1.4768	37

where;

r = Correlation coefficient

dff = degree of freedom

tt = t-test statistics

p-value = significance level of t-test

APPENDIX XXIII: Bivariate correlation of log transformed relative *dfrA1* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Correlations between *dfrA1* and metals, Co and Se were weak and insignificant.

Gene	Metals	r	p<0.05	tt	dff
dfrA1	Cu	0.4231933	0.007269	2.8411	37
dfrA1	Zn	0.4904477	0.001526	3.4233	37
dfrA1	Pb	0.440228	0.005038	2.9823	37
dfrA1	Mn	0.4380429	0.005286	2.964	37
dfrA1	Fe	0.4326729	0.005941	2.9192	37
dfrA1	Al	0.4360499	0.005522	2.9474	37
dfrA1	Co	0.1117165	0.4983	0.68383	37
dfrA1	Cr	0.3138679	0.05168	2.0108	37
dfrA1	Ni	0.3605044	0.02416	2.3509	37
dfrA1	Cd	0.3607676	0.02405	2.3529	37
dfrA1	Se	-0.03575692	0.8289	-0.21764	37

where;

r = Correlation coefficient

dff = degree of freedom

tt = t-test statistics

p-value = significance level of t-test

APPENDIX XXIV: Bivariate correlation of log transformed relative *tetA1* gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Correlations between *tetA* and metals Co and Se were weak and insignificant

Gene	Metals	r	p<0.05	tt	dff
tetA	Cu	0.3812625	0.01663	2.5086	37
tetA	Zn	0.4363889	0.005481	2.9502	37
tetA	Pb	0.3870408	0.01493	2.5533	37
tetA	Mn	0.4152003	0.008579	2.776	37
tetA	Fe	0.4046144	0.01062	2.6913	37
tetA	Al	0.4249681	0.007002	2.8557	37
tetA	Co	0.06113022	0.37254	0.37254	37
tetA	Cr	0.4205282	0.007685	2.8194	37
tetA	Ni	0.3210024	0.04631	2.0617	37
tetA	Cd	0.3540934	0.027	2.3031	37
tetA	Se	-0.02887669	0.8615	-0.1757	37

where;

r = Correlation coefficient

dff = degree of freedom

tt = t-test statistics

p-value = significance level of t-test

APPENDIX XXV. Bivariate correlation of log transformed relative *bla*$_{CTX-M-1}$ gene abundance with heavy metals from sampling sites. Correlation was performed at 95% level of confidence. Correlation analysis between *bla*$_{CTX-M-1}$and Co was weak and insignificant.

Gene	Metals	r	p<0.05	tt	dff
bla$_{CTX-M-1}$	Cu	0.5531564	0.00026	4.0389	37
bla$_{CTX-M-1}$	Zn	0.5286823	0.0005404	3.7886	37
bla$_{CTX-M-1}$	Pb	0.5217404	0.0006584	3.7201	37
bla$_{CTX-M-1}$	Mn	0.5645173	0.0001815	4.1601	37
bla$_{CTX-M-1}$	Fe	0.5112098	0.0008816	3.6181	37
bla$_{CTX-M-1}$	Al	0.5090805	0.0009341	3.5977	37
bla$_{CTX-M-1}$	Co	0.2516675	0.1222	1.5817	37
bla$_{CTX-M-1}$	Cr	0.5243361	0.0006118	3.7456	37
bla$_{CTX-M-1}$	Ni	0.4460461	0.004427	3.0315	37
bla$_{CTX-M-1}$	Cd	0.4460461	0.004427	3.0315	37
bla$_{CTX-M-1}$	Se	0.3629875	0.02314	2,3696	37

where;

r = Correlation coefficient

dff = degree of freedom

tt = t-test statistics

p-value = significance level of t-test