ISOLATION, CHARACTERIZATION AND ANTIBIOGRAM OF SOME ENTERIC PATHOGENIC BACTERIA FROM WASTE DUMPS IN ZARIA METROPOLIS, NIGERIA

BY

YOMI MARIE CAROLE NYANDJOU

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCES
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

MAY, 2017

ISOLATION, CHARACTERIZATION AND ANTIBIOGRAM OF SOME ENTERIC PATHOGENIC BACTERIA FROM WASTE DUMPS IN ZARIA METROPOLIS, NIGERIA

BY

YOMI MARIE CAROLE NYANDJOU

B.Sc. Microbiology, UNIUYO (1998), M.Sc. Microbiology, UNIUYO (2006)

(Ph.D/SCI/29080/2012-2013)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

MAY, 2017

DECLARATION

I declare that the work in this dissertation entitled ‘‘**Isolation, Characterization and Antibogram of some Enteric Pathogenic Bacteriafrom Waste Dumps in Zaria Metropolis, Nigeria**.’’ was performed by me in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Thesis dissertation was previously presented for another degree or diploma at this or any other Institution.

Yomi Marie Carole Nyandjou Signature_____Date_____

CERTIFICATION

This dissertation entitled **''Isolation, Characterization and Antibiogram of some Enteric Pathogenic Bacteria from Waste Dumps in Zaria Metropolis, Nigeria.''** by Yomi Marie Carole NYANDJOU meets the requirement governing the award of the degree of Doctor of Philosophy in Microbiology of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Prof S.E. Yakubu
Chairman, Supervisory Committee
Department of Microbiology,
Ahmadu Bello University, Zaria **Signature_____Date_____**

Prof I.O. Abdullahi
Member, Supervisory Committee
Department of Microbiology,
Ahmadu Bello University, Zaria **Signature_____Date_____**

Prof D.A. Machido
Member, Supervisory Committee
Department of Microbiology,
Ahmadu Bello University, Zaria **Signature_____Date_____**

Prof I.O. Abdullahi,
Head,
Department of Microbiology,
Ahmadu Bello University, Zaria **Signature_____Date_____**

Prof. A.Z. Abubakar,
Dean, School of Postgraduate Studies,
Ahmadu Bello University, Zaria **Signature_____Date_____**

DEDICATION

I dedicate this work to God Almighty and to my lovely husband and my departed mother. Their inspiration, discipline and admonitions towards hard work and perseverance are the foundations that bore the fruit of this work.

ACKNOWLEDGEMENT

I am deeply grateful to my supervisors Prof S.E. Yakubu, Prof I.O. Abullahi and Prof D.A. Machido, whose guidance and wise counsels steered this work to its completion. To the IITA staff especially Mrs Iwu, Nwagbara Victoria; Miss Yemi Tokwe together with Mr Y.Y Pala of the Department of Veterinary Medicine, ABU, Zaria who guided the molecular aspect of the study, my gratitude is unequivocally bequeathed. I acknowledge my father in the Lord, Pastor Yusuf Inuwa for being there for me at any time of spiritual need during the entire period of the project. To all my colleagues in the Department of Microbiology, friends and classmates whose contribution towards this accomplishment is unforgetable, I treasure the fond memories of the time we had together and your cheering words that made the going a lot easier. My deep appreciation goes to my dear husband Prof C.S. Ajinomoh whose constant challenge, comfort and encouragement propelled me towards completion of this work and to my lovely children Adeiza, Anase, Desiree and Patrick who endured my long stays in the laboratories and library.

I am particularly grateful to Dr. E.E. Ella for his contribution towards the success of this project. My sincere thanks are also due to Dr. H.M. Raji for the construction of the phylogenetic trees and her contribution towards the success of this work, to Mr A.M. Shitu and the entire laboratory Staff of the Department of Microbiology, Ahmadu Bello University, Zaria for their support and co-operation throughout the period of this work. My special regards go to my sweet brothers and sisters, Nyamsi Jean, Doumi Claudine, Ngahan Richard, Wandji Gilbert and Ndean Brigitte for their moral support.

To the Generator of Diversity (GOD) is all the glory. He made the microbes with such intricacy and design. It was such a marvel, attempting to unravel the art of His creation, wherein His glory is inscribed.

TABLE OF CONTENTS

Cover Page……...i

Fly Leaf……...ii

Title Page……...iii

Declaration……iv

Certification……....v

Dedication……..vi

Acknowledgements……...vii

Table of Contents……..…viii

List of Tables……...……xiv

List of Figures…….....xvi

List of Plates……...xvii

List of Appendices……...xix

Abbreviations and Acronyms……xx

Abstract……xxi

CHAPTER ONE

1.0 INTRODUCTION……..1

1.1 Background to the Study…….....1

1.2 Statement of Research Problem……...5

1.3 Justification of the Study……..7

1.4 Aim of the Study…….....9

1.5 Objectives of the Study…….9

CHAPTER TWO

2.0 LITERATURE REVIEW……10

2.1 Waste…….....10

2.2 Waste Dumps or Landfills……...11

2.3 Solid Waste Disposal-……..…....12

2.4 Solid Waste Management …….....13

2.5 Impact of Solid Waste Dump on the Environment.....15

2.5.1 Need for Environmental and Health Impact.....17

2.5.2 Types of Environmental and Health Hazards.....18

2.6 The Significance of Environmental and Health Impact Assessment (EHIA).....19

2.6.1 Community Health Implications.....19

2.6.2 Public Information and Participation.....20

2.7 Benefits of Waste Management.....20

2.8 Bacterial Biofilms.....21

2.8.1 Bacterial Biofilm Infections.....22

2.8.2 Public Health Perspective.....24

2.9 Microbial Degradation of Solid Waste.....24

2.9.1 Biodegradation of Cellulosic Materials.....25

2.9.2 Pathogenic Bacteria in Waste Dumps.....27

2.9.3 Health Implication of Pathogenic Bacteria in Waste Dump Sites.....27

2.9.4 *Vibrio cholerae*: The Causative Agent of Cholera.....29

2.9.5 *Salmonella*.....39

2.9.6 *Escherichia* coli.....49

2.10 Antimicrobial Resistance (AMR).....54

2.10.1 Resistance is an Emerging Problem.....55

2.10.2 Mechanism of action of antibiotics.....58

2.11 Molecular Methods for Detection of Bacteria.....60

2.11.1 Polymerase Chain Reaction (PCR)…….....61

2.12 Virulence Genes in Bacteria.....62

2.13 Isolation of Genomic DNA (gDNA).....62

2.13.1 Lysis.....64

2.13.2 Removal of Protein, Carbohydrate, RNA.....64

2.14 PCR as a Tool in Bacterial Taxonomy.....64

2.15 Identification of Bacteria using 16S rRNA Gene 65

2.16 GenBank.....67

CHAPTER THREE

3.0 MATERIALS AND METHODS 69

3.1 Study Area.....69

3.2 Study Design.....70

3.3 Sample Size.....70

3.4 Study Locations.....72

3.5 Solid Waste Composition from Different Settlements in Zaria Metropolis……....72

3.6 Culture Media…….....77

3.7 Collection of Sample……....77

3.8 Determination of Temperature and pH…….....78

3.8.1 Soil Temperature.....78

3.8.2 pH……..…….....78

3.9 Media Preparation.....78

3.10 Bacteriological Analysis.....79

3.10.1 Enumeration of Aerobic Bacteria.....79

3.10 2 Isolation of *Salmonella* species.....80

3.10.3 Isolation of *Vibrio* species.....80

3.10.4 Isolation of *Esherichia coli* O157:H7…….....80

3.10.5 Characterization of bacteria…….81

3.10.6 Biochemical Characterization of the Isolates……..81

3.11 Identification of the Isolates using the Microgen Identification System Software.....85

3.11.1 Identification of *E. coli* serogroup O157:H7 using the M44 MICROGENR *E. coli* O157:H7 Agglutination Kit……...87

3.11.2 Characterization of *Vibrio cholerae*.....88

3.12 Antibiotic Susceptibility Testing of the Isolates.....89

3.12.1 Antibiotics Used.....89

3.12.2 Standardization of Bacterial Inoculum…….....89

3.12.3 Disc Diffusion Susceptibility Test……..…89

3.13 Molecular Characterization of Isolates.....90

3.13.1 Bacterial Cell Suspension.....90

3.13.2 Genomic DNA Extraction Using ZR Genomic DNA TM- Tissue MiniPrep kit.....91

3.13.3 Agarose Gel Electrophoresis.....92

3.13.4 PCR Amplification of 16S rDNA for Virulent and Resistant Genes **.....93**

3.13.5 PCR Reaction 95

3.13.6 Gel Electrophoresis and Gel Visualization.....95

3.13.7 Purification of PCR Products.....95

3.13.8 PCR Amplification of 16S rRNA Genes Sequencing of 16S rRNA Genes.....96

3.13.9 Sequence Analysis.....96

3.14 Analyses of Data.....96

CHAPTER FOUR

4.0 RESULTS.....98

4.1 Temperature and pH of Waste Dumps.....98

4.2 Total Viable Aerobic Bacterial Counts.....101

4.3 Isolation and Characterization of Enteric Bacteria from Waste Dumps.....104

4.4 Antibiotic Susceptibility Patterns of the Isolates.....116

4.5 Detection ofVirulence and Antibiotic Resistance Genes in the Isolates.....124

4.6 Alignment and Phylogeny of 16S rRNA Sequencing of the Isolates.....145

CHAPTER FIVE

5.0 DISCUSSION.....151

5.1 Temperature and pH of the Waste Dump Soil Samples.....151

5.2 Mean Aerobic Bacterial Counts of Soils from Waste Dumps.....153

5.3 Occurrence of Pathogenic Bacteria in Waste Dumps in Zaria Metropolis.....155

5.3.1 Health Problems Associated with Enteric Pathogenic Bacteria.....160

5.4 Antibiotic Susceptibility of the Isolates Obtained from

Waste Dump Sites.....162

5.5 Virulence and Antibiotic Resistance Genes……..169

5.6. Phylogeny of the Isolates.....176

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS.....180

6.1 Conclusion.....180

6.2 Recommendations.....182

REFERENCES.....184

APPENDICES.....213

LIST OF TABLES

Table	Title	Page
2.1	The Typical Waste Generation in Some Urban Cities in Nigeria	16
2.2	Partial List of Human Infections Involving Biofilms	23
2.3	The Cholera Pandemics	32
2.4	Species and Subspecies in the *Salmonella* Genus	41
2.5	Antigenic Formulae of some *Salmonella* serotypes	44
2.6	World Health Organization's Categorization of Antimicrobials of Critical Importance to Human Medicine	57
2.7	Some genes Used that have been Used to detect *Salmonella* spp., *E. coli* O157:H7 and *Vibrio cholerae*…	63
3.1	List of Primers and Primer Sequences used in Study	94
4.1	Variation of Temperature and pH of Soil from Waste Dumps in Zaria Metropolis with Seasons	99
4.2	Mean Temperature and pH of Soil from Waste Dumps in Zaria Metropolis	100
4.3	Variation of Total Viable Bacterial Load of Soil from Waste Dumps in Zaria Metropolis with Season	102
4.4	Total Viable Aerobic Bacterial Count with respect to Study Locations…….	103
4.5	Correlation of Temperature, pH and Bacterial Counts…….	105
4.6	Morphological and Biological Characterization of Pathogenic Bacteria Isolated from Waste Dump Soil Samples	107
4.7	Occurrence of Bacterial Isolates fromWaste Dump Soils at various Sampling Locations	112
4.8	Occurrence of Target Enteric Pathogenic Bacteria from Waste Dumps in Zaria Metropolis (N=520)	113
4.9	Occurrence of Enteric Pathogenic Bacterial Isolates from Waste Dump Soils at various Sampling Locations	115

4.10 Seasonal Variation in the Frequency of Occurrence of *E. coli* O157:H7, *S. enterica* and *Vibrio cholerae* non-O1 based on Sample Locations……....117

4.11 Antibiotic Susceptibility Patterns of Bacterial Isolates from Waste Dump Sites in Zaria Metropolis……...118

4.12 Antibiotic Resistance Pattern of Enteric Bacteria Isolated from Waste Dump Soil Samples……..123

4.13 Multiple Antibiotics Resistant Patterns of Bacterial Isolates From Waste Dump Samples.....125

4.14 Occurrence of Antibiotic Resistance Genes in Relation to Study Locations.....138

4.15: *Salmonella enterica* Virulence Genes in respect to Study Location.....142

4.16 *E. coli* O157:H7 Virulence Genes in respect to Study Location 143

4.17 *Vibrio cholerae* non-O1 Virulence Genes in respect to Study Location.....144

4.18 Sequence Analysis of 16S rRNA Genes of Bacteria Isolated from Waste Dump Sites in Zaria Metropolis.....147

LIST OF FIGURES

Figure	Title	Page
2.1	Global Mortality from Diarrhea in Children under the Age of 5 in 2010	50
3.1	Solid Waste Disposal Dump Sites in Zaria Metropolis	71
4.1	Principal Component Analysis of the Temperature and pH of the Soil Samples in relation to Bacterial Counts	106
4.2	Proportion of Bacterial Isolates from Waste Dump Soils at Various Sampling Locations	111
4.3	Susceptibility of *Salmonella enterica* from Waste Dumps in Zaria Metropolis	119
4.4	Susceptibility of *Vibrio cholerae* from Waste Dumps in Zaria Metropolis	121
4.5	Susceptibility of *E. coli* O157:H7 from Waste Dumps in Zaria Metropolis	122
4.6	Distribution of Multiple Antibiotics Resistance (MAR) with respect to Study Locations	126
4.7	Percentage Occurrence of Multiple Antibiotic Resistance Index (MARI) among the Target Isolates	127
4.8	Percentage Distribution of Target Isolates Possessing Virulence Genes in relationtoSeasons	146
4.9	A Maximum Parsimony Tree showing Phylogenetic Affiliation of 16S rRNA Sequences from Species of *E. coli* Obtained from Waste Dump Soils in Zaria Metropolis	149
4.10	A Maximum Parsimony Tree showing Phylogenetic Affiliation of 16S rRNA Sequences from Species of *Salmonella* Obtained from Waste Dump Soils in Zaria Metropolis	150

LIST OF PLATES

Plate	Title	Page
I	Solid Waste Dump Site at Rail Way tract at Yan Katako, Sabon-Gari	73
II	Waste Dump Site at Fly-over bridge behind the Motor Park, Kwangila	74
III	Waste Dump Site at Agoro in Tudun-Wada	75
IV	Kusfa Solid Waste Dumpsite around Residential Building in Zaria City	76
V	Sub-Cultured Distinct Colonies	109
VI	Gel Electrophoresis of Extracted DNA of Isolates	128
VII	Amplicon of bla_{TEMl} gene of *Salmonella enterica* Isolated from Waste Dump Soils	130
VIII	Amplicon of bla_{TEM1} Gene *E. coli* O157:H7 Isolated from Waste dump Soils	131
IX	Amplicon of tetA Gene of *Salmonella enterica* Isolated from Waste Dump Soils	132
X	Amplicon of tetB Gene of *Salmonella enterica*	133
XI	Amplicon of tetA Gene *E. coli* O157:H7 Isolated from Waste Dump Soils	134
XII	Amplicon of Stx2 Gene of *E. coli* O157:H7 Isolated from Waste Dump Soils	135
XIII	Amplicon of hlyA Gene of *E. coli* O157:H7 Isolated from Waste Dump Soils	136
XIV	Amplicon of InvA Gene of *Salmonella enterica* Isolated from Waste Dump Soils	137
XV	Amplicon of Stn Gene of *Salmonella enteric* Isolated from Waste Dump Soils	140
XVI	Amplicon of toxR Gene of *Vibrio cholerae* non-O1 Isolated from Waste Dump Soils	141

LIST OF APPENDICES

Appendix	Title	Page
I	Images from Triple Sugar Iron (TSI), by Isolates	213
II	Images from Microgen Identification Test Kits, Presumptive *E. coli* isolates (P1 & P2) and Presumptive *Salmonella* isolates (P3 & P4)	214
III	Identification of *Salmonella* and *Escherichia coli* using Microgen™ Enterobacteriaceae GNA- ID System	215
IV	Identification of the Bacterial Isolates by MICROGEN GNA+GNB Test Kit	216
V	Isolation Rate of Bacteria in Waste Dump Samples	217
VI	Zone Diameter Interpretative Standards for Bacterial Isolates	218
VII	Images from Antibiotic Resistance (P1 & P2) and Subseptibility (P3 & P4) by Isolates	219
VIII	Percentage Susceptibility of *Salmonella* spp, *E coli* O157:H7 and *Vibrio Cholerae* non-O1 Isolated from Waste Dumps Samples	220
IX	Antibiotic Resistance Profile of Target Organisms Isolated from Waste Dump Samples	221
X	Antibiotic Resistance Patterns and Multiple Antibiotic Resistance (MAR) Index of the Bacterial Isolates from Waste Dump Soil Samples	222

ABBREVIATIONS AND ACRONYMS

BLAST	Basic Local Alignment Search Tool
CDC	Centers for Disease Control and Prevention
CVM	Center for Veterinary Medicine
DBJ	Data Bank of Japan
^{o}C	Degree Celsius
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine-tetraacetate
EHEC	Entero-haemorrhagic *E. coli*
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
gDNA	Genomic DNA
G +ve	Gram Positive
G –ve	Gram Negative
GDP	Gross Domestic Product
ML	Molecular Ladder
MAR	Multiple Antibiotic Resistance
MARI	Multiple Antibiotic Resistance Index
MMDB	Molecular Modeling Database
NARMS	National Antimicrobial Resistance Monitoring System
NC	Negative Control
NCBI	National Center for Biotechnology Information
ONPG	Ortho-Nitrophenyl-β-galactosidase
PCR	Polymerase Chain Reaction
rRNA	Ribosomal RNA

ABSTRACT

Refuse dumpsites are found littered both within and on the outskirts of cities in Nigeria; due to poor and ineffective waste management, poised public health concerns and add strain on diminishing land space as well as harboring enteric pathogenic bacteria. This study is aimed at assessing the Temperature and pH conditions of the waste dump sites, estimation of the total aerobic bacterial counts, isolation and characterization of Gram negative aerobic enteric pathogenic bacteria. Five hundred and twenty (520) soil samples were collected from sixteen waste dumps in four locations and a control site in Zaria metropolis. The locations were Tudun-Wada, Zaria city, Sabon-Gari, Samaru and Kabama in the dry and in the wet seasons. Pathogenic bacteria were isolated from the waste dump soil samples on selective culture media. Pure isolates were identified by Gram staining and characterized using both conventional biochemical and Microgen identification, and molecular techniques. Bacterial isolates were subjected to antibiotic susceptibility testing using agar disc diffusion method. Target isolates were molecularly characterized by PCR amplification of the virulence and antibiotic resistance genes, and the closest match of the 16S rRNA genes were determined by similarity searches on the Genbank database. The data for refuse dumps conditions which included temperature and pH were analysed using descriptive statistics. Statistical Analysis System (SAS) version 9.2 package was used in the analysis of data on the bacterial counts at $p = 0.05$. Zaria City had the highest mean temperature ($34.1^{o}C$), pH (8.3) and plate counts ($3.3x10^{8}\pm1.3x10^{7}$cfu/g)**.** Bacteriological analysis of the soil samples gave a total of 189 (36.3%) isolates among which 65(12.5%) were identified target isolates. Of these 65 target isolates, 36(6.9%) were *Salmonella enterica,* 22(4.2%) *Escherichia coli* O157:H7 and 7(1.4%) *Vibrio cholerae* non-O1 isolates. Dry season had the highest occurrence of the isolates 37 (14.2%) while the wet season has the least 29 (11. 2%). *Escherichia coli* O157:H7 and *Salmonella enterica* were 100% susceptible to Gentamicin (30µg) and Chloramphenicol (30µg) respectively. *Vibrio cholerae* non-O1 isolates were 85.7% susceptible to Ofloxacin (10µg). Most isolates were resistant to Ampicillin (10µg), Amoxycillin-clavulanic acid (30µg) and Tetracycline (30µg). The *Vibrio cholerae* non-O1 were 100% resistant to both Ampicillin (10µg) and Cotrimoxazole (25µg). Virulence genes, InvA and Stn was demonstrated in 6(100%) and 5(83.33%) respectively in *Salmonella enterica,* Stx and hlyA in 5(71.43%) and 4(57.14%) respectively of *E. coli* O157:H7 and toxR in 6(100%) of *Vibrio cholerae* non-O1 by PCR. Similarly antibiotic resistant genes tetA, tetB and bla_{TEM1} was demonstrated in 2(33.33%), 1(16.66%) and 2(33.33%) respectively of *Salmonella enterica*, tetA

and bla_{TEM1} in 4(66.66%) and 1(16.66%) of *E. coli* O157:H7. The BLAST search yielded 13.3% each of the isolates having 98% and 97% homology with *Salmonella enterica* and *E. coli* O157:H7 respectively. Five isolates (33.3%) had 96% identity, 4 had 95% and 2 had 94% identity with the organisms in the GenBank. A total of 6 *Salmonella* serovars and 7 enterohaemorrhagic *E. coli* were identified among which 5 *Salmonella enterica* subsp. *enterica* serovar Typhi, one *Salmonella enterica* subsp. *enterica* serovar Typhimurium, 6 enterohaemorrhagic *E. coli* serogroup O157:H7 and one *E. coli* serogroup O83:H1. The predominant *Salmonella* serovars were the typhoidal *Salmonellae* and the predominant *E. coli* seroroup were the enterohaemorrhagic *E. coli* serogroup O157:H7. The most potent antibiotics against the enteric pathogenic bacteria isolates were Gentamicin (30µg) and Chloramphenicol (30µg) followed by Nitrofurantoin (30µg) and Ofloxacin (10µg). Ampicillin (10µg) was the least effective against the isolates. Zaria city was the location with the highest prevalence 26 (25.0%) and multidrug resistant isolates. The presence of multidrug resistant enteric bacterial pathogens suggests that the waste dump soil environment were contaminated with faecal matters and that antibiotics were disposed off indiscriminate. Proper policy should be put in place for waste handlers and those who live in the vicinity of the dumps to safeguard their lives.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Waste dumps refer to areas or land where material wastes from several sources and processes are deposited (Odeyemi, 2012). Solid waste disposal is crucial for the maintenance of both human and environmental health. Notwithstanding, the waste dumps are indiscriminately placed in developing countries (Arigbede and Yusuf, 2010; Odeyemi, 2012). It is needless to say that waste management has been rigorously assessed in terms of the potential danger to the immediate environment and public health. Efficient waste management is fundamental to the sustenance of physical and social infrastructure as well as the enhancement of the socio-economic well being of any community (Arigbede and Yusuf, 2010). However, poor sanitation continues to be a leading cause of health problems especially in developing countries where it is estimated that about 80% of ill health are water and sanitation related; and about 15% of all child deaths under the age of 5years results from diarrheal diseases (World Health Organization /United Nations Chieldren's Education Fund, 2004).

Human societies generate large amounts of waste. Many things are discarded everyday ranging from ordinary rubbish to old newspapers, packaging, cleaning materials, and many different kinds of junk (Osunwoke and Kuforiji, 2012). With urban industrialization, social development and population increases, solid waste production is growing rapidly, making garbage pollution a serious problem (Mbata, 2008). Microorganisms such as bacteria and fungi rapidly populate materials using their components as their sources of nutrition for growth and multiplication, many of these

microorganisms have been found to be harmful to man. Indiscriminate waste dumping breeds these microorganisms and causes a health hazard to the human population.

Trends towards the use of waste dump as a preferred method of waste management might be altered if risks were adequately addressed and analyzed. Massive waste dump as an efficient, safe and economic method of solid waste is based on the supposition that such wastes are removed regularly and that the waste stream can be effectively managed. Failure to remove wastes, as and when due allows for the wide spread of litters in the environment (Achudume and Olawale, 2007). An observable fact is that, as the wastes lie, decomposition takes place and microbial pathogens of all kinds thrive therein. Bartram *et al.* (2003) and Onweremadu *et al*. (2009) discovered that bacterial pathogens in wastes that lack leachaete collection system are source of pathogens to the soil. These pathogens when increased in population, pose great risk to human health (Achudume and Olawale, 2007).

The isolation of pathogenic bacteria from waste dumps connotes a serious health risk to waste handlers, scavengers and people living in the vicinity of the dumps. This risk is further exacerbated by the widely reported cases of waste dumps bacterial pathogens resistant to several antibiotics. For example, in a study conducted on the antibiogram status of bacterial isolates from air around dump sites, all the isolated organisms were resistant to most of the antibiotics against which they were tested (Odeyemi, 2012). Pathogenic bacteria that may be associated with faecal contamination include pathogenic strains of *Escherichia coli*, *Salmonella* species, *Shigella* species and *Vibrio cholerae.* In addition to these organisms causing human disease, resistance to antibiotics has complicated the health problem (Glynn *et al*., 1998; Ajayi and Akonai, 2003). Most microorganisms found in the waste can be transmitted by inhalation and contact (exposure

to the dumpsite), with infection occurring in the respiratory tract, in skin lesion and mucus membrane (Awisan *et al*., 2011). Members of the faecal Coliform group are used as indicators of the sanitary quality of the environment especially the Gram negative non spore forming rods (Achudume and Olawale, 2007; WHO, 2013). Therefore, microbial contamination of waste by human or animal excreta is hazardous and of public health significance. Due to inadequate and lack of government authorized waste disposal infrastructure in most rural and urban areas, most of the population dump waste are in open spaces and drainage channels. These wastes are largely untreated and poorly managed and might serve as shelter and food source for rodents, flies and insect vectors. These are known to transmit various pathogenic agents of diseases including, amoebic and bacillary dysentery, typhoid fever, plague and cholera. A good percentage of these infections are caused by bacteria which can be found in these refuse dumps and may cause diseases (Arigbede and Yusuf, 2010; Odeyemi *et al*., 2011; Ogunrinola and Adepegba, 2012).

One of the most important steps towards controlling bacterial infections in the 21st century is the development and the use of antibiotics (Nesa *et al.,* 2011). There is worldwide concern about the appearance and rise of bacterial resistance to commonly used antibiotics. In this regard, programmes for monitoring resistance have been implemented in many countries for the purpose of protecting the health of humans as well as animals (Li *et al*., 2010). However, the subsequent appearance and spread of antibiotic resistance in pathogenic organisms had made many currently available antibiotics ineffective (Kam *et al*., 2007). Globally, antibiotic resistance among many bacterial isolates had increased a great deal in both developed and developing counties. For instance, high rates of resistance of *Salmonella* serovars, *Escherichia coli* and *Vibrio*

cholerae strains to conventional antibiotics such as ampicillin, chloramphenicol, tetracycline, cotrimoxazole (trimethoprim-sulfamethoxazole), streptomycin and other newer antibiotics (quinolones and extended-spectrum cephalosporins) have been reported with increasing frequency in many parts of the world (Su *et al*., 2004; Hendriksen *et al*., 2008; Li *et al*., 2010; Heuer *et al.*, 2011). In addition, multidrug- resistance bacteria are now encountered frequently worldwide and rates of multidrug resistance had increased considerably in recent years (Ikpeme *et al*., 2011; Gordana *et al*., 2012; Gregory *et al*., 2013; Adekanle *et al*., 2014).

The emergence of antibiotic resistant pathogens threatened the discovery of potent antibiotics (Sivakumar *et al.*, 2012) for the treatment of infections and is threatening to become a serious public health problem (Kimura, 2005). Antibiotic resistance in pathogenic bacteria had resulted in increased morbidity and mortality as well as health care costs worldwide (Sivakumar *et al.*, 2012; Kwaga, 2016).

In recent years, more rapid and precise methods have been developed for bacteria identification from various sources. Recent molecular advances in microbiology have greatly improved the detection of bacterial pathogens in the environment. Bacterial identification also may be improved by Genetic identification systems (Hoorfar and Radstro, 2000) and several Polymerase Chain Reaction (PCR) assays have been developed by targeting various bacteria genes such as Stx1, Stx2 (Tahamtan *et al*., 2010; Ding *et al*., 2011; Jalil *et al*., 2011), bla_{TEM1}, bla_{CMY}, bla_{SHV}, bla_{PSE-1} or bla_{CTX} (Chen *et al.*, 2004; Mooljuntee *et al*. 2010; Momtaz *et al*., 2012; Adesoji *et al.*, 2015). Stn (Moore and Feist, 2007), hylA (Pathmanathan *et al.*,2003; Ohud *et al.*, 2012 ; Kargar and Homayoon, 2015), InvA (Moussa *et al*., 2010 ; Smith *et al*., 2015), OmpU, OmpW

(Rivera *et al*., 2003 ; Alam *et al*., 2006), toxR (Singh *et al*., 2002; Rivera *et al*., 2003) and tetA, tetB (Randall *et al*., 2004) etc.

1.2 Statement of Research Problem

Zaria Metropolis is an urban city densely populated with a lot of economic and social activities such as markets, institutions and industries. This has created a steady growth in population and human activities which result in the indiscriminate disposal of waste as waste is directly linked to human development. There are no facilities or equipment to adequately manage the waste or evacuate it by environmental health officers (Interim Management Committee, 2011). Waste management agencies are saddled with the responsibility of clearing these wastes and effort to privatise waste disposal has not been very successful in Nigeria. This makes the evacuation difficult and is often not carried out or done once sporadically. The remnants are usually burnt or dumped indiscriminately on open plots of land; along the streets or on the drainages which are sometimes close to sources of drinking water or play ground of children and their proximity often violate the requirement stipulating a distance of 300m.

A greater proportion of households in the metropolis dispose waste in open dumps (or spaces). Environmental pollution is one of the greatest global concerns due to the threat it poses to human existence (Interim Management Committee, 2011). The main source of drinking water in the Metropolis is bore hole water. Some people drink from well, some obtain their drinking water from commercial tanker supply. During water shortage, some inhabitants resort to nearby streams and rivers for various household activities such as cooking and washing. Water closet facilities are available in a few urban houses while pit latrine and the pail system are still being used in some areas. People defaecate on farm

lands, along road ways, street and areas which are swept by public sweepers and these end up in the solid waste.

These indiscriminate disposals sometimes contribute to flooding or outbreak of disease epidemic such as gastroenteritis which is a common feature in this city during rainy seasons. Similarly, several items containing residual antimicrobial agents are disposed off in dumpsites, placing a pressure on the microbial flora and a potential for development of resistance in these microorganisms. Globally about 700,000 people die of resistance infections each year, and by 2050 it is projected that 10 million lives annually and cumulatively 100 trillion of US dollars of economic output will be at risk due to rise in drug-resistant infection and reducing global gross domestic product (GDP) by up to 3.5% to (The O'Neil Report, 2016) as cited by Kwaga (2016).

While resistance to single antibiotic occurs, development of multi-drug resistant bacterial pathogens has made treatment of the disease they cause more difficult (Carraminana *et al.,* 2004; Okeke *et al*., 2007). Infections caused by these resistant pathogens have resulted in significant morbidity and mortality and escalating health care cost worldwide (Sivakumar *et al.,* 2012; Sule *et al*., 2012). The evolutionary prowess of microorganisms presents serious challenges to successfully stopping the development of antimicrobial resistance (Stephan and Mathew, 2005). Even the heterotrophic pathogens that were once thought to be harmless are now of serious public concern (Bartram *et al.,* 2003). This concern is worsened by the fact that the so called indicators of the sanitary quality of the environment, the faecal coliforms, are unreliable indicators of the presence of a number of key pathogenic agents (Achudume and Olawale, 2007).

1.3 Justification of the Study

Poor sanitation, poor treatment of waste, as well as indiscriminate refuse dumps, introduce pathogenic microorganisms into the environment, infecting and killing many people. The goals of clean environment has to be achieved with the still growing population, given the number of waste dumps in Nigeria, owing to the fact that the right to clean environment is part of human rights and the march towards the achievement of vision 20-20-20 (The vision of the Nigeria State to become one of the twentieth largest economies in the world by the year 2020) (Abraham, 2011; Valentine, 2012). The safe disposal and treatment of solid waste therefoere is critical for public health. Accordingly, solid waste must be dealt with quickly to avoid further microbial proliferation and development of serious health risks which may affect public health and quality of life (WHO, 2011). But the waste management has not been rigorously assessed in terms of it potential denger to the immediate environment and public health (Achudume and Olawale, 2007). For example, poliomyelitis, a sickness associated with dirty environment on which millions of naira have been spent by the government to eradicate has resurfaced in some local government in Zaria, an indication that there is poor sanitation and waste management (Interim Management Committee, 2011).

The United Nation System in its 2001 progress report on sanitation and the World Health Organisation/United Nations Children's Fund Joint Monitoring Programme (2012), have described Nigerian Cities as some of the dirtiest, most unsanitary and least aesthetically pleasing in the World due to inadequate waste management and ranked Nigeria third behind China and India on the list of countries with the largest population without access to improved drinking water and inadequate waste management respectively. The presence of refuse dumps on the road of Zaria is an unfortunate development that needs the prompt

attention of the authorities. If care is not taken, most roads in Zaria would not be motorable in the nearest future due to refuse dumps and human waste. Osei and Duker (2008) recommended a buffer distance of 500m within which refuse should not be cited from residence, but studies on waste dumps in different parts of Zaria metropolis have shown that this is not the case (Arigbede and Yusuf, 2010; Stanley *et al*., 2012; Uba *et al.,* 2013).

Effective waste management is one of the global concerns associated to population explosion. As population density increases, solid waste production simultaneously rises thereby earlier effective waste management methods rapidly become inadequate (Rmadass and Palaniyandi, 2007). Many bacterial pathogens have been reported to occur in waste dumps and cause infections and public health risks (Obire *et al*., 2002; Noah *et al.,* 2008; Mbata, 2008; Wachukwu *et al.,* 2010; Osunwoke and Kuforiji, 2012). Odeyemi (2012) while studying Antibiogram Status of Bacterial isolates from air around dumpsite of Ekiti State, reported that the mean total bacterial counts obtained from the dumpsites (1.375×10^2 cfu/g) and residential area close to the dumpsites (1.244×10^2 cfu/g) were relatively higher than that of the sites free from waste dumps (6.14×10^1 cfu/g). The highest mean bacterial counts observed were in two dumpsites samples. Antibiotic resistance has also been reported in bacterial pathogens from waste dumps in Nigeria (Ajayi and Akonai, 2003; Scarpino and Quinn, 2010; Ikpeme *et al.,* 2011; Odeyemi, 2012).

Despite the importance of microorganisms in the decomposition of organic matter, the bacterial pathogens in the soil and wastes are not considered as public health concern (Adekanle *et al*., 2014). Therefore, there is need to isolate and characterize bacteria of public health significance such as *Salmonella* spp., *Esherichia coli* O157:H7 and *Vibrio*

cholerae in order to build local knowledge about health issues and resistance trends in dumpsites.

Antibiotic resistance patterns of these pathogenic bacteria may vary locally and regionally and can also change rapidly with time; as such they need to be monitored closely because of their public health implications.

1.4 Aim of the Study

The study was aimed at isolating, characterizing and determining the antibiotic susceptibility patterns of some enteric pathogenic bacteria present in waste dumps in Zaria Metropolis, Nigeria.

1.5 Objectives of the Study

The objectives of this study were to:

1. Determine the temperature and pH conditions in the refuse dump sites
2. Determine the total viable aerobic bacterial counts in soil samples from the refuse dump sites
3. Isolate and characterize *Escherichia coli* O157:H7, *Salmonella* species and *Vibrio cholerae* from different waste dump sites in Zaria metropolis using biochemical procedures.
4. Determine the antibiogram of the isolates to commonly used antibiotics.
5. Detect virulence and antibiotic resistance genes in the isolates using the Polymerase Chain Reaction (PCR) technique.
6. Confirm the identity of the isolates using 16S rRNA and subsequently determine their evolutionary relationship using phylogenetics.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Waste

Waste is any substance, solution mixture or article for which no direct use is envisaged but which is transported for reprocessing, dumping, elimination by incineration or other methods of disposal (Oviasogie *et al*., 2010). Wastes may be generated during the extraction of raw materials, the processing of raw materials into intermediate and final products, the consumption of final products, and other human activities (Olufunmilayo and Kelechi, 2016). In Nigeria, the sources of solid waste are commercial, industrial, household, agricultural and educational establishments (Babayemi and Dauda, 2009). Waste could be in the form of rubber, plastic, metal, paste, oil, organic matter and other similar commodities. It is solid, liquid or gaseous, renewable or non-renewable, degradable or non-degradable (Thermal Energy for Renewables, 2006). Gaseous waste is normally vented to the atmosphere, either with or without treatment depending on composition and the specific regulations of the country involved. Liquid wastes are commonly discharged into sewers or rivers, which in many countries is subject to legislation governing treatment before discharge. In many parts of the world such legislation either does not exist or is not sufficiently implemented, and liquid wastes are discharged into water bodies or allowed to infiltrate into the ground.

Solid wastes, the subject of this chapter, include all non liquid wastes generated by human activity and a range of solid waste material resulting from the disaster, such as: general domestic garbage such as food waste, ash and packaging materials; human faeces disposed of in garbage; emergency waste such as plastic water bottles and packaging from other emergency supplies and fallen trees and rocks obstructing transport and

communications (Gouveia and Ruscitto, 2010). Solid wastes also include textiles, glass, metals, wood, street sweepings, landscape and tree trimmings as well as general wastes from parks, beaches, and other recreational areas (Alonso and Themelis, 2011). Solid wastes are mainly disposed of to waste dump or landfill, because landfill is the simplest, cheapest and most cost-effective method of disposing of waste (Evironmental Research Foundation (ERF), 2011). In whatever form, waste is nuisance when left unchecked and poses serious challenges to both human health and environment.

2.2 Waste Dumps or Landfills

Waste dumps as defined earlier, are areas or land where material waste from several sources and processes are deposited (Odeyemi, 2012). They are generally safely constructed to minimise any form of negative externality, (e.g. pollution of ground water via leaching) to the surrounding areas. According to ERF (2011); "A secure landfill is a carefully engineered depression in the ground (or built on top of the ground, resembling a football stadium) into which wastes are put. The aim is to avoid any hydraulic (water-related) connection between the wastes and the surrounding environment, particularly groundwater''. Basically, a landfill is a bathtub in the ground; a double-lined landfill is one bathtub inside another.Three types of landfills are normally used for solid waste disposal and they are: secured or sanitary landfills, controlled landfills and open dumps. As defined above, secured or sanitary landfills are highly lined at the base to prevent infiltration by percolating liquids, controlled landfills are waste dumps where the refuse are merely covered with soil, and in open dumps there is no standard for refuse dumping (Gouveia and Ruscitto, 2010).

Rapid urbanization, high population density and quest for improvement in standard of living are factors responsible for the generation of large quantities of wastes in most

Nigerian Cities. These wastes (Municipal Solid Wastes) as observed by Ojo (2008) are generated mostly in Urban Centres.

In most low- to medium-income developing nations, almost 100 per cent of generated waste goes to landfill. Even in many developed countries, most solid waste is landfilled. For instance, within the European Union, although policies of reduction, reuse, and diversion from landfill are strongly promoted, more than half of the member states still send in excess of 75 per cent of their waste to landfill (e.g. Ireland 92 per cent), and in 1999 landfill was still by far the main waste disposal option for Western Europe (ERF, 2011). Landfill is therefore likely to remain a relevant source of groundwater contamination for the foreseeable future (Nwaokwe, 2004).

2.3 Solid Waste Disposal

Many countries in Africa do not have efficient waste collection and disposal services, which often results in both environmental and health problems for the people (Ahmed, 2011). Solid waste disposal in developing countries like Nigeria faces lots of challenges due to lack of sanitary landfill in most Rural and Urban Centres and to inadequate equipement and funds challenging the agencies responsible for the evacuation of these wastes (Obire *et al* 2002; Arigbede and Yusuf, 2010; Stanley *et al*., 2012; Uba *et al.*, 2013). In most developing countries, solid waste disposal is of serious concern especially due to rapid urbanization and decrease in land space. Roadsites, ditches, drainages, water bodies, empty plots of land, farms, wetlands, uncompleted buildings, etc. are sites observed for dumping of household wastes in most Nigerian Cities. This unsanitary and indiscriminate indulgence of the public due to the ineffectiveness of waste management by the Government poses serious health risk to human (Falase, 2004; Ojo, 2008). Some of the wastes are inert materials such as glass, metals, plastics, and ceramics, but large

amounts are the biodegradable wastes (Boulter *et al*., 2002). Waste disposal poses threat to man, animals and the soil. Like chemical hazards etiologic agents might be dispersed in the environment through water and wind. Poisonous plants, insects, animals and indigenous pathogens are biologic hazards that might be encountered at the waste site (Obire *et al*., 2002). Though there are available methods of waste disposal, such as composting, landfill and incineration, open dump continues to be the only method available in Nigeria. The nonchalant attitude of the people on issues concerning waste management and environmental best practices has become a major source of worry. Waste are left on the streets (open dump) for days or weeks, without proper sorting before they are disposed to the final dump sites or relocated to open lands (Mbata, 2008).

2.4 Solid Waste Management (SWM)

Waste management is the organized and systematic channeling of waste through pathways to ensure that they are disposed off with attention to acceptable public health and environment safeguards (Oghenekohwo and Akporehwe, 2015). Proper waste management is not only regarded as a political tool and an indicator of good government policy, it is also an important element for good health (Oyedele, 2009). The consequences resulting from improperly managed wastes include its serving as reservoir of pathogens, habitat for pests such as rats, flies and mosquitoes, reduction of usable land area of the society, obstruction of motorable roads and general nuisance and societal problems in residential areas (Oyedele, 2009). Other negative aspects associated with unmanaged solid waste include negative impact on the value of properties surrounding it, acid rain and contamination of aquifers or water table (Ahmed, 2011). Municipal Solid Waste Management (MSWM) in developing countries like Nigeria faces lots of challenges due to weak economies, inabilities to enforce environmental legislation, and poor

administrative capacities (Muniafu and Otiato, 2010). In most developing countries, SWM is of serious concern especially due to rapid urbanization (Glawe *et al*., 2005; Erdogan *et al*., 2008). If not properly undertaken, inadequate management of wastes can cause harm to human health and the environment (United Nations Environmental Policy, UNEP, 2004; Khajuria *et al*., 2008).

Per capita daily municipal waste generation ranges from 2.75 to 4.0 Kg in high income countries and 0.5 to 0.8 Kg in countries with low incomes (Countreau, 1982; World fact sheet, 2001; Zurburgg, 2002). The quantity of the wastes generated is enormous, with an average household rate of 0.44 to 0.66kg per capita (Solid Waste Report, 2004; Ogwueleka, 2009). In Oyo State, Afon and Okewole (2007) estimated that 50.90 ton of waste was generated daily while Ogwuleke (2009) estimated higher quanties of 8518 ton/day for Lagos, 5222 ton/day for Kano, 4513 ton/day for Ibadan, 3418 ton/day for Kaduna, 3927 ton/day for Port Harcourt, 808 ton/day for Makurdi, 2804 ton/day for Onitsha, 400 ton/day for Nsukka and 492 ton/day for Abuja. Only half of the estimated waste volume generated daily in Nigeria is collected. MSW generation in Sabon-Gari, Zaria approximately 2kg composite wastes per day, about 80.18% of wastes are kept in bins for disposal, while 46.85% are burned (Stanley *et al*., 2012). However, wastes are rarely evacuated by the agencies responsible. Indiscriminate dumping of wastes in Zaria has been documented to negatively affect the health of thousands of Zaria residents. Waste management is viewed as one of Zaria Metropolis key environmental concerns (Stanley *et al*., 2012). Table 2.1 shows the list of typical waste generation rate in some cities in Nigeria and the various agencies that are responsible for the cities.

2.5 Impact of Solid Waste Dump on the Environment

Proper waste management is not only regarded as a political tool and an indicator of good government policy, it is also an important element for good health (Oyedele, 2009). The consequences resulting from improperly managed wastes include its serving as reservoir of pathogens, habitat for pests such as rats, flies and mosquitoes, reduction of usable land area of the society, obstruction of motorable roads and general nuisance and societal problems in residential areas (Oyedele, 2009). Other negative aspects associated with unmanaged solid waste include negative impact on the value of properties surrounding it, acid rain and contamination of aquifers or water table. For instance, air pollution from open burning, due to emission of green house gases such as methane and carbon dioxide; the air emissions and leachates generated as a result of decomposition of waste may contaminate air, surface and groundwater sources; fire hazards and explosions cause public health risks as well. In addition, scattering of wastes by wind and scavenging by birds, animals and waste pickers creates aesthetic nuisance. Malodour emanating due to the degradation of the waste in the dumpsite has nuisance effect and decreases the economic and social values in the locality. Pathogenic microorganisms and harmful chemicals in solid waste can be introduced into the environment when the waste is not properly managed (Wai-Ogosu, 2004; Ogbonna *et al.,* 2006).

There are a number of major risks and impacts of solid waste on the environment. Obire *et al*. (2002) opine that improper disposal of untreated municipal solid wastes is not only harmful to human's health but also constitute a threat to ecological environment. In many dumpsites, the waste is directly increasing global concern over the public health impacts attributed to environmental pollution, in particular, the environmental quality and human health risks associated with the waste dumps. The World Health Organization estimate

Table 2.1: The Typical Waste Generation in Some Urban Cities in Nigeria

City	Population	Agency	Tonnage per month	Density (Kg/m3)	(Kg/m3) Kg/Capita/day
Lagos	8,029,200	Lagos State Management Authority	255, 556	294	0.63
Kano	3,348,700	Kano State Environmental Protection Agency	156,676	290	0.56
Ibadan	307,840	Oyo State Environmental Protection Commission	135,391	330	0.51
Kaduna	1,458,900	Kaduna State Environmental Protection Agency	114,433	320	0.58
Port Harcourt	1,053,900	Rivers State Environmental Protection Agency	117,825	300	0.60
Makurdi	249,00	Urban Development Board	24,242	340	0.48
Onitsha	509,500	Anambra State Environmental Protection Agency	84,137	310	0.53
Nsukka	100,700	Enugu State Environmental Protection Agency	12,000	370	0.44
Abuja	159,900	Abuja Environmental Protection Agency	14,785	280	0.66

Source: (Ogwueleka, 2009)

that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution (WHO, 2011).

2.5.1 Need for Environmental and Health Impact

Epidemiological studies have shown that a high percentage of workers who handle refuse, and of individuals who live near or on disposal sites, are infected with gastrointestinal parasites, worms and related organisms (Scarpino *et al*., 2010). Contamination of this kind is likely at all points where waste is handled. It is also known that vector insects and rodents can transmit various pathogenic agents (amoebic and bacillary dysentery, typhoid fever, salmonellosis, various parasites, cholera, yellow fever, plague and others) (Arigbede and Yusuf, 2010; Odeyemi *et al*., 2011). During the last decade of the 19th century as well as during the 5 initial years of the 20th century, millions of people died due to Bubonic Plague in India, which had linkages to poor management of solid waste. A study by the US Public Health Service has demonstrated the relationship of 22 human diseases to improper solid waste management (Mfasunwon *et al*., 2010).

The organic fraction of municipal solid waste is an important component, not only because it constitutes a sizable fraction of the solid waste stream, but also because of its potentially adverse impact upon public health and environmental quality. A major adverse impact is due to its attraction of rodents and vector insects for which it provides food and shelter. Impact on environmental quality takes the form of foul odors, land, water, air and noise pollution (Nwaokwe, 2004; Grisey *et al*., 2010).These impacts are not confined merely to the disposal site, also, they pervade the area surrounding the site and wherever the wastes are generated, spread or accumulated. Unless an organic waste is appropriately managed, its adverse impact will continue until it has fully decomposed or otherwise

stabilized. Uncontrolled or poorly managed intermediate decomposition products can contaminate air, soil and water resources.

2.5.2 Types of Environmental and Health Hazards

2.5.2.1 Communicable Disease

Houseflies may be important in the transmission of enteric infections, particularly those responsible for infantile diarrhoea and dysentery. Disease transmission by houseflies is greatest where inadequate refuse storage, collection and disposal (leading to increased breeding) is accompanied by inadequate sanitation (Osei and Duker, 2008). Thus flies gain greater access to human faeces and then to food. Refuse must be collected daily to prevent flies breeding.

2.5.2.3 Injury

People collecting rubbish may be injured by sharp objects including glass, metal and wood. These may lead to puncture wounds and lacerations which may become infected and cause serious morbidity. Composted solid waste can cause injury to farmers as sharp objects are not always properly removed.

2.5.2.4 Aesthetics Aspects

Foul odour is emitted at the disposal site due to continuous decomposition of organic matter and emission of methane, hydrogen sulphide, ammonia, etc. The problem is intensified if proper mitigation measures are not adopted. Odour is also emitted at the collection points if quick removal of wastes is not practised. Spreading of the wastes in the area adjacent to the dustbin due to activity of ragpickers, cause degradation of aesthetic quality. Uncontrolled disposal and open burning of wastes at the landfill sites

create poor vision. Domestic rats, birds and other scavenging animals act as reservoirs for many organisms transmissible to people, including plague, forms of typhus, leptospirosis, trichinosis, psittacosis, *Salmonella* infection and bovine tuberculosis. Chemical control of both houseflies and rodents is not very effective because of widespread resistance. The essential basis of control remains denial of access to food and harborage, by covered storage and efficient removal. Mosquitoes, vectors of dengue and yellow fever, breed greatly in discarded containers that trap rainwater. Culex mosquitoes, vectors of filariasis, breed in polluted stagnant water. Such breeding sites often occur where drains are choked by solid waste.

2.6 The Significance of Environmental and Health Impact Assessment (EHIA)

The significance of Environmental and Health Impact Assessment is aimed at improving the information support for proper management of municipal solid waste. Infrequent collection and rapid decomposition of wastes provide an attractive feeding and breeding site for flies, rats and other scavengers. Human and animal faecal matter or hospital wastes are often mixed with the refuse. Vectors and pathogens multiply. Domestic and on occasion industrial, solid wastes are disposed of in open spaces within residential areas. Collection and disposal of refuse can consume up to 50% of a municipal operating budget. In many otherwise good systems, only 50-70% of the refuse is regularly collected. The problem is organizational rather than technical. Refuse disposal is often a non-profit making business and thus is treated as an unwanted side-effect of development.

2.6.1 Community Health Implications

There are implications that could be derived from the above analysis in relations to community development. First, it is always said that “health is wealth”. Therefore, a

healthy community built on effective and efficient waste management is a factor critical to sustainable development. Secondly, the presence of waste materials with positive value represents potential source of livelihood for the poor. This was true for medieval cities and for rapidly industrializing cities of Europe and North America in the 19th century, and also applies to developing countries today (Wilson, 2007).

2.6.2 Public Information and Participation for EHIA

According to (WHO, 2011), the public should be made aware on the following aspects for environment and health surveillance**:**

(i) As far as possible faecal matter should not be allowed to mix with municipal refuse.

(ii) Hospital and municipal wastes should be handled separately.

(iii) Burning of refuse should not be permitted.

(iv) Discharging of waste into drains and open areas should be prohibited by law.

(v) Solid waste should be handled once and its contact with workers minimised as much as possible.

(vi) Efforts should be made to remove solid waste from habitations regularly.

(vii) Regular medical check up of personnel handling waste should be carried out.

2.7 Benefits of Waste Management

Most development activities are expected to have a beneficial effect on human health by increasing the resources available for food, education, employment, water supply, sanitation and health services (Oghenekoho and Akporehwe, 2015). Proper waste management is not only regarded as a political tool and an indicator of good government policy, it is also an important element for good health (Oyedele, 2009). Proper

management of municipal solid waste should have minimum effects on environment and health impacts. It is a development activity and is expected to have a beneficial effect on human health by increasing the resources available for the aforementioned. It is then true that the individuals in the community must appreciate the environmental elements of the community which definitely define the status personalized of the community in the development process.

2.8 Bacterial Biofilms

A biofilm is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix. Noncellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix (Donlan, 2002). Biofilms may form on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems (Rodney, 2002). Bacterial biofilms consist of microcolonies of bacteria that have developed into organized communities with functional heterogeneity. Microorganisms within biofilms display features distinct from their planktonic counterparts (Donlan, 2002; De Beer and Stoodley, 2006; Vu *et al*., 2009), such as increased adherence to surfaces, high population densities (around 10 cells per ml of hydrated biofilm), enhanced production of extracellular polymeric slime matrix (glycocalyx), wide range of physical, metabolic and chemical heterogeneities, elevated tolerance to antimicrobial agents, higher level of nutritional interactions between microorganisms, higher order of communication through quorum sensing, less susceptibility to host defence mechanism, and organisms in biofilm may display some novel phenotype(s).

Formation of sessile communities and their inherent resistance to antimicrobial agents are at the root of many persistent and chronic bacterial infections (Greenberg *et al*., 1999). Biofilms constitute a protected mode of growth that allows survival in a hostile environment. Sessile biofilm communities can give rise to nonsessile individuals, planktonic bacteria that can rapidly multiply and disperse. Biofilm cells may be dispersed either by shedding of daughter cells from actively growing cells, detachment as a result of nutrient levels or quorum sensing, or shearing of biofilm aggregates (continuous removal of small portions of the biofilm) because of flow effects. Research over the past few decades has now clearly established that 99.9% of all known microorganisms in natural settings are attached to surfaces, due to the nutritional and protective benefits associated with life in an adherent population (Cowan, 2011). Once bacteria attach to a surface, they produce complex exopolymers—containing polysaccharides, proteins, and nucleic acids (Branda *et a*l., 2005; Steinberg and Kolodkin-Gal, 2015) which help both to preserve their attachment to the surface and to maintain bacterial cells in close proximity to each other. As the number of bacteria present in a specific area increases, this adherent population is referred to as a biofilm (Omar *et al.*, 2017).

2.8.1 Bacterial Biofilm Infections

Until the relatively recent development of vaccines and antibiotics, human societies have been beset by acute epidemic infectious diseases caused by the planktonic cells of such specialized pathogens as *Vibrio cholerae* and *Yersinia pestis* (Greenberg *et al.*, 1999). More than half of the infectious diseases that affect mildly compromised individuals involve bacterial species that are commensal with the human body or are common in our environments. These biofilm infections may be caused by a single species or by a mixture of species of bacteria or fungi (Table2.2).

Table 2.2 Partial List of Human Infections Involving Biofilms

Infection or disease	Common biofilm bacterial species
Dental caries	Acidogenic Gram-positive cocci (e.g. *Streptococcus*)
Periodontitis	Gram-negative anaerobic oral bacteria
Musculoskeletal infections	Gram-positive cocci (e.g., staphylococci)
Biliary tract infection	Enteric bacteria (e.g., *Escherichia coli*)
Bacterial prostatitis	*E. coli* and other Gram-negative bacteria
Native valve endocarditis	Viridans group streptococci
Cystic fibrosis pneumonia	*P. aeruginosa* and *Burkholderia cepacia*
Meloidosis	*Pseudomonas pseudomallei*
Nosocomial infections	
Sutures	*Staphylococcus epidermidis* and *S. aureus*
Exit sites	*S. epidermidis* and *S. aureus*
Arteriovenous shunts	*S. epidermidis* and *S. aureus*
Contact lens	*P. aeruginosa* and Gram-positive cocci
Urinary catheter cystitis	*E. coli* and other Gram-negative rods
Hickman catheters	*S. epidermidis* and *C. Albicans*
Central venous catheters	*S. epidermidis* and others
Mechanical heart valves	*S. aureus* and *S. epidermidis*
Biliary stent blockage	A variety of enteric bacteria and fungi
Orthopedic devices	*S. aureus* and *S. epidermidis*

Source: Greenberg *et al.*, (1999)

2.8.2 Public Health Perspective

Clinical and public health microbiologists' recognition that microbial biofilms are ubiquitous in nature has resulted in the study of a number of infectious disease processes from a biofilm perspective. Cystic fibrosis, native valve endocarditis, otitis media, periodontitis, and chronic prostatitis all appear to be caused by biofilm-associated microorganisms. A spectrum of indwelling medical devices or other devices used in the healthcare environment have been shown to harbor biofilms, resulting in measurable rates of device-associated infections (Donlan, 2002). Biofilms of potable water distribution systems have the potential to harbour enteric pathogens, *L. pneumophila*, nontuberculous mycobacteria, and possibly *Helicobacter pylori*. What is less clear is an understanding of how interaction and growth of pathogenic organisms in a biofilm result in an infectious disease process. Characteristics of biofilms that can be important in infectious disease processes include a) detachment of cells or biofilm aggregates may result in bloodstream or urinary tract infections or in the production of emboli, b) cells may exchange resistance plasmids within biofilms, c) cells in biofilms have dramatically reduced susceptibility to antimicrobial agents, d) biofilm-associated gram-negative bacteria may produce endotoxins, and e) biofilms are resistant to host immune system clearance (Rodney, 2002).

2.9. Microbial Degradation of Solid Waste

Microbial degradation of solid waste implies the breaking down of organic components of waste to inorganic form by the microorganisms, which can readily serve as nutrient for a variety of other organisms. When wastes are dumped on land, soil microorganism such as bacteria, fungi and worms (helminthes) readily colonize the waste carrying out

degradation or transformation of degradable (organic) materials in the waste. Similarly, mesophilie, thermophilic and their thermo-tolerant bacteria in the waste use the constitutuents as nutrient, thus detoxifying the materials as their digestive process, breakdown complex organic molecules into simpler less toxic molecules (Boulter *et al*., 2002). Bacteria are the dominant population in the entire waste degradation process (Rebollido *et. al*., 2008). A variety of such waste microbes are widespread in the environment. They are found in abundance in the soil habitat (10^8-10^9 g^{-1} of soil) (Hartel, 1999; Van Elsas *et al.*, 2011). Of the waste degrading microorganisms, the aerobic populations are preferred because of their efficiency in degradation process. During the process, microorganisms use the crude organic matter as source of food thereby producing heat, carbon dioxide, water vapor and humus (Tiquia, 2005). Most of these microbes are potential human pathogens (such as *Salmonella* spp, *Escherichia coli*, Fungi and Protozoan) and may cause severe health hazards (Obire *et al*., 2002).

2.9.1 Biodegradation of Cellulosic Materials

Cellulose is a polymer of 100-14,000 glucose units linked by β-1,4 glycosidic bonds and is the main component of plant cell walls. As such, it is one of the most abundant polymers on Earth (Beguin and Aubert, 1994). Bacteria have been found flourishing in natural environments that are considered to be extreme for life on Earth, for example Cellulosic materials are the only renewable resources available in large quantities which need to be properly utilized to meet our needs of energy, chemicals, food and feed for a long-range solution (Perez *et al*., 2002).

Basically, the cellulose is the most widely distributed skeletal polysaccharide and represents about 50% of the cell wall material of plants. Beside hemicellulose and lignin,

cellulose is a major component of agricultural wastes and municipal residues (Perez *et al*., 2002). The cellulose and hemicellulose comprise the major part of all green plants and this is the main reason of using such terms as 'cellulosic wastes' or simply 'cellulosics' for those materials which are produce especially as agricultural crop residues, fruit and vegetable wastes from industrial processing, and other solid wastes from canned food and drinks industries (Gianfreda *et al*., 1999; George *et al*., 2001). The ability of cellulolytic microorganisms to degrade cellulose vary greatly with the physico-chemical characteristics of the substrate, such as: (a) the size and permeability of cellulolytic enzymes and other reagent molecules, which are involved in relation to the size and surface properties of the grown fibrils and the space between microfibrils and cellulose molecules from amorphous region; (b) the degree of crystallinity of cellulose; (c) the unit cell dimensions of cellulose; (d) the stereoscopic conformation and rigidity of the anhydroglucose units; (e) the degree of polymerization of cellulose molecules; (f) the nature of components with which cellulose is associated; (g) the nature, concentration and distribution of substituted groups (Leschine, 1995; Messner *et al*., 1998). The crystallinity degree of cellulose is one of the most important structural parameters which affect the rate of enzymatic degradation by hydrolysis. MessnerTherefore, the rate of degradation should be a function of the surface properties of cellulose which makes possible the access of enzymes to polymeric molecules (Paice *et al.*, 1993; Messner *et al*., 1998).

Most of the cellulolytic microorganisms belong to eubacteria and fungi, even though some anaerobic protozoa and slime molds able to degrade cellulose have also been described. Cellulolytic microorganisms can establish synergistic relationships with non-cellulolytic species in cellulosic wastes. The interactions between both populations lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic

conditions, and carbon dioxide, methane and water under anaerobic conditions (Beguin and Aubert, 1994; Leschine, 1995).

2.9.2 Pathogenic Bacteria in Waste Dumps

The number of pathogenic organisms in MSW in developing and non-developed countries is found to be higher due to improper waste management and lack of public conveniences in most Urban Centres (Obire *et al*., 2002). Pathogenic bacteria that may be associated with faecal contamination include pathogenic strains of *Escherichia coli*, *Salmonella* species, *Shigella* species and *Vibrio cholerae* (Achudume and Olawale, 2007; WHO, 2013).These are pathogens and, hence capable of causing infection and pose great risk to human health and animals when increased in population (Onweremadu *et al*., 2009).

2.9.3 Health Implication of Pathogenic Bacteria in Waste Dump Sites

Many countries in Africa do not have efficient waste collection and disposal services, which often results in both environmental and health problems for the people. Because these wastes are not properly disposed off, they constitute serious health problems, such as dissemination of infectious diseases to man and animals living within the vicinity (Lorentz *et al*., 2000). The use of waste dump as a preferred method of solid disposal is unscientific and constitutes a nuisance to the public; hence, the resultant effect is pollution. When waste is dumped on land, microorganisms such as bacteria and fungi proliferate. Most of these microorganisms are potential human pathogens and may cause severe health hazards (Wachukwu *et al.*, 2010). Bartram *et al.* (2003) and Onweremadu *et al*. (2009) discovered that bacterial pathogens in wastes that lack leachaete collection system are source of pathogens to the soil. These pathogens when increased in population, pose great risk to human health (Achudume and Olawale, 2007).

The microbial community of soil may be expected to vary considerably, depending on the type of waste and other factors. Mbata (2008); Oviasogie *et al*. (2010)**;** Wachukwu *et al.* (2010) Osunwoke and Kuforiji (2012) identified many bacteria general including *Arthrobacter* spp., *Bacillus* spp. *Micrococcus* spp., *Poteus* spp., *Pseudomonas* spp., *serratia* spp., *staphylococcus aureus, staphylococcus* spp., *Streptococcus, Esterichia coli, Klebsiella* spp., *streptococcus* spp., *Salmonella* spp., *Vibrio* spp., in waste.

Staphylococcus is an opportunistic pathogen in that it causes infections most commonly at sites of lowered host resistance, for example, damaged skin or mucous membranes (Arora, 2004). *Staphylococcus aureus* has been associated with several diseases, especially superficial infections, osteomyelitis, septicaemia and otitis media (Mandell *et al*., 1995). Klebsiellae in general, are more frequently involved in hospital-associated urinary tract infections, wound and burn infections and as secondary invaders in other respiratory infections. In fact, they are the most frequently encountered gram-negative pathogen causing nosocomial infections of the lower respiratory tract and are second only to *Escherichia coli* as a cause of primary bacteraemia by gram-negative organisms. They may also cause meningitis and diarrhoea (Harris and Paxman, 1982). *Bacillus anthracis* and *Bacillus cereus* have been incriminated in human and animal disease. *Bacillus anthracis* is one of the highly pathogenic microorganisms known to mankind. Infections by other members of the genus are rare; however, they occasionally cause gastrointestinal icaemia in the immunocompromised and acute purulent meningitis.

Aeromonas may cause cellulitis and wound infections, acute diarrhoeal disease, septicaemia in immunodeficient subjects and infections of the ear, meningities and urinary tract (Arora, 2004). *Streptococci* are more likely to give rise to septicaemia. *Streptococcus pyogenes* causes tonsillitis and pharyngitis, scarlet fever, impetigo, rheumatic fever, glomerulonephritis, as well as streptococcal toxic shock syndrome. *Enterococci* may cause urinary tract infections, wound infections, infective endocarditis, biliary tract infections, peritonitis, suppurative abdominal lesions and septicaemia. *Serratia* can lead to serious nosocomial infections particularly in the newborn, the debilitated or the patient receiving immunosuppressive drugs. These include infections of wound, urinary and respiratory tracts; meningitis, endocarditis, septicaemia and endotoxic shock (Henderson, 1999).

2.9.4 *Vibrio cholerae*: The Causative Agent of Cholera

Cholera is a highly epidemic diarrhoeal disease which continues to devastate many developing countries where socioeconomic conditions are poor, sanitary systems and public hygiene are detrimentary, and safe drinking water is not available (Theron *et al*., 2000). Infection is characterised by vomiting and rice-like diarrhoea (Morin *et al.*, 2004). The infection dose of cholera is high and has been reported to be 10^5- 10^8 organisms (Hunter, 1997). Cholera remains a major public health problem with significant mortality wherever it occurs, despite the fact that it is a simple and inexpensive disease to treat when adequate medical supplies and knowledgeable, committed health workers are available (Sack *et al.*, 2003).

2.9.4.1 Introduction of Cholera in Nigeria

Cholera as a disease is not new in Nigeria. Cholera was introduced into Nigeria on the 28th December, 1970 when three bacteriologically continued cases were reported in Lagos State (Oshinkalu, 1971). Sporadic cases of cholera out breaks in different parts of the country are being reported nearly every year. However, cholera epidemic has not yet left Nigeria; there have been lots of documented cases in Nigeria. For instance, in 1971 cholera epidemic was reported in the former Cross River State and the riverine area of Calabar, Oron, Itu, and Eket were the worst affected. In 1981 there was a cholera epidemic report in Lagos and available evidence indicated that the infection was probably introduced by one of the many visitors who troop in from villages for a festival (Bitto *et al*., 1992).

2.9.4.2 Distrution and Spread in Africa-Nigeria

Cholera was first reported in the Bangal area of India, now East Pakistan and West Bengal Province of India (WHO, 1970). From here it latter spread to Europe and America; and finally to Africa. For instance, in 1961, *Vibrio cholerae* El Tor biotype occurred in an epidemic rate in Celebes, and with the introduction of cholera into Guinea, West Africa in July, 1970, the need for a plan to contain the disease especially in the virgin soil became necessary, and in most parts of West Africa, cholera spread East wards into the neighbouring countries of Sierra Leone, Liberia, Mali, Cote Devoire, Ghana, Upper Volta, Togo, Benin Republic, Niger and Nigeria (WHO, 1970). The World Health Organization urgently sponsored a meeting of all the Anglophons to curb the impending invation of the territory by cholera.

In Nigeria the first National Conference on Cholera was held in Lagos in December, 1970 and subsequent meeting on the same subject at Ibadan in March, 1974. In 1991 there was a cholera epidemic which occurred in Nigeria resulting in a totalof 56,352 confirmed cases of cholera and 7,289 deaths (WHO, 1990). In the same year, there were reports of cholera in Kano, and an abnormal increase in cases of gastroenteritis in Cross River State and Uyo, Akwa Ibom State. This prompted the Nigeria Health Autorities to alert the Communities of a possible cholera outbreak. *Vibrio cholerae* non-O1 and *Vibrio parahaemolyticus* were isolated from acute diarrhoeal patients in these areas. They suggested that there is probably an ecological niche which supports *Vibrio cholerae* non-O1 and *Vibrio parahaemolyticus* in the Cross River Basin of Nigeria.

2.9.4.3 Epidemiology of Vibrio cholerae

The epidemiology of cholera has been dominated by its tendency to spread through the world (Table 2.3) in pandemics (Hunter, 1997). During the 19th century, cholera spread repeatedly from its original reservoir or source in the Ganges Delta in India to the rest of the World, before receding to South Asia. Six pandemics were recorded that killed millions of people across Europe, Africa and the America (WHO, 2008). The seventh pandemic started in 1961, in Asia (Lan and Reeves, 2002). The eight pandemic first due to a non-O1 strain, first appeared in 1991 in India and has spread more rapidly to Bangladesh, China, Thailand, Nepal, and Malaysia (Hunter, 1997).

As of 25 December 2008, a total of 26497 cases, including, 1518 deaths due to cholera, were reported in Zimbabwe. The cholera outbreak was reported to be closely linked to the lack of safe drinking water, poor sanitation, declining health infrastructure, and reduced numbers of health care staff reporting to work (WHO, 2008). The outbreak took on a sub-regional dinention with cases being reported from neighbouring countries like Botswana

Table 2.3: The Cholera Pandemics

Pandemic	Organism	Origine	Duration	Affected regions
First	O1-Classical	Bangladesh	1817-1823	India, SE Asia, Middle East, Afria
Second	O1-Classical	Bangladesh	1826-1851	India, SE Asia, Middle East, Africa, Europe, America
Third	O1-Classical	Bangladesh	1852-1859	India, SE Asia, Middle East, Africa, Europe, America
Fourth	O1-Classical	Bangladesh	1863-1879	India, SE Asia, Middle East, Africa, Europe, America
Fifth	O1-Classical	Bangladesh	1881-1896	India, SE Asia, Middle East, Africa, Europe, America
Sixth	O1-Classical	Bangladesh	1899-1923	India, SE Asia, Middle East, Africa, Europe, America
Seventh	O1-EL TOR	Indonesia	1961	India, SE Asia, Middle East, Africa, Europe, America
Eighth	O139	India	1991	India, SE Asia

Adopted from Hunter (1997)

and South Africa. In South Africa as of 26 December 2008, 1279 cumulative cases and 12 deaths had been recorded with the bulk of the cases (1194) in the Limpopo province (WHO, 2008). Among the 193 currently recognised O serogroups of *Vibrio cholerae*, only O1 and O139 have caused epidemics of cholera (Fraga *et al*., 2007).

2.9.4.4 Ecology of Vibrio cholerae

The general assumption by most workers, was that *Vibrio cholerae* was an organism whose normal habitat was the human gut and/or intestine, and incapable of surviving for more than a few days outside the gut (Osei and Duker, 2008). According to Osei and Duker (2008), *Vibrio cholerae* is usually found in aquatic and terrestrial environment. However, other studies suggest that the natural habitat of *Vibrio cholerae* is the aquatic environment (Rivera *et al*., 2003; Colwell, 2004; Percival *et al.*, 2004; Fraga *et al*., 2007). It is strongly aerobic although literature has it that it is a facultatively anaerobic organism. According to Cheesbrough (2006), *Vibrio cholerae* can be found in fresh and brackish water, shellfish and other seafoods. Humans are the only known reservoir of cholera. An acute case of cholera usually excretes 10^6-10^8 Vibrios per millilitre in faeces, which contaminates its immediate environment (Deb *et al*., 1990). Deb *et al*. (1990) further stated that *Vibrio cholerae* O1 probably depends on the human intestinal tract as it primary multiplication site, but the probability of an extra-human reservoir cannot be roled out.

2.9.4.5 Morphology of Vibrio cholerae

Vibrio cholerae is a short, slightly curved Gram-negative rod; it is strongly aerobic in nature although literature has it that it is a facultatively anaerobic with a single polar flagellum which makes the organism actively motile. It may occur singly or in chains

which have the appearance of short spirals or s-shaped forms with size of about 1.5-3µ in length and 0.4-0.6 µ in breath. Cultures that have been maintained for a long time on agar often lose the curved form. Colonies on agar medium are similar to those of other enteric bacilli but may be distinguished from those of *E.coli* by their thin, opalescent appearance. They are 1-2mm in diameter, low, convex, and grayish yellow in colour, with a finely granular consistency which is accentuated under low magnification (Cheesbrough, 2006).

2.9.4.6 Serological Identification of Vibrio cholerae

Vibrio cholerae O1 can be divided serologically into three sub-types depending on the possession of three antigens: A, B, and C. Ogawa possesses antigens A and B; Inaba has antigens A and C and Hikojima has all three antigens (A, B and C) (Hunter, 1997). The O1 serogroup is further divided into two distict biotypes designated Classical and El Tor (Boyd and Waldor, 1999).

The sharp distinction between serogroups which can cause cholera and those which are not associated with cholera is related to the observation that more than 95% of the strains belonging to serogroups O1 and O139 produce the cholera toxin (CT). Strain belonging to the serogroups non-O1 and non-O139; have been implicated to severe human gastroenteritis (Faruque *et al*., 2003). They produce other extracellular products such as the heat-stable toxin NAG-ST (coded by the gene stn and sto), the hemolysin hlyA and the pore forming toxin RtAA which may be responsible for the pathogenesis of these strains (Fraga *et al*., 2007).

2.9.4.7 Biotypes (Biovars) of Vibrio cholerae

This is a classification based on physiological or biological characteristics. It emphasizes phenotypic manifestations. The *Vibrio cholerae* O1 bacteria can be classified into two

groups these include the biotype El Tor and biotype Classical. It is important to note that some non-O1 *Vibrio cholerae* are phylogenetically related to the El Tor cholera (Udo, 1996). The chlassification of *Vibrio cholerae* O1 into El Tor and Classical is based on the following:

(i) El Tor biovar shows positive Voges Proskauer reaction. Classical biovar shows negative Voges Proskauer reaction

(ii) When chicken red blood cells or sheeps red cells (1drop) are mixed with colonies of *Vibrio* species (loopful) in the presence of 1 drop of normal saline on a slide, there is agglutination of the El Tor biovar while there is no agglutination of Classical biovar.

(iii) When a 5ml polymyxim B is placed on a plate culture of El Tor biovar and Classical biovar and incubated at 37^{o}C overnight, the culture examined after incubation will show sensitivity of Classical biovar to polymyxim B but the El Tor biovar will be resistant to it.

(iv) Murkejee phage 4 causes lysis of Classical biovar but does not cause lysis of El Tor biovar.

(v) Vibriostatic agent 0/129 compound 2, 4- diamino 6,7-disoprophylpteridine is resisted by El Tor biovar but the Classical biovar is sensitive to it.

(vi) *Vibrio cholerae* produces haemolyses. This causes lysis of red blood cell. Usually the *Vibrio cholerae* O1 haemolysis more readily the red cells of the O group of human. There is little or no effect on the B and AB red blood cell. The El Tor biovar are 100% haemolytic while the Classical biovar are 100% non haemolytic (Udo, 1996).

2.9.4.8 Serotypes

Biochemical reactions typical of *Vibrio cholerae* are biotyped using the polyvalent O1 Antiserum, as described by Cheesbrough (2006). *Vibrio cholerae* O1 agglutinate the polyvalent O1 while *Vibrio cholerae* non-O1 does not. Serotyping is therefore a classification besed on biochemical reaction or characteristics.

2.9.4.9 Pathogenesis of Vibrio cholerae

The pathogenesis of the disease involves the ingestion of *Vibrio cholerae*, motile vibrios, chemotactic attraction, penetration of mucus layer on the intestinal surface, adherence to receptors in the mucus gel, chemotaxis into deeper intervillous spaces, adherence to the epithelial cell surface, and production of cholera toxin. After ingestion of *Vibrio cholerae,* the organisms multiply in the small intestine and produce a potent enterotoxin. The enterotoxin binds to the ganglioside of the epithelial cells of the small intestine and stimulates the adenylate cyclase which in turn causes an increase in intracellular cyclic Adenosine monophosphate (AMP). As a result, there is out pouring of huge amounts of isotonic fluid into the bowel lumen and when this small intestinal secretion exceeds the absorptive capacity of the colon, water diarrhoea starts. This loss of fluid and electrolytes from the body leads to the dehydration which is the main cause of death in cholera. *Vibrio cholerae* is present in large numbers throughout the entire length of the gastro-intestinal tract from mouth to anus during natural disease (Sack *et al*., 2003).

2.9.4.10 Pathogenic potential of Vibrio cholerae

The pathogenicity of *Vibrio cholerae* is enhanced by it ability to produce a potent protain enterotoxin that activate the enzyme adenylcyclase (Alam *et al*., 2006). The toxin when elaborated by the organism is reported to act upon the luminal surface of the small bowel

mucosal cells thus stimulating adenylcyclase activity in the gut leading to the production of accumulated amount of Cyclic Adenosine Monophosphate (cAMP).

$$\text{ATP} \xrightarrow{\text{Adenylcyclase}} \text{Cyclic AMP}$$ (Pierce *et al*., 1971).

The organism also secretes a toxin which attaches tightly to the enterocyte and increases the activity of adenylate cyclase and consequently the 3' 5'-cyclic monophosphate concentration.

2.9.4.11 Transmission and Spread of Vibrio cholerae

Factors such as inadequate sanitation, lack of personal and food hygiene, use of polluted water in preparing food, ingestion of uncooked or inadequately cooked shelfish and other sea food collected from polluted coastal waters and the use of untreated sewage as fertilizer contribute to the transmission of *Vibrio cholerae* (Theron *et al*., 2000; Fraga *et al*., 2007; Osei and Duker, 2008). During outbreaks, the mode of transmission may reflect person to person spread in addition to contaminated food and water by flies and other insects that have come in contact with patient's faeces or seawage contaminated with patients faeces. The vectors latter contaminate food or water for healthy people. Spread of cholera infection therefore occurs through consumption of contaminated water and food. In a study conducted in Calcutta slum, a highly endemic area, it was shown that contamination of underground water sources and drainage pipe lines often results in explosive outbreaks of cholera (Deb *et al*., 1970). In urban agglomeration, low lying areas usually get water-logged due to improper drainage facilities. This accumulated water gets easily contaminated due to indiscriminate defaecation leading to explosive epidemics.

2.9.4.112 Treatment of Cholera

Control of cholera will include proper curative measures. Cholera generally is managed by prompt replacement of the large quantity of water and electrolyte that are lost in diarrhoea. This will require volume of isotonic polyelectrolyte sodium in excess of one litre per hour (WHO, 1970). ORT, or ORS- Rehydration therapy or solution given orally; the World Health Organization has recommended ORS which contains 20g glucose, 3.5g sodium chloride, 2.5g $NaCO_3$ and 1.5g potassium chloride dissolved in one litre of drinking water (WHO, 1970). Antibiotic therapy like Tetracycline will shorten the duration of diarrhea and reduce fluid losses. Because of the plasmid mediated antibiotic resistance in some strains of *Vibrio cholerae*; antibiotic therapy is usually reserved for cases where fluid replacement is not able to stall diarrhoea (Salami, 1972).

2.9.4.13 Public Health Activities, Sanitation and Faecal Disposal

There is a general agreement over the point that improvement of environmental sanitation coupled with that of personal hygiene can effectively reduce or even eradicate cholera. Experiences during the current 7^{th} pandemic of cholera have shown that high sanitation and personal hygiene standard have made certain countries non-receptive to cholera infection (WHO, 1990). The principles of control measure should include provision of sanitary excreta disposal, safe water supply, domestic and food hygiene, disinfection of infected materials etc. The improvement of environmental sanitation is considered economical in the long run (WHO, 1970). The improvement of both samitation and personal hygiene should go side by side in order to achieve the goal. Isolated implementation of one component is not likely to give the desired result.

2.9.5 *Salmonella*

Salmonella is an important cause of food borne infections with a broad host spectrum. It remains a threat to public health and there are reports of increase in its incidence (Nazemi *et al*., 2012). Salmonellosis can result in a number of disease symptoms including gastroenteritis, bacteraemia, typhoid fever and focal infections.Typhoid and paratyphoid fevers are infections caused by *Salmonella*, which are transmitted by faecal oral route (WHO, 2008). Millions of human cases are reported worldwide every year and the disease results in thousands death (WHO, 2005). The United States alone reported an estimated 1.4 million total cases of non-thyphoidal *Salmonellae* per year (Sukhnanand *et al.*, 2005; Patchanee *et al*., 2008; Garry *et al*., 2009). Although infections with non-typhoidal Salmonellae usually cause self-limiting diarhoeal illness, serious sequelae, including meningitis, septis, and death may occur, especially among infants and elderly persons (Akinyemi *et al*., 2007). The large number of outbreaks in developed and developing countries produced by these bacteria indicates its importance and impact (Bell and Kyriakides, 2002). Salmonellosis is not only responsible of a large number of illnesses but also there is a cost associated with these outbreaks which in United States has been estimated to range from $600 million to $3.5 billion each year (Ter-Hsin *et al*., 2005).

In recent years problems related to *Salmonella* have increased significantly, both in terms of incidence and severity of cases of human salmonellosis. While some developed countries have managed to reverse the increasing incidence of human salmonellosis, the situation is different in developing countries of Africa and Asia where salmonellosis continues to be a cause to public health with an estimated incidence of 33 million cases each year (Sood *et al*., 1999). Socio-demographic factors (age, education, income), environmental and sanitation factors (poor sanitation and poor access to drinking water)

and climatic factors (rainfall, temperature and humidity) are thought to be related to incidence and special distribution of diarrhoea (WHO, 2007). The frequency and gravity of these infections are affected by hygiene condition, malnutrition, and the excessive use of antibiotics that select for multiresistant strains (Centres for Disease Control and Prevention, 2008).

In Nigeria, morbidity associated with illnesses due to *Salmonella* continues to be on the increase and, in some cases, resulting in death. New concerns have been identified. Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials, including first-choice agents for the treatment of humans, such as chloramphenicol and cotrimoxazole, and the third-generation cephalosporins, have emerged and are threatening to become a serious public health problem (Akinyemi *et al.*, 2007). Although Fluoroquinolones have been forced to be efficatious both in vitro and in vivo in the treatment of severe *Salmonella*-associated illnesses, strains with reduced susceptibility to ciprofloxacin among travelers have been reported in some parts of the globe (Hakanen *et al.,* 2001). Multi-drug resistance to critically important antimicrobials is compounding the problems.

2.9.5.1 Salmonella Taxonomy

Salmonella belongs to the family Enterobacteriaceae (Guthrie, 1991). The genus *Salmonella* contains two species; *S. enterica* and *S. bongori* (Table 2.4), which was formerly subspecies V. Six subspecies are differentiated within *S. enterica* based on their biochemical and genomic characteristics, a Roman numeral and a name are used for the designation of these six subspecies as follows: I, *S. enterica* subsp. *enterica*; II, *S. enterica*

Table 2.4: Species and Subspecies in the *Salmonella* Genus

***Salmonella* species**	**Subspecies**	**Number of Serovars**
Salmonnella enteric	Enterica	1,478
	Salamae	498
	Arizonae	94
	Diarizonae	327
	Houteane	71
	Indica	12
Salmonella bongori		21
Total		2,501

Source: Card, (2009).

subsp. *salamae* ; IIIa, *S. enterica* subsp. *arizonae* ; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. Enteric* subsp. *houtenae,* and VI, *S. enterica* subsp. *indica*) (Brenner *et al.,* 2000). By newer convention, names are retained only for subspecies *enterica* serovars, and these names are no longer italicized. The first letter is a capital letter "*S*" followed by the serovar names of subspecies *enterica* (e.g. Typhimurium or Montevideo). At the first citation of the serotype the genus name is given followed by the word "serotype" or the abbreviation "ser" followed by the serotype name. This project follows the abbreviated modern naming system, i.e. *S.* Typhimurium rather than the more complete nomenclature *S. enterica,* subsp. *enterica* serovar Typhimurium (Brenner *et al.,* 2000, Molbak *et al*., 2006).

The antigenic formulae are also used to name *Salmonella* serotypes. This designation includes: (i) subspecies designation (subspecies I trough VI), (ii) O (somatic) antigens followed by a colon, (iii) H (flagellar) antigens (phase 1) followed by a colon, and (iv) H antigens (phase 2, if present) (Brenner *et al.,* 2000). The nomenclature detailed above is internationally accepted based on recommendations of the WHO Collaborating Center (Molbak *et al*., 2006). By newer convention, names are retained only for subspecies *enterica* serovars, and these names are no longer italicized. The first letter is a capital letter "*S*" followed by the serovar names of subspecies *enterica* (e.g. Typhimurium or Montevideo). At the first citation of the serotype the genus name is given followed by the word "serotype" or the abbreviation "ser" followed by the serotype name. This project follows the abbreviated modern naming system, i.e. *S.* Typhimurium rather than the more complete nomenclature *S. enterica,* subsp. *enterica* serovar Typhimurium (Brenner *et al.,* 2000; Molbak *et al*., 2006).

The antigenic formulae are also used to name *Salmonella* serotypes (Table 2.5). This designation includes: (i) subspecies designation (subspecies I trough VI), (ii) O (somatic) antigens followed by a colon, (iii) H (flagellar) antigens (phase 1) followed by a colon, and (iv) H antigens (phase 2, if present) (Brenner *et al.,* 2000).

2.9.5.2. Salmonella Features

Salmonella are facultative anaerobic, gram negative, small rods, motile (Bell and Kyriakides, 2002; Molbak, 2006). Temperature for growth ranges from 8°C to 45°C, strains can stand pH between 4- 9 and is able to grow at water activities above 0.94. *Salmonella* is heat labile so the organism can be inactivated at ordinary cooking temperatures (> 70 °C) although the cooling time and values for temperature and time could change depending on the serotype and the food matrix. In addition *Salmonella* has

been shown to tolerate up to 20% salt concentration (Guthrie, 1991; Bell and Kyriakides, 2002). Under freezing conditions (from -23°C to -18°C) this microorganism is able to survive as long as seven years (Bell and Kyriakides, 2002). The difficulty in controlling *Salmonella* is due to its ability to survive extreme environmental conditions (Guthrie, 1991).

The biochemical characteristics of *Salmonella* indicate that they are able to reduce nitrates to nitrites, produce gas from glucose (not always), produce hydrogen sulfide on triple-sugar iron agar, and they are usually able to use citrate as the sole carbon source (Bell and Kyriakides, 2002; Molbak *et al*., 2006). *Salmonella* can be further subdivided by phage typing, this method in conjunction with serotyping, pulse field electrophoresis (PFGE), determination of antibiotic resistance patterns and plasmid profiling are methodologies

Table 2.5: **Antigenic Formulae of some *Salmonella* Serotypes**

Serotype	Serogroup	Somatic antigen (O)	Fagella (H) antigens	
			Phase 1	**Phase 2**
***S*. Paratyphi A**	A	1,2,12	A	(1,5)
***S*. Typhimurium**	B	1,4, (5),12	I	1,2
***S*. Agona**	B	4,12	F,g,s	-
***S*. Derby**	B	1,4, (5),12	F,g	(1,2)
S. Typhi	D	9,12, (Vi)	c	1,2
***S*. Enteritidis**	D	1,9,12	g,m	(1,7)

Source: Card (2009).

that provide significant information for the assessment of *Salmonella* prevalence and epidemiology (Molbak *et al*., 2006).

2.9.5.3 Serological Identification (Kauffman White scheme)

The scheme used worldwide for serological identification of *Salmonella* serovars was first proposed by White and expanded by Kauffman (Le Minor and Popoff, 1987). The list of 2,501 *Salmonella* serotypes is maintained and annually updated by the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France (Brenner *et al*., 2000). The Kauffman- White scheme (KW) is based on the antigenic structure of *Salmonella* serotypes (Helmuth, 2000). The antigenic properties and variations of the O (surface polysaccharide) and H (flagellar) antigens from each serovar are summarized and described in what is known as the antigenic formulae (Mortimer *et al.,* 2004; Wattiau *et al*., 2008). The structure of each microbial cell is dependent of a variety of antigenic molecules, which are at the time dependent of many determinant groups (chemical groups). Thus it is the chemical make up and the arrangement of these determinant groups that assign the immunological specificity of the antigen (Guthrie, 1991). The cross absorption of antisera is used to reveal the antigenic structure of *Salmonella* (Helmuth, 2000). The composition and structure of polysaccharides, which constitute a part of the structure of the cell surface, allow for recognition and differentiation of O antigens (Guthrie, 1991). In the KW scheme O antigens are indicated in brackets when they are easily modified by mutation, otherwise they are underlined when these factors are determined by bacteriophages or plasmids (Helmuth, 2000). H antigens are present in the flagella, they are composed of protein subunits called flagellin that are typically diphasic and thought to help the bacteria to survive host immune responses (Helmuth, 2000). A capsular polysaccharide found in

some serovars (Typhi, Paratyphi C and Dublin) is termed "The virulence (Vi) antigen". This factor first needs to be heated at 100 °C for 60 minutes to remove the capsule; otherwise it would not be agglutinable with anti-O antiserum (Helmuth, 2000). Serological typing of *Salmonella enterica* serovars requires, over 150 O and H antigens and more than 250 antisera (Cai *et al*., 2005; Wattiau *et al*., 2008). The problem with this conventional method is that it is laborious, time consuming, and cannot differentiate within serovars (Nashwa *et al*., 2009). It also depends on the availability of hundreds of antisera, needs highly trained personnel, consumes high volumes of reagents, and a minimum of three days is required to identify.

2.9.5.4 Salmonella Infection

Infections caused by *Salmonella* serotypes can produce enteric fever, gastroenteritis, and bacteraemia or septicaemia conditions (Guthrie, 1991, Monteville and Matthews, 2008). *Salmonella* Typhi and Paratyphi are responsible for causing enteric fever (Guthrie, 1991). The period of incubation for this infection ranges from 8 to 28 days and the common symptoms include fever, diarrhoea, abdominal pain, headache (Monteville and Matthews, 2008). Certain cases of Salmonellosis are quite severe and often need antimicrobial therapy for treatment; thus, resistance to antimicrobial drugs is of great concern *Salmonella* species are becoming increasingly resistant, making it more difficult to treat patients with severe infections. This makes multidrug resistant *Salmonella* an important subject area of research. The antibiotics of choice for treatment of enteric fever are chloramphenicol, ampicillin or trimethoprim-sulfamethoxazole (Monteville and Matthews, 2008). When the infection is due to the consumption of a food item contaminated with non-typhoid *Salmonella* strains, the disease is often self-limiting in healthy individuals. Symptoms appear 8 to 72 hours after ingestion, and are less severe

than in the previous case, and non-bloody diarrhea and abdominal pain disappear within 5 days (Meneses, 2010).

The treatment is based more on fluid and electrolyte replacement than on antibiotic use. Infections caused by nontyphoid *Salmonella* serotypes can also evolve into systematic infections followed by chronic conditions (Monteville and Matthews, 2008). Salmonellosis occurs when the bacteria have been able to survive the low pH in the stomach and reach the mucosa in the small intestine in adequate numbers to cause infection. Epithelial cells localized in the mucose midlayer are responsible of cover completely the *Salmonella* cells, which drive an inflammatory response (Guthrie, 1991). The infection could progress to acute levels, depending on the serotype causing the illness.

2.9.5.5. Salmonella Detection

There are four steps for the recovery of injured *Salmonella* cells from the environment. First the pre-enrichment, where buffered peptone water or lactose broth can be used, followed by growth on a non-selective broth. This is followed by enrichment in selective broth, such as Rasspaport- Vasilliadis (RV) broth, Selenite Cysteine Broth (SC), or tetrationate broth (TT). Finally the subsequent isolation is done on selective Brillant green agar, Bismuth sulfite agar, Hektoen agar (HA) or Xylose Lysine Desoxycholate (XLD) (Molbak *et al*., 2006).

Some strains of *Salmonella* could have a different reaction to the combinations of inhibitory substances, incubation temperatures, selective enrichment broths and media (Cardinal *et al*., 2005). Some *Salmonella* serotypes (*S*. Anatum, *S*. Tennessee, *S*. Newington and S. Senftenberg) are lactose positive cultures (Bell and Kyriakides, 2002),

for that reason it is important not to rely only on lactose to distinguish *Salmonella* from other microorganisms present in the food matrix, but to utilize alternative selective media such as Mannitol Lysine Crystal Violet Brillant Green (MLCB) or Bismuth Sulphite Agar (Bell and Kyriakides, 2002).

There are a wide variety of methods commercially available for *Salmonella* detection and identification. These include the use of antibodies to *Salmonella* antigens (Enzyme-Linked Immuno-sorbent assay (ELISA), immuno-chromatography, chemiluminescent immunoassay, antibody coated dipsticks, latex agglutination), electrical conductance methods, and polymerase chain reaction (PCR) (Bell and Kyriakides, 2002; Molbak *et al*., 2006). The principle limitation is that all of these techniques need a pre-enrichment step to reach detectable numbers of cells in the sample (10^4 -10^5 cells/ml). This factor makes it very difficult to develop a truly rapid method for detection and identification of *Salmonella* that would allow processing the sample in a normal 8-hours work day (Bell and Kyriakides, 2002, Molbak *et al*., 2006). The capability to serotype or fingerprint is of importance for surveillance, inspection, and investigation of outbreaks. *Salmonella* subtyping can be accomplished by biotyping, phage typing, antibiotic resistant patterns, pulse field gel electrophoresis, and ribotyping (Berge *et al.*, 2004). The development of DNA-based methods for detection of *Salmonella*, have allowed for novel approaches in this field. The foundation of these methods is the hybridization of two complementary single –stranded molecules (one in the form of a probe, primer, DNA fragment or oligonucleotides developed in the laboratory and the other strand corresponds to the target microorganism) to obtain double- stranded nucleic acid molecules under defined physical and chemical conditions. Other diagnostic tools for *Salmonella* are the DNA microarrays. These are biochips, which enables hybridization by the presence of immobilized

oligonucleotides to a solid base. Results can be analyzed automatically with use of an appropriate device (Molbak *et al*., 2006). Numerous probes can be placed on a DNA chip and that number is expanding because of the continued growth of fully sequenced organisms (Molbak *et al*., 2006).

2.9.6 *Escherichia coli*

Escherichia coli is known as a harmless commensal of the gastrointestinal tract in warm-blooded animals and is used as the colloquial laboratory workhorse. However, there is an alternate side to *E. coli* afforded through gene gain and loss that enable it to become a highly diverse and adapted pathogen. Pathogenic *E. coli* can cause a broad range of human diseases that span from the gastrointestinal tract to extraintestinal sites such as the urinary tract, bloodstream, and central nervous system (Kaper *et al*., 2004; Croxen and Finlay, 2010). Diarrhoeal illness causes much mortality worldwide, particularly in children under the age of 5 (WHO, 2012) (Figure 2) and particularly in countries in sub-Saharan Africa and South Asia, whose children suffer many diarrhoea-related deaths. While there are many etiological agents responsible for diarrhea, pathogenic *E. coli* is a major contributor. Data from the Global Enteric Multi-Center Study (GEMS), one of the largest case-control studies aiming to understand the burden of pediatric diarrhoeal disease in sub-Saharan Africa and South Asia (Levine *et al*., 2012), illustrate that enterotoxigenic *E. coli* and *Shigella* are among two of the four main causative agents of moderate to severe diarrhea among children in these areas (Kotloff *et al*., 2013). In addition, increased fatality rates are associated with enteropathogenic *E. coli* and certain enterotoxigenic *E. coli* strains, thus underlining the significant role of pathogenic *E. coli* in the global health burden of diarrhoeal disease (Figure 2.1).

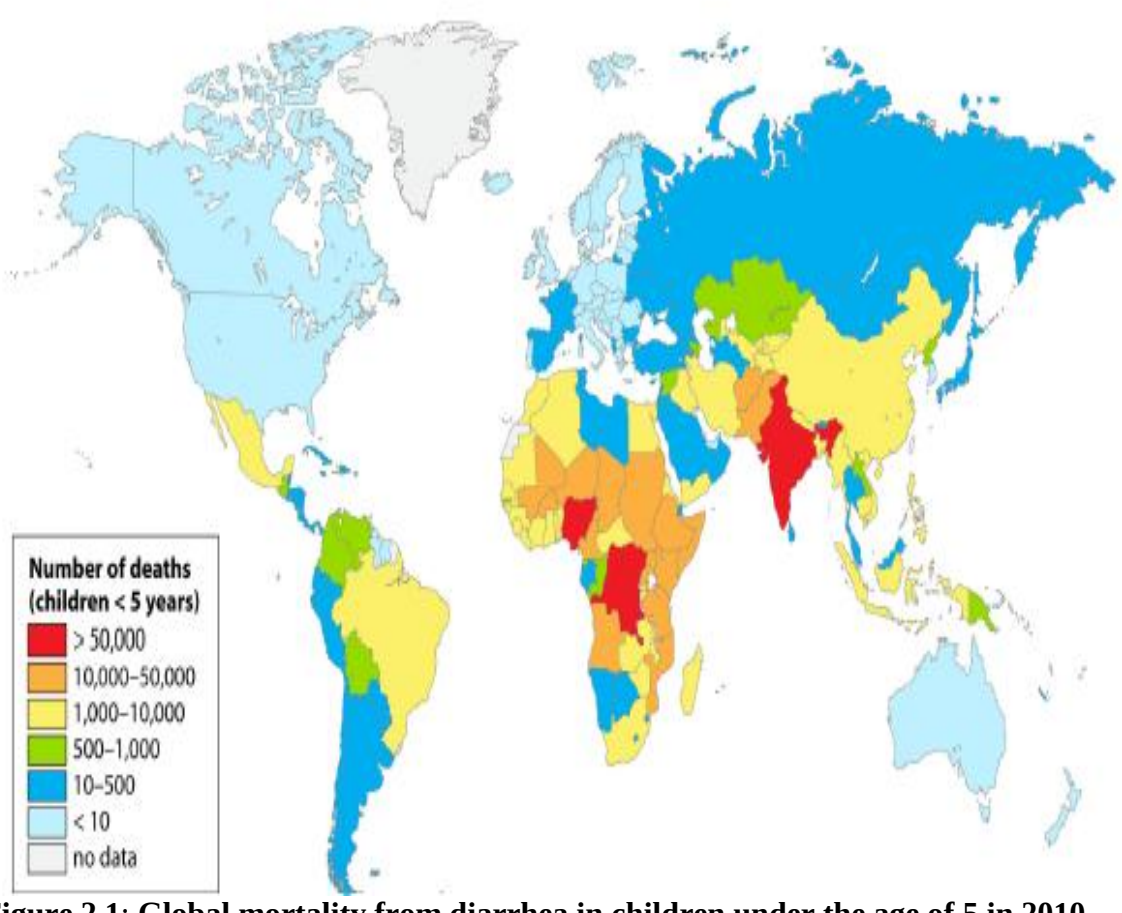


Figure 2.1: **Global mortality from diarrhea in children under the age of 5 in 2010**

Estimates of diarrhea-specific mortality among children under 5 for each country reflect high mortality in developing countries, with the highest tolls present in countries in sub-Saharan Africa and South Asia. Many etiological agents, including pathogenic *E. coli*, are responsible for diarrhea-related mortality in these children. Recent work published by GEMS found significant child mortality associated with EPEC and ETEC infections in developing countries (Kotloff *et al*., 2013).

Source data for the map: World Health Organization (WHO, 2012).

The *E. coli* scientific and clinical communities have made great strides in understanding *E. coli* microbiology, pathogenesis, ecology, and interactions with its host. These advances are essential for novel approaches to vaccines and treatments that prevent some of the serious sequelae and complications associated with *E. coli*-induced diarrheal illness. While various pathotypes contribute to diarrhoea, the clinical symptoms and outcomes, site and mechanism of colonization, and disease can differ. In most parts of the world, STEC O157:H7 is the most common serotype that causes human illness. However, it is becoming evident that non-O157 STEC strains also cause significant human illness.

2.9.6.1 Taxonomy of Escherichia coli

E. coli belongs to the family Enterobacteriaceae having the pathogenic capacity to cause significant diarrheal and extraintestinal diseases (Matthew *et al*., 2013). There are six major diarrheagenic *E. coli* pathotypes: enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC) (e.g., enterohemorrhagic *E. coli* [EHEC]), *Shigella*/enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), and enterotoxigenic *E. coli* (ETEC), as well as a new pathotype, adherent invasive *E. coli* (AIEC), in the context of detection, diagnosis. The most common EHEC serogroup is O157:H7 and has been the subject of many studies, especially for molecular mechanisms of pathogenesis. While STEC O157:H7 has been classified as an adulterant in beef since 1994, the U.S. Department of Agriculture (USDA) has recently declared 6 more EHEC serogroups, i.e., O26, O45, O103, O111, O121, and O145 (Anonymous, 2012), as they are the most commonly found non-O157 STEC strains associated with severe illness in humans.

2.9.6.2 Features of Escherichia coli

E. coli is a Gram-negative, oxidase-negative, rod-shaped bacterium from the family *Enterobacteriaceae*. It is able to grow both aerobically and anaerobically, preferably at 37°C, and can either be non-motile or motile, with peritrichous flagella. *E. coli* is readily isolated from environment samples by plating on selective media. The change in pH due to lactose fermentation can be used to differentiate between lactose-fermenting and non-lactose-fermenting strains, as lactose-positive *E. coli* colonies will appear red or pink on media such as MacConkey agar. Not all *E. coli* strains, particularly *Escherichia coli* belong to the large group of Gram negative rods referred to as enterobacteria. They are naturally found in the intestinal tract, in soil and water.

2.9.6.3 Shiga toxin

Shiga toxin is the main characteristic that defines STEC and is the key virulence factor in STEC causing hemolytic uremic syndrome (HUS). Due to its clinical significance and ability to cause disease, it has been the subject of many investigations. Shiga toxins can be classified into two types, Stx1 and Stx2, with

Stx1 having 3 subtypes (a, c, and d), and Stx2 having 7 (a to g) (Scheutz *et al*., 2012). STEC can carry a single variant, stx1 or *stx*2, both *stx*1 and *stx*2, or a combination of *stx*2 subtypes (e.g., *stx*2a and *stx*2c). Both *stx*1- and *stx*2-containing STEC can lead to HUS; however, *stx*2 is more often associated with severe disease (Boerlin *et al*., 1999). Both Stx1 and Stx2 are encoded on prophages that are integrated into the chromosome. Shiga toxin-carrying phages can become lytic during bacterial stress, and it is believed that Stx1/Stx2 is released from lysed bacterial cells during the lytic cycle of the phage (Neely and Friedman, 1998).

2.9.6.4 Lineages

There are four different clonal lineages of STEC: EHEC 1, which includes O157:H7 and SFO157:NM; EHEC 2, which contains non-O157 STEC serotypes such as O111:H8 and O26:H11; STEC 1, which contains LEE-negative STEC serotypes such as O113:H21 and O91:H21; and STEC 2, which includes serotypes O45:H2 and O103:H2/H6 (Whittan *et al*., 1993). STEC O157:H7 strains have been further classified into 2 different lineages (lineages I and II), based on lineage-specific polymorphism assay 6 (LPSA-6) (Yang *et al*., 2004), and 9 different clades, based on single nucleotide polymorphism (SNP) analysis (Manning *et al.,* 2008).

2.10.6.5 Incidence of STEC

In the United States, there were 463 STEC O157:H7 (0.97 per 100,000 populations) and 521non-O157 STEC (1.10/100,000) cases reported by FoodNet in 2011 (CDC, 2012). Hospitalization rates due to these cases were over 2-fold higher for STEC O157:H7 (43.4%) than for non-O157 STEC (18%). Similarly, the case-fatality rate for O157:H7 was about 2-fold higher than that for non-O157 STEC. What is promising is the fact that the incidence of STEC O157:H7 has dropped 42% in 2011 compared to the incidence in 1996 to 1998 (CDC, 2012). Increased incidences of non- O157 STEC were identified in a statewide study in Washington between 2005 and 2010; the authors suggest that this increase may be due to changes in testing (Stigi *et al.,* 2012). The incidence in Canada, reported by the National Enteric Surveillance Program (NESP), has also improved for STEC O157:H7 cases in 2010 (1.18/100,000) compared to the incidences in 2005 (2.28/100,000) and 2006 (3.0/ 100,000) (NESP, 2010). NESP reported that the non-O157 STEC incidence has not changed in the last 10 years. In Australia, the overall incidence of

STEC illness from 2000 to 2010 was reported to be 0.4/100,000, with a slight increase in incidence over this time (Vally *et al*., 2012). In Europe, the European Center for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) report on STEC incidences from 24 European Union member states; they reported that the overall incidence of STEC in 2009 for the European Union was 0.75/100,000. Ireland and Denmark had incidence rates significantly higher than those in other countries (5.33 and 2.90/100,000, respectively) (ECDC and EFSA, 2011). It should be noted that the ECDC/EFSA recommends not comparing incidences between countries due to differences in detection.

2.10 Antimicrobial Resistance (AMR)

Antimicrobial resistance refers to the resistance of microorganisms to an antimicrobial agent to which it was previously sensitive. It is a consequence of the use, particularly the misuse of antimicrobial medicines and develops when a microorganism mutates or acquires a resistance gene. Inappropriate and irrational uses of antimicrobial medecines provide favourable conditions for resistant microorganisms to emerge, spread and persist (WHO, 2011).

AMR is considered one of the most important global threats to human health in the 21st century (Liu *et al*., 2016). Many factors contribute to the emergence of antimicrobial resistant microorganisms, but the use of antimicrobials in medicine and agriculture is considered the most important factor (United State Food and Drug Administration, 2015). AMR threatens the effective prevention and treatment of an ever increasing range of infections caused by bacteria, parasites, viruses and minor injuries can kill and is a real possibility in the 21st century. Often antibiotic resistance mechanism/genes acquired by bacteria are associated with mobile genetic elements (MGEs) that mediate their exchange

between pathogens, or between commensal and pathogenic bacterial populations (Woolhouse and Ward, 2013; Beatson and Walker, 2014).

During the past decade, the increase and spread of antibiotic resistance has become a major concern worldwide (Kwaga, 2016). Antibiotic resistant bacteria are estimated to kill 23,000 people each year in the United States of America (Kennedy, 2013). Globally about 700,000 people die of resistant infections each year, and by 2050 it is projected that 10 million lives annually and cumulatively 100 trillion of US dollars of economic output will be at risk due to rise of drug-resistant infection and reducing global GDP by up to 3.5% (The O'Neil Report, 2016). While resistance to single antibiotic occurs, development of multi-drug resistant bacterial pathogens has made treatment of the disease they cause more difficult (Carraminana *et al.,* 2004; Okeke *et al*., 2007). Infections caused by these resistant pathogens have resulted in significant morbidity and mortality and escalating health care cost worldwide (Sivakumar *et al.,* 2012; Sule *et al*., 2012). Dealing with resistant infections, currently costs the U.S economy $20 billion annually (The Economist, 2016). The emergence and rise of antibiotic resistance worldwide, cannot be explained only by the increasing modern use of antibiotics in humans, but involves a complex interaction in an ecosystem comprising microbial communities, antibiotics and antibiotic resistance genes (Rolain *et al*., 2012; Thanner *et al*., 2016).

2.10.1 Resistance is an Emerging Problem

Resistance to antimicrobials and particularly multidrug resistance is an emerging problem in Enterobacteriaceae and Vibrionaceae for developing and developed countries (Schwarz and White, 2005; Odeyemi, 2012). Resistant microorganisms have emerged as a result of improper use of antibiotics in human health as well as in agricultural practices (Khachatourians, 1998). For example, in United States it has been reported that most of

the antibiotics produced are fed to farm animals as growth promoters and to obtain a better meat to feed ratio (Goldman, 2004). In the pork and poultry industry low levels of bacitracin, chlortetracycline, erythromycin, lincomycin, neomycin, oxytetracycline, penicillin, streptomycin, tylosin or virginiamycin are administrated in each ton of feed (Khachatourians, 1998). Over the time these low doses of antimicrobials confer the ability of microorganisms to evolve mechanisms of defense, therefore making them less susceptible to the effect of the drug and contributing to treatment failure.

Salmonella, E. coli and Vibrio cholerae have been widely documented to possess resistance to several antibiotics used in medical treatment. For instance, antibiotic-resistant *Salmonella* accounted for an annual mortality estimate of 4,760 deaths in the U.S alone (Khachatourians, 1998). Antibiotic resistance has an important social and economic impact, and there is a need for stronger scientific and public health efforts to better regulate, control and monitor the use and abuse of antimicrobials (Helmuth, 2000). Due to the concern over increasing resistance, the CDC, FDA-CVM, (Center for Veterinary Medicine) and USDA-FSIS (Food Safety and Inspection Service and Agricultural Research Services) established the National Antimicrobial Resistance Monitoring System (NARMS) for Enteric Bacteria to monitor antimicrobial resistance among foodborne enteric bacteria isolated from humans and foods (CDC, 2009). In 2006, the NARMS published a list containing the categories of antimicrobials of importance for human health (Table 2.6); antimicrobials in this list are classified based on whether the evaluated antimicrobial is unique or one of the few alternatives for treatment of human diseases (NARMS, 2006). This report also details two multidrug resistant patterns. 5.5 % of non-Typhi *Salmonella* are thought to be resistant to sulfamethoxazole/sulfisoxazole, ampicillin, chloramphenicol, streptomycin, and tetracycline (ACSSuT) (NARMS, 2006).

Table 2.6: World Health Organization's Categorization of Antimicrobials of Critical Importance to Human Medicine

	Categorization of Antimicrobial	Subclass
Critically Important	Aminoglycosides	Amikacin
		Gentamicin
		Streptomycin
	Aminopenicillins	Ampicillin
	B-Lactamase inhibitor combinations	Amoxicillin-clavulanic acid
	Cephalosporins(3rd generation)	Ceftriaxone
	Ketolides	Telithromycin
	Macrolides	Azithromycin
		Erythromycin
	Quinolones	Ciprofloxacin
		Nalidixic acid
Highly Important	Aminoglycosides	Kanamycin
	Cephalosporin (1st generation)	Cephalothin
	Cephamycins	Cefoxitin
	Folate pathway inhibitor	Trimethoprim-sulfamethoxazole
	Phenicols	Chloramphenicol
	Sulfonamides	Sulfamethoxazole
		Sulfisoxazole
Important	Tetracyclines	Tetracycline
	Lincosamides	Clindamycin

Source: (NARMS, 2006).

This percentage is lower than the 8.8% observed in 1996 due to the overall reduction of resistance in some serovars. However other *Salmonella* serovars appear to be acquiring resistance to these antibiotics, such as *S.* Newport. Evaluated in 2006, the resistance of this serotype increased 6% from 1996. According to the CDC (2009), a second multidrug resistant pattern (ampicillin, streptomycin, chloramphenicol, sulfamethoxazole or sulfisoxazole, tetracycline, amoxicillin clavulanic acid, and ceftiofur) was not detected in any serotype in 1996. In 2006, a 2% increase was observed among non- typhi *Salmonella* serovars (NARMS, 2006).

2.10.2 Mechanism of Action of Antibiotics

Ampicillin is part of the beta- lactam antibiotics, and belongs to the penicillin group. Its main difference with other beta-lactams is the presence of an amino group. The interaction of penicillin-binding protein with the bacterial cell wall results in the disruption of synthesis of the bacteria cell wall (Brook, 2009). The principal mechanism for β –lactam antibiotic resistance is the acquisition or hyperexpression of β-lactamases (Schwacz and White, 2005, Brook, 2009).

The use of chloramphenicol in the European Union and North America is exclusively for non-food-producing animals (Guardabassi and Courvalin, 2006). Chloramphenicol blocks the formation of the peptide bond between amino acids by inactivating the peptidyltransferase reaction, and this mechanism of action makes chloramphenicol a highly effective protein synthesis inhibitor (Schwacz and White, 2005). Enzymatic inactivation by chloramphenicol acetyltransferase is the leading mechanism of resistance to chloramphenicol in both gram-positive and gramnegative bacteria (Schwacz and White, 2005; Guardabassi and Courvalin, 2006). In addition, other mechanisms have been

proposed and include: target site mutations, permeability barriers, phosphotransferase inactivation and some effux systems (Schwacz and White, 2005).

Ciprofloxacine is part of the quinolone antibiotics. It is mostly effective against gram-negative bacteria by inhibiting a subunit of DNA gyrase. This has been demonstrated to be the mechanism of action of all antimicrobials belonging to the quinolone group (Helmuth, 2000; Emmerson and Jones, 2003). Antibiotic resistance occurs when an altered target is developed (Helmuth, 2000).

Gentamicin belongs to the aminoglycosides group. Amyglycosides target bacterial ribosome (30S unit) which results in the misreading of the genetic code during synthesis of protein resulting in growth inhibition (Helmuth, 2000; Schwacz and White, 2005). Modifying enzymes and reduction in uptake are the main resistance mechanism developed by bacteria resistant to this drug (Helmuth, 2000).

Cotrimoxazole belongs to the sulfonamides group and these antimicrobials work by affecting the DNA, RNA and protein synthesis. The major factors responsible for the resistance are the permeability barrier and/or efflux pumps, natural insensitivity target enzymes and changes in the target enzymes (Mascaretti, 2003)

Tetracycline inhibits microbial growth by inhibiting the elongation step of protein synthesis. When it forms a complex with a divalent cation in the cytoplasm, the antibiotic binds reversibly to the 16S ribosomal RNA (rRNA) of prokaryotes near the ribosomal acceptor a site, thus preventing binding to aminoacyl-tRNA to this site. Four mechanisms have been identified to confer resistance:

i) energy-dependent efflux (this mechanism does not allow tetracycline to get into the cytoplasm) ii) Tetracycline molecule inactivation iii) rRNA mutations and iv) Ribosomal protection (Schwacz and White, 2005).

Tetracycline's active efflux is the major mechanism of bacterial resistance. Transporter proteins, located in the cytoplasmatic membrane, mediate energy-dependent efflux of the tetracycline, allowing tetracycline- resistant cells to lose the accumulated drug faster than susceptible cells do (Mascaretti, 2003). These proteins are encoded by naturally occurring genetic units which include all genes involved in resistance (tetracycline determinants). These genetic units confer resistance by removing tetracycline from the cytoplasmatic matrix (Mascaretti, 2003).

Continuous monitoring of pathogens and their acquisition of antibiotic resistance genes is important to human health, but few laboratories testing for the presence of pathogens in food samples are able to provide this relevant information (FAO, 2010). Therapeutic options become limited when multidrug resistant pathogens are encountered, which constitutes an emerging public health issue worldwide (Grimont and Weill, 2007).

2.11 Molecular Methods for Detection of Bacteria

Molecular methods have been investigated to provide increased specificity and sensitivity for the detection of low cell numbers compared to culture based methods (Harwood *et al.*, 2004). Molecular methods are mostly independent of culturability of bacteria, and require no additional conformational steps (Mumy and Findlay, 2004). Among the different molecular methods, PCR has proved very useful for detecting low amounts of a specific DNA against a large background of prokaryotic and eukaryotic cells, and organic materials present in environmental samples (Lleo *et al*., 2005). DNA has to be isolated and purified before it is amplified with PCR. Bacterial DNA purification consequently becomes an indispensable preliminary step for any PCR reaction (Burtsher *et al*., 2003). The availability of effective DNA extraction methods is essential for environmental microbiology methods (Lemarchand *et al.*, 2005).

2.11.1 Polymerase Chain Reaction (PCR)

PCR is a rapid, oligonucleotide primer-directed *in vitro* method for replicating defined DNA sequences from target organisms (Lee *et al.,* 2009). The Polymerase Chain Reaction has been used to detect a wide variety of microorganisms directly in clinical specimen including bacteria in blood, cerebrospinal fluid (CSF), and tissue. It is also been used to characterize antimicrobial resistance genes and virulence genes in bacterial isolates. PCR has been used to determine the evolutionary relationship of bacteria by analyzing nucleotide sequences of various genes, including 16S/23S rRNA, housekeeping genes, and invasion genes (Fukushima *et al*., 2002). In particular, 16S rRNA sequences have been widely used to construct bacterial phylogenetic relationship or to detect pathogenic bacteria. This can be a single gene, just a part of a gene, or a non-coding sequence. Most PCR methods typically amplify DNA fragments of up to 10 kilo base pairs (kb). Some PCR methods can copy DNA fragments of up to 40kb in size which is still much less than the total nuclear DNA content of eukaryotic cell – for comparison, the haploid genome of a human cell consists of about three billion DNA base pairs (3Gb). With the development of the polymerase chain reaction technique, it is now possible to amplifiy any piece of DNA.

PCR, as currently practiced, requires several basic components. As a consequence of the speed, specificity and sensitivity of the PCR, the procedure has become one of the most widely used assays for direct detection of low levels of pathogenic microbes in environmental samples as opposed to the cultural methods (Theron and Cloete, 2004).

The efficiency of PCR detection however depends on several factors. The selection of an appropriate DNA extraction method and the pre-PCR procedures are very crucial factors (Chomvarin *et al*., 2007). Since PCR analyses are based on detection of intact nucleic

acids rather than intact viable cells, the possibility exist that positive pathogen PCR amplifications may arise from either dead cells or non-infectious cells (Chomvarin *et al.*, 2007). It will also not be possible to assess the viability of the detected bacteria.

PCR primers have been reported that allow for specific detection of a range of targets which include species, serogroup and virulence genes. Table 2.6 indicates some of the reported gene targets for detection of species, serotype and virulent *Salmonella* spp, *E. coli* O157:H7, and *Vibrio cholerae*.

2.12 Virulence Genes in Bacteria

Reflecting a complex set of interactions with their host, most pathogenic bacteria require multiple genes for full virulence. Many of these genes are found in the 'pathogenecity islands' in the chromosome, which confer specific virulence traits and may have been acquired by horizontal transfer from other organisms. Collectivelly, these packages of virulence cassettes together with a highly conserved plasmid are essential for instance for *Salmonella* pathogenicity (Marcus *et al*., 2000). A pathogenicity island is considered to be a stable insertion of a large region of DNA, containing virulence genes, into a site in the bacterial chromosome. This region usually contains complex virulence determinants, which appear to be acquired in a single step. Table 2.7 are example of some bacterial virulent genes reported in literature**.**

2.13 Isolation of Genomic DNA (gDNA)

The isolation and purification of DNA from cell is one of the most common procedures in contemporary molecular biology and embodies a transition from cell biology to the molecular biology; from *in vivo* to *in vitro*, as it were. The purification method must eliminate any contaminant that would interfere with subsequent steps. This depends,

Table 2.7: Some Genes Used that have been Used to Detect *Salmonella* spp., *E. coli* O157:H7and *Vibrio cholerae*

Type of species	Gene name	Function	Reference
***Salmonella* spp.**	lnvA	Inner membrane protein	Smith *et al*. (2015)
	Stn	Putative virulence factor	Masayuki *et al*. (2012)
***E. coli* O157:H7**	Stx1	Virulence factor	Tahamtan *et al*. (2010)
	Stx2	Virulence factor	Kargar and Homayoon (2015)
	hylA	Plasmid-encoded Haemolysin	Kargar and Homayoon (2015)
Vibrio cholerae	toxR	Central regulatory protein	Singh *et al*. (2002)
	ompW	Outer membrane protein	Alam *et al*. (2006)

of course, on what is planed to do with the DNA once isolated. PCR will tolerate a reasonable degree of contaminant so long as the contaminants do not inhibit the thermostable DNA polymerase or degrade DNA. DNA isolation methods are designed to break cells and denature proteins using specific reagents. The phenol/chloroform reagent, due to the cost of hazardous waste disposal, is not only dangerous but expensive. Hence many laboratories are deviating from previous procedure which involves the use of phenol/chloroform.

2.13.1 Lysis

Cell walls and membranes must be broken to release the DNA and other intracellular components. This is usually accomplished with an appropriate combination of enzymes to digest the cell wall (usually lysozyme) and detergents to disrupt membranes. The ionic detergent Sodium Dodecyl sulphate (SDS) could be used to lyse *E. coli* at 80^{o}C.

2.13.2 Removal of Protein, Carbohydrate, RNA

The Ribo Nucleic Acid (RNA) is usually degraded by the addititon of RNAase. The resulting oligoribinucleotides are separated from the DNA on the basis of their higher solubility in non-polar solvents (usually alcohol/water). Proteins are subjected to chemical denaturation and/or enzymatic degradation. Varieties of DNA isolation kits have been used such as ZR Fungal/bacterial DNA kit from Zymo Research Corp, ZR Genomic DNA TM- Tissue MiniPrep kit, Qiagen DNeasy Kit, etc.

2.14 PCR as a Tool in Bacterial Taxonomy

The Polymerase Chain Rection (PCR) is used to amplify specific region of a DNA strand. This can be a single gene, just a part of a gene, or a non-coding sequence. Most PCR methods typically amplify DNA fragments of up to 10 kilo base pairs (kb). Some PCR methods can copy DNA fragments of up to 40kb in size which is still much less than the total nuclear DNA content of eukaryotic cell – for comparison, the haploid genome of a human cell consists of about three billion DNA base pairs (3Gb).

With the development of the polymerase chain reaction technique, it is now possible to amplifiy any piece of DNA. PCR, as currently practiced, requires several basic components.

The PCR is carried out in small reaction tubes (0.2-0.5 ml volumes), containing a reaction volume typically of 15-100ul, that are inserted into a thermal cycler. This is a machine that heats and cools the reaction tubes within it to the precise temperature required for each step of the reaction. Information about the DNA sequences flanking the region of interest is used in the design of primers that are required for the polymerase enzyme at the start of the *in vivo* DNA amplification process. The resulting PCR fragment is to be cloned; one can expedite the cloning steps by adding DNA sequences that are recognition sites for specific restriction enzymes in the primers.

2.15 Identification of Bacteria using 16S rRNA Gene

Molecular techniques, especially those based on comparative analysis of 16S rRNA gene sequences, have been widely used and proven to be effective for studying prokaryotes in nature. To date, very few molecular studies of microbial diversity in full-scale landfills have been conducted (Huang *et al*., 2002; Chen *et al*., 2003; Huang *et al*., 2004).

Ribosomal RNA (rRNA), the central component of the ribosome, the protein manufacturing machinery of all living cells, is the most conserved (least variable) gene in all cells and has little lateral genetic transfer (Marsh, 1999). For this reason, genes that encode the rRNA (rDNA) are sequenced to identify an organism's taxonomic group, calculate related groups, and estimate rates of species divergence.

Many thousands of rRNA sequences are known and stored in specialized databases such as GenBank, Ribosomal Database Project (RDP-II) (Cole *et al.*, 2003) and the European small subunit (SSU) ribosomal database (Wuyts *et al.*, 2002). A 70S ribosome is composed of 30S and 50S subunits. About two thirds of the mass of 30S subunit is 16S rRNA which comprises about 1500 nucleotides. 16S-rRNA is the main structural component of 30S ribosome. Many methods for investigating microbial diversity are based on 16S-rRNA gene sequence analysis. Although highly conserved between bacteria, the selected regions of the 16S are unique to bacteria and their presence can be used to define an organism as a bacterium. PCR primers can therefore be designed to anneal to conserved regions within 16S rRNA and will amplify the RNA from a wide variety of sources. However, sequence variations within other regions of the 16S rRNA from different bacteria do exist. These permit more detailed taxonomic classification of different bacteria, including speciation (Relman, 1993). The amplified sequences are analyzed by several methods, including cloning and sequencing.

The results of 16S-rRNA-gene-sequence studies have provided the strongest evidence that the microbial populations in nature contain many surprises (Amann *et al*., 1995; Amann and Kühl, 1998) as many new sequence types are found that do not correspond to sequences in the databases (Hugenholtz and Pace, 1996). Molecular analysis of microbial communities has provided evidence that unexploited microbial diversity exists in many

environments (Amann *et al.,* 1995; Delong, 1996; Hugenholtz and Pace, 1996). In as much as the molecular techniques will undoubtedly detect numerous unique microbes, it is still considered complementary to culturing technique. None of the two approaches can be considered to be superior to the other (Brambilla *et al*., 2001; Dees and Ghiorse, 2001).

2.16 GenBank

Convergence of complementary technologies, exemplified by DNA amplification and sequencing, genome sequencing and annotation, proteome analysis, and phenotypic inventorying has resulted in the establishment of huge databases (Benson *et al*., 2004). These databases can be mined in order to generate useful information such as the identification and characterization of organisms and the identity of biotechnology targets (Dahl *et al.,* 2005). The knowledge from complementary databases can be used to facilitate answers to complex questions involving sequence, biochemical, physiological, taxonomic, and ecological information of the sort poised in exploitable biology. GenBank (Benson *et al.*, 2004) is a comprehensive public database of nucleotide sequences and supporting bibliographic and biological annotation, built and distributed by the National Center for Biotechnology Information (NCBI). NCBI builds GenBank primarily for the submission of sequence data from authors and from the bulk submission of expressed sequence tag (EST), genome survey sequence (GSS) and other high-throughput data from sequencing centers. Direct submissions receive a quality assurance review that includes checks for vector contamination, proper translation of coding regions, correct taxonomy and correct bibliographic citations.

GenBank incorporates sequences submitted to the EMBL data library (Stoesser *et al.,* 2003) in the UK and the DNA Data Bank of Japan (DDBJ) (Miyazaki *et al.*, 2003) to

ensure a uniform and comprehensive collection of sequence information. Each GenBank entry includes a concise description of the sequence, the scientific name and taxonomy of the source organism, bibliographic references and a table of features (listing areas of biological significance, such as coding regions and their protein translations, transcription units, repeat regions and sites of mutation or modification). The sequence records in GenBank are accessible via Entrez, a robust and flexible database retrieval system that accesses DNA and protein sequence data, genome mapping data, population sets, phylogenetic sets, environmental sample sets, gene expression data, the NCBI taxonomy, protein domain information, protein structures from the Molecular Modeling Database, MMDB (Chen *et al.,* 2003) and MEDLINE references via PubMed. NCBI offers the BLAST (Basic Local Alignment Search Tool), family of programs to locate regions of similarity between a query sequence and database sequences (Altschul *et al.,* 1997). BLAST is a set of programs designed to perform similarity searches on all available sequence data. Scientists frequently use such searches to gain insight into the function and biological importance of gene products (Altschul *et al.,* 1990; NCBI BLAST Information, 2003). BLAST searches may be performed on the NCBI‟ s website. Specialized sequence analysis programs including multiple sequence alignment and inference of phylogenies using CLUSTALW (Thompson *et al*., 1994) are among interactive sequence analysis resources based on the European Molecular Biology Open Software Suite (EMBOSS) (http://www.emboss.org/) which are also freely accessible.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Zaria Metropolis is located on the high plains of Northern Nigeria, 652.6 meters above the sea level, and 950 km away from the coast. It lies approximately on latitudes 11°07□ N to11°51□ Nand longitudes 7°43□ E to 7°45□ Eand is presently one of the most important cities in Northern Nigeria (Uba *et al.,* 2013). It has a total land area of 300km^2 and constitutes four major settlements; Zaria City, Tudun Wada, Sabon Gari and Samaru (Zaria at a glance, 2013). It is a very large, heterogeneous city with a population of 975,228. Zaria is second in size only to the State capital, Kaduna. It has attracted such a large population partly because of the presence of many educational institutions which attract people all over Nigeria for academic and employment purposes. It may also be due to a lot of economic and social activities. For instance, Zaria is home to Ahmadu Bello University (A.B.U), the largest University in Nigeria and the second largest on the African continent, the base for the Nigerian College of Aviation Technology (About us NCAT, 2010).

Other important Institutions in Zaria are Nigerian College of Aviation Technology, Federal College of Education, Nigeria Institute of Leather Research Technology. This leads to further increase in population size. This has created a steady growth in population and human activities resulting in changes in the population's consumption patterns. Consequently, there is more heterogeneous composition of solid waste and an immense increment in its generation rate as waste is directly linked to human development. However, there is no government authorized waste disposal infrastructure in Zaria (Arigbede and Yusuf, 2010).

Zaria Metropolis is characterized by a tropical continental climate with two main seasons; a pronounced dry season, lasting up to seven months (October to May) During this season, a cool period is usually experienced between November and February. This emanates from the influence of the North-eastern winds (the Harmattan) which control the tropical continental air mass coming from the Sahara. The wet season lasts from May to September/October, with long-term annual rainfall of 1,040mm in about 90 rain days (Zaria at a glance, 2013). Figure 1 shows solid waste disposal dump sites in Zaria Metropolis.

3.2 Study Design

This study was a cross sectional study. Each section in each settlement was captured to cover the metropolitan and a comparative analysis was done and prevalence recorded.

3.3 Sample Size

The sample size for this study was determined using the prevalence of 17.2% as reported by ((Wachukwu *et al*., 2010).

$$n = \frac{Z^2 pq}{L^2}$$ Where, Z = 1.96 for 95% confidence interval

p = Prevalence rate

q = 1 – p

L= Allowable error (5%)

n= Number of samples

$$= \frac{(1.96)^2 \times 0.172 \times 0.828}{(0.05)^2}$$

= 219

For the purpose of this research, 260 soil samples were collected during dry season and 260 samples during wet season in all the settlements in Zaria Metropolis.

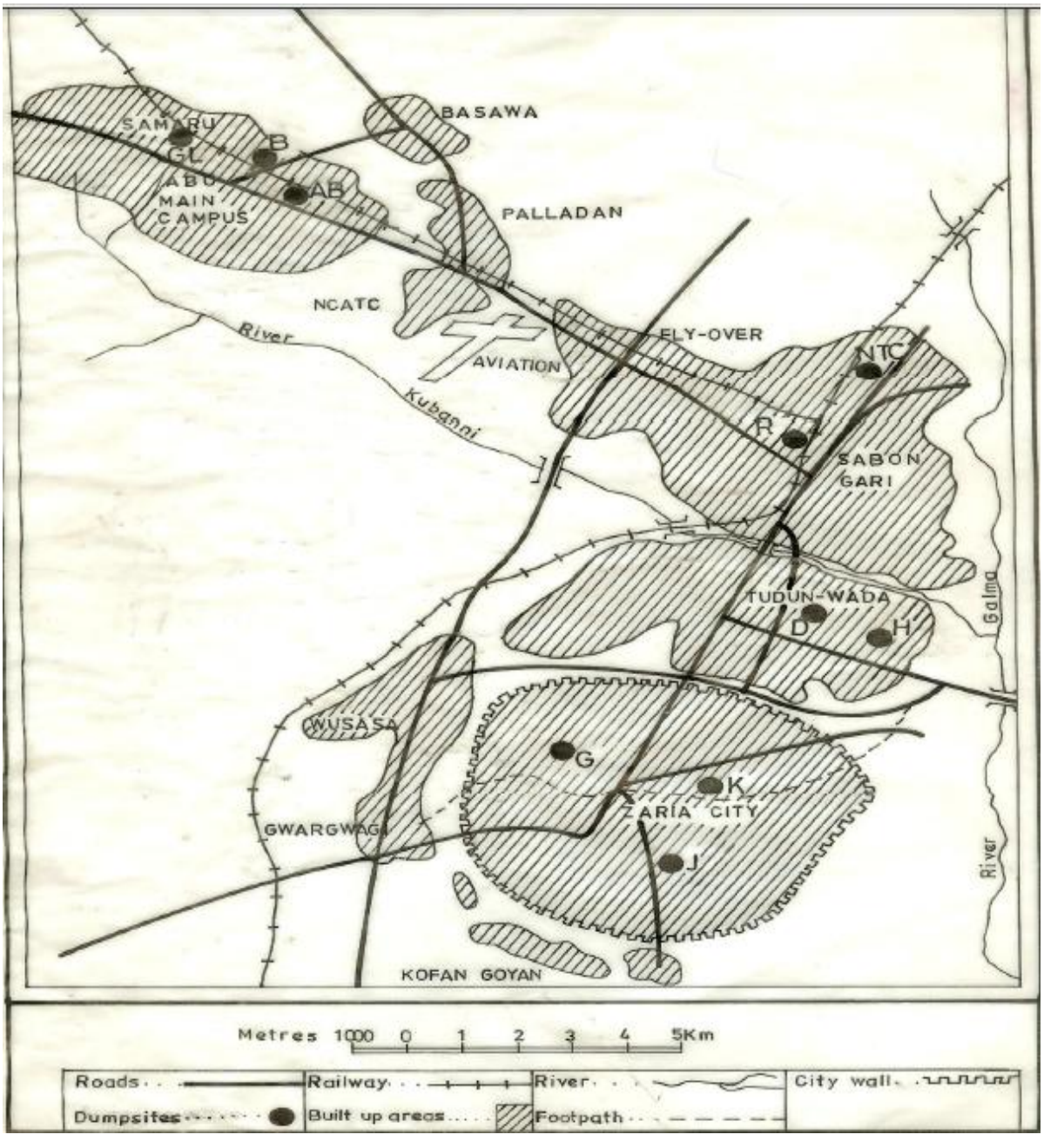


Figure 3.1: Solid Waste Disposal Dump Sites in Zaria Metropolis

Source: Modified from Zaria Topographic map and field work 2005

3. 4 Study Locations

The soil samples were collected from four refuse dumpsites in each location in Zaria Metropolis, as well as from an area free of waste (Control Site). The sampling site located in Zaria City is referred to as, location ZC, the second waste dump site is located in Tudun-Wada (Settlement TW), location SG is in Sabon-Gari, while the fourth waste dump site is located in Samaru (location SA), the last sampling site is situated in Kabama area and was used as the Control Site (CS).

3.5 Solid Waste Composition from Different Locations in Zaria Metropolis

The composition of solid wastes found in SG and SA included papers and cartons, food remnants, glass and bottles, plastic and polythene, tin and metals, ashes and dust, textile and rags, aluminium and other minerals (Plate I & II).

Waste at TW dump sites include both degradable (paper wastes, food and agricultural wastes, sewage etc) and non biodegradable wastes (plastics, nylon, aluminum and other metal containing substances) (Plate III).

Location ZC is composed of the following solid wastes: plastics (straws, plastic paper bags), cardboard, dry leaves, Styrofoam, tin cans, paper, wood, ash, sacks, glass bottles, batteries, clothes, food wastes, diapers , disposable napkins and sanitary towels, clinical waste as hypodermic needles and syringes, domestic human origin wates and large amount of human and live-stock faeces (Plate IV).

The Control Site (CS) did not contain any waste at all; it however supported a little vegetation.



Plate I: Solid Waste Dump Site at Rail Way tract at Yan Katako, Sabon-Gari



Plate II: Waste Dump Site at Fly-over bridge behind the Motor Park, Kwangila



Plate III: Waste dump Site at Agoro in Tudun-Wada



Plate IV: Kusfa Solid Waste Dumpsite around residential building in Zaria City

3.6 Culture media

Isolation media commonly used for the isolation of enteric bacterial pathogens were employed in this study. They consisted of *Salmonella Shigella* Agar (SSA) and Xylose Lysine Desoxycholate (XLD) for the isolation of *Salmonella*; Thiosulphate-Citrate-Bile-Salt-Sucrose (TCBS) agar used for the isolation of *Vibrio cholerae* and Eosin Methylene Blue (EMB) agar for the isolation of *Escherichia coli* enrichment broths (Selenite F Broth , Alkaline peptone water and Tryptic soy Broth) (Obi *et al*., 2003).

3.7 Collection of Sample

A total of 520 soil samples were collected within the perimeter of refuse collection points as adopted by Isirimah *et al*. (2005). Sampling was designed such that 260 soil samples were collected in 6 months (November, 2014 to May, 2015) in dry season and in 6 months (June, 2015 to December 2015) in wet season. For the five study sites, 52 samples each were collected at 3days interval per week from October 2014 to November, 2015. A total of 16 refuse dump sites were selected. Four (4) refuse dumps were located in each settlement to cover the metropolitan and a Control/uncontaminated site was located in Kabama in an area without dumping activities.

At each sampling site, surface debris was removed and soil was dug to a depth of 15cm using a hand trowel. Soil was then scooped into a sterile low density polythene bag and transported in cool boxes to the Laboratory in the Department of Microbiology, Ahmadu Bello University, Zaria for analysis. Samples were stored at 4^{o}C if not analysed immediately.

3.8 Determination of Temperature and pH

Soil physicochemical analysis was carried out to determine Temperature (^{o}C) and pH *in situ* using thermometer and pH meter respectively.

3.8.1 Soil Temperature

Using mercury in-glass thermometer (10^{o}C-110^{o}C), waste dump soil temperature was determined by directly dipping thermometer bulb into the soil. Temperature (^{o}C) readings were collected in duplicates at each sampling spot, and recorded.

3.8.2 pH

Ten grams of the soil sample was suspended in 10ml distilled water and manually stirred using a flame sterilized glass rod. The resultant mixture was then allowed to stand for 30mins at room temperature. Using standardized pH meter (Orion 3 Star model No 115220 VAC) calibrated with buffer (pH 4 and 9), the electrode was inserted into the mixture and the reading taken and recorded.

3.9 Media preparation

The media were prepared according to manufacturer's instructions. Briefly, enrichment medium for *Salmonella* was prepared by first dissolving 19.0g of Selenite F Broth powder in 750ml distilled water. Four grams (4g) of Selenite F Broth was dissolved separately in 250ml distilled water. The resultant solutions were mixed together, then warmed to dissolve and distributed into test tubes. Sterilization of the prepared broth was carried out by heating to boil using a burnsen burner flame for 10mins

Xylose Lysine Desoxycholate (XLD) agar for the isolation of *Salmonella* was prepared by suspending 55.2g of Xylose Lysine Desoxycholate (XLD) agar in 1liter of distilled

water. The mixture was then brought to boil using heating mantle with frequent agitation until completely dissolved.

Alkaline peptone water was used as enrichment medium for *Vibrio* spp and was prepared by dissolving 30g of alkaline peptone water powder in 1liter of distilled water. The mixture was then dispensed in test tubes and wasSterilized by autoclaving at 121^{o}C for 15 minutes. Preparation of Thiosulphate-Citrate-Bile-Salt-Sucrose (TCBS) agar was carried out by suspending 89g of Thiosulphate-Citrate-Bile-Salt-Sucrose (TCBS) agar powder in 1 litre of distilled water and boiled to completely dissolve.

The culture media for *E. coli* strains were prepared by dissolving 30g of Tryptic soy broth in 1liter of distiled water.The mixture was then dispensed in test tubes and sterilized by autoclaving at 121^{o}C for 15mn. Eosin Methylene Blue (EMB) agar was prepared by dissolving 36g of in 1000ml distilled water and heat gently to completely dissolve.

3.10 Bacteriological Analysis

3.10.1 Enumeration of Aerobic Bacteria

For each sample, 25g of soil was suspended in 225ml sterile distilled water, and mixed using a flame sterilized glass rod. The resultant stock solution was then serially diluted ten fold by taking 1ml into 9ml sterile peptone water, up to a dilution of 10^{-8}. Then 0.1ml of the mixture was spread on the surface of Nutrient agar amended with Nystatin (1mg/ml) to suppress the growth of fungi

and Brain Hearth Infusion (BHI) agar using a sterile glass rod; and then incubated at 37^{o}C for 24h. Distinct colonies formed were then counted and expressed in colony forming units per gram of soil (cfu/g).

3.10 2. Isolation of *Salmonella* species

For the isolation of *Salmonella* species, 1ml of the stock solution was inoculated in 9ml selenite-F-broth and incubated at 37^{o}C for 24h for enrichment. The enriched samples were then plated on XLD and *Salmonella-Shigella* Agar (Oxoid) and incubated at 37^{o}C for 24h. Small red and /or colourless colonies with black centres were further subcultured on Nutrient agar.

3.10.3 Isolation of *Vibrio* species

For *Vibrio* species, 1ml soil dilution was enriched in 9ml alkaline peptone water (pH 8.6) and incubated at 37^{o}C for 8h. A loopful of the enriched peptone water culture (taken from the surface) was streaked on Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar (Difco) and incubated at 37^{o}C for 24h. Distinct yellow (sucrose -fermenting) and blue-green (non-sucrose fermenting) colonies ranging from 2mm to 3mm in size were subcultured on nutrient agar plates to obtain pure cultures. Pure cultures were then preserved on Nutrient agar slants.

3.10.4 Isolation of *Esherichia coli* O157:H7

E. coli O157:H7 was isolated from the soil samples as described by Stigi *et al*. (2012). One ml of each suspension was added to a tube containing 9ml sterile tryptone soy broth. The tube was then incubated at 44^{o}C for 24 hours. After this, a loopful of the broth was streaked on plates of Eosin Methylene Blue (EMB) agar and incubated at 44^{o}C for 24 hours. Mixed cultures were re-streaked on sterile EMB agar plates until pure colonies characterized by greenish sheen, small with dark centres were obtained (Stigi *et al*., 2012).

3.10.5 Characterization of bacteria

3.10.5.1 Colonial morphology

The distinct colonies were differentiated using the conventional morphological criteria based on colour, size, margin, elevation, consistency, transparency and sheen. Microscopy and Gram's stain were performed to determine the cellular morphology and Gram reaction of the bacteria. This further distinguished the bacterial isolates into Gram positive and negative groups with their respective cellular structures and aggregations.

3.10.5.2 Gram's Stain

On a clean dry slide, a drop of sterile distilled water was placed and a smear of the test organism was prepared by emulsifing a loopful of the bacterial culture on the slide. It was then allowed to air dried and heat fixed by passing through a burnsen flame. This was stained with crystal violet (primary stain) and allowed to stand for 60 seconds, followed by addition of Lugol's iodine and allowed for another 60 seconds. It was washed off under slow running tap water. Then decolorized with 95% alcohol for 3 seconds and was immediately rinsed with the slow runing tap water. It was further counter-stained with dilute safrarnin for 30 seconds then drained and blot dried. The slide was then examined using x100 oil immersion objective. A violet or purple colour indicated a Gram-positive while a pink/red colour indicated a Gram-negative organism.

3.10.6 Biochemical Characterization of Isolates

Biochemical tests, such as, indole, methyl red, Voges-Proskauer, Triple sugar iron, oxidase, Urease and citrate utilization test and motility test were carried out according to standard procedures (Cheesbrough, 2006).

3.10.6.1 Indole test

The test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan, to indole which accumulates in the medium to give a red colour with P-dimethyl aminobensaldehyde. The test organism was grown in sterile 5ml peptone water at 35^0C for 2days to give optimum accumulation of indole and 0.5ml of Kovac's reagent was added. The development of a brilliant red colour ring in the reagent layer above the broth within 1 minute indicated a positive result; no colour change indicated a negative result, that is, the indole reagent retains its yellow colour (Cheesbrough, 2006).

3.10.6.2 Methyl Red test

Single colony from the pure culture of the test organism was inoculated in 5 ml of sterile MR-VP broth and incubation at 37ºC for 48h. After this, 3 – 5 drops of methyl red solution was added and observed for colour formation. Development of red or yellow colour indicated positive or negative result, respectively (Cheesbrough, 2006).

3.10.6.3 Voges -Proskauer (V-P) test

The test was performed by adding alpha-naphthol and KOH to the Voges-Proskauer broth inoculated with bacteria. The test organisms were grown in 3 ml of sterile MR-VP broth at 37ºC for 48 h and then 0.6 ml of 5% alpha-naphthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine was added per ml of broth culture. The broth was then vigorously shaken to aerate and allowed to stand for 5-10 minutes. Development of pink-red colour indicated positive result (Cheesbrough, 2006).

3.10.6.4 Citrate utilization test

This test demonstrates the ability of certain bacteria to metabolize citrate, the removal of which creates an alkaline condition in the medium. The shift in pH turns bromothymol blue indicator in the medium from green to blue. Using a wire loop, a loopfull amount of growth of the tsst organism from nutrient agar was inoculated on Simmons' citrate agar slant in a bijou bottle and incubated at 37^{o}C for 24 to 72h. The development of a deep blue colour indicated a positive reaction (World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC and P/USAID, 2003).

3.10.6.5 Oxidase test

The oxidase test was carried out using filter paper method. About 2 or 3 drops of Kovac's oxidase reagent were added to a piece of filter paper in a Petri dish. The filter paper was allowed to absorb the reagent and a platinum loop was used to pick a portion of the test colony and smeared onto the moistened filter paper. A purple colour formation at the region of bacteria smeared within 10 seconds indicated positive reaction (World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC and P/USAID, 2003).

3.10.6.6 String test for the identification of Vibrio cholerae

A loopfull of the test bacteria from a 24h culture on nutrient agar plate was emulsified in a drop of 0.5% sodium taurocholate on a glass slide. The liquid was observed for a mucoid 'string' within 2mins when the loop is pulled upward.

3.10.6.7 Motility test

A premixed commercially available motility medium was prepared according to manufacturers' instruction.The medium was brought to boil, dispensed into 10ml test tube and sterilized by autoclaving at 121^{o}C for 15 minutes. Sterilized medium was cooled in an upright position on racks.Using a sterile inoculating needle, the motility medium was inoculated with well isolated colony of the test organism by making a fine stab to a depth of about 1-2 cm short of the bottom of the tube. The tubes were then incubated at 37^{o}C for 24 hours. The line of inoculation was not sharply defined and the rest of the medium was somewhat cloudy if the organism was motile. The growth of non-motile organisms was restricted to the line of inoculation which became sharply defined and the rest of the medium remained clear (World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC and P/USAID, 2003).

3.10.6.8 Urease production test

Urease agar was prepared according to manufacturers' instruction and sterilized by autoclaving at 121^{o}C for 15min. Then 100ml of the filter sterilized urea base was aseptically added to the cooled agar solution at 50^{o}C. It was mixed and 5ml was dispensed into strile test-tubes and placed at slanting position during cooling until solidified.

The urea agar slant in the test-tubes was inoculated with a small amount of growth of the test organism and incubated at 37^{o}C for 24 h. The development of a bright pink or red colour indicated a positive reaction (World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC

and P/USAID, 2003). Escherichia coli and Salmonella are urease negative and were used as a control.

3.10.6.9 Triple Sugar Iron (TSI) test

The TSI is a composite medium. It was prepared according to manufacturers' instruction and boiled to dissolve. It was then dispensed into test-tubes and sterilized at 121^{o}C for 15min. The sterile molten agar was then cooled in slating position. Using a sterile inoculatihg needle, the surface of TSI agar was streaked with the test organism and the butt was stabbed. The cap of tubes were loosely screwed and then incubated aerobically at 37^{o}C for 24 hours. Formation of hydrogen sulphide was determined by the blackening of the whole butt or a streak or ring of blackening at the slant butt junction. When the organism utilized glucose (glucose fermentation), the butt became yellow. If no other sugar was fermented, the slant became red while the butt became yellow and this was referred to as an ALKALINE/ACID or K/A reaction and if gas formed, K/AG in addition to glucose fermentation if lactose or sucrose or both sugars fermented, both the butt and the slant became yellow, designated as A/A or A/AG (**Appendix I**). Thus, A/A or A/AG meant that either glucose or lactose had been fermented or glucose and sucrose had been fermented or glucose, lactose and sucrose had been fermented(World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC and P/USAID, 2003).

3.11 Identification of the Isolates using the Microgen Identification System Software

The biochemical identification of the organisms was carried out after incubation of the organisms on the MICROGEN GNA+B-ID test kit consisting of ninety six (96) microwell test strips containing dehydrated substances for biochemical tests. The substrates were

first inoculated with 3drops (approximately 100ul) of a bacterial suspension prepared from a pure colony of a 24 hours culture of the organism evenly suspended in sterile physiological saline. Oxidase positive organisms were identified by inoculating both GN A and GN B microwell test strips. Wells 1, 2, 3, 9 (GN A microwell test strip counting from the tabbed end), 13 and 24 (GN B microwell test strip) were overlaid with 3 drops of mineral oil. The inoculated microwell test strips were then incubated for 24 hours at 37^{o}C for Enterobacteriaceae and 48 h for the oxidase positive isolates and the results were read with the aid of a colour chart after addition of the following reagents: Two drops of Kovac's reagent were added to well 8 and results read and recorded after 60 seconds. Formation of a red colour indicated a positive result. For well 10, One drop of VP l reagent and 1 drop of VP ll reagent were added and read after 30 minutes. Positive result was shown by the formation of a deep pink/red colour. In well 12, one drop of TDA reagent was added and read after 60 seconds. Formation of a cherry red colour indicated a positive result (World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC and P/USAID, 2003).

For oxidase positive organisms, nitrate reduction test was further performed on well 7 by addition of 1 drop of Nitrate reagent A and 1 drop of Nitrate reagent B. The development of a red colour after 60 seconds indicated reduction of nitrate to nitrite. A small amount of zinc powder was added to indicate whether nitrate was completely reduced to nitrogen gas if there was no colour change or if well 7 remained colourless after addition of Nitrate reagents A+ B. Therefore, Red= Positive, Colourless/yellow= Negative, after addition of zinc powder, Colourless/yellow= Positive, Red= Negative (**Appendix II**).

For GN B strip, all positive reactions were also recorded with the aid of the colour chart. Specific wells were read as follows: The gelatin well 13 was read after 24 hours for

Enterobacteriaceae and after 48 hours for oxidase positive isolates. A positive gelatine liquefaction was shown by black particles visible throughout the well.The arginine well was interpreted after 24 hours incubation for Enterobacteriaceae, positive and negative reactions were indicated by a Yellow and Green/Blue colour respectively and after 48 hours for oxidase positive organisms: a positive and negative reactions were indicated by a Blue and Yellow/Green colour respectively.The results for oxidase, nitrate reduction and motility were included to form a 9 digit Octal Code.

The isolates were then identified using the Microgen Identification System Software(MID-60) made of substrates organized into set of 3 reactions with each substrate assigned a numerical value (1, 2, or 4) and the sum of the positive reactions for each triplet forming a single digit of the Octal Code that was used to determine the identity of the isolate. The Octal Code was then entered into the Microgen Identification System Software which generated report of the five most likely organisms in the selected database. The software generated an identification based on probability, % probability and likelihood with analysis of the quality of differentiation recorded.

3.11.1 Identification of *E. coli* serogroup O157 using the M44 MICROGEN^{R} *E. coli* O157:H7 agglutination kit

A smooth suspension of the test organism grown on Sorbitol MacConkey agar at 37^{o}C for 24 h was prepared on two wells of an agglutination slide. The slide was rocked gently for 30 seconds and observed for agglutination. If there was no agglutination in either well, a drop of MicrogenR *E. coli* O157:H7 Test Latex (M44a) was added to one well and one drop of control Latex (M44b) to the other. The slide was then rocked gently for 2 mins.

An obvious agglutination only in the well containing the test latex indicated a positive result.

3.11.2 Characterization of *Vibrio cholerae*

Once *Vibrio cholerae* has been identified, it is important to establish the correct strain and this was done with slide agglutination using *Vibrio cholerae* O1 and *Vibrio cholerae* O139 Antisera on typical colonies subcultured on Nutrient Agar as described by the (Standard methods, 2005).

3.11.2.1 Serological identification of Vibrio cholerae using the Vibrio cholerae Antisera

Serological type of *Vibrio cholerae* is based on its O somatic antigens. A certain amount (3 colonies) of an overnight bacterial growth on Nutrient agar at 37^{o}C was suspended in 0.5 ml physiological saline and use antigenic suspension. A drop of antiserum and physiological saline (30ul) as a control were placed onto a clean glass slide partitioned into several parts with a glass pencil. The antigenic suspension was placed onto the serum and the physiological saline on the glass slide. The reagents were then mixed by tilting the glass slid back and forth for 1 min to see if there was agglutination which was grossly observed with light through the slide. Only strong agglutination observed within 1min in the reaction with each serum was regarded as positive. Delayed or weak agglutination was regarded as negative (Cheesbrough, 2006).

Due to the fact that serotyping with live cells may be impossible for some strains of *Vibrio cholerae* O1, all the test organisms with negative polyvalent sera were retested by heating antigen suspension as follows. A certain amount of bacterial growth was suspended in 3ml physiological saline and heated at 121^{o}C for 15 minutes. The heated solution was then centrifuged at 900rpm for 20 mins, the supernatant was discarded and

the precipitate was then suspended with 0.5ml physiological saline and used as heated cell suspension. Polyvalent sera that showed negative results with the heated antigen suspension were identified as *Vibrio cholerae* non-O1.

3.12 Antibiotic Susceptibility Testing of the Isolates

Antibiotics susceptibility assessment of pure bacterial isolates using the disc diffusion method was adopted (Obi *et al.*, 2007).

3.12.1 Antibiotics used

The organisms were tested in vitro for susceptibility to the following commonly used antibiotics: Ampicillin (10µg), Amoxycillin-clavulanic acid (30µg), Chloramphemicol (30µg), Cefotaxime (30µg), Ciprofloxacin (10µg), Norfloxacin (10µg), Gentamicin (30µg), Ofloxacin (10µg), Tetracycline (30µg), Nitrofurantoin (30 µg) and Sulphamethozazole/Trimethoprim (25µg) with concentrations as recommended by the National Committee for Clinical Laboratory Standards (CLSI, 2014).

3.12.2 Standardization of Bacterial Inoculum

Using a sterile wire loop, three discrete colonies that were freshly grown for 24 hours on Nutrient agar at 37^{o}C were picked and emulsified in sterile 10mL normal saline. The prepared turbidity was matched with 0.5 McFarland standards to have an equivalent suspension (1.5×10^{8} cells/ml).

3.12.3 Disc Diffusion Susceptibility Test

A sterile swab was used to inoculate the suspension by streaking on the prepared and dried Mueller-Hinton agar plate evenly. It was then allowed to stand for 3 minutes. Sterile forceps were used to place the disc; the plate was then incubated at 37^{o}C for 24 hours. By

using a ruler on the underside of the plate, the diameter of each zone of inhibition was measured in millimeters. Zone diameter for standards was compared with Clinical and laboratory standards Institute, CLSI, 2014) published limits; each isolate was classified as Sensitive (S), Intermediate (I) or Resistant (R) (WHO, 2004; Tagoe *et al*., 2011). Organisms that were observed to be resistant to at least four different antibiotics were classified as being multidrug resistant (Ezekiel *et al*., 2011).

3.13 Molecular Characterization of Isolates

Molecular techniques were employed to identify each of the segregated individual bacteria selected.

3.13.1 Bacterial cell Suspension

The preparation of the bacterial cell suspension was carried out using the method described by Dubey (2009). Luria and Bertani broth media was prepared with the following constituents: peptone (10g), NaCl (5g), 1N NaOH (10ml), yeast extract (5g), distilled water (1litre), pH 7.0 (adjusted with NaOH solution) and sterilized at 121^0C for 15 mins. The isolates from stored slants were subcultured onto the same medium and under the same incubation condition as before. Discrete colonies were picked from the fresh culture plates, placed into 3ml Luria and Baertani (LB) broth medium and incubated at 37^oC for 24h. Bacterial cells were then harvested by centrifugation at 8000rpm (6800xg) in a refrigerated micro centrifuge at 4^oC for 30 seconds in an Eppendorff's tube. The supernatant were decanted and cells harvested (Dubey, 2009).

3.13.2 Genomic DNA Extraction Using ZR Genomic DNA TM- Tissue MiniPrep kit

Genonic DNA extraction was carried out using the method described by DNeasy Blood and Tissue Handbook (2006): The harvested cell pellets were dislodged and 20µl of proteinase K was added and mixed thoroughly by vortexing. The mixture was further incubated at 55^{o}C for 3h and vortexed occasionally using a thermo-mixer until the cells were completely lysed and properly mixed to prevent clogging of the DNeasy Mini spin column. Two hundred microlitres (200µl) of buffer AL was added to the sample and mixed thoroughly by vortexing. This was further accompanied by the addition of 200µl of absolute ethanol (96-100) and mixed again by vortexing to yield a homogenous solution. This mixture was then dispensed`into DNeasy Mini spin column placed in a 2ml collection tube and centrifuged at 8000rpm for 1 min. The flow-through and collection tube were then discarded. The DNeasy Mini spin column was then transferred into a new 2ml collection tube and 500µl buffer AW1 was added and centrifuged again at 8000rpm for 1 min before the flow-through was discarded for the third time. DNeasy Mini spin column was placed in 2ml collection tube and 500µl buffer AW2 was added and centrifuged at 14000rpm for 3minutes to dry the DNeasy membrane in order to remove the residual ethanol that might interfere with subsequent reactions. The flow-through and collection tube were removed and discarded carefully so that the column does not come into contact with the flow-through which could result in carryover of ethanol. The used DNeasy Mini spin column was now placed in a clean 2ml micro-centrifuge tube and 200µl buffer AE was introduced onto the DNeasy membrane to elute, and increase the final DNA concentration and yield in the elute. The column was then incubated at room temperature (30^{o}C+ 2^{o}C) for 1min before being centrifuged at 8000rpm for 1minutes. The elution of the membrane was then repeated twice to ensure increased overall DNA yield

in the same micro-centrifuge tube previously used, but care was taken to prevent dilution of the first eluate inserted at the red bands to ensure easy view of the To ascertain that plasmid DNA was actually extracted, the eluent was subjected to agarose gel electrophoresis, and viewed using a U.V illuminator.

3.13.3 Agarose Gel Electrophoresis

To ascertain that plasmid DNA was actually extracted, the eluent was subjected to agarose gel electrophoresis and it was prepared as follows:

The agarose gel was prepared by combining 1.0g of agarose in ten times concentration (10x) of Trisacetate ethylene diamine tetra-acetate (2ml 10x TAE) buffer and 98ml distilled water in a 250ml Beaker flask and heating in a microwave for 2minutes until the agarose was dissolved. 20µl ethidiumbromide (5.0mg/ml) was added to the dissolved agarose solution as dye and mixed. The gel was then poured onto a mini horizontal gel electrophoresis tank and the casting combs well while filling the plasmid DNA. It was then allowed to set for 30minutes. The casting combs were carefully removed after the gel had completely solidified, one time concentration (1x) TAE electrophoresis buffer was then added to the reservoir until the buffer covered the agarose gel. Five microlitres (5µl) of gel loading dye (Bromophenol blue) was added to 15µl of each sample with gentle mixing. Twenty microlitres (20µl) of the sample was loaded onto the wells of the gel, the mini horizontal electrophoresis gel set up was covered and the electrodes connected running from cathode (-) to anode (+). Electrophoresis was carried out at 100mV for 45 minutes to allow easy separation of sample based on molecular weight. At the completion of the electrophoresis, the gel was removed from the buffer (Tris) and was viewed under a trans-illuminator UV light of wavelength 302nm. The band pattern of the DNA fragments was then photographed with a Polaroid camera**.** It was then documented using an

electrophoresis gel documentation system. Electrophoresis was employed to identify the number of plasmid copies present in different isolates. However, standard DNA molecular weight markers were used to estimate the plasmid size (1kb ladder, 1kb plus DNA ladder and lambda DNA/Mlu 1diges).

3.13.4 PCR Amplification of 16S rDNA for Virulent and Resistant Genes

Amplification of virulent and resistant 16S rDNA fragments was carried out using Dream Tag^{TM} DNA polymerase, which is an enhanced multiplex PCR application as described by DNeasy Blood and Tissue Handbook (2006):

It ensures higher sensitivity, longer PCR products and higher yield compared to conventional Taq DNA. Dream Taq^{TM} DNA polymerase uses the same reaction set-up and recycling condition as conventional TaqTM DNA polymerase. Extensive optimization of reaction is not required. The enzyme is supplied with optimized Dream Taq^{TM} buffer, which includes 20mM $MgCl_2$. DreamTaq^{TM} DNA polymerase generates PCR products with 3'-dA overhangs. The enzyme is inhibited by dUTP but can incorporate modified nucleotides.

Dream Taq^{TM} PCR master mix (2X) was vortexed and centrifuged for 30 seconds at 8000rpm after thawing. The thin-walled PCR tube was then placed on an ice pack and the following components were added for each isolate for single reaction: (a) Dream Taq^{TM} PCR master mix (b) Forward primers (c) Reverse primers (d) Template DNA, Taq buffer, dNTP (e) 5µl of the nuclease-free water as a negative control. The samples were vortexed gently and spined down. The primers that were used for PCR are listed in Table 3.1.

Table 3.1: List of primers and primer sequences used in study

Gene	Primer sequence (5'-3')	Size (bp)	Reference
hlyA	GCA TCA TCA AGC GTA CGT TCC AAT GAG CCA AGC TGG TTA AGC T	534	Paton and Paton (1998)
InvA	GCT GCG CGC GAA CGG CGA AG TCC CGG CAG AGT TCC CAT T	389	Murugkar *et al.* (2003)
Stn	TTA GGT TGA TGC TTA TGA TGG ACA CCC CGT GAT GAA TAA GAT ACT CAT AGG	750	Lee *et al*. (2009)
stx1	ACA CTG GAT GAT CTC AGT GG CTG AAT CCC CCT CCA TTA TG	614	Jalil *et al*. (2011)
stx2	CCA TGA CAA CGG ACA GCA GTT CCT GTC AAC TGA GCA CTT TG	1181	Ding *et al*. (2011)
bla_{TEM1}	GCG GAA CCC CTA TTTG ACC AAT GCT TAA TCA GTG AG	643	Olesen, *et al*. (2004)
tetA	CGA GCC ATT CGC GAG AGC GCC TCC TGC GCG ATC TGG	517	Lapierre *et al*.(2006)
tetB	CTC AGT ATT CCA AGC CTT TG ACT CCC CTG AGC TTG AGG GC	415	Hendriksen *et al*. (2008)
toxR	CCT TCG ATC CCC TAA GCA ATA C AGG GTT AGC AAC GAT GCG TAA G	779	Rivera *et al*. (2003)
OmpW	ACG CTG ACG GAA TCA ACC AAA G GCG AAG TTT GGC TTG AAG TAG	336	Alam *et al*. (2006)
16S rRNA	AGA GTT TGA TCM TGG CTC AG AAG GAG GTC WTC CAR CCG CCA	1500	Integrated DNA Technology, USA

3.13.5 PCR Reaction

The DNA samples were loaded onto PCR machine and the 16S rRNA gene amplification was done at PCR conditions of 94^{o}C (pre-heating) for 1min, (denaturing at 94^{o}C for 1min, annealing temperature of 55^{o}C for 1min, and extension at 72^{o}C for 1min) 30 cycles and final extension at 72^{o}C for 9min. The PCR products were store at 0^{o}C awaiting purification after dispensing 5 µl of electrophoresis (Yamamoto, 2002).

3.13.6 Gel Electrophoresis and Gel Visualization

The 5µl of the PCR products was mixed with 2µl of loading dye and the mixture loaded into the wells on the electrophoresis gel. The loaded products were run at 100V on agarose gel (1.5% w/v gel in x1 TAE buffer) for 15 minutes. 5µl ethedium bromide in about 300ml water was used to stain the bands for 30 minutes on rocking shaker. The bands were viewed under UV lamp to establish amplification of target genes and photographed.

3.13.7 Purification of PCR Products

Positive PCR products were purified using QIAquick purification kit (Qiagen USA) according to the manufacturer's instructions as follows: 5 volumes of phosphate buffer (PB) was added to 1 volume of PCR samples in QIAquick spin columns. The spin columns were placed into 2ml collection tubes and centrifuged at 8000 rpm for 60 seconds to bind DNA onto membrane base. The flow through was discarded and the columns were centrifuged again for 60 seconds. 750µl of buffer PE was added to the spin columns and re-centrifuged for another 60seconds to wash. The flow through was then discarded and the columns replaced into the same collection tubes and centrifugation done for 1 minute to remove residual ethanol in the buffer PE. The columns were then placed in

clean 1.5µl centrifuge tubes and 50µl elution buffer (EB)(10mM Tris. HCl, pH 8.5) added, incubated for 1 minute and then centrifuged for another 1 minute. 5µl of the purified products were loaded onto gel tank and centrifuged at 8000 rpm for 60 seconds to confirm the presence of the purified products. These products were stored at -20^{o}C.

3.13.8 PCR amplification of 16S rRNA genes sequencing of 16Sr RNA Genes

The purified products were sequenced using Big DyeTM Terminator V3.1 cycle sequencing kits; an ABI 3130xl automated capillary Genetic Analyzer (Applied BioSystems) to obtain 16S rRNA partial sequences. The universal Primers as used in the PCR reaction were employed in the initial partial sequencing. Each pair of the partial sequences obtained were further aligned using Promiga program to enable the designing of the internal primers for complete 16S rRNA gene sequencing.

3.13.9 Sequence Analysis

The sequences were compared with those in the GenBank database using the BLAST program for the initial identification of the isolates. Identified isolates were grouped according to their BLAST identities and the sequences were aligned using MEGA 4 software (Tamura *et al.,* 2007) multiple sequence alignment program to give the pairwise percentage identity.The organisms with high percentage similarities were considered similar or closely related. Subsequently, the aligned sequences were subjected to phylogenetics relationships using the MEGA 4 software.

3.14 Analyses of Data

The data for refuse dump site conditions which included temperature and pH were analyzed using descriptive statistics. Statistical Analysis System (SAS) version 9.2

package was used in the analysis of data on the bacterial colony forming units. ANOVA analysis and Duncan's multiple range test were employed to verify the differences in mean by seasons and by locations ($P \leq 0.05$). The degree of relationship between temperature, degree of acidity and the bacterial counts of soil samples from the waste dumps was established using Pearson Correlation. Chi-square (χ^2) was employed to establish association between the bacterial isolates, seasonal variation and the dump sites. The results are expressed as Mean ±SE. Data presentation was made using tables and graphs.

CHAPTER FOUR

4.0 RESULTS

4.1 Temperature and pH of waste dumps

Temperature and pH readings of the soil samples from the waste dumps from various locations were taken and their range calculated. The temperature of the soil samples from all the sampling sites ranged between 25^{o}C and 37^{o}C for the dry season and between 22^{o}C and 36^{o}C for the wet season while the pH for all the sample sites ranged from pH 6.21 to 9.45 in the dry season and between 6.02 and 9.34 in the wet season (Table 4.1).

The mean values of the temperature and pH of the soil sample from the waste dumps from various locations were compared (Table 4.2). There were slight variations between the waste dump temperatures at the various sampling locations in the four dump locations with that of Zaria City being highest and the one for Samaru lowest as displayed in Table 4.2. Of the five sampled locations, Zaria City had the highest sample temperatures with mean temperature of 34.13^{o}C, followed by Sabon-Gari, Tudun-Wada and Samaru with mean temperatures of 31.82^{o}C, 30.53^{o}C and 29.51 ^{o}C respectively.The lowest sample temperatures were observed in the Control Site with mean temperature of 27.23°C (Table 4.2).

There were varied pH values for samples obtained at each of the 520 sampling spots of the five locations as summarized in Table 4.2 with the majority showing alkaline pH. The lowest pH obtained was 6.02 and the highest 9.45. The highest pH readings were observed from samples obtained from Zaria City (8.27) and acidic pH ranges were mostly noted in the Control Site (6.21). Zaria City samples notably had marked higher pH values compared to the other sampling locations. Cumulatively Tudun-Wada, Sabon-Gari and Samaru samples had no significant pH differences. However there was a difference in

Table 4.1: Variation of Temperature and pH of Soil from Waste Dumps in Zaria Metropolis with Seasons

Sampling Locations	Season	No. of samples analyzed	Temp. range	pH range
Sabon-Gari	Dry	52	27 – 36	6.21 - 9.34
	Wet	52	26 – 36	6.02 - 9.34
Samaru	Dry	52	27 – 36	6.21 – 9.34
	Wet	52	27 – 34	6.21 – 9.34
Tudun-Wada	Dry	52	25 – 37	6.17 – 9.38
	Wet	52	27 – 36	6.21 – 9.34
Zaria City	Dry	52	25 – 37	6.33 – 9.45
	Wet	52	27 – 36	6.21 – 9.34
Control Site	Dry	52	27 – 34	6.21 – 8.34
	Wet	52	22 – 33	6.21 – 9.34
Total Range	Dry	260	25 – 37	6.21 – 9.45
	Wet	260	22 – 36	6.02 – 9.34

KEY:

pH: Degree of acidity or alkalinity

Temp: Temperature in degree Celsius

Table 4.2: Mean temperature and pH of soil from waste dumps in Zaria metropolis

Sampling Locations	No. of samples analyzed	Mean Temperature (^{o}C)	Mean pH
Sabon-Gari	104	31.82	7.26
Samaru	104	29.51	7.21
Tudun-Wada	104	30.53	7.16
Zaria City	104	34.13	8.27
Control Site	104	27.23	6.51
Total Mean	**520**	**30.64**	**7.30**

KEY:

pH: Degree of acidity

(^{o}C): Degree Celsius

mean pH of ZC in comparison with other locations but all averaged at alkaline range (Table 4.2).

A comparison of the mean values of the temperature and pH of the soil samples in the dry and wet seasons showed that their values are significantly different (Table 4.2). Individually the 520 spots of the five sampling locations showed no characteristic temperature and pH patterns.

4.2 Total Viable Aerobic Bacterial Counts

The Duncan Multiple Range Test, performed on the total aerobic bacterial counts obtained at the different sampling locations on 520 samples showed the mean value for each location as well as the standard error, the variation between the locations and the two seasons (Dry and Wet) at 0.05 levels (Table 4.3).

A comparison of the mean bacterial counts of the soil samples in the dry and rainy seasons was carried out and results are presented in Table 4.3. The highest mean bacterial counts were recorded in the dry season whereas the lowest counts were recorded in the wet season. Statistical analysis using ANOVA revealed a statistical significant difference ($p<0.05$) between the mean total bacterial counts of the dry and the wet seasons.

The mean values for the total aerobic bacterial counts ranged as follows: Sabon-Gari ($2.7x10^8$cfu/g), Samaru ($2.1x10^8$cfu/g), Tudun-Wada ($2.8x10^8$cfu/g), Zaria City ($3.3x10^8$cfu/g) and Control Site ($1.7x10^8$cfu/g) with Zaria city having the highest number of counts followed by Tudun-Wada and the Control Site having the least number of counts (Table 4.4).

Table 4.3: Variation of Total Viable Bacterial Load of soil from Waste Dumps in Zaria Metropolis with Season

Sampling Location	Season	No. of sample analysed	Mean ABC±SE(cfu/g)/season
Sabon-Gari	Dry	52	$2.97x10^{8} \pm 1.26x10^{7c}$
	Wet	52	$2.39x10^{8} \pm 9.30x10^{6b}$
Samaru	Dry	52	$2.14x10^{8} \pm 1.07\ x10^{7d}$
	Wet	52	$2.16x10^{8} \pm 1.10x10^{7b}$
Tudun-Wada	Dry	52	$3.10x10^{8} \pm 1.37x10^{7b}$
	Wet	52	$2.46x10^{8} \pm 1.17\ x10^{7b}$
Zaria City	Dry	52	$4.13\ x10^{8} \pm 1.75x10^{7a}$
	Wet	52	$2.50x10^{8} \pm 1.18x10^{7a}$
Control Site	Dry	52	$1.73x10^{8} \pm 7.20x10^{6e}$
	Wet	52	$1.68x10^{8} \pm 6.80x10^{6c}$
Total Mean	Dry	260	$2.8x10^{8} \pm 7.6x10^{6}$
	Wet	260	$2.2x10^{8} \pm 4.9x10^{6}$

Analysis of variance (ANOVA), Duncan multiple range test, $P \leq 0.05$, p-value= 0.00, df= 4 In each category, values with different subscript are significantly different.

KEY:

df =Degree of freedom

ABC- Aerobic bacterial counts

SEM- Standard error of the mean.

Table 4.4 Total Viable Aerobic Bacterial Count with Respect to Study Locations

Sampling location	No. of sample analysed	Mean ABC±SE(cfu/g)/location
Sabon-Gari	104	$2.7x10^{8} \pm 8.3x10^{6b}$
Samaru	104	$2.1x10^{8} \pm 7.8x10^{6c}$
Tudun-Wada	104	$2.8x10^{8} \pm 9.5x10^{6b}$
Zaria City	104	$3.3x10^{8} \pm 1.3x10^{6a}$
Control Site	104	$1.7x10^{8} \pm 4.7x10^{6d}$
Total Mean	520	$2.5x10^{8} \pm 4.7x10^{6}$

Analysis of variance (ANOVA), Duncan multiple range test, $P \leq 0.05$, p-value= 0.00, df= 4

In each category, values with different subscript are significantly different.

KEY:

df =Degree of freedom**,** ABC- Aerobic bacterial counts, SEM- Standard error of the mean.

There was a significant variation between the bacterial counts at various locations, but the counts at locations ZC and SA as well as ZC and CS were significantly different from those counts from locations SG and TW. This infers that locations SA and CS supported the least bacterial counts in comparison with all the other locations.

The degree of relationship among the temperature, pH and the bacterial counts of soil samples from the waste dumps was established (Table 4.5). Statistical analysis using Pearson correlation test revealed that temperature and pH were highly correlated to bacterial counts. However, temperature was highly correlated ($r = 0.653$) to bacterial count than pH ($r = 0.579$).

The results of the principal component analysis revealed that temperature has 65.4% influences on microbial activities while pH had only 18.7% (Figure 4.1).

4.3 Isolation and Characterization of Enteric Bacteria from Waste Dumps

For the characterization of the bacteria, physical differentiation of the bacterial colonies was done based on physical characteristics (colony colour, size, elevation, margin, shine, transparency and consistency as well as the bacterial Gram reaction and microscopic appearance). Morphological and biochemical characterization of pathogenic bacteria isolated from waste dump soil samples are presented in Table 4.6. Isolates that appeared red-pink with black centred on XLD agar plate were selected as presumptive *Salmonella* for biochemical characteristics. Isolates that were able to convert triple sugar iron (reducing sugars) to alkaline slant and acid butt with hydrogen sulfide (H_2S) and gas or no gas production, Indole, urease, Voges-Proskauer and Oxidase negative and showed methyl red, and citrate positive were taken as presumptive *Salmonella.*

Table 4.5: **Correlation of Temperature, pH and Bacterial Counts**

	Temperature	**pH**	**Bacterial counts**
Temperature	1		
pH	0.579**	1	
Bacterial counts	0.653**	0.561**	1

Pearson Correlation. The estimation is restricted maximum likelihood. Colour separation was used to detect the significant level.

**= Highly significant

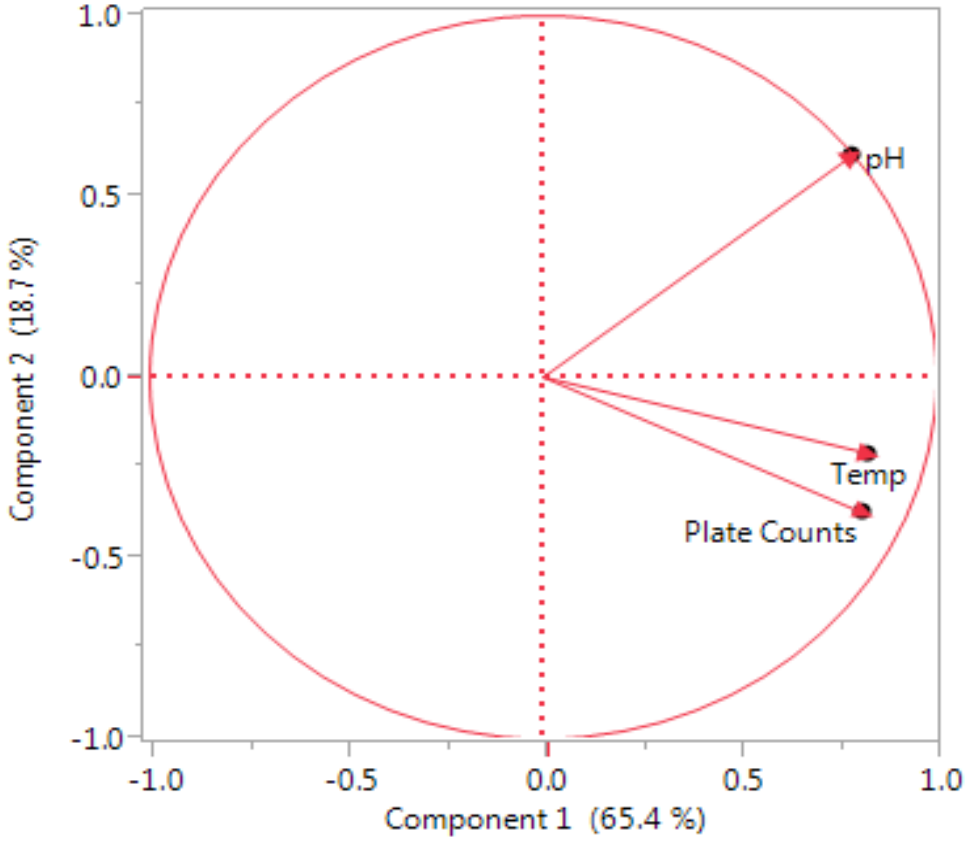


Figure 4.1: Principal Component Analysis of the Temperature and pH of the Soil Samples in Relation to Bacterial Counts

KEY:

pH- Degree of acidity/alcalinity

TEMP- Temperature in degree Celsius (^{o}C)

COUNT- Bacterial counts in cfu/g

Table 4.6: Morphological and Biological Characterization of Pathogenic Bacteria Isolated from Waste Dump Soil Samples

Microscopic examination & gram reaction	**Biochemical reactions**											**Presumptive Identity**
	Colonies	Motility	Growth in PW freeNaCl	VP	MR	Citrate	Urease	Indole	Oxidase	String	TSI	
Gram negative short rods	Shiny green with black centres and purity on EMB agar plate	+	+	−	+	−	−	+	−	NA	A/G+A	*Escherichia coli*
Gram negative slender rods	Red-pink black centred on XLD agar plate	+	+	−	+	+	+	−	−	NA	K/A,G + weak H_2S	*Salmnella* spp.
Gram negative short rods		+	+	−	+	−	−	−	−	NA	K/A+ weak H_2S	*Salmonella* Typhi
Gram negative slender rods	Red with black centre on XLD agar plate	+	+	−	+	+	−	−	−	NA	K/G+H_2S	*Salmonella* Pullorum
Gram negative slender rods		+	+	−	+	+	−	+	−	NA	K/A+H_2S	*Salmonella* Arizonae
Gram negative slender rods	Red with black centre on XLD agar plate		+	−	+	+	−	−	−	NA	K/A,G +H2S	*Salmonella* Typhimorum
Gram negative short curved rods	Greyish yellow on TCBS agar plate	+	+	+	−	D	−	+	+	+	K/A	*Vibrio cholera*
Gram negative short curved rods	Green on TCBS agar plate	+	−	−	−	D	−	+	+	+	K/A	*Vibrio vulnificus*
Gram negative short curved ros	Green on TCBC agar plate	+	−	−	−	D	+	+	+	+	K/A	*Vibrio parahaemolyticus*
Gram negative short curved rods	Yellow on TCBC agar plate	+	+	+	−	D	−	+	+	+	K/A	*Vibrio alginolyticus*

KEY:

D=Different strains give different results, A=Acid reaction, K=Alkaline raction, G=Gas production, H_2S=Hydrogen sulphide, NA=Not applicable

Isolates that showed greenish metallic sheen on Eosin Methylene Blue agar plate were selected for biochemical characteristics as *Escherichia coli*. Furthermore, isolates that were able to convert triple sugar iron (reducing sugars) to acid slant and acid butt with gas production, tryptophan to indole and showed methyl red positive, produced negative result of urease, citrate, oxidase and Voges-Proskauer tests were identified as *E. coli*.

In this research, all short curved rod, Gram-negative, yellow or greyish yellow (sucrose fermenting colonies) and green (non-sucrose fermenting colonies) on TCBS, immobilized in distilled water but remain motile in peptone water, oxidase-positive, positive or weak string test reaction were presumptively identified as *Vibrio* spp.

A total of 221 distinct colonies were differentiated from different source materials from the dump sites that grew on the different culture media at the various temperature conditions. These distinct colonies were sub-cultured on the same media and at the same temperature conditions as before (Plate V) in order to obtain pure colonies.

The Gram reaction and microscopic examination on the pure colonies revealed that over 85% of these isolates were Gram negative and the rest Gram positive. Microscopic examination further revealed that rod shaped structures were the most predominant cell type among the bacterial isolates. The other cell types were cocci and filamentous bacteria.Together with the colony types, these physical characteristics were considered in the assortment in which 189 bacterial colonies from the previous 221 isolates were further selected for additional characterization. The details of the description codes used for naming of the isolates based on the sample treatment, incubation temperatures and the culture media used are tabulated in **Appendix III**



Plate V: Sub-Cultured Distinct Colonies: Colonies plated from primary culture medium plates onto secondary plates based on their differences in morphological characteristics to obtain pure colonies: P1 Metallic sheen green, reducing sugars *Escherichia coli* colonies on Eosin Methylene Blue (EMB) agar. P2 Yellow, sucrose fermenting *Vibrio cholerae* colonies on Thiosulphate Citrate Bile-salt Sucrose (TCBS) agar. P3 Red-pink black centred H_2S producing *Salmonella* colonies on Xylose Lysine Deoxycholate (XLD) agar.

The proportion of diverse isolates obtained from the different dump sites varied with Zaria city yielding higher isolates than Tudun-Wada, Sabon-Gari and Samaru (Figure 4.2). All the isolation media (XLD, EMB and TCBS) yielded greater proportion of diverse bacterial isolates as displayed in Figure 4.2.

Biochemical characterization from the 520 samples analysed using MICROGEN GNA and MICROGEN GNA+GNB test kit, revealed a total of 189 bacterial isolates amongst which, 36 *Salmonella* species, 67 *E. coli*, 26 *Vibrio* spp. and 60 Other isolates which include *Acinetobacter* spp.**,** *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus mirabilis* etc. (Table 4.7).

The prevalence of the various bacterial species differed significantly ($P<0.05$) throughout the sampling period. All target organisms were isolated in the various sampling locations except in the control site (Table 4.8).

Out of the 26 *Vibrio* spp. isolated, *Vibrio algynolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulmificus* and *Vibrio cholerae* accounted for 9 (39.1 %), 4 (17.4 %), 3 (13.1 %) and 7 (30.4 %) respectively.

On the basis of the agglutination with the Polyvalent O1 and O139 antiserum, out of the 7 *Vibrio cholerae* identified, *Vibrio cholerae* non-O1 accounted for 7(100%). Other serotypes were not identified. A prevalence of 1.35% of *Vibrio cholerae* non-O1 was recorded.

Out of the 36 *Salmonella* identified, *S.* Pullorum, *S.* Arizonae , *S.* Typhi and *Salmonella* species accounted for 5(13.89%), 7(19.44%), 3(8.33%) and 21(58.33%) respectively. A prevalence of 6.92% of *Salmonella* species was obtained.

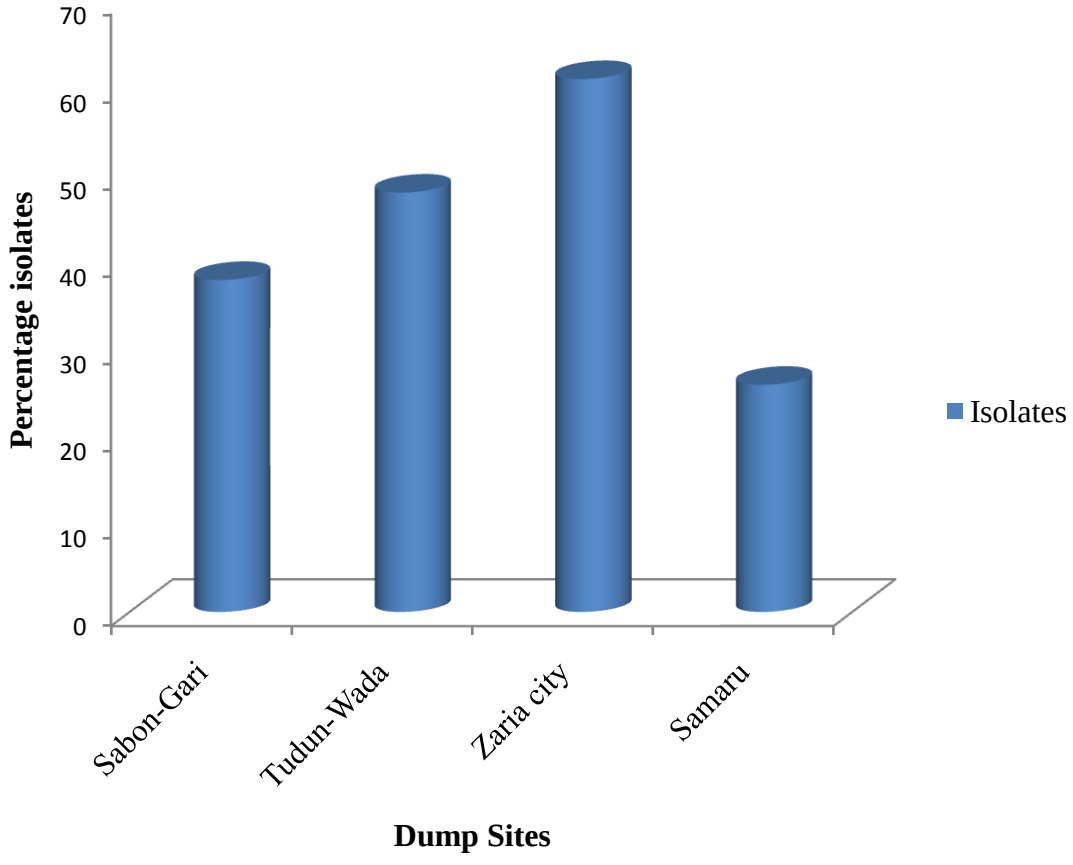


Figure 4.2: Proportion of Bacterial Isolates from Waste Dump Soils at Various Sampling Locations

Table 4.7: Occurrence of Bacterial isolates from Waste Dump Soils at Various Sampling Locations

Bacteria spp. Isolated	Locations					Total Frequency N=520(%)
	SG N=104(%)	SA N=104(%)	TW N=104(%)	ZC N=104(%)	C S N=104(%)	
Acinetobacter iwoffii	0(0.00)	0(0.00)	1(0.96)	0(0.00)	0(0.00)	1(0.19)
A. Baumannii	0(0.00)	1(0.96)	0(0.00)	2(1.92)	0(0.00)	3(0.58)
Citrobacter sakazakii	1(0.96)	1(0.96)	0(0.00)	0(1.92)	1(0.96)	3(0.58)
Citrobacter freundii	2(1.92)	0(0.00)	1(0.96)	1(0.96)	0(0.00)	4(0.77)
Enterobacter Liquefaciens	1(0.96)	2(1.92)	1(0.96)	2(1.92)	0(0.00)	6(1.15)
***E. coli* O157:H7**	5(4.81)	3(2.88)	5(4.81)	9(8.65)	0(0.00)	22(4.23)
***Salmonella* spp.**	4(3.85)	2(1.92)	6(5.77)	7(6.73)	0(0.00)	19(3.65)
***S.* Typhi**	0(0.00)	1(0.96)	1(0.96)	2(1.92)	0(0.00)	4(0.77)
***S.* Arizonae**	2(1.92)	1(0.96)	0(0.00)	3(2.88)	0(0.00)	6(1.15)
***S.* Pullorium**	1(0.96)	2(1.92)	3(2.88)	1(0.96)	0(0.00)	7(1.35)
Hafnia alvei	0(0.00)	0(0.00)	2(1.92)	1(0.96)	1(0.96)	4(0.77)
Non-O157 *E. coli*	9(8.65)	7(6.73)	11(10.57)	14(13.46)	4(3,85)	45(8.65)
Proteus mirabilis	3(2.88)	2(1.92)	5(4.81)	4(3.85)	2(1.92)	16(3.08)
Morganella morganii	0(0.00)	0(0.00)	1(0.96)	1(0.96)	0(0.00)	2(0.38)
***Pseudomonas* spp.**	1(0.96)	1(0.96)	2(1.92)	1(0.96)	1(0.96)	6(1.15)
***V. cholerae* non-O1**	1(0.96)	0(0.00)	3(2.88)	3(2.88)	0(0.00)	7(1.35)
V.parahaemolyticus	1(0.96)	2(1.92)	3(2.88)	2(1.92)	0(0.00)	8(1.53)
Vibrio vulmificus	2(1.92)	1(0.96)	1(0.96)	2(1.92)	0(0.00)	6(1.15)
Vibrio alginolyticus	1(0.96)	1(0.96)	2(1.92)	1(0.96)	0(0.00)	5(0.96)
Klebsiella oxytoca	0(0.00)	2(1.92)	1(0.96)	2(3.85)	1(0.96)	6(1.15)
Klebsiella pneumonia	2(1.92)	0(0.00)	1(0.96)	0(0.00)	1(0.96)	4(0.77)
Klebsiella ozaenae	1(0.96)	1(0.96)	2(1.92)	1(0.96)	0(0.00)	5(0.96)
Total	**37(35.56)**	**30(28.85)**	**52(50.0)**	**59(56.73)**	**11(10.58)**	**189(36.35)**

Total $p<0.05$

KEY:

N=Total number of samples analyzed

ZC- Zaria city, TW- Tudun-Wada, SG- Sabon-Gari, SA- Samaru, CS- Control Site

Table 4.8: Occurrence of Target Enteric Pathogenic Bacteria from Waste Dumps in Zaria Metropolis (N=520)

Isolated targets	Number of positive samples	Prevalence (%)
***Escherichia coli* O157:H7**	22	4.23
Salmonella enterica	36	6.92
***Vibrio cholerae* non-O1**	7	1.35
Total	65	12.50

KEY:

N= Total number of samples

Using the *E. coli* O157:H7 Latex agglutination Kit, out of the 67 *E. coli* identified, *E. coli* O157:H7 accounted for 22(32.84%) while Non-O157:H7 *E. coli* accounted for 45(67.16 %). A prevalence of 4.23% of *E. coli* O157:H7 was recorded (Table 4.8).

Out of the 520 soil samples, 104 samples were collected from each location, 22 yielded positive for *E. coli* O157:H7 giving a prevalence of 4.23%, 36 yielded positive for *Salmonella* species giving a prevalence of 6.92% and 7 yielded positive for *Vibrio cholera* non-O1 giving a prevalence of 1.35%.

The highest occurrence of the target isolates was obtained in ZC 26(25%), followed by TW and SG, 18(17.31%) and 13(12.50%) respectlvely. SA 8(7.69%) had the least bacterial isolates (Table 4.9). *Salmonella* spp. was isolated from 14, 10, 7 and 5 samples each in ZC, TW, SG and SA respectively whereas *E. coli* was isolated from 5, 9, 5 and 3 in SG, ZC, TW and SA respectively and *Vibrio cholerae* non-O1 was isolated from 3 samples each in ZC and TW and from 1 sample in SG while none was isolated from SA (Table 4.9).

From the five hundred and twenty samples that were collected, 260 were collected respectively in dry and wet seasons. *Salmonella enterica* was isolated from 22(8.46%) and 14(5.38%) samples in the dry and wet season respectively and *Vibrio cholerae* non-O1 from 3(1.15%) and 4(1.54%) in the dry and wet season respectively. The study showed that there was an equal distribution of *E. coli* O157:H7 in both seasons 11(4.23%). The highest isolation rate was obtained in the dry season (37) giving a prevalence of 14.23% of the target isolates while the lowest was obtained during the wet season (29) giving a prevalence of 11.15%.

Table 4.9: Occurrence of Enteric Pathogenic Bacterial Isolates from Waste Dump Soils at Various Sampling Locations

Sampling Location	No. of Samples analyzed	*Escherichia coli* O157:H7 Number (%)	*Salmonella enterica* Number (%)	*V. cholerae* non-O1 Number (%)	Total No. of isolates/location Number (%)
Sabon-Gari	104	5(4.81)	7(6.73)	1(0.96)	13(9.6)
Samaru	104	3(2.88)	5(4.81)	0(0.0)	8(7.7)
Tudun-Wada	104	5(4.81)	10(9.62)	3(2.88)	18(17.3)
Zaria city	104	9(8.65)	14(13.50)	3(2.88)	26(25.0)
Control Site	104	0(0.00)	0(0.00)	0(0.00)	0(0.0)
Total	520	22(4.23)	36(6.92)	7(1.35)	65(12.5)

Statistical analysis using Chi-square (χ^2) revealed a statistical significant difference ($p<0.05$) in the occurrence of the target isolates between the two seasons (Table 4.10). However, there was no statistical significant difference in the occurrence of *E. coli* O157:H7 between the dry and the wet seasons ($p>0.05$).

4.4 Antibiotic Susceptibility Patterns of the Isolates

The susceptibility of 21 isolates of *Salmonella enterica*, 18 isolates of *E. coli* O157:H7 and 7 isolates of *Vibrio cholerae* non-O1 to 10, 10 and 8 commonly used antibiotics respectively is presented in Table 4.11. Antibiotic susceptibility patterns of these pathogenic isolates as presented in the aforementioned Table show susceptibility of the bacterial isolates to some commonly used antibiotics.

Salmonella enterica were 100% susceptibility to Gentamicin. In addition they were susceptible to Chloramphenicol (85.71%), Nitrofurantoin (90.48%), Ciprofloxacin (76.19%), Cotrimoxazole 66.66%, Ofloxacin (61.90%) and Cefotaxim (57.14%).

Nineteen percent, 14% and 5% intermediate susceptibility was observed for Amoxicillin-clavulanic acid, Ofloxacin, Ciprofloxacin, Cotrimoxazole and Cefotaxim. These isolates were 95% resistant to Ampicillin. Similarly, they were resistant to Amoxicillin-clavulanic acid (71%), Tetracycline (57%), Cefotaxim (38%) Cotrimoxazole (29%), Ofloxacin (19%) and Nitrofurantoin, Chloramphenicol (10%). Gentamicin was found to be the most effective drug on *Salmonella* spp. with 100% susceptibility followed by Chloramphenicol (90%) Ciprofloxacin (76%), Cotrimoxazole (67%) and Cefotaxim (57%) (Figure 4.3).

Vibrio cholerae were 100% resistant to Ampicillin and Cotrimoxazole. Similarly, they were resistant to Tetracycline (86%), Chloramphenicol (43%), Gentamicin and Norfloxacin (29%).

Table 4.10: Seasonal Variation in the Frequency of Occurrence of *E. coli* O157:H7, *Salmonella enterica* and *Vibrio cholerae* non-O1 based on Sample Locations

Sampling Location	Season	No. of samples collected	*E.coli* O157:H7 Number (%)	*Salmonella enterica* Number (%)	*Vibrio chlorae* Number (%)	Total No of Isolates/ location/ season
Sabon-Gari	**Dry**	52	2(3.85)	4(7.69)	0(0.00)	6(11.54)
	Wet	52	3(5.77)	3(5.77)	1(1.92)	7(13.46)
Samaru	**Dry**	52	1(1.92)	3(5.77)	0(0.00)	4(7.69)
	Wet	52	2(3.85)	2(3.85)	0(0.00)	4(7.69)
Tudun-Wada	**Dry**	52	3(5.77)	6(11.54)	1(1.92)	10(19.23)
	Wet	52	2(3.85)	4(7.69)	2(3.85)	8(15.38)
Zaria City	**Dry**	52	5(9.62)	9(17.31)	2(3.85)	16(30.77)
	Wet	52	4(7.69)	5(9.62)	1(1.92)	10(19.23)
Control Site	**Dry**	52	0(0.00)	0(0.00)	0(0.00)	0(0.00)
	Wet	52	0(0.00)	0(0.00)	0(0.00)	0(0.00)
TOTAL	**Dry**	**260**	11(4.23)	22(8.46)	3(1.15)	37(14.23)
	Wet	**260**	11(4.23)	14(5.38)	4(1.53)	29(11.15)

Chi-square(χ^2)

χ^2= 24.00, General association= 0.03*(Dry season)

$\chi^{2\ =}$21.67, General association=0.06^{ns} (Wet season)

df= 12

KEY:

χ^2 =Chi-square; df= Degree of freedom; %= Percentage isolation frequency

P-value (Dry season) = 0.02*

P-value (Wet season) = 0.04

**=significant at p≤0.05, ns=not significant (p≥0.05).

Table 4.11: Antibiotic Susceptibility Patterns of Bacterial Isolates from Waste Dump Sites in Zaria Metropolis

Waste dump pathogens	No. of isolates	Percentage (%) susceptibility										
		AMP (10µg)	AMC (30µg)	CHL (30µg)	CTX (30µg)	CIP (10µg)	CN (30µg)	F (30µg)	NOR (10µg)	OFX (10µg)	SXT (25µg)	TE (30µg)
***Salmonella* spp.**	**21**	1(4.76)	2(9.52)	18(85.71)	12(57.14)	16(76.19)	21(100)	19(90.48)	NT	13(61.90)	14(66.66)	9(42.86)
***E. coli* O157:H7**	**18**	0(0.00)	2(11.11)	18(100)	13(72.22)	13(72.22)	15(83.33)	11(61.11)	10(55.55)	NT	12(66.66)	2(11.11)
***Vibrio cholerae* non-O1**	**7**	0(0.00)	NT	2(28.57)	3(42.86)	NT	5(71.43)	NT	2(28.57)	6(85.71)	0(0.00)	1(14.29)
Total	**46**	**1(2.17)**	4(10.26)	38(82.61)	28(60.87)	29(74.36)	41(89.13)	30(76.92)	12(48.00)	19(67.86)	26(56.52)	12(26.09)

KEY:

AMP-Ampicillin, AMC-Amoxycillin-clavulanic acid, CHL-Chloramphenicol, CTX- Cefotaxim, CIP-Ciprofloxacin, CN- Gentamicin, F-Nitrofurantoin, NOR- Norfloxacin, OFX- Ofloxacin, SXT-Sulphamethoxazole-trimethoprim, TE-Tetracycline, NT-Not Tested

Antibiotics applied Using Clinical Laboratory Standard Institute (CLSI, 2014).

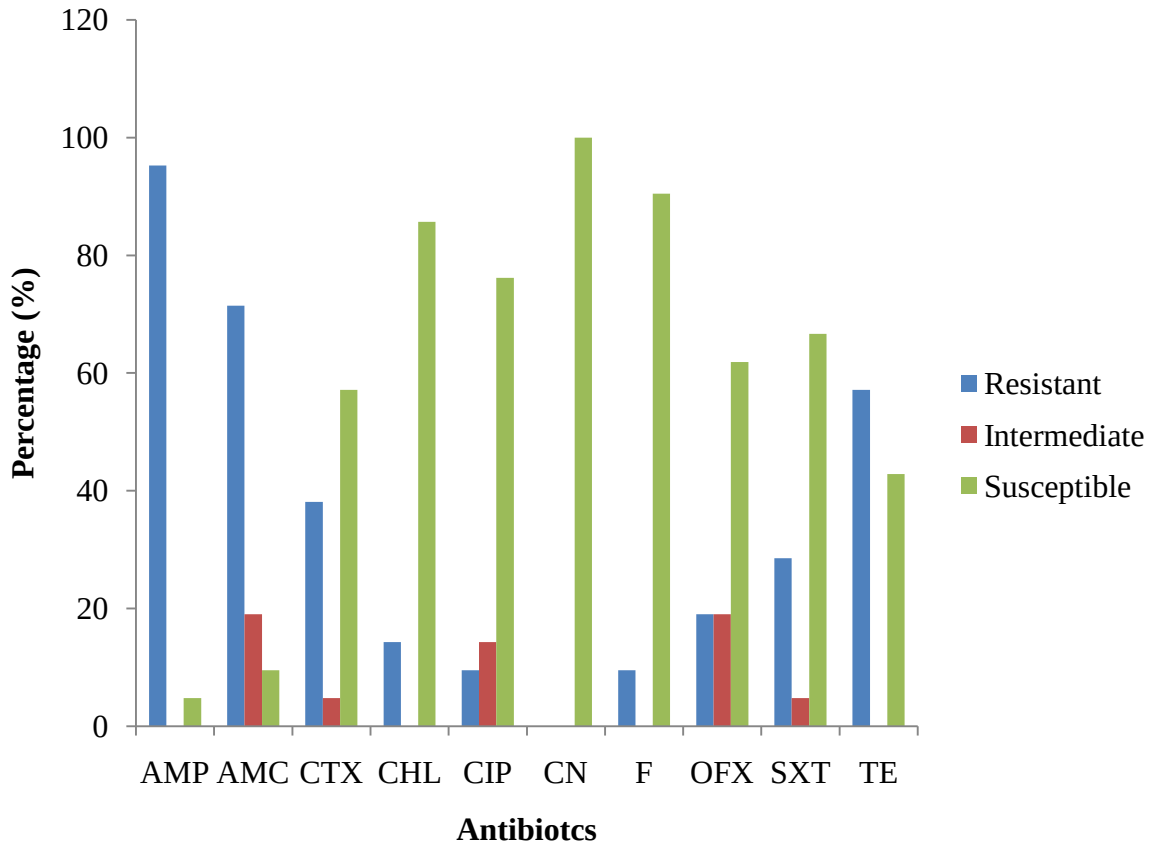


Figure 4.3: Susceptibility of *Salmonella enterica* from Waste Dumps in Zaria Metropolis

KEY:

AMP-Ampicillin

AMC-Amoxycillin-clavulanic acid

CTX-Cefotaxime

CHL-Chloramphenicol

CIP-Ciprofloxacin

CN-Gentamicin

F-Nitrofurantoin

OFX-Ofloxxacin

SXT-Sulphamethoxazole/trimethoprim

TE-Tetracycline

57%, 43% and 29% intermediate susceptibility was observed for Cefotaxim, Norfloxacin and Chloramphenicol respectively. There were no 100% effective drugs on *Vibrio cholera*e non- O1. Ofloxacin and Gentamicin appear to be the most effective drugs with 86%, 79% susceptibility respectively (Figure 4.4).

E. coli O157:H7 strains were 94% resistant to Ampicillin. In addition the strains were resistant to Amoxicillin clavulanic acid and Tetracycline (89%), Cotrimoxazole and Norfloxacin (33%), Cefotaxim (28%), Nitrofurantoin (22%) and Gentamicin (17%). Sixteen percent, 11% and 6% intermediate susceptibility was observed for Nitrofurantoin, Ciprofloxacin, Norfloxacin and Ampicillin respectively. Chloramphenicol was found to be the most effective antibiotic on *E. coli* O157:H7 strains with 100% susceptibility followed by Gentamicin (83%), Ciprofloxacin and Cefotaxim (72%), Cotrimoxazole (67%) and Norfloxacin (56%) (Figure 4.5).

A total of 65.2% of the isolates were multidrug resistant. Multidrug resistance was taken as resistance of isolate to at least four different antibiotics or more than two different classes of antibiotics. High resistance values were recorded in Ampicillin, Amoxicillin-clavulanic acid and Tetracycline, while low resistance values were recorded in Chloramphenicol, Ciprofloxacin, and Nitrofurantoin. However, Gentamicin, Ofloxacin, Ciprofloxacin and Chloramphenicol displayed better performing index with 10.9%, 17.9%, 12.8% and 13.0% isolates resistance, respectively. The bacterial isolates were resistant to 1-7 antibiotics and twenty five different phenotypic resistance profiles were observed among them. The resistance pattern of the isolates showed that 2 isolates were resistant to one antibiotic, 5 were resistant to two antibiotics, 7 were resistant to three antibiotics, 13 were resistant to four antibiotics, 6 were resistant to five antibiotics and 10 were resistant to six antibiotics while 1was resistant to seven antibiotics (Table 4.12).

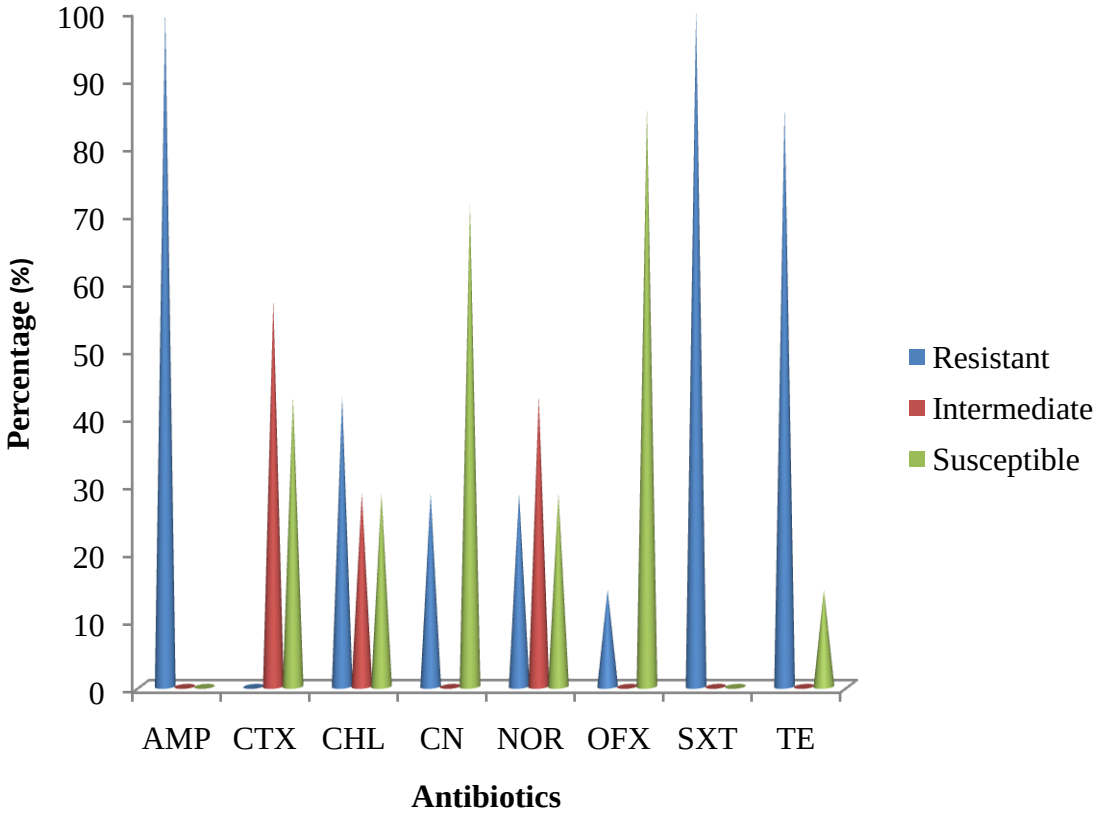


Figure 4.4: Susceptibility of *Vibrio cholerae* from Waste Dumps in Zaria Metropolis

KEY:

AMP-Ampicillin

AMC-Amoxycillin-clavulanic acid

CTX-Cefotaxime

CHL-Chloramphenicol

CIP-Ciprofloxacin

CN-Gentamicin

F-Nitrofurantoin

NOR-Norfloxacin

SXT-Sulphamethoxazole/trimethoprim

TE-Tetracycline

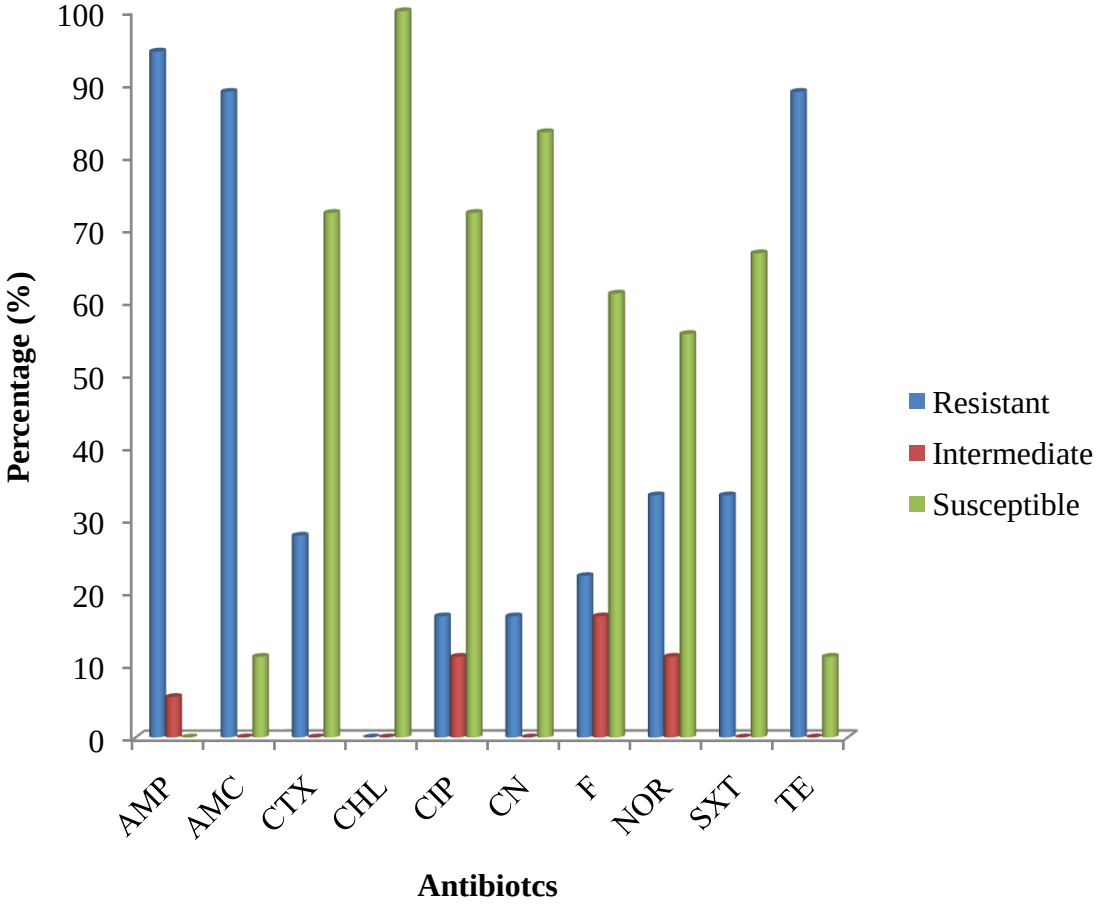


Figure 4.5: Susceptibility of *E. coli* O157:H7 from Waste Dumps in Zaria Metropolis

KEY:

AMP-Ampicillin

AMC-Amoxycillin-clavulanic acid

CTX-Cefotaxime

CHL-Chloramphenicol

CIP-Ciprofloxacin

CN-Gentamicin

F-Nitrofurantoin

NOR-Norfloxacin

SXT-Sulphamethoxazole/trimethoprim

TE-Tetracycline

Table 4.12: Antibiotic Resistance Pattern of Enteric Bacteria Isolated from Waste Dump Soil Samples

Resistance phenotype	No. of bacterial isolates with pattern			
	***Salmonella enterica* (%)**	***Escherichia coli* O157:H7 (%)**	***Vibrio cholerae* non-O1 (%)**	**Number of isolates resistant**
AMP	1	1	0	2
AMP,TE	0	1	0	1
AMP,SXT	0	0	1	1
AMP,AMC,TE	0	6	0	6
AMP,AMC,SXT	2	0	0	2
AMP,AMC,OFX	1	0	0	1
AMP,SXT,TE	0	0	1	1
AMP,CTX,SXT	2	0	0	2
AMP,CIP,TE	1	0	0	1
AMP,AMC,CIP	0	4	0	4
AMP,AMC,CTX	1	0	0	1
AMP, AMC,CIP,TE	5	0	0	5
AMP,AMC,CTX,TE	2	0	0	2
AMP,AMC,SXT,TE	1	0	0	1
AMP,AMC,CTX,CHL	1	0	0	1
AMP,CN,SXT,TE	0	0	2	2
AMP,AMC,CTX,NOR	0	2	0	2
AMP,AMC,CTX,OFX.	1	0	0	1
AMP,AMC,OFX,SXT,TE	1	0	0	1
AMP.SXT,NOR,CHL,TE	0	0	2	2
AMP,AMC,CTX,F,TE	0	1	0	1
AMP,OFX,SXT,TE,CHL	0	0	1	1
AMP,AMC,CTX,TE,CHL	1	0	0	1
AMP,AMC,CIP,NOR,SXT,TE	0	1	0	1
AMP,AMC,CTX,NOR,F,TE	0	1	0	1
AMP,AMC,SXT,CN,NOR,TE,F	0	1	0	1
Total	20	18	7	45

KEY:

AMP-Ampicillin, AMC-Amoxicillin-clavulanic acid, CHL-Chloramphenicol, CTX- Cefotaxim, CIP-Ciprofloxacin, CN- Gentamicin, NOR- Norfloxacin, OFX- Ofloxacin, SXT-Sulphamethoxazole-trimethoprim, TE-Tetracycline.

Multiple antibiotic resistance (MAR) pattern of the bacterial isolates ranged from 4(28.26%) to 7(2.17%) with a total of 30(65.22%) isolates exhibiting this trend. Highest multiple resistance was observed among *E.coli* O157:H7 isolates followed by *Salmonella enterica* and *Vibrio cholerae* non-O1.

The bacterial isolates were MAR to 4-7 antibiotics and seventeen different phenotypic resistance profiles were observed among them (Table 4.13).

The distribution of multiple antibiotics resistance in study locations is shown in (Figure 4.6). Zaria city had the highest (26.09%) occurrence of isolates with MAR, followed by Tudun-Wada (21.74%) and SG (13.04%) with SA having the least (4.34%).

The occurrence of Multiple Antibiotics Resistance Index (MARI) ranged from 0.1 – 0.7. Isolates with 0.4 had the highest occurrence of 83%, followed by isolates with 0.5 (59%), 0.6(55%), 0.3(45%), 0.2 (33%) and 0.1(10%). Isolates with 0.7 MARI had the least occurrence of 5% (Figure 4.7).

4.5 Detection of Virulence and Antibiotic Resistance Genes in the Isolates

Selected 24 bacterial isolates obtained after conventional characterization and using Microgen identification kits were subjected to molecular characterization through DNA extraction, PCR amplification and then, sequencing of the 16S rRNA. Genomic DNA was extracted from each of the 24 bacteria isolates; gel picture of the extracted DNA of the isolates is shown in Plate VI Their 16S rDNAs were PCR amplified and the amplification products assessed through gel electrophoresis. Of the products assessed, many produced bands at the expected base pair.

Table 4.13: Multiple Antibiotics Resistant Patterns of Bacterial Isolates from Waste Dump Samples

No. of antibiotics	Resistance phenotype	No. of bacterial isolates with pattern			
		***Salmonella enterica* (%)**	***Escherichia coli* O157:H7 (%)**	***Vibrio cholerae* non-O1 (%)**	**Total No. of resistant isolates**
4	AMP,AMC,SXT,TE	1(2.17)	-	-	1
	AMP,AMC,CIP,TE	2(4.35)	-	-	2
	AMP,AMC,CTX,TE	2(4.35)	2(4.35)	-	4
	AMP,SXT,CN,TE	-	-	2(4.35)	2
	AMP,AMC,F,TE	1(2.17)	-	-	1
	AMP,AMC,SXT,CTX	2(4.35)	-	-	2
	AMP,AMC,OFX,CTX	1(2.17)	-	-	1
5	AMP,AMC,CTX,F,TE	-	2(4.35)	-	2
	AMP,AMC,CTX,CHL,TE	1(2.17)	-	-	1
	AMP,SXT,OFX,CHL,TE	-	-	1(2.17)	1
	AMP,SXT,NOR,CHL,TE	-	-	2(4.35)	2
6	AMP,AMC,CIP,NOR,SXT,TE	-	3(6.52)	-	3
	AMP,AMC,NOR,CTX,F,TE	-	1(2.17)	-	1
	AMP,AMC,SXT,CN,F.TE	-	3(6.52)	-	3
	AMP,AMC,SXT OFX,NOR,TE	-	2(4.35)	-	2
	AMP,AMC,CIP,SXT,CHL,TE	1(2.17)	-	-	1
7	AMP,AMC,OFX,SXT,CTX,CHL,F	1(2.17)	-	-	1
Total (%)		12(26.09)	13(28.26)	5(10.87)	30(65.22)

KEY:

AMP-Ampicillin, AMC-Amoxycillin-clavulanic acid, CHL-Chloramphenicol, CTX- Cefotaxim, CIP-Ciprofloxacin, CN- Gentamicin, NOR- Norfloxacin, OFX-Ofloxacin, TE-Tetracycline SXT-Sulphamethoxazole-trimethoprim.

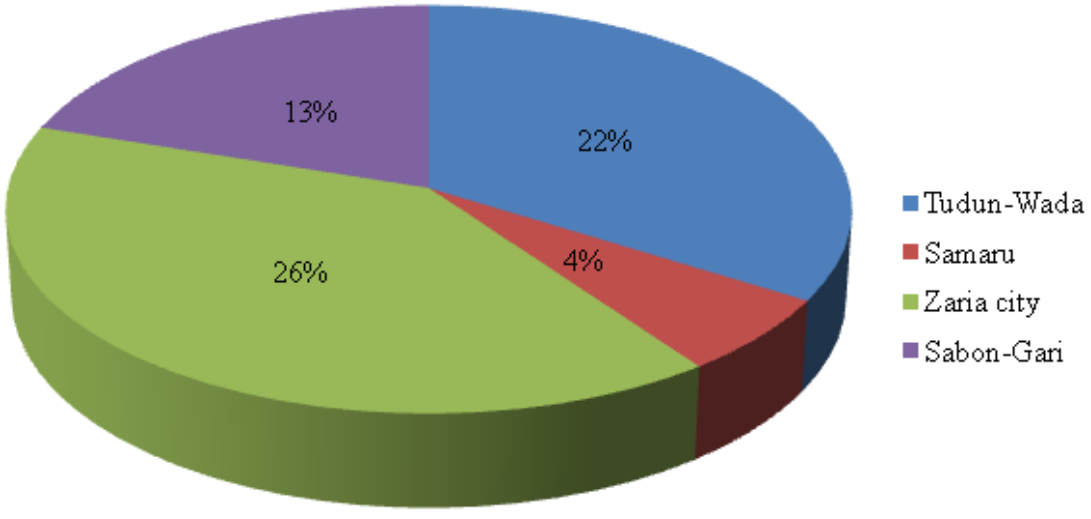


Figure 4.6: Distribution of Multiple Antibiotics Resistance (MAR) with Respect to StudyLocations

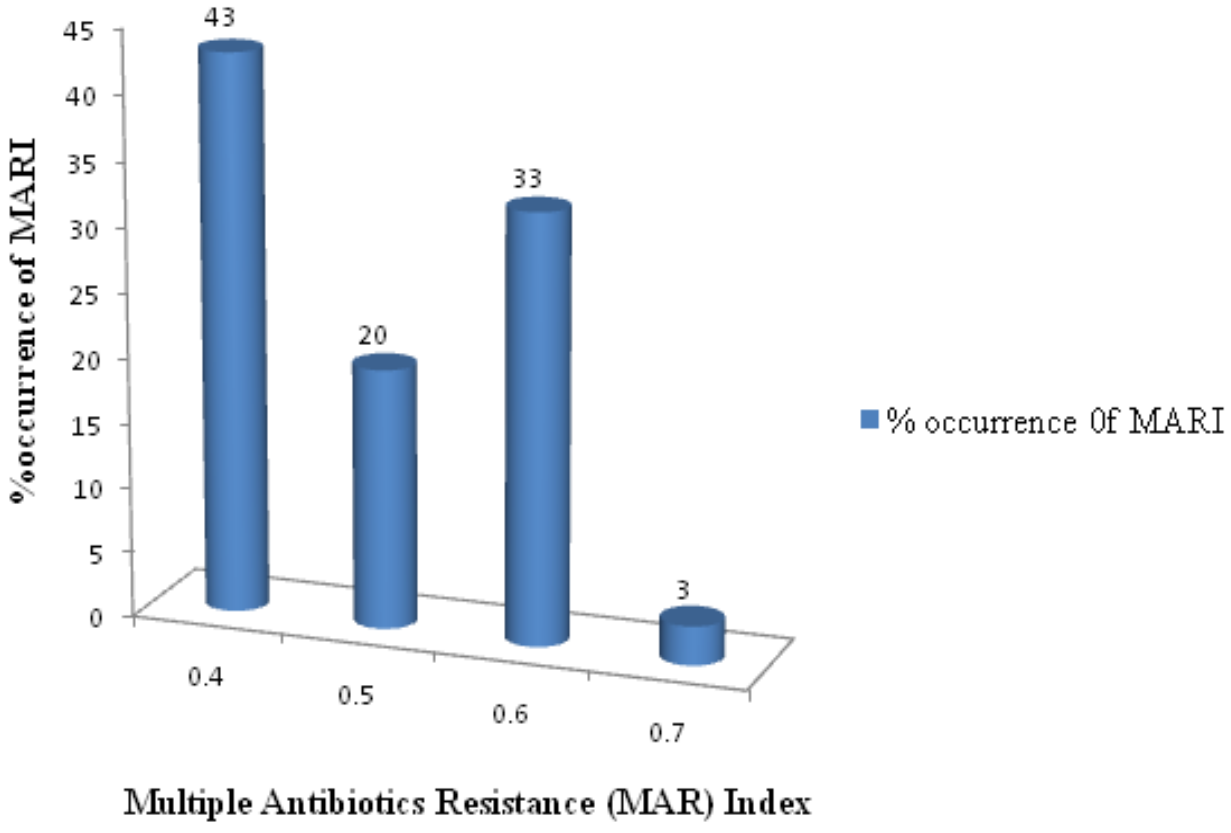


Figure 4.7: Percentage Occurrence of Multiple Antibiotic Resistance Index (MARI) among the Target Isolates

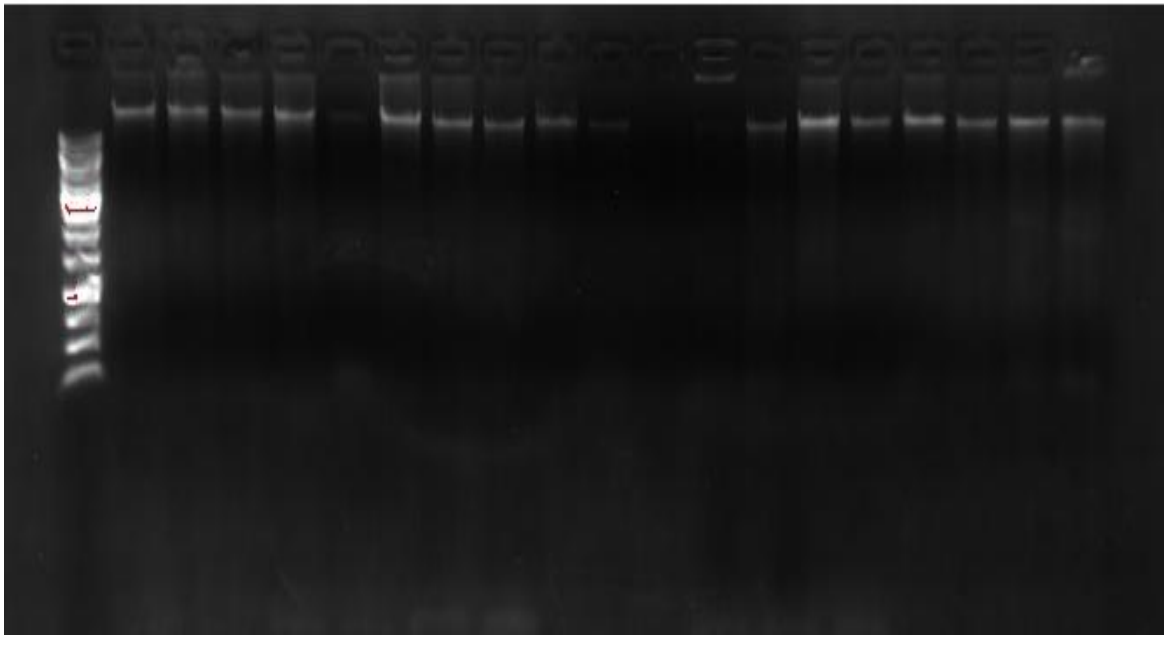


Plate VI: Gel Electrophoresis of extracted DNA of isolates

Molecular lader=1kb

Plate VII and VIII represent the detection of the β-lactamase gene bla_{TEM1}, the gene which amplified at 643pb. Two *Salmonella* isolates and one *E. coli* isolate with multiple antibiotic resistance carried $bla_{TEM\ 1}$ gene as shown on the agarose gel electrophoresis (S2 and S6) and (E3) respectively.

Plate IX; X and XI are the agarose gel electrophoresis showing amplicons for tetA and tetB, the genes which amplified at 517bp and 415bp respectively. E2, E4, E6, E7, S6 and S7 showed positive amplicons for tetA while only S1 showed positive amplicon for tetB. A 1kb plus ladder (Invitrogen) was used as a molecular maker. The aforementioned genes in *Vibrio cholerae* isolates showed no amplification at the expected base pairs; 643bp for bla_{TEM1}, 517bp for tetA and 415bp for tetB. The binds above the expected amplicon size are unspecific.

The PCR amplification of the Shiga toxin stx1 gene in *E. coli* isolates showed no amplification at the expected amplicon size of 614bp. However there were positive amplifications at 1181bp for stx2 and at 534bp for hlyA genes (Plates XII). A prevalence of 57.1% of both genes put together was recorded. A 1kb plus ladder (Invitrogen) was used as a molecular maker.

The shika toxin stx2 and the hlyA were found to be present in the *E. coli* O157:H7 isolates; the Stn and the InvA genes responsible for invasion were found to be present in the *Salmonella* isolates, the toxR gene was found to be present in *Vibrio cholerae* non-O1. Two resistance genes conferring resistance to two categories of antibiotic including β-lactams and Tetracyclines were identified. The PCR results were consistent with the antibiotic phenotypes. A 1kb molecular marker plus ladders (Invitrogen), a negative control (Nuclease-free water) and primers were used.

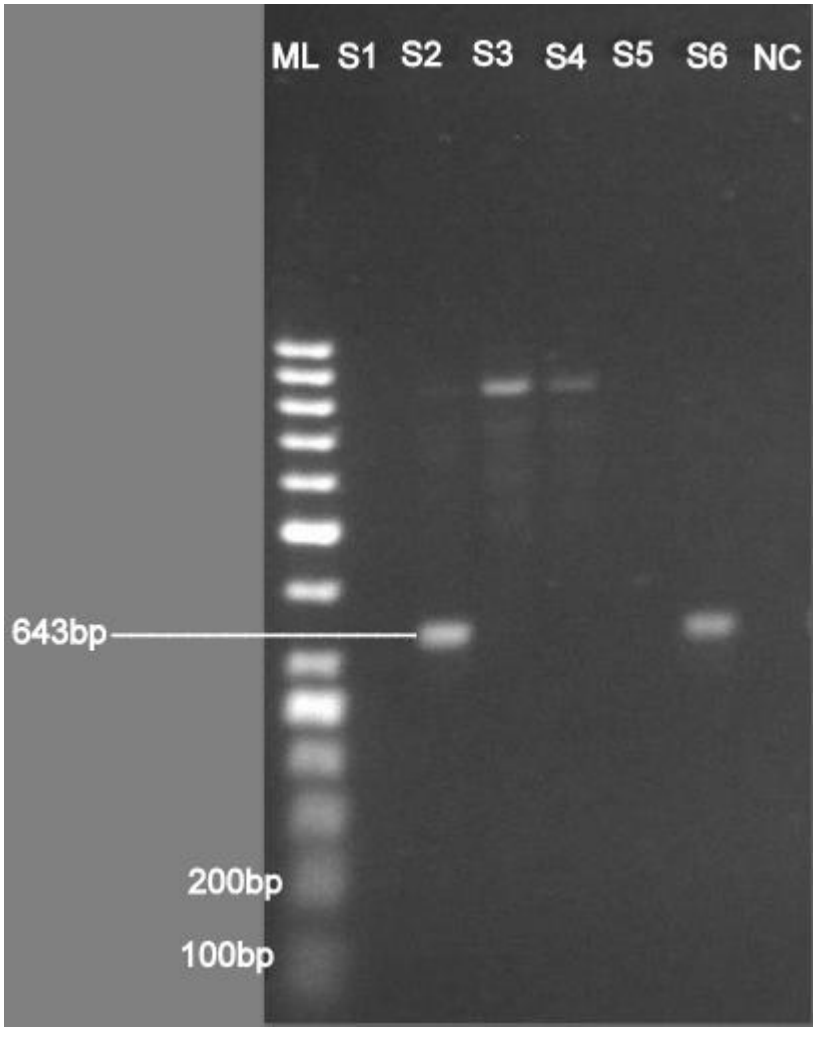


Plate VII: Amplicon of bla_{TEMI} Gene of *Salmonella enterica* Isolated from Waste Dump Soils

KEY:

Size: 643bp

ML: Molecular ladder (1kb)

NC: Nuclease-free water (Negative control)

S1- S6: *Salmonella enterica* isolates.

Positive for lane S2 and S6

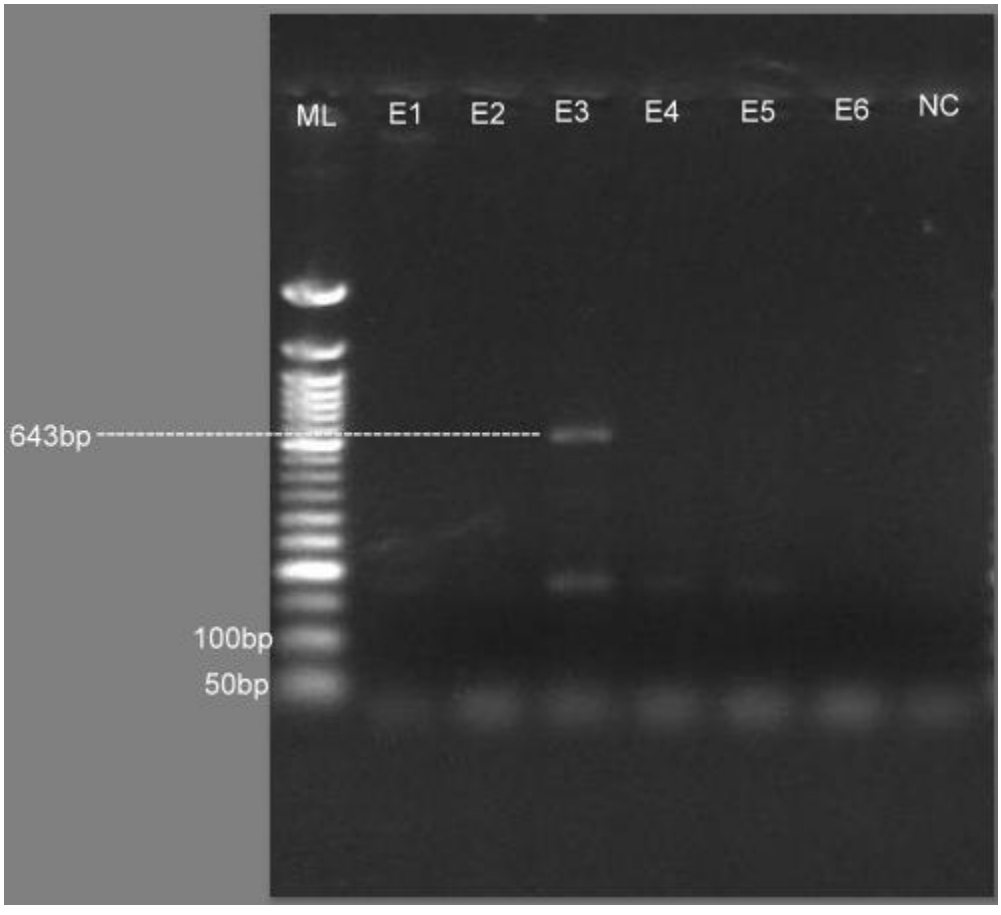


Plate VIII: Amplicon of bla_{TEM1} Gene *E. coli* O157:H7 isolated from Waste Dump Soils

KEY:

Size: 643bp

ML: Molecular ladder (1kb)

NC: Nuclease-free water (Negative control)

E1- E6: *E. coli* O157:H7 isolates

Positive for lane E3

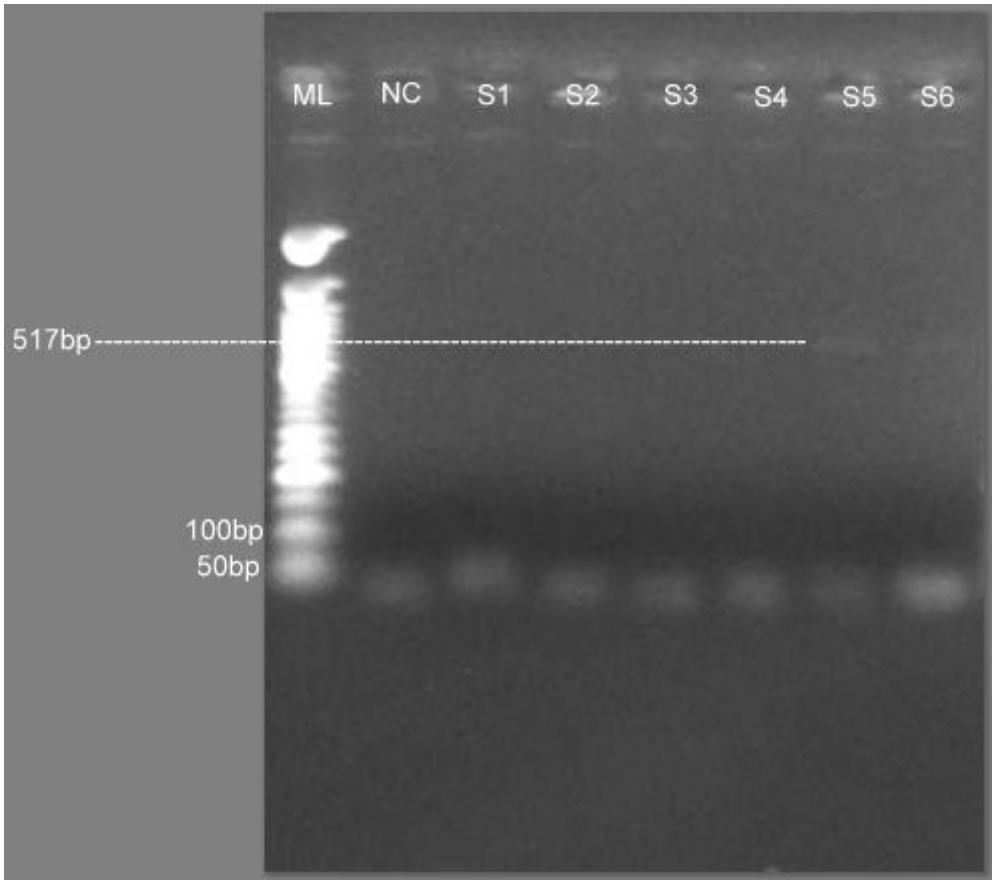


Plate IX: Amplicon of tetA Gene of *Salmonella enterica* Isolated from Waste Dump Soils

KEY:

Size: 517bp

ML: Molecular ladder (50bp)

NC: Nuclease-free water (Negative control)

S1- S6: *Salmonella enterica* isolates

Positive for lanes S5 and S6

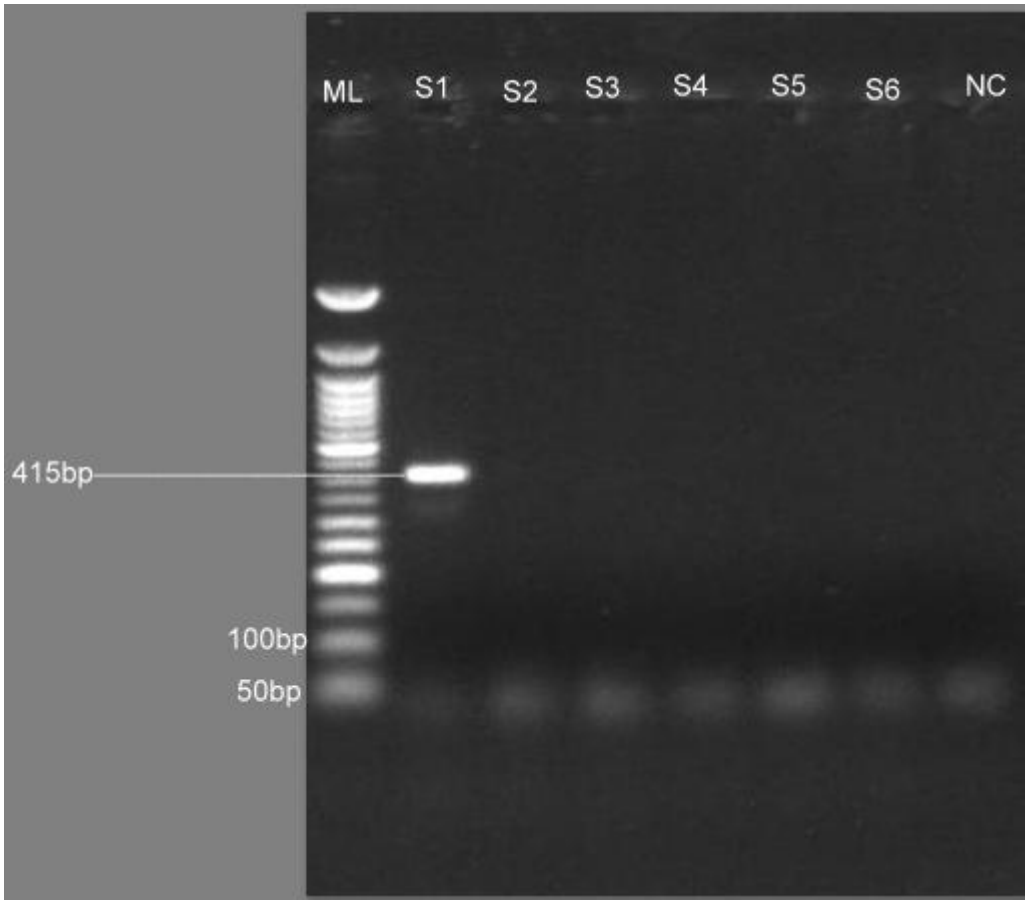


Plate X: Amplicon of tetB Gene of *Salmonella enterica* Isolated from Waste Dump Soils

KEY:

Size: 415bp

ML: Molecular ladder (50bp)

NC: Nuclease-free water (Negative control)

S1- S6: *Salmonella enterica* isolates

Positive for lane S1

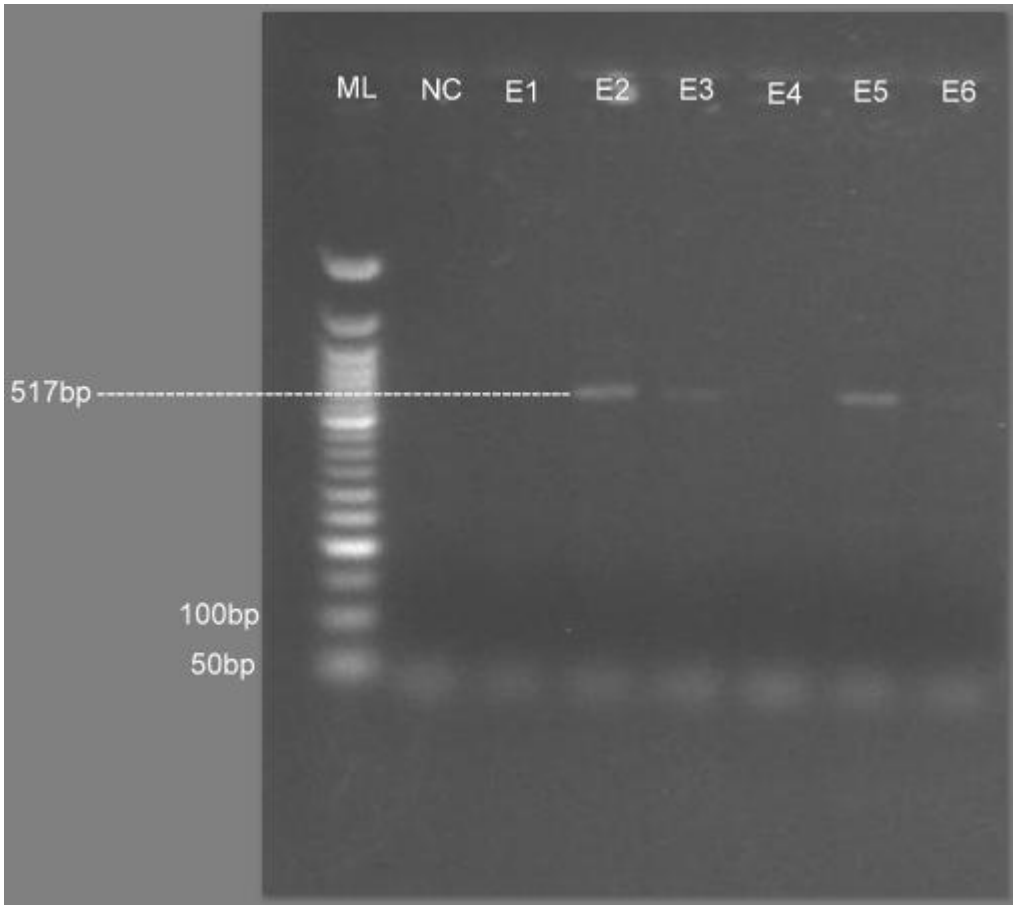


Plate XI: Amplicon of tetA Gene *E. coli* O157:H7 Isolated from Waste Dump Soils

KEY:

Size: 517bp

ML: Molecular ladder (50bp)

NC: Nuclease-free water (Negative control)

E1- E6: *E. coli* O157:H7 isolates

Positive for lane E2, E3, E5 and E6

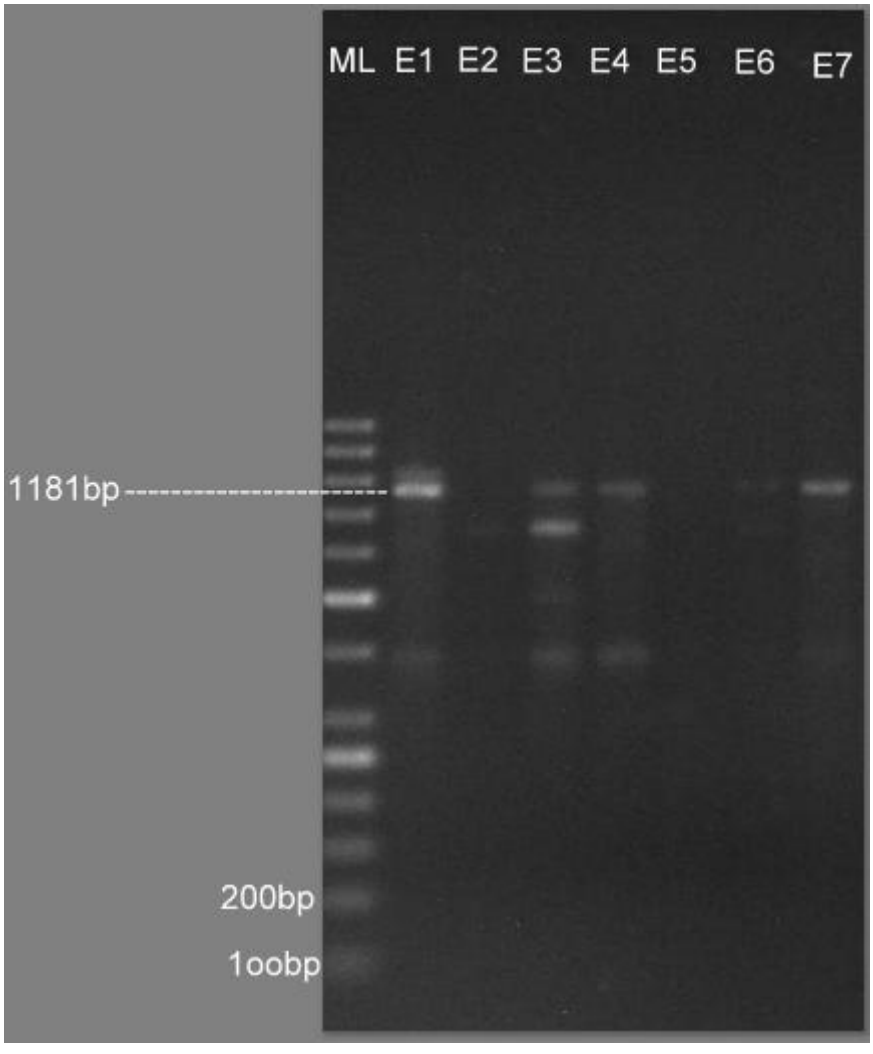


Plate XII: Amplicon of Stx2 Gene of *E. coli* O157:H7 Isolated from Waste Dump Soils

KEY:

Size: 1181bp

ML: Molecular ladder (1kb)

E1- E7: *E. coli* O157:H7 isolates

Positive for lane E1, E3, E4, E6 and E7

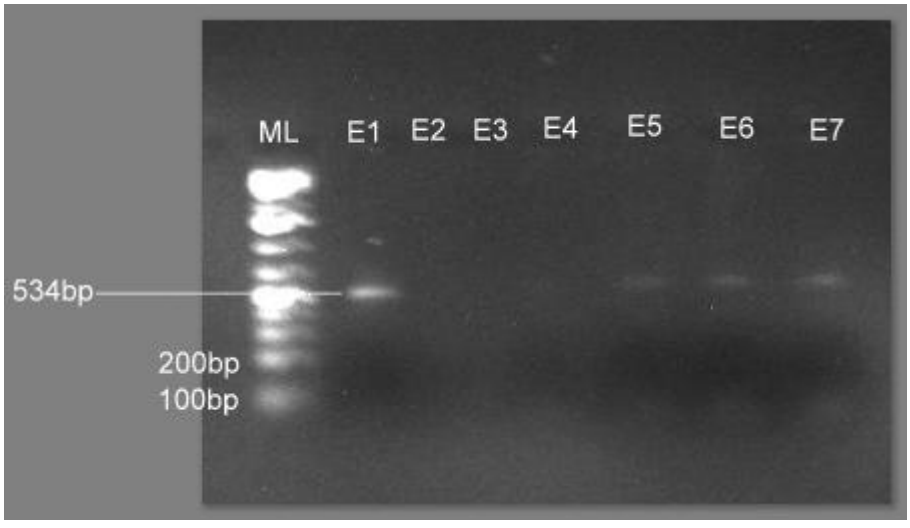


Plate XIII: Amplicon of hlyA Gene of *E. coli* O157:H7 Isolated from Waste Dump Soils

KEY:

Size: 534bp

ML: Molecular ladder (1kb)

E1- E7: *E. coli* O157:H7 isolates

Positive for lane E1, E5, E6 and E7

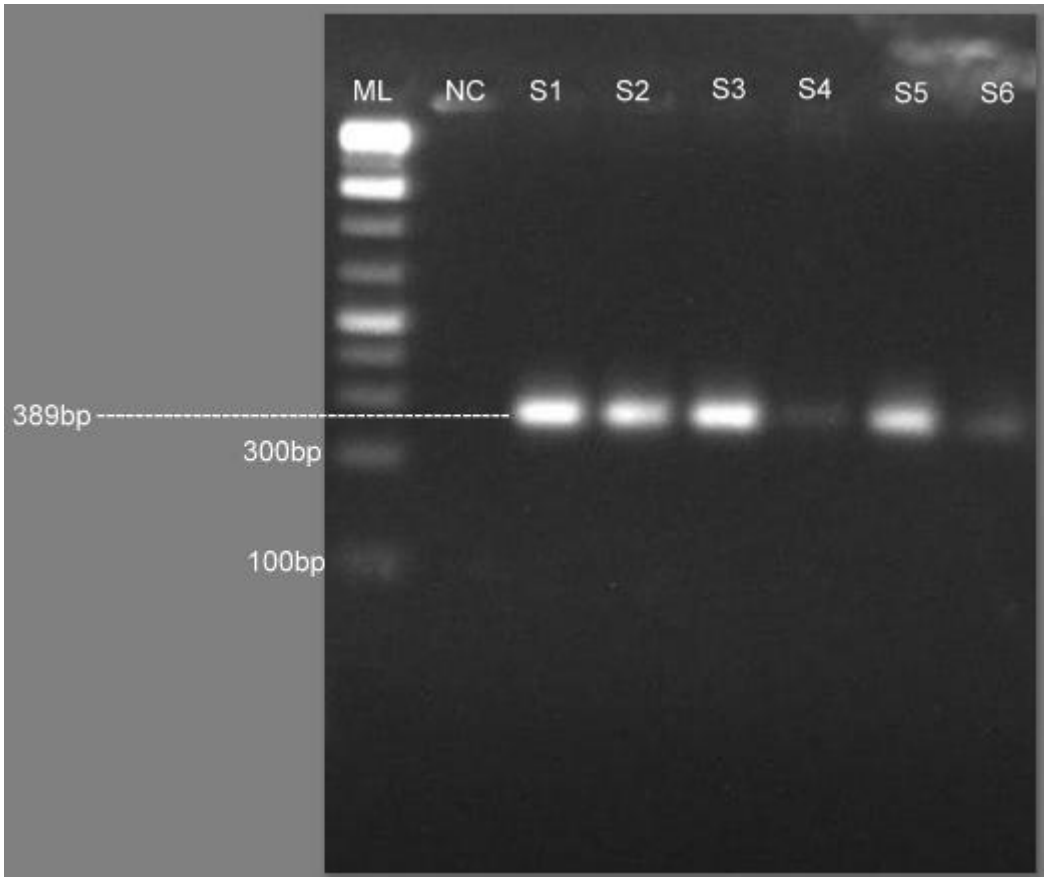


Plate XIV: Amplicon of InvA Gene of *Salmonella enterica* Isolated from Waste Dump Soils

KEY:

Size: 389bp

ML: Molecular ladder (1kb)

NC: Nuclease-free water (Negative control)

S1- S6: *Salmonella enterica* isolates

Positive for lane S1, S2, S3, S4, S4 and S6

Table 4.14: Occurrence of Antibiotic Resistance Genes in Relation to StudyLocations

Locations	Resistant genes	Number of isolates screened	*Salmonella enterica*	*Escherichia coli* O157:H7	*Vibrio cholerae non O1*	Total gene/ Location
			Number and percentage isolates positive			
Sabon-Gari	tetA	6	0(0.0)	1(16.7)	0(0.0)	2(11.1)
	tetB	6	0(0.0)	0(0.0)	0(0.0)	
	bla_{TEM1}	6	1(16.7)	0(0.0)	0(0.0)	
Samaru	tetA	6	0(0.0)	1(16.7)	0(0.0)	1(5.3)
	tetB	6	0(0.0)	0(0.0)	0(0.0)	
	bla_{TEM1}	6	0(0.0)	0(0.0)	0(0.0)	
Tudun-Wada	tetA	6	1(16.7)	0(0.0)	0(0.0)	2(11.1)
	tedB	6	0(0.0)	0(0.0)	0(0.0)	
	bla_{TEM1}	6	1(16.7)	0(0.0)	0(0.0)	
Zaria city	tetA	6	1(16.7)	2(33.3)	0(0.0)	5(27.8)
	tetB	6	1(16.7)	0(0.0)	0(0.0)	
	bla_{TEM1}	6	0(0.0)	1(16.7)	0(0.0)	

KEY:

bla_{TEMl} = ß-lactamase gene

tetA and tetB = Tetracyclines genes

The negative controls gave no bands. Some other bands showing above some specified base pair sizes can be considered to be non-specific bands.

Plates XV and XVI showed the agarose gel electrophoresis of the PCR targeting the InvA and the Stn genes in some *Salmonella enterica* isolates. All the isolates produsedamplicons for InvA gene whereas only five out of six isolates prodused amplicons for Stn gene at the expected amplicon sizes of 389bp and 750bp respectively giving 6(100%) genotype InvA and 5(83.3%) genotype Stn. A 1kb plus ladder (Invitrogen) was used as a molecular maker.

The PCR amplification of toxR gene in *Vibrio cholerae* non-O1 isolates showed positive amplifications for this gene in all the isolates at 779bp given 6(100%) genotype toxR (Plate XVII). However, there was negative amplification at 336bp for OmpW gene. A 1kb plus ladder was used as a molecular maker.

The occurrence of multiple antibiotics resistance among bacterial isolates in relation to the presence of antibiotic resistance genes is shown in Table 4.14. Two *Salmonella* isolates and one *E. coli* O157:H7 isolates with multiple antibiotics resistance carried bla_{TEM1}gene, 2 *Salmonella* isolates carried tetA gene, 1 carried tetB gene while four *E. coli* isolates

harboured tetA genes. However, these genes were not detected in the *Vibrio cholerae* isolates.

The results of the isolates that possess virulence genes in relation to study locations are presented in Tables 4.15, 4.16 and 4.17). All the locations possess isolates with virulence gene, with Zaria city having the highest isolates with virulence genes followed by Tudun-Wada and Sabong-Gari. Samaru is the location with the least isolates that possess virulence genes.

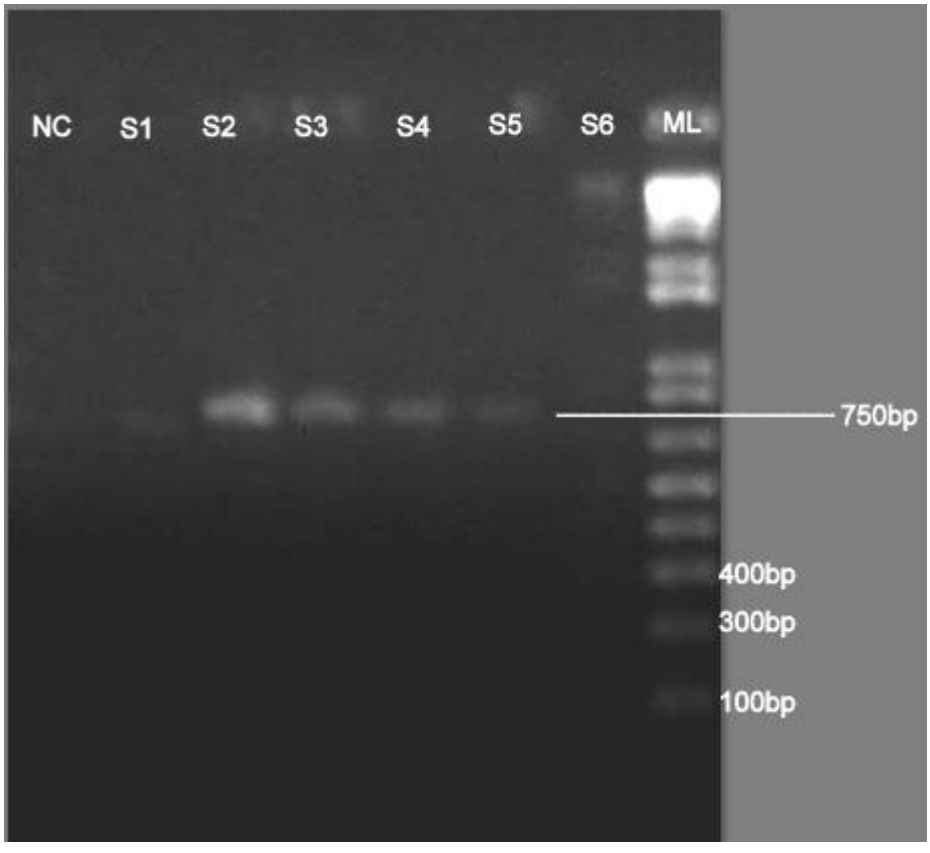


Plate XV: Amplicon of Stn Gene of *Salmonella enterica* Isolated from Waste Dump Soils

KEY:

Size: 750bp

ML: Molecular ladder (1kb)

NC: Nuclease-free water (Negative control)

S1- S6: *Salmonella enterica* isolates

Positive for lane S1, S2, S3, S4 and S5

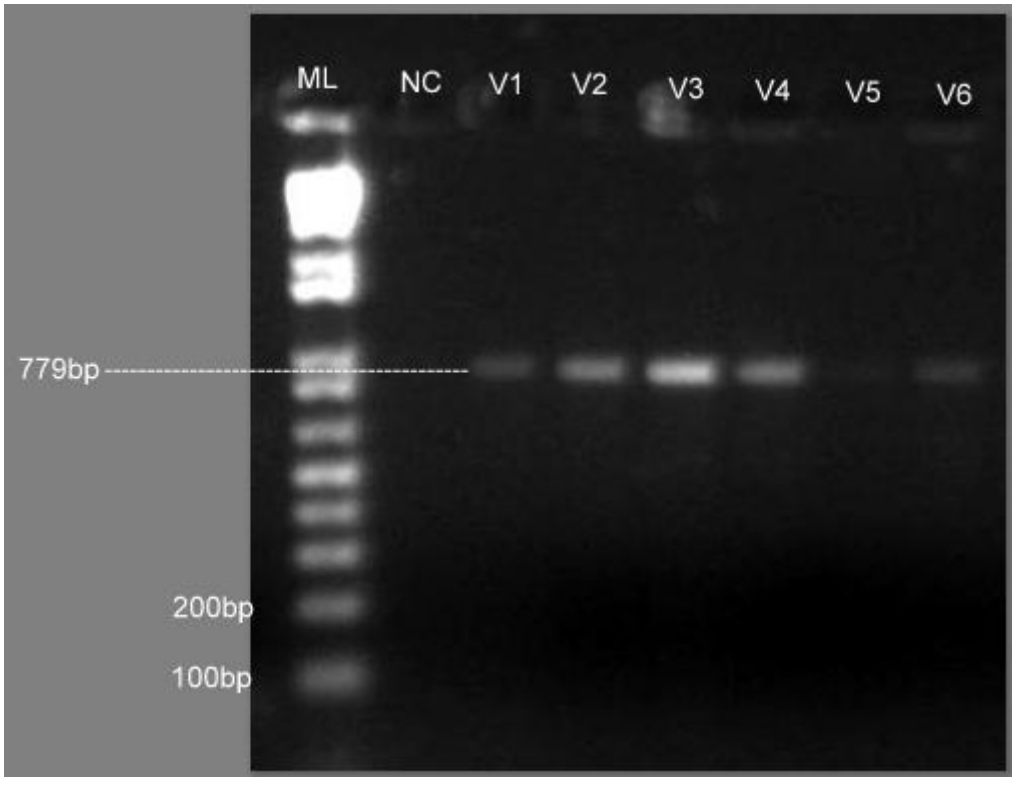


Plate XVI: Amplicon of toxR Gene of *Vibrio cholerae* non-O1 Isolated from Waste Dump Soils

KEY:

Size: 779bp

ML: Molecular ladder (1kb)

NC: Nuclease-free water (Negative control)

V1- V6: *Vibrio cholerae* non-O1 isolates

Positive for lane V1, V2, V3, V4, V5 and V6

Table 4.15: *Salmonella enterica* Virulence Genes in respect to Study Location

Isolate code/No.	Identity	Location	InvA	Stn
ZC72	*Salmonella enterica* ser. Typhi	Zaria city	+	+
TW85	*Salmonella enterica* ser. Typhimurium	Tudun-Wada	+	+
SG17	*E. coli* O83:H1	Sabon-Gari	+	_
ZC102	*Salmonella enterica* ser. Typhi	Zaria city	+	+
SA81	*Salmonella enterica* ser. Typhi	Samaru	+	+
TW42	*Salmonella enterica* ser. Typhi	Tudun-Wada	+	+

KEY:

SA- Samaru

SG- Sabon-Gari

TW- Tudun-Wada

ZC- Zaria city

+ = Gene present

- = Gene absent

Table 4.16: *E. coli* O157:H7 Virulence Genes in respect to Study Location

Isolate code/No.	Identity	Location	Stx1	Stx2	hylA
ZC6	*E. coli* O157:H7	Zaria city	-	+	+
SG84	*E. coli* O157:H7	Sabon-Gari	-	+	-
ZC62	*E. coli* O157:H7	Zaria city	-	+	-
TW8	No significant similarity found	Tudun-Wada	-	-	+
SA39	*E. coli* O157:H7	Samaru	-	+	+
ZC44	*E. coli* O157:H7	Zaria city	-	-	-
TW77	*E. coli* O157:H7	Tudun-Wada	-	+	+

KEY:

SA- Samaru

SG- Sabon-Gari

TW- Tudun-Wada

ZC- Zaria city

+ = Gene present

- = Gene absent

Table 4.17: *Vibrio cholerae* non-O1 Virulence Genes in respect to Study Location

Isolate code/No.	Identity	Location	OmpW	ToxR
TW92	*Vibrio cholerae* non-O1	Tudun-Wada	-	+
ZC29	*Vibrio cholerae* non-O1	Zaria city	-	+
TW14	*Vibrio cholerae* non-O1	Tudun-Wada	-	+
SG62	*Vibrio cholerae* non-O1	Sabon-Gari	-	+
ZC41	*Vibrio cholerae* non-O1	Zaria city	-	+
TW103	*Vibrio cholerae* non-O1	Tudun-Wada	-	+

KEY:

SA- Samaru

SG- Sabon-Gari

TW- Tudun-Wada

ZC- Zaria city

+ = Gene present

- = Gene absent

The seasonal variation in the occurrence of the virulence genes in the isolates is shown in Figure 4.8. High percentage occurrence (85%) of virulence genes was observed in the dry season while low percenttage virulence genes were observed in the wet season.

4.6 Alignment and Phylogeny of 16S rRNA Sequencing of the Isolates

The PCR products were purified. Only 14 purified samples were selected for the sequencing, among which 7 *Salmonella enterica* and 7 presumptive *E. coli* O157:H7 samples. The sequencing results on the 5' and the 3' ends of the 16S rRNAs for the 14 selected isolates using their respective forward and reverse primers are summarized in Table 4.18 where BLAST results reveal the identity of the sequenced isolates after BLAST on the NCBI (National Centre for Biotechnology Information) website. The result shows the description of the isolates' genus, species, subspecies and serovars, the maximum score of isolates among probable variants the e-value (standard error), the percentage probability of identy and the accession number of the closest organism in the GenBank.

The BLAST result revealed a total of 5 *Salmonella* serovars and 7 Enterohaemorrhagic *E. coli* among which 4 *Salmonella enterica* subsp. *enterica* serovar Typhi, 1 *Salmonella enterica* subsp. *enterica* serovar Typhimurium, 6 Enterohaemorrhagic *E. coli* serogroup O157:H7 and 1 *E. coli serogroup* O83:H1. However, 2 sequencing reactions yielded no sequences at all as shown in Table 4.18. A prevalence of 57.1% of *Salmonella* Typhi, 12.5% of *Salmonella Typhimurium*, 85.7% of *E. coli* O157:H7 was recorded.

The predominant *Salmonella* serovars were the typhoidal *Salmonellae* and the predominant *E. coli* seroroup were the Enterohaemorrhagic *E. coli* serogroup O157:H7.

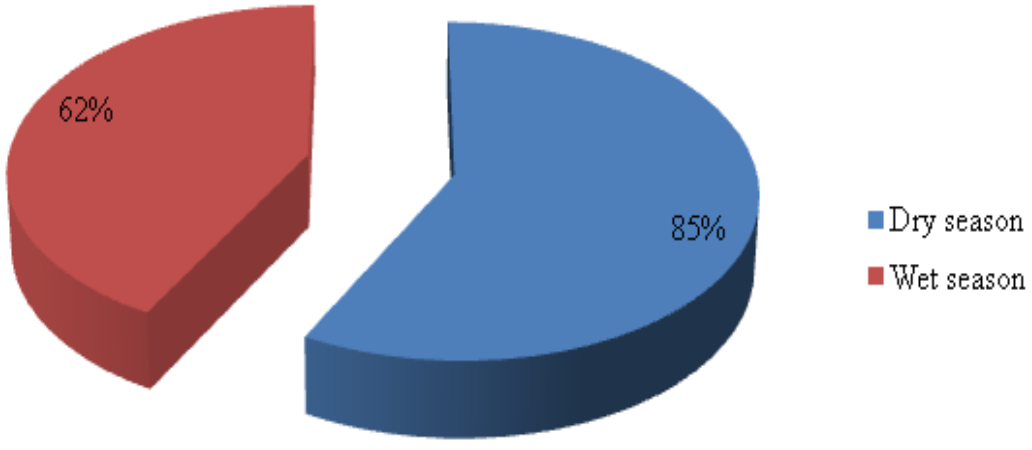


Figure 4.8: Percentage Distribution of Target Isolates Possessing Virulence Genes in relation to Seasons

Table 4.18: Sequence Analysis of 16S rRNA genes of Bacteria Isolated from Waste Dump Sites in Zaria Metropolis

DNA from soil samples	Cloest match from Genbank database	Sequence Identity (%)	Accession Number	E-Value
TW(E7)	*E. coli* O157:H7	96	NC 002695.1	0.00
SG(E1)	*E. coli* O157:H7	97	NC 002695.1	0.0
ZC(E3)	*E. coli* O157:H7	95	NC 011750.1	0.0
SA(E5)	*E. coli* O157:H7	95	NC 002695.1	3e-133
SG(E2)	No significant similarity found			
ZC(E4)	*E. coli* O157:H7	94	NC 011750.1	0.00
TW(E6)	*E. coli* O157:H7	96	NC 002695.1	0.00
ZC(S7)	*Salmonella enterica* subsp. *enterica* serovar Typhi	96	NC 003198.1	0.00
TW(S1)	*Salmonella enterica* subsp. *Enterica* serovar Typhimurium	95	NC 00307.1	0.00
SG(S5)	No significant similarity found			
TW(S6)	*Salmonella enterica* subsp. *Enterica* serovar Typhi	98	NC 003198.1	0.00
TW(S4)	*E. coli* O83:H1	96	NC 017634.1	0.00
SA(S3)	*Salmonella enterica* subsp. *enterica* serovar Typhi	96	NC 003198.1	0.00
ZC(S2)	*Salmonella enterica* subsp. *enterica* serovar Typhi	95	NC 003198.1	0.00

KEY: SA- Samaru, SG- Sabon-Gari, TW- Tudun-Wada, ZC- Zaria city

Their percentage identity scores range from 94% to 98%. All the sequences that were subjected to BLAST revealed e- values of 0.00 (Table 4.18).

The phylogenetic tree between the 16S rRNA sequences from species of *E. coli* constructed using a Maximum Parsimony is shown in Figure 4.9. All isolates showed evolutionary relationship as shown in the rooted tree and have a common node. However, there was no significant similarity found with isolates E2 and S5.

Diversity among isolates is also shown on the tree which separates the isolates into numerous clades based on differences in their nucleotide sequences. The bootstrap of the phylogenetic tree from the Maximum Parsimony method results (Figure 4.9) with the values at the nodes of the branches indicates the level of consistency of the tree. The higher the value the more reliable is the node.

Figure 4.10 shows the phylogenetic tree between the 16S rRNA sequences from species of *Salmonella* constructed using a Maximum Parsimony method. All isolates clustered together. However, there was no significant similarity found with isolate S5. Diversity among isolates is also shown on the tree which separate the isolate into numerous clades based on differences in their nucleotide sequences. Accession numbers of sequences from Genbank are in parentheses.

The trees were rooted with *Geitlerinema* sp., (from the phylum: Cyanobacteria); accession number of reference strain from the Genbank is indicated in parantheses. Bootstrap values carried out for 500 replicates; the numbers at the nodes indicate the bootstrap value in percent. Bar signifies nucleotide divergence.

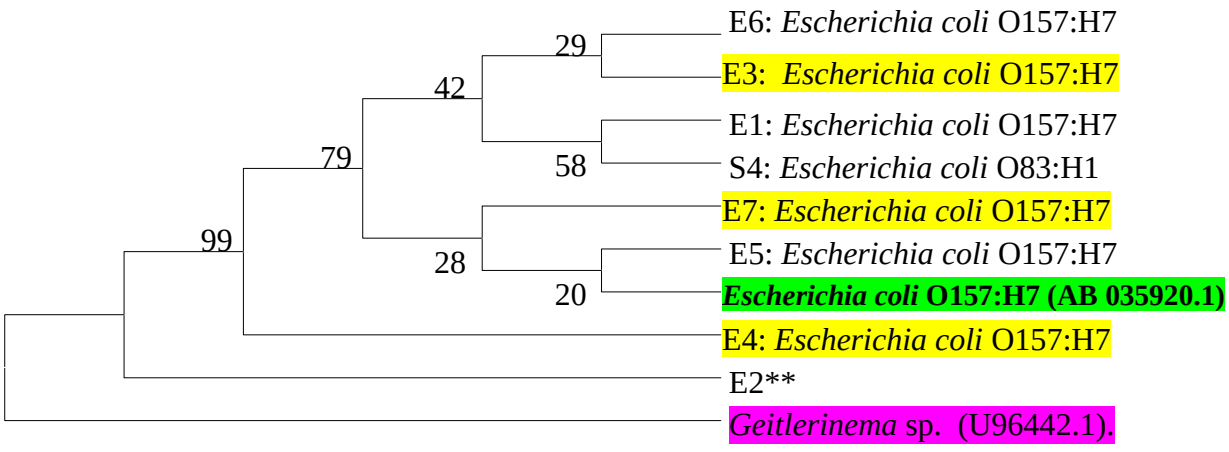


Figure 4.9: A Maximum Parsimony Tree showing Phylogenetic Affiliation of 16S rRNA Sequences from species of *E. coli* obtained from Waste Dump Soils in Zaria Metropolis

The tree wer rooted with *Geitlerinema* sp., (from the phylum: Cyanobacteria); accession number of reference strain from the Genbank is indicated in parantheses. Bootstrap values carried out for 500 replicates; the numbers at the nodes indicate the bootstrap value in percent.

KEY:

E6- *E. coli* O157:H7 obtained from Tudun-Wada sample

E3, E7and E4 - *E. coli* O157:H7 obtained from Zaria city samples

E1- *E. coli* O157:H7 obtained from Sabon-Gari sample

E5- *E. coli* O157:H7 obtained from Samaru sample

S4- *E. coli* O83:H1 obtained from Tudun-Wada sample

***Escherichia coli* O157:H7 (AB 035920.1) –** Reference Strain

Geitlerinema sp- Organism that was used to root the tree

**No close match found in Genbank database

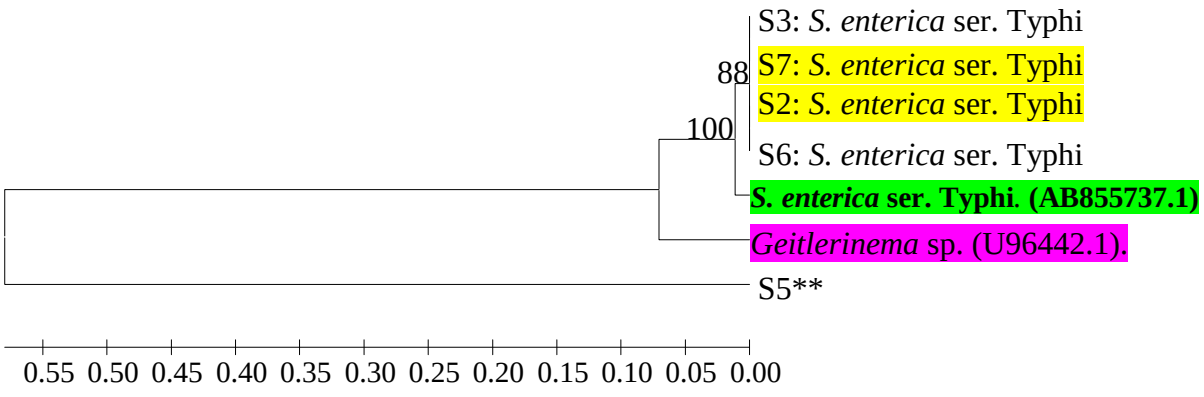


Figure 4.10: A Maximum Parsimony Tree showing Phylogenetic Affiliation of 16S rRNA Sequences from species of *Salmonella* obtained from Wate Dump Soils in Zaria Metropolis

The tree wer rooted with *Geitlerinema* sp., (from the phylum: Cyanobacteria); accession number of reference strain from the Genbank is indicated in parantheses. Bootstrap values carried out for 500 replicates; the numbers at the nodes indicate the bootstrap value in percent. Bar signifies nucleotide divergence.

KEY:

S3- *Salmonella enterica* serovar Typhi obtained from Samaru sample

S2, S7- *Salmonella enterica* serovar Typhi obtained from Zaria City sample

S6- *Salmonella enterica* serovar Typhi obtained from Tudun-Wada sample

***S. enterica* ser. Typhi. (AB855737.1)** – Reference Strain

Geitlerinema sp- Organism used to root the tree

**No close match found in Genbank database

Bar signifies nucleotide divergence

CHAPTER FIVE

5.0 DISCUSSION

5.1 Temperature and pH of the Waste Dump Soil Samples

In the assessment of the environment from which the samples were sourced, slight disparity between the dump temperatures at various sampling locations was observed. This discrepancy may in part have been a result of diurnal variations as the sampling was done at different times of the day, the dumps being distanced apart from each other. Previous researches have indicated the existence of different microenvironments within the waste dump piles that have varied physical compositions (Atchley and Clark, 1979; McKinley and Robie, 1984; Foster, 1988; Elaigwu *et al*., 2007). These dissimilar environments result from the non-homogeneity of the wastes and different microbial activities taking place in these micro-niches. Besides the physical nature of the wastes, other factors like different moisture contents at the various sampling spots would also influence the microbial activities at the sampling spots.The presence of these microenvironments in the dump sites was probably responsible for the temperature variations at the various sampling locations.

Since the sampling was done at a depth of about 0 to 15 cm below the surface, the layers of waste materials shielded the inner portions from the direct atmospheric temperatures. This, coupled also with the insulating nature of the waste materials explains the independence of the waste temperatures from the ambient. The temperature of the soil samples from all the sampling sites ranged between 27^{o}C and 36^{o}C for the dry season and between 23^{o}C and 36^{o}C for the wet season. These values fall within the mesophilic range of temperatures for most pathogenic bacteria whose optimum temperature for growth is 37^{o}C with upper and lower temperature limits of 40-50^{o}C and 15-20^{o}C respectively

(Arora, 2004). Hagerty *et al.* (1973), reported that during initial composting development, the mesophilic flora predominate and are responsible for most of the metabolic activities that occur. This increased microbial activity elevates the temperature of the compost, with the subsequent replacement of mesophilic population by thermophilic flora. In all the cases the temperature of the dump sites were found to be higher than that of the Control Site. The elevated temperatures in the waste dumps noted have also been reported by other authors (Hagerty *et al.*, 1973; Bach, 1987; Yeşiller *et al.,* 2005*;* Tognetti *et al*., 2007) and are due to biological decomposition. The insulation of the metabolic heat and its trapping within the waste heap has been known to elevate the temperatures in the waste dump piles from the ambient to levels in excess of 70 or 80°C (Jeris and Regan, 1973; Epstein *et al*., 1976; Tansey and Brock, 1978). Such elevated temperatures were however not recorded in any sampling spots or locations from the waste dumps in this study. It indicates that the thermophilic activity at this depth or at the time of sampling was not optimum. This further indicates that our values of temperature (23 ^{o}C to 36^{o}C) are lower than the values of the aforementioned authors. However, the values in this work are higher when compared to the values obtained by Obire *et al*. (2002) (27^{o}C – 28^{o}C) and Adekanle *et al.* (2014*)* (25.1^{o}C- 25.9^{o} C). The differences in the temperature values between this and earlier studies could be explained when factors such as the physical nature of the wastes and different moisture contents at the various sampling spots and locations are considered.

The pH levels of the sampled spots within the dumps were not indicative of the entire waste masses. The varied pH of the samples at different sampling locations attests to the existence of different micro-environments within each of the dump sites. The large number of these difference niches in the dumps could have been occasioned by the very

heterogeneous nature of the waste mass, the varied source of the materials, the diverse microbial population and activity as well as the stage of the waste occurring at each location (at the times of sampling) as has also been reported by Pavoni *et al*. (1975), Atchley and Clark (1979) and Holm-Nielsen *et al*. (2006). The highest pH values recorded in this study (9.56) could have been due to the age of the waste. Pavoni *et al*. (1975) mentioned that in the first 2 to 3 days of composting, the pH drops to 5.0 or less and then begins to rise to about 8.5 for the remainder of the aerobic process. Similar alkaline pH values for waste dumps have also been observed in other studies (Aziz *et al*., 2010; Osunwoke and Kuforiji, 2012; Zainol, 2012; Adekanle *et al*., 2014). However, Obire *et al*. (2002) reported a shorter range of pH values of 5.4 - 7.9. Hagerty *et al.* (1973) reported that the initial pH of solid waste is between pH 5.0 and 7.0 for refuse which is about 3 days old. The difference in pH for ZC, TW, SG and SA samples could be attributed to the nature of the wastes found in these sampling locations and the age of the waste as earlier reported.

5.2 Mean Aerobic Bacterial Counts of Soils from Waste Dumps

Bacterial growth depends upon various physicochemical conditions such as media, pH, temperature, incubation period, carbon source etc. The mean bacterial counts of soils from waste dumps ranged between 3.0 x 10^7 cfu/g and 6.40 x 10^8 cfu/g. This was higher than that recorded in Mexico by Flores-Tena *et al*. (2007) (3.0x10^8 cfu/g); (Oviasogie *et al*., 2010) in Benin City, Nigeria (4.0x10^5 cfu/g to 10.0x10^5 cfu/g) and (Adekanle *et al.*, 2014) in Osun State **(**112x10^5 cfu/g to 187x10^5 cfu/g). However, Osunwoke and Kuforiji, (2012) reported a range of 1.0x10^5 cfu/g to 7.0x10^{17} cfu/g in Ota, Ogun State, Nigeria. The higher bacterial counts recorded in this study could be attributed to the higher degree of pH recorded for the soil (pH 6.21 - 9.34) and the type of waste generated in the refuse dumps.

It was observed that ZC had the highest bacterial counts of all the sample locations (Table 4.2). This could be as a result of the high degree of acidity recorded in this location (8.27) and the waste composition (large amount of human and animal faeces). It was noted that most streets in this location lack public conveniences and when nature calls, any waste dump around becomes latrine. Also, the nearby clinics dispose their hospital wastes in that refuse dump site. This implies that there were enough nutrients to support large microbial populations as obtained from stations: TW, SG and SA (Table 4.2).

Statistical analysis using analysis of variance (ANOVA) at 5% significance level revealed a significant difference between the bacterial counts of locations ZC and SA as well as between ZC and CS (Table 2). This infers that location SA and the CS supported the least bacterial populations in comparison with all other sampling sites, whereas location ZC recorded the highest bacterial count. This could be attributed to the kind of waste generated, the temperature and pH recorded in each dump location. This study is at variance with the report of Obire *et al.* (2002) who found that there was no statistical significant difference in the number of bacteria and fungi at 5% level of significance among the four stations of the waste dumpsite studied.

The mean bacterial counts were significantly higher in the dry season than in the wet season ($P< 0.05$). The decrease in microbial load in the wet season could be due to the migration and infiltration to the lower layer caused by rain and decrease in the quantity of organic matter present for microbial use due to flushing caused by rain (Flores-Tena *et al*., 2007).

Temperature and pH affect bacterial counts. The study revealed a strong correlation between temperature, pH and bacterial counts. This is in consonance with the findings of

Elaigwu *et al*. (2007) who reported that temperature and pH of soil affect all soil properties that is, chemical, physical and biological. However, there was a strong association between temperature and bacterial counts (r= 0.653). This is in agreement with the findings of Hassen *et al*. (2002) who reported that under aerobic conditions, temperature is the major factor that determines the rate of microbial metabolic activities. Foday *et al.* (2013) opined that high temperatures speed up the rate of bacterial action on biodegradable organic material.

The results of the principle component analysis revealed that temperature is the major factor with (65.4%) on microbial activities as compared to (18.7%) pH. This validates the results of the correlation analysis and confirmed the early assumption made by Hagerty *et al.* (1973) who opined that during initial composting development, the mesophilic flora predominate and are responsible for most of the metabolic activities that occur. This increased microbial activity elevates the temperature of the compost.

5.3 Occurrence of Pathogenic Bacteria in Waste Dumps in Zaria Metropolis

Soil environment, by its nature, presents a theatre of ecological diversity and evolutionary adaptation. Many anthropogenic activities have been known to be major causes of pollution of soil. Most of the population under study dump their wastes in open spaces and drainage channels.These areas are largely untreated and poorly managed. Most of these dump sites are situated very close to residences. Indiscriminate waste disposal and inadequate waste management account for the major source of microbial pollution and contamination of soil, air, land and water (Nwaokwe, 2004; Grisey *et al*., 2010).

Pathogenic bacteria isolated from soils at dumpsites showed prevalence of *Salmonella enterica* (6.92%), *Vibrio cholerae* non-O1 (1.35%) and *E. coli* O157:H7 (4.23%). Other

bacterial species such as *Citrobcter* spp. (3.70%), *Proteus* spp. (11.64%), *Klebsiella* spp. (5.82%), *Enterobacter* spp. (3.17%), *Escherichia coli* (35.45%) etc. were also isolated, which implied that the waste dump soils were heavily contaminated. The isolation of these pathogenic bacteria in these waste dumps is not surprising since the alkaline pH and mesophylic range of temperature recorded for the soil samples in the study (pH 6.21 - 9.34) and (23 ^{o}C - 36^{o}C) respectively would favour the proliferation of many bacterial genera. This is in line with the report of Arora, (2004) who opined that most of the medically important bacteria can grow at neutral or slightly alkaline pH and to the findings of Osunwoke and Kuforiji, (2012) who reported that high pH would favour the proliferation of bacteria than that of fungi.

Salmonella enterica and *Escherichia coli* O157:H7 were isolated from all the four waste collection sites while *Vibrio* spp. were isolated from all except location SA. This shows that sanitation and waste management is generally poor in all parts of Zaria Metropolis. The presence of these pathogens may be due to domestic wastes which usually form the main component of wastes at the dumpsites. The isolation rate of *Salmonella enterica* (6.92%), *Escherichia.coli* O157:H7 (4.23%). and *Vibrio cholerae* (1.35%) in this study is lower when compared to the study conducted in Adim community, Cross River State, Nigeria with these values: *Vibrio cholerae*, 14.7%, *Salmonella paratyphi,* 12.5%, *Salmonella typhimurium,* 8.8% and *Shigella sonnei,* 6.3%; Udo and Nfongeh (2005); Ikpeme *et al.* (2011) in Cross River State, Nigeria,with these values: *Salmonella* spp. (47.02%), *Shigella* spp (21.63%), *Vibrio cholerae* (13.10%), *Escherichia coli* (58.37%). The differences in the prevalence between this and earlier studies could be explained when factors such as differences in study time, sample size, nature of the waste, environmental and geographical variation are considered. The isolation of these pathogens

at dumpsites directly confirms the presence of faecal wastes at various dumpsites since it was common practice to dump human excreta in the sites which could also be used as latrines at night.

The dumpsite on its own offers a rich source of organic matter and factors that favour the abundance of these organisms. The high number of bacteria from the family Enterobacteriaceae and considerable number of faecal coliform bacteria is an indication that the environment is hazardous and constitutes serious health risk and threat to both the waste workers and residents of the nearby community. According to Scarpino and Quinn (2010), people in close proximity to the dumpsite complained of serious odours emanating from the site, personal discomfort due to the odours, loss of sleep, possible allergic manifestations and respiratory difficulties. Of the genera of organisms isolated, *Escherichia coli* had the highest frequency of occurrence (Table 4.8), this agrees with the findigs of Lewis and Gattie (2002) who reported that *Escherichia coli* is able to withstand competition from the other indigenous microorganisms with higher growth rates and to the findings of Van Elsas *et al*. (2011) who reported high incidence of *Escherichia coli* in their study.

Majority of *Vibrio cholerae* strains in the environmental reservoir have been observed to belong to the non-O1/non-O139 serogroups (Smritikana *et al*., 2012). The ability of *Vibrio cholerae* to survive within and outside the aquatic environment makes cholera a complex health problem to manage (Osei and Duker, 2008). Cheesbrough (2006) opined that cholera is endemic in parts of Africa, South and Southeast Asia where sanitation and hygiene are inadequate and water supplies are contaminated and not treated.

The results of this study revealed that waste dumps in Zaria metropolis are associated with enteric pathogenic bacteria such as *Salmonella enterica*, *Escherichia coli* O157:H7 and *Vibrio cholerae*. The occurrence of these pathogenic Gram-negative bacteria in the waste dumps suggest that there is a significant sanitary risk, especially to the waste workers, scavengers and those leaving in close proximity to the dumps. There is a risk for these people to develop gastrointestinal infections. However, diseases such as bacteremia, thyphoid fiver, meningitis, urinary tract infections, cholera, respiratory infections and other opportunistic infections can also occur, especially for immunosuppressed individuals. The cattle, dogs and chicken living nearby are also at risk. There are farms very close (less than one kilometre) to the waste dumps and even cattle graze and drink a few meters away from some of these waste dumps. Several strains isolated from these waste dumps are pathogenic for bovine, pigs and birds. These animals could also be a potential risk as pathogen dispersers. Much of the pathogenic bacteria are transmitted by infected hosts. However several pathogenic microorganisms are transmitted by environmental carriers. Osei and Duker (2008) hypothesized a high rate of contact of these pathogens through flies. Flies are attracted by the odour emanating from refuse dumps, especially the common housefly. This fly lives in close association with man feeding on all kinds of human food, garbage and excreta, and will travel no farther from its breeding site (refuse dumps) to the nearest resting place. The indiscriminate feeding habits (feeding on filth and human food) of this fly species combined with its structural morphology (presence of hair and sticky pads) make them ideally suited to carry and disseminate pathogenic microorganisms (Greenberg, 1976; Fotedar, 1992; Kobayashi *et al*., 1999). Research has proven that the common housefly (*Musca domestica vicina*) and flies in general are mechanical vectors of many kinds of pathogens such as bacteria

(Cohen *et al*., 1991), protozoa (Fotedar *et al*., 1992), viruses (Ogata *et al*., 1961), and helminth eggs (Sulaiman *et al*., 1988; Dipeolu, 1982). Fotedar, (2001) undertook a study to ascertain the vector potential of the domestic housefly as a carrier of *Vibrio cholerae* in Delhi, India, where an outbreak of cholera was encountered. Viable *Vibrio cholerae* was isolated from six (60%) of the pooled fly samples, which confirmed that there were potentially contaminated mechanical vectors among the flies. Some published reports have also shown that fly control measures can be effective in reducing the incidence of diarrhoea (Cohen *et al*., 1991, Chavasse *et al*., 1999). Where high fly populations and poor hygiene conditions prevail, or where pathogens can grow within fly-contaminated food, the potential exists for transmitting pathogens with a high infectious dose (e.g., *Vibrio cholerae, Salmonella* spp.) (Gordon, 2005). Studies also show that *Vibrio cholerae* is able to colonize and multiply within some flies like the *Drosophila melanogaster* (Blow *et al*., 2005). Although *Vibrio cholerae* infection is dose dependent (about 10^8 cells) (Levine *et al*., 1981), and the number of bacterial cells flies can carry is not clear, flies can contaminate food in which the cholera vibrios could multiply and reach an infective dose. It is worth noting that this study does not prove fly and animal transmission of these pathogens to humans, but only gives an indication of their possible involvement in transmission. Therefore, further studies are required to emphatically prove this hypothesis.

The present study has also shown that seasonal influence can affect microbial proliferation. During the dry season 36 target bacteria were isolated from the dumpsites soil. By contrast, only 29 target bacteria were isolated in the wet season, this is not supprising in view of the fact that the bacterial counts were also high in the dry season than in the wet season. High incidence of bacterial species was also recorded by Flores-Tena, (2007) in Mexico during the dry season (9 bacterial species) than during the wet

season (6 bacterial species). Foday *et al*. (2013) reported that during summer (dry season) high temperature speed up microbial activities**.** It was observed that in the dry season, the number of *E. coli* O157:H7 was 11 and in the wet season, the munber of isolated strains was similar. This suggests a great adaptative potential for these strains and confirmed the earlier assumption made by Lewis and Gattie, (2002). However, the isolation rate of *Salmonella enterica* was higher in the dry season than in the wet season and that of *Vibrio cholerae* non-O1 was higher in the wet season than in the dry season. This suggests that bacteria of the waste dump sites respond differently to seasonal influence. This is in-line with the report of Obire *et al.* (2002) who opined that variation of climatic conditions such as wet and dry seasons, may selectively favour certain physiological types. It has also been stated that under aerobic conditions, temperature is the major factor that determines the types of microorganisms, their species diversity and the rate of their metabolic activities (Hassen *et al*., 2002).

5.3.1 Health Problems Associated with Enteric Pathogenic Bacteria

The occurrence of 46 pathogenic bacteria in waste dumps in Zaria metropolis is a matter of public healths:

Infection caused by *Vibrio cholerae* can proceed to dehydration, electrolyte disturbances, renal failure, hypovolaemic shock and subsequent death if immediate and proper management is not effected (Cheesbrough, 2006). A large number of *Vibrio cholerae* non-O1/non-O139 strains are able to produce cholera like toxins, i.e., heat-stable enterotoxin, thermostable direct haemolysin, hemaagglutinin/ protease, shiga-like toxin play significant role in the enteropathogenecity.

Salmonella is the most common causative agent of a wide range of human diseases such as enteric fever, gastroenteritis, endocardiatis and bacteraemia (Nazzal *et al*., 2012). *Salmonella* spp. are capable of causing typhoid fever and food poisoning (Wachukwu *et al*., 2002).

Infection caused by *S.* Typhi can lead to inflammation and ulceration of the intestine, epistaxis, intestinal haemorrhage and perforation (anaemia may occur with increase in white cell count), toxaemia, renal failure and subsequent death in untreated late typhoid (Cheesbrough, 2006). *S.* Typhi can also cause osteomyelitis and typhoid arthritis particularly in immumocompromised individuals. *S.* Typhimurium causes bacteraemia and septicaemia in young children and immunosuppressed. *S.* Typhimurium is also reported as causing neonatal meningitis (Although infections with non-typhoidal Salmonellae usually cause self-limiting diarhoeal illness, serious sequelae, including meningitis, septis, and death may occur, especially among infants and elderly persons (Akinyemi *et al*., 2007).

Enterohaemorrhagic *E. coli* (EHEC) refers to a subset of Shiga toxin-producing *Escherichia coli* (STEC) strains found to cause human and sometimes animal disease. STEC causes life-threatening haemorrhagic diarrhoea, haemorrhagic colitis (HC) and can progress to haemolytic uremic syndrome (HUS) with renal failure, thrombotic hospitalization and intensive care and subsequent death if proper management is not effected (Terrance *et al.*, 2002; Cheesbrough, 2006; Marie *et al*., 2011)**.** *Escherichia coli* is one of the organisms that cause Urinary Tract Infection (UTI) and gastroenteritis in children.

Mortalities can occur with these bacterial organisms in the form of secondary infections. The number of these types of infections can directly link to dumpsite thereby leading to death.

Osunwoke and Kuforiji (2012) opined that truly pathogenic forms may survive in waste. In line with this statement, all the bacterial genera reported in this study have been reported by Wachukwu *et al*. (2002) and Cheesbrough (2006) as potential pathogens. That is, they are capable of causing diseases. It is important that regulatory framework is developed.

5.4 Antibiogram of the Isolates Obtained from Waste Dumpsites

The importance of susceptibility testing cannot be over emphasized. It provides guidance to selective use of drug for therapy and information on the spectrum of an antimicrobial agent. Degree of susceptibility can assist in determining the length of therapy and choice of antimicrobial agents with fewer side effects (Edward and Ewing, 2003). Therefore, it is of great importance to monitor antibiotic resistance among pathogenic bacterial isolates from waste dump soils to detect emerging resistant pathogens and antibiotic resistance trends.

From the results obtained in this study, antibiotics resistant bacteria were widespread as nearly all the isolated organisms were resistant to most of the antibiotics against which they were tested. The origin of this resistance can be traced to the faecal constituent of the waste dumps produced by people or animals that have been treated indiscriminately with various antibiotics and also to antibiotics production naturally by soil microorganisms. Similar result was obtained by (Odeyemi, 2012) in a study conducted on antibiogram status of bacterial isolates from air around dumpsite of Ekiti State in Nigeria who reported

resistance of the organisms to the entire panel of antibiotic against which they were tested. A high level of resistance has been found with members of the family Enterobacteriaceae and Vibrionaceae which are believed to have increased the incidence of pathogenic strains of bacteria with acquired antibiotics resistance (Odeyemi, 2012). This may be due to either the in-trinsic resistance of many microorganisms to antibiotics or acquired resistance of the organisms enabled by the transfer of drug resistance plasmids among members of the isolates (Ajayi and Akonayi, 2003; Lamrani *et al*., 2010). Since antibiotics in animal feeds promote animal growth, improved efficiency of feed conversion to body weight, and may also affect disease prophylaxis among the confined microbes in those animals and their subsequent impact on human health, it has increased its indiscriminate use (Scarpino *et al*., 2010).

The variation in the susceptibility and resistance level of the bacterial isolates to different classes of antibiotics in this study could be due to differences in species from location to location. Antibiotic susceptibility patterns vary regionally and geographically and have been reported to change rapidly over time (Mengistu *et al*., 2014). The source of isolation and variability of strains among the same species may influence antibiotic susceptibility test. Although antibiotics have been very effective in the treatment of enteric bacterial infections and play a crucial role in reducing mortality, the progressive increase in antibiotic resistance among enteric pathogens in

developing counties is becoming a cause for concern (Mengistu *et al*., 2014). All target isolates obtained in this study are considered pathogenic and therefore represent a hazard for public health but the rate of resistance to antibiotics varies among them.

High incidence resistance was evident against Ampicillin, Amoxycillin-clavulanic acid and Tetracycline on *Salmonella enterica* and *E. coli* O157:H7 isolates, against Ampicillin,

Cotrimoxazole and Tetracycline on *Vibrio cholerae* non-O1 isolates (Table 4.10), indicating that resistance to these antimicrobial agents is wide spread in Zaria metropolis. These antibiotics are commonly used to prevent diseases in human beings and animals. Therefore, enteric bacteria pathogens entering into the soil with human or animal faeces with antibiotic resistant plasmids might have contributed to the prevalence of the resistance in genes in the waste dump soil environment which is concurrent with the reports of Ajayi and Akonai (2003); Flores-Tena *et al.* (2007); Ikpeme *et al.* (2011); Odeyemi (2012). At the same time, there has been a significant increase in the level of antibiotic residues entering the environment (Flores-Tena *et al.,* 2007; Moura *et al*., 2010). The observed high resistance of the isolates to ß- lactams observed in the study agrees with the findings of Olorunmola *et al*. (2012); Omojowo and Omojasola, (2013) and Akingbade *et al.,* (2014).

Resistance of the *Salmonella* isolates to various antibiotics has been demonstrated in previous studies. Boyen *et al.* (2008) and Duru *et al*. (2014) reported a high resistance by *Salmonella* to ß- lactam class of antibiotics namely: ampicillin, augmentine and ceftriazone. These findings are consistent with the results in this study where 68% of the *Salmonella* isolates showed resistance to the β-lactam class of antibiotics (Ampicillin, Amoxicillin-clavulanic acid and Cefotaxime).

Resistance of *Vibrio cholerae* non-O1 isolates to Sulfamethoxazole/Trimethoprim (100%) drugs in this study is concomitant with the study of Lamrani *et al*. (2010) who find non-O1 *Vibrio cholerae* strains resistant to sulfamethoxazole (75%), followed by Streptomycin (62%), and Cephalothin (60%) and Trimethoprim (49%). However, resistance to Chloramphenicol (43%) by the non-01 *Vibrio cholerae* isolates in this study is contrary to

the findings of Lamrani *et al*. (2010) who reported all the non-01 *Vibrio cholerae* isolates susceptible to Chloramphenicol.

Vibrio cholerae frequently change their characteristics regarding antibiotic susceptibility, producing serious problem in health management and this is due to presence of drug resistant gene which is solely responsible for the transmission and spread of the multidrug resistant properties among the pathogen (Lamrani *et al*., 2010).

The high resistance of *E.coli* isolates to Tetracycline (88.9%) may be attributed to it being the most commonly available antibiotic used as growth promoter and routine chemoprophylaxis among livestock in Nigeria (Olatoye, 2010; Reuben and Owuna, 2013; Olukosi *et al.,* 2016). This is worrisome considering the fact that Tetracycline is a first line drug in Nigeria, and as in most developing countries, people with gastrointestinal infections readily purchase it across the counter for self-medication (Chigor *et al.*, 2010). This study is in-line with the findings of Olukosi *et al.* (2016) who found *E. coli* isolates resistant to 80% tetracycline. The highest antimicrobial resistance observed was to the Penicillin families (Ampicillin, Amoxycillin).The resistances of the bacterial isolates to commonly used antibiotics as obtained in this study indicate the potential dangers they may pose to the public health. It has been reported that major epidemics in the World have been linked withantibiotic resistant pathogens (Levy, 2001).

Antibiograms of the target pathogens isolated revealed resistance to preferred drugs. All isolates showed high resistance to Ampicilln (95.7%), Amoxycillin-clavulanic acid (79.5%) and Tetracycline (73.9%) while Ciprofloxacin (12.8%), Chloramphenicol (17.9%) and Gentamicin (20.0%) had low resistance percentages for the isolates. The low resistance or susceptibility of the isolates to fluoroquinolones, phenicols and aminoglycosides observed in the study means that these chemotherapeutic agents are still

effective in the treatment of diseases caused by these pathogens which may be as a result of the relatively high cost of ciprofloxacin and chloramphenicol thereby limiting their indiscriminate use as few individuals can afford to buy them. Gentamicin (10μg) is administered parenterally and therefore cannot be easily taken without the aid of a skilled medical personnel. However, Ampiciline (10 μg), Amoxycillin-clavulanic acid and Tetracycline which are relatively cheaper showed lesser activities against the isolates because of their indiscriminate use as many individuals can afford them.

Salmonella enterica in the study showed 100% susceptibility to the aminoglycoside Gentamicin; however, it has been reported that in vitro susceptibility of *Salmonella* spp. and *Shigella* spp. to aminoglycosides may appear active but are actually not effective clinically and should not be reported as susceptible (CLSI, 2014).

E. coli O157:H7 showed low resistance to Cotrimoxazole (33.3%). This conforms to the findings of Schroeder *et al.* (2002) who reported that among *E. coli* O157:H7 isolated from various sources between 1985 and 2000, 19 (10%) were resistant to this drug and to the study of Momtaz *et al*. (2012) who found *E. coli* isolates resistant to 45.6% Sulfamethoxazole. However, this research is contrary to the findings of Adejuwon *et al*. (2011) and Ali *et al*. (2011) who reported *E. coli* sensitive to most of the antibiotics among which were Cotrimoxazole and Tetracycline used in this study. The observed high resistance of the isolates to some of the antibiotics in this study is not suprising as factors that could promote or encourage development of antibiotic resistance such as non-adherence to treatment strategies, sub-standard drugs, over-the-counter sale of potent antibiotics and self medication are present in developing countries including Nigeria. Therefore it is of great importance to monitor antibiotic resistance among isolates such as

Salmonella, E.coli and Vibrio cholerae from waste dump soils to detect emerging resistant pathogens and antibiotic resistance trends.

Multiple-antibiotic-resistance (MAR) has been defined in this study as resistance of isolate to at list four different antibiotics or more than two different classes of antibiotics (Ezekiel *et al*., 2011) and was calculated as the ratio of number of antibiotics to which an organism is resistant to total number of antibiotics to which an organism is exposed (Furtula *et al*., 2013). Zaria city had the highest percentage occurrence of MAR (26 %) obviously because the location had the highest number of isolates. Most of the resistant isolates showed the AmpAmcSxtTe -resistance pattern. These antibiotics belong to the β-lactams, Sulfonamides and Tetracycline classes of antibiotics. The isolates showed 17 resistance patterns, and were MAR to 4-7 antibiotics.

It was observed that 65.2% of the targets isolates were multidrug resistant in the study. This agrees with the findings of Ikpeme *et al*. (2011), Odeyemi, (2012), Adekanle *et al*. (2014) who reported multiple resistance among the isolates in their study. Multi-drug resistant pathogenic bacteria have emerged in recent time presumably due to the extensive use of chemotherapeutic agents in both humans and animals (Miriagou *et al*., 2006). Antibiotics are used in livestock production, disease promotion and as growth promoting feed additives in veterinary medicine (Swartz, 2002; Van *et al*., 2007). Due to the extensive use of antibiotics in both humans and animals, the normal flora of the intestine are disrupted resulting in the emergence of antibiotic resistant enteric pathogens including *Vibrio cholerae*, *Salmonella* and *E.coli* and prolonged faecal shedding of these organisms into the environment (Threlfall, 2002). Multy drug resistance (MDR) is a cause for concern in both clinical and veterinary medicine because it limits the therapeutic options available for treatment.

The fact that several bacterial species are known to be resistant to a wide array of antibiotics was confirmed by Aseffa *et al. (*1997). Multiple antibiotic resistance in *E.coli* isolates may be partly due to the spread of genetic elements including plasmids, transposons and integrons (Zhao *et al*., 2001). The marked resistance of strains of *Salmonella, and Vibrio cholerae* to Ampicillin and Tetracycline as shown in the present study agrees with the findings of Ash *et al.* (2002) and Gregory *et al*. (2013) working on rivers in the United States. Similarities in antibiograms among isolates from both environmental sources indicate a possible infiltration of pathogens from dumpsite soils to water sources.

The multiple antibiotic resistance among bacterial isolates from the various study locations is frightening because such organisms can become endemic within the environment and pose serious public health threats. They may also act as reservoirs which will contribute to the maintenance and spread of antibiotic resistance genes (Guardabassi *et al.*, 2004; Constanzo *et al.*, 2005).

Multiple antibiotic resistance is becoming increasingly widespread and therefore, antimicrobial agents are becoming less and less effective. Consequently, majority of the older antibiotics (penicillin, tetracycline, ampicillin, and cloxacillin) have been rendered ineffective while the efficacy of the newer antibiotics is being increasingly droped. The rip-ple effects of these developments connotes that for certain strains, there are few or no effective antibiotic (Obi *et al.*, 2007). These reports are in agreement with that of our study indicating no 100% effective drugs on the *Vibrio cholerae* non-O1. Persistent multiple drug resistance of most isolates to appropriate drugs of choice are of great public health concern and calls for periodic monitoring of antibiograms to detect possible changing patterns. Many of the isolates from this study were observed to have patterns of

resistance that indicate that the genes responsible were acquired from other organisms, such as resistances to antibiotics of different classes.

The public health significance of these findings is that antibiotics resistance of the target isolates from waste dumps may colonize the human population via environmental carriers, water sources and the food chain since it was observed that some farms were located just few meters away from the waste dumps, some cattle graze and drank waterfrom ponds few meters away, and some houses overlapping the waste dumps (Plate II and plate IV).

5.5 Virulence and Antibiotic Resistance Genes

The Polymerase Chain Reaction (PCR) is a technique for the *in vitro* amplification of specific segments of DNA. Rapid detection and identification of pathogenic organisms is essential for public health, and conventional PCR which is relatively less expensive and easier to use than other PCR-based assay, provides rapid means to identify microorganisms from culture and a variety of samples. Other amplification methods such as Real time PCR, Nested PCR, Rapid PCR, Multiplex PCR etc. have been employed for detection of pathogenic bacteria such as *Vibrio* species, *Salmonella* species, *E. coli* strains (Moore and Feist, 2007; Hyong *et al.,* 2008; Lee *et al.,* 2009).

Among the 30 multiple-antibiotics resistant targets isolates, three resistance genes conferring resistance to two cathegories of antibiotics, including Tetracyclines, β-lactams, were identified. The PCR results were consistent with the antibiotic susceptibility phenotypes (Table 4.13). For example, the Tetracycline tetA and tetB genes were detected in the Tetrcycline-resistant isolates. This aggrees with the report of Adesoji *et al*. (2015) who opined that the tetracycline resistance is a common and developing aspect of resistance among bacteria and the tetracycline resistance genes including tetA and tetB

have been detected from environmental samples. Also, bla_{TEMl}, β-lactamase gene conferring resistance to β-lactam antibiotics was detected in 3 isolates, all of which were resistant to Ampicillin, Amoxicillin-clavulanic acid and Cefotaxime. Similar findings were made by Mooljuntee *et al*. (2010) and Momtaz *et al*. (2012). However, several other isolates with similar resistance pattern gave negative amplifications with these genes in this study. This could be due to the fact that there are several kinds of β-lactamase genes such as bla_{CMY}, bla_{SHV} gene, bla_{PSE-1} gene or bla_{CTX} genes which were not covered in this study but have been known to confer similar resistance in these isolates (Chen *et al.*, 2004; Mooljuntee *et al*., 2010; Momtaz *et al*., 2012; Adesoji *et al.*, 2015). Other genetic element conferring antibiotic resistance is located on movable genetic elements such as plasmid and may not be detectable in the genomic DNA of the organism. Guerra *et al*. (2000) opined that for an accurate characterization of pathogenic bacteria other features such as virulence, antimicrobial resistance and plasmid profiles should be determined and related, because virulence and resistance genes can be plasmid-mediated.

Seven isolates of *Salmonella* and *E. coli* put together all of which were resistant to Tetracyline showed positive amplification with the tetA and tetB gens. However, several other isolates that were resistant to this antibiotic gave negative amplification with these genes. Gene encoding for tetracycline resistance and multidrug resistance in Gram negative bacteria have been found associated with mobile genetic elements that encode specific resistance genes (Ikpeme *et al*., 2011).

The prevalence of tetA gene was higher (33.3% and 66.7% genotype tetA) than tetB gene (16.7% and 0% genotype tetB) for *Salmonella* and *E. coli* isolates respectively in this study (Table 4.13). This is in consonance with the findings of Koo and Woo, (2011), who opined that tetA gene can be spread more easily in the environment. However our result is

in contrast with the findings of Skockova *et al*. (2012), who identified genotype tetB as the most prevalent tetracycline determinant with a wide host range due to the fact that it resides on highly mobile genetic elements that readily transfer between different bacteria genera. The presence of these genes might have occurred as a result of inappropriate antibiotics usage or antibiotics production naturally by soil microorganisms.

In this study, *Salmonella* and *E. coli* isolates, one each (16.7%) were able to be detected genotypically for the tetB and $bla_{TEM\ 1}$ genes respectively which is an indication of differences between the genotypical and the phenotypical methods. This trend seems to parallel what was obtained by Koo and Woo, (2011).

The presence of genes that confer resistance to these antibiotics (ß-Lactamase and tetracyclines) was high (17.5% - 50.0%), indicating that *Salmonella* and *E. coli* isolates originating from waste dumps could be a reservoir of antimicrobial resistance.

The observed binds above the expected amplicom size may be as a result of unspecific binding due to un-optimization of the PCR or the primers. It was observed that Zaria city had the highest percentage resistant gene probably because the location has the highest number of isolates.

Shiga toxin-producing *Escherichia coli* (STEC) strains are human pathogens linked to hemorrhagic colitis and hemolytic uremic syndrome (HUS). Shiga toxin is considered to play a prominent role in the pathogenesis of STEC and is presently classified into two broad types, Stx1 and Stx2, which are similar in their molecular structure and enzymatic activity but are antigenically distinct (Kawano *et al*., 2008). Shiga toxins (Stx1 and Stx2) are the major virulence factors of these strains (Tahamtan, 2010). Human infection caused by STEC O157:H7 depends on mamy bacterial virulence factors including Stx1, Stx2,

enterohemorrhagic *E. coli* (EHEC) – haemolysin, and intimin among others (Wang *et al*., 2008). But the most important factor is Stx2 (Kawano *et al*., 2008).

E. coli O157:H7 strains differ in their ability to produce Stx (Cornick *et al*., 2002). The Shiga toxin type 1 (Stx1) gene was not detected in any of the *E. coli* O157:H7 isolates whereas the Shiga toxin type 2 (Stx2) gene was present in 5 of the *E. coli* O157:H7 isolates, giving 71.4% genotype Stx2. Mazhaheri *et al.* (2005) reported that loss of Stx gene in serial cultures is seen after long term shedding of the organism. Going by the above report, it is possible that the Stx1 gene was lost during subsequent subculturing of the isolates. There are also possibilities that they could be having plasmids or satellite DNA that give them these unexpected features. Our reports are in-line with the findings of Ludwig *et al.* (2001) and Shekarforoosh *et al.* (2008) who reported 71% and 93.1% prevalence of Stx2 gene in their isolates respectively and the absence of Stx1 gene.

Nurmohammad and Phillip, (2003) opined that almost all *E. coli* O157:H7 strains possess Stx2. Kargar and Homayoon (2015) reported that within the human pathogens linked to hemorrhagic colitis and hemolytic uremic syndrome-associated strains, those produising Stx2 appear to be more commonly responsible for serious complications; Tahamtan *et al*. (2010) found Stx2 about 400 times more toxic than Stx1. Some studies have revealed that strains possessing only Stx2 are potentially more virulent than strains harboring Stx1 or even strains carrying both Stx1 and Stx2 (Nataro and Kaper, 1998; Ludwig *et al.*, 2002). However this result is in contrast with the findings of Jomezadeh *et al.* (2009) who reported the presence of Stx1 in 35.5% and Stx2 in 49.1% of human isolates and Tahamtan *et al*. (2010) who detected genotypes Stx1 and Stx2 in 15(10.27%) and 78(53.42%) of environmental isolates in their study respectively. Notwithstanding, *E. coli*

O157:H7 strains without virulent factors have also been isolated (Barkocy-Gallagher *et al.,* 2004), but isolates without these major virulence factors are not considered pathogenic. There are several other kinds of Stx2 genes such as Stx2c, Stx2d, Stx2e, Stx2f and Stx2g which were not covered in this study but are known to be present in STEC O157:H7 (Beutin *et al*., 2008; Prager *et al*., 2009).

The enterohaemolysin (EhlyA), also called enterohaemorrhagic *E.coli* haemolysin (EHEC- hlyA), encoded by the hlyA gene is another virulence factor of *E. coli*. The identification of *E. coli* O157:H7 in this research using hlyA gene seems to contradict the findings of Paton and Paton, (1998), who opined that the hlyA gene is not confined to serogroup O157. Pradel *et al.* (2001) suspected the gene to have a role in pathogenecity, because it occurred in the majority of the pathogenic STEC strains tested and were reactive to sera of patients with HUS.

Several genes have been targeted for the detection of *Salmonella* serovars from culture, environmental, food and clinical samples.

Salmonella enterotoxin (Stn) is a putative virulence factor and causative agent of diarrhea (Chopra *et al*., 1999; Masayuki *et al*., 2012). The Stn gene assists the organism in expressing its virulence in the host cells (Murugkar *et al*., 2003).

Salmonella enterotoxin Stn gene was found to be present in 83.3% of the *Salmonella* isolates. This implies that the gene is conserved among the *Salmonella* isolates. The presence of Stn gene in the *Salmonella* isolates is consistent with the findings of Moore and Feist, (2007) and Lee *et al.* (2009) who reported that *Salmonella* enterotoxin is widely distributed among *Salmonella* serovars irrespective of their serotypes and source of isolation.

The InvA gene encodes a protein in the inner membrane of bacteria which is responsible for invasion into epithelial cells of the host's intestine (Darwin and Miller, 1999). The InvA gene which is located on the *Salmonella* pathogenecity Island 1 is reported to assist the organism in expressing its virulence in the host cells and is highly conserved in *Salmonella* spp. and encodes a type lll secretion system that exports protein in response to contact of bacteria with epithelial cells of the intestine (Soto *et al*., 2006; Moussa *et al.*, 2010; Smith *et al.*, 2010).

The InvA gene was found to be present in the *Salmonella* isolates. The identification of the *Salmonella* isolates in this study using the InvA gene is in consonance with the findings of Ammari *et al*. (2009), Karami *et al*. (2010), Shanmugasamy *et al.* (2011) and Ohud *et al*. (2012) who reported the presence of the gene in *Salmonella* spp. It was observed that the isolates were 100% genotype InvA by PCR in this study suggesting that the gene is highly conserved among *Salmonella* spp. Murugkar *et al*. (2003), Moussa *et al.* (2010) and Shanmugasamy *et al.* (2011) also observed that the InvA gene is widely distributed among *Salmonella* spp. and is particularly used for the detection of *Salmonella* in PCR assays. Amplification of InvA gene has now been recognized as an international standard for the detection of the genus *Salmonella* (Malorny *et al.*, 2003).

The toxR gene located in the larger chromosome of *Vibrio cholerae* organism is the transmembrane regulatory protein required for expression of virulence factors in the human diarrheal pathogen *Vibrio cholerae* and is known as a gene encoding a transmembrane DNA binding regulatory protein in *Vibrio* species. Its role is to regulate ompU and ompT porins in *Vibrio cholerae* pathogenesis (Provenzano *et al*., 2001).

The toxR gene was found to be present in the *Vibrio cholerae* non-O1isolates. The presence of toxR gene in the *Vibrio cholerae* isolates in this study agrees with the findings of Karaolis *et al*. (1998) and Provenzano *et al*. (2001), who verified the presence of the toxR gene in all *Vibrio cholerae* studied, regardless of serogroup or source of isolation. It was observed that *Vibrio cholerae* non-O1 were 100% genotype toxR by PCR in this study. This provides some evidence to support the hypothesis that the toxR genes are universally distributed in the family Vibrionaceae and their primers are sensitive and specific for *Vibrio cholerae* strains (Osorio and Klose, 2000; Bisweswar *et al*., 2000). Provenzano *et al*. (2001) found all of the *Vibrio cholerae* O1, O139, and non-O1/non-O139 strains, regardless of whether they were toxigenic or non-toxigenic possessing the toxR regulatory sequence, among which, *Vibrio cholerae* non-O1 in this study. The difference in toxR sequences among *Vibrio* species has been used as an effective marker for the identification of *Vibrio* species (Ludwig *et al*., 2001).

The OmpW is an outer membrane protein located in the smaller chromosome. Although its precise function in *Vibrio cholerae* is not yet known, it may play a role in the adherence process, which is likely to facilitate the survival of the organism within the host or in the environment or both.

The OmpW gene was not detected in this study. This is at variant with the findings of Bisweswar *et al.* (2000), who demonstrated that, although the toxR primers are sensitive and specific for *Vibrio cholerae* strains, the primers targeted to OmpW are better suited due to the unique presence of the gene with conserved sequence in *Vibrio cholerae.*

The results of the sites of isolates that possess virulence genes revealed that all the locations present isolates with virulence gene. This shows that waste dumps in Zaria

metropolis contain organisms of public health significance and connotes a public health threat and indicates the contamination of the dumpsites by human and/or animal faecal matter confirming our hyphothesis. *Salmonella* Typhi is strictly a human pathogen that causes invasive fever (typhoid fever). Most other *Salmonella* serotypes cause mainly gastrointestinal symptoms without systemic invasion (Braden, 2006).

The result of the seasonal variation in the occurrence of virulence genes revealed that there is a significant seasonal difference in the levels of distribution of virulent genes-carrying bacterial isolates in this study. The results revealed decrease in the number of virulence genes- carrying bacteria during the wet season and high percentage of positive bacterial isolates in dry seasons. This is not surprising since the highest percentage occurrence of the isolates was also observed during the dry season. These results are in agreement with those studies indicating that STEC shedding has seasonnal variations by cattle in different countries (Bonardi *et al*., 1999). Tahamtan, (2010) also observed a marked decrease in the prevalence of STEC from October, which in Iran is the beginning of fall, to the end of spring (June).

These findings are important for public health. Therefore, emergency cautions are necessary to decrease the incidence of virulence and antibiotic resistant genes- carrying bacteria in the waste dump environments. In order to achieve this, wastes should be removed regularly and the waste stream effectively managed and also, there should be more prudent use of antibiotics in both human and veterinary medicine.

5.6. Phylogeny of the Isolates

DNA extraction and subsequent molecular processes and analyses realized success resulting in the positive amplification and sequencing of 16S rRNA genes from the target

isolates. The few PCR negative and unsuccessful sequencing reaction outcomes must have resulted from the organisms unique sub-optimal sequencing condition requirements. Sequencing analysis resulted in further identifications of the bacteria up to species level. Different species of bacterial populations have been detected in waste dumps from previous studies (Bartram *et al.*, 2003; Onweremadu *et al*., 2009; Odeyemi, 2012; Osunwoke and Kuforiji, 2012). It has been reported that the use of nearly complete or complete 16S rRNA gene sequence fragments (as used in this study) greatly minimizes inherent biases associated with the amplification of ribosomal genes using universal bacterial primers (Partanen *et al.*, 2010*).* Concerning the proportions of the isolates identified, the *Proteobacteria* (especially the Enterobacteriaceae) formed the vast majority. Lewis *et al*. (2002) also detected similar predominance of Enterobacteriaceae at over 74% and the other members at about 26% as was comparatively detected in this study. The predominance of Gram negative bacteria, both Enterobacteriaceae (to a larger extent) and Vibrionaceae (to a lesser proportion), in waste dumps has been reported elsewhere (Flores-Tena, 2007; Osei and Duker, 2008; Ikpeme *et al.*, 2011) and was attributed to faecal contamination which conform to the findings of this work since as reported earlier it was common practice to dump faeces in the dumpsite waste which could also be used as latrine at night.

Proteobacteria are a class of several medically and scientifically important bacteria, such as the Enterobacteriaceae (*Escherichia coli*) and Vibrionaceae. An exceeding number of important pathogens belong to the proteobacteria, such as *Salmonella* (Thypi and typhoid fever), Thyphimurum (bacteraemia, septicaemia and neonatal meningitis), *Vibrio* (cholera), and *Escherichia coli* (Urinary Tract Infection and gastroenteritis) (Brenner *et al.*, 2005; Cheesbrough, 2006).

As demonstrated in the present study, the genetic diversity of bacteria of public health significance in waste dumps in Zaria metropolis is considerable. The clustering of the 16S rRNA sequences into a collection of phenotypes on the basis of sequence similarity gave rise to the phylogenetic trees displaying a good array of related clusters of *Salmonella* serovars and *E.coli* serogoups. This attests to quality of the PCR amplification and the accuracy in the sequencing techniques of the 16S rRNA genes of the target isolates as used in this study. The actual identified isolates were cultured and are available. As noted, there was no significant similarity found with isolates E2 and S5. In addition, E4 and E7 did not cluster with the expected members of their group in the phylogenetic tree. White *et al*. (2010) had demonstrated the fundamental limitation of hierarchical clustering strategies for 16S rRNA analyses in which some species fail to correctly cluster with their homogeneous groupings on the phylogenetic tree, irrespective of similarity threshold chosen. They explained that these discrepancies arise from the concept that ‘‘species” is borne out of gross morphological and phenotypic traits of microorganisms, and therefore cannot be precisely mapped to fine-scale molecular measurements. The report of our study and earlier studies could be explained with the fact that serogroup does not correspond to genotype. However isolates S4 (*E. coli* O83:H1) a presumptive *Salmonella enterica* showed good match with E1 of serogroup O157. This is in line with the result got by Fukushima *et al*. (2002) where *E. coli* isolates with O serogroups associated with EPEC and EHEC appearing in different pathotypes are grouped in one cluster.This confirms the earlier assumption made by White *et al*. (2010). Furthermore, the rate of evolution varies across the trees of life. There was no particular trend of grouping of the bacterial isolates in terms of their sites of isolation. Isolates belonging to the same genus but obtained from different dump sites (Samaru, Sabon-Gari, Tudun-Wada and Zaria

City) also grouped together without showing any site specific patterns as the clusters consisted of isolates drawn from the varied sampling dump sites. Also to consider is the existence of many clusters (3clusters with the *E. coli* O157:H7 and 2 with the *Salmonella* serovars) caused probably by nutation. Another reason is the existence of root which implies that, isolates from within the same cluster are likely to arise from the same ancestral origin.

CHAPTER SIX

6.0 CONCLUSION AND RECOMENDATION

6.1 Conclusion

The occurrence of bacteria of public health significance in waste dumps has received much attention in recent years. To this end, the findings of this study have demonstrated that the temperature and pH existing at the waste dump sites are optimum for growth and proliferation of the bacterial isolates.

From the study conducted, the highest bacterial count was found in Zaria city while the lowest was found in Samaru. A statistical significant difference with P-value < 0.05 was found between locations ZC and SA as well as between locations ZC and CS.

A prevalence of 6.92%, 4.23% and 1.35% was obtained for *Salmonella enterica, E.coli* O157:H7 and *Vibrio cholerae* non-O1 respectively.

The Highest occurrence of bacterial isolates was observed in the dry season 37(14.2%) while the low occurrence was observed in the wet season 29(11.2%) and there was a statistical significant difference in the occurrence of the bacterial isolates between the two seasons.

Salmonella enterica showed highest sensitivity to Gentamicin followed by Nitrofurantoin, Chloramphenicol and Ciprofloxacin. *E. coli* O157:H7 isolates were observed to be highly susceptible to Chloramphenicol, followed by Gentamicin, Cefotaxim and Ciprofloxacin. However, high resistance to Ampicillin, Amoxycillin-clavulanic acid and Tetracycline was observed among these organisms. For the *Vibrio cholerae* non-O1 isolates, high resistance to Ampicillin, Cotrimoxazole and Tetracycline was observed, however high susceptibility was observed to Ciprofloxacin followed by Gentamicin.

The most effective drugs in the study were Gentamicin on *Salmonella enterica* and Chloramphenicol on *E. coli* O157:H7 as no single resistance was observed against them, while there was no 100% effective drug on *Vibrio cholerae* non-O1 isolates.

A total of 65.2% of the isolates were multidrug resistant. In addition, it was observed that 28%, 26% and 11% of *E. coli* O157:H7, *Salmonella enterica* and *Vibrio cholerae* non-O1 isolates respectively were multidrug resistant. The bacterial isolates were resistant to 1-7 antibiotics and seventeen different phenotypic resistance profiles were observed among them. Most of the resistant isolates showed the AmpAmcCtxSxtTe -resistance pattern.

The virulence genes InvA and Stn of *Salmonella enterica* were found in 6(100%) and 5(83.33%) respectively of the isolates, Stx and hlyA genes in 5(71.43%) and 4(57.14%) respectively of *E. coli* O157:H7 isolates and toxR gene in 6(100%) of *Vibrio cholerae* non-O1 isolates. Similarly antibiotic resistant genes tetA, tetB and bla_{TEM1} were demonstrated in 2(33.33%), 1(16.66%) and 2(33.33%) respectively of *Salmonella enterica*, tetA and bla_{TEM1} in 4(66.66%) and 1(16.66%) of *E. coli* O157:H7.

Sequencing analysis was used to successfully identify *Salmonella* Typhi, *Salmonella* Typhimurium, *E. coli* O157:H7 and *E. coli* O83:H1, hence it is a useful tool for confirmation of the identity of bacteria pathogens.

The occurrence of pathogenic bacteria isolated and identified in soil from Zaria Metropolis waste dumps, suggest that there is a significant sanitary risk, especially to the refuse handlers, rag pickers and people leaving within the vicinity of such dumps. The results further suggest a misuse of antibiotics, a fact that necessitate the control of antibiotics use in Zaria Metropolis in both humans and animals.

6.2 Recomendation

1. Since the dump sites are a home to many harmful microorganisms, proper policy should be put in place for waste handlers and those who live in the vicinity of the dumps to safeguard their lives.
2. Further studies should be extended to cover other Local Government areas so as to identify some other serovars not identified in this study and examine the health implications of such dumps to the surrounding residents.
3. Extensive study on the antibiotic susceptibility pattern and drug resistance mechanisms of *Salmonella enterica, E. coli* O157:H7 and *Vibrio cholerae* should be conducted.
4. Public health organizations and other relevant bodies should embark on public awareness and enlightenment campaigns to enlighten individuals on the hazards of indiscriminate waste disposal and the open dump system of waste disposal. The public should be enlightened on the following aspects for environment and health surveillance**:**

(i). Faecal matter should not be allowed to mix with municipal refuse

(ii). Hospital and municipal wastes should be handled separately

(iii). Discharging of waste into drains and open areas should not be practised.

(iv). Efforts should be made to remove solid waste from habitations regularly

5. Public health officials and town planners should implement the critical (minimum) distance within which refuse dumps should not be sited (away from inhabitants of communities).
6. Government should provide the city with public conveniences in order to discourage open defaecation at waste dump sites.

7. Further studies on the molecular mechanisms of acquiring resistance, pathogenicity and virulence should be conducted for *Salmonella enterica*, *E. coli* O157:H7 and *Vibrio cholerae.*

8. Public health may be ensured from these pathogenic agents at waste dumpsites by prompt removal of waste and proper management (mechanical sorting and excavating) methods. A number of infections can directly link to waste dump pathogens thereby leading to death. It is important that regulatory framework is developed and most importantly to curb this menace if health for all by the year 2020 and vision 20-20-20 is to be attained.

REFERENCES

About Us'. (2010). Nigerian College of Aviation Technology. Retrieved 2010-03-27

Abraham, W.R. (2011). Mega cities sources for pathogenic bacteria in rivers and their fate downstream. *International Journal of Microbiology,* **79:**8292.

Achudume, A.C. and Olawale, J.T. (2007). Microbial pathogens of public health significance in waste dumps and common sites. A paper (Ph.D.Thesis). Institute of Ecology and Environmental Studies Obafemi Awolowo University, Ile-lfe, Nigeria.

Adejuwon, A.O., Bisi-Johnson, M.A., Agboola, O.A., Fadeyi, B.O. and. Adejuwon, A.O. (2011). "Antibiotics sensitivity patterns of *Escherichia coli* and *Aerobacter aerogenes* isolated from well water in ile–ife, Nigeria". *International Journal of Medicine and Medical Sciences*, **3**(5):155–160.

Adekanle, M.A., Oluremi, A.S. and Akindele, A.A. (2014). Bacteria isolated from waste dump sites soil at Osogbo, Osun State. *Journal of Microbiology and Biotechnology Research,* **4**(3):66-69.

Adesoji, A.T., Ogunjobi, A.A., Olatoye, I.O. and Call, D.R. (2015). Prevalence of tetracycline resistance genes among multi-drug resistant bacteria from selected water distribution systems in south-western Nigeria. *Annals of Clinical Microbiologyand Antimicrobials*, **14**:35-38.

Afon, A.O. and Okewole, A. (2007). Estimating the quantity of solid waste generation in Oyo, Nigeria. *Waste Management Research,* **25**:371-379.

Ajayi, A.O. and Akonai, K.A. (2003). Antibiotics sensitivity profile of microorganisms in Lagos Lagoon, Nigeria. *African Journal of Biotechnology,* **6**:79-84.

Akingbade, O., Balogun, S., Ojo, D., Akinduti, P., Okerentugba, P.O., Nwanze, J.C. and Okonko, I.O. (2014). Resistant plasmid profile analysis of multidrug resistant Escherichia coli isolated frm urinary tract infections in Abeokuta, Nigeria. *African Health Sciences*, **14**(4):821-828.

Akinyemi, O.K., Bamiro, S.B. and Coker, O.A. (2007). Salmonellosis in Lagos Nigeria: Incidence of Plasmodium falciparum associated Co- infection, patterns of antimicrobial resistance and emergence of reduced susceptibility to fluoroquinolone. *Journal of Health Population and Nutrition*, **25**(3):351-358.

Alam, M., Sulta, M., Nair, B., Sack, D.A., Siddique, A.K., Ali, A., Huq, A. and Cowell, R.R. (2006). Toxigenic *Vibrio cholerae* in the environment of Mathbaria, Bangladesh. *Applied and Environmental Microbiology*, **72**:2849-2855.

Ali, H., Bacha, K. and Ketema, T. (2011). "Bacteriological quality and antimicrobial susceptibility of some isolates of well water used for drinking in jimma town, southwest Ethiopia." *Ethiopian Journal of Education and Sciences*, **6**(2):95-108.

Alonso, M.G.V.P. and Themelis, N. (2011). Generation and disposition of municipal solid waste in Mexico and potential for improving waste management in Toluca municipality. *Waste-to-Energy Research and Technology Council (WTERT).*

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, **215**:403–410.

Altschul S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Miller, W. and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST. a new generation of protein database search programmes. *Nucleic Acids Research*, **25**:3389–3402.

Amann, R.I., Ludwig, W.and Schleifer, K.H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**,143–169.

Amann, R.I. and Kuhl, M. (1998). *In situ* methods fornassessment of microorganisms and their activities. *Current Option in Microbiology*, **1**:352-358.

Ahmed, A.B. (2011).Insect vectors of pathogens in selected undisposed refuse dumps in Kaduna Towm, Northern Nigeria. *Science World Journal,* **6**(4):21-24.

Ammari, S., Laglaoui, A., En-nanei, L., Bertrand, S., Wildemauwe, C., Said, B.S. and Abid, M. (2009). Isolation, drug resistance and molecular characterization of Salmonella isolates in Northern Morocco. *Journal of Infecton in Developing Countries*, **3**(1):41-49.

Anonymous**. (**2012). Shiga-toxn producing *Escherichia coli* in certain raw beef products. *Federal Registration*, **77:**31975-31981.

Arigbede, Y.A. and Yusuf, O.R. (2010). Waste disposal among underage children and the location of waste disposal infrastructure in Samaru, Zaria. A paper presented during the 50thAnnual Conference of the Association of Nigeria Geographers holding at the Kogi State University, Anyingba, Nigeria.

Arora, D.R. (2004). Textbook of microbiology. 2nd Eds. C.B.S Publishers and Distributors. p. 686.

Aseffa, A., Gedhi, E.and Asmelash, J. (1997).Antibiotic resistance of prevalent *Salmonella* and *Shigella* strains in North West Ethiopia. *East African Medical Journal*, **74**:708-713.

Ash, R.J., Mauck, B. and Morgan, M. (2002). Antibiotic resistance of Gram negative bacteria in rivers, United States of America. *Emerging Infectious Diseases*, **8**:7-12.

Atchley, S.H. and Clark, J.B**.** (1979). Variability of temperature, pH, and moisture in an aerobic composting process. *Applied Environmental Microbiology*, **38**:1040-1044.

Awisan, J.,Venchito, D.J., Angelica, B., Lizlotte, C. and Deanna, M.F. (2011). Aerobic pathogenic and opportunistic bacteria of public health significance in Ireland dumpsites soil. *SLU Research Journal,* **42**(1):27-37,

Aziz, H.A., Yusoff, M.S. and Bashir, M.J.K. (2010). Leachate characterization in semi-aerobic and anaerobic sanitary landfills. A comparative study. *Journal of Envnvironmental Managagement*, **91**:2608-2614.

Babayemi, J.O. and Dauda, K.T. (2009). Evaluation of solid waste generation, categories and disposal options in developing countries: a case study of Nigeria. *Journal of Applied Science andEnvironmental Management*, **14**(1):83-88.

Bach, P.D., Nakasaki, K., Shoda, M. and Kubota, H. (1987). Thermal balance in composting operations. *Journal of Fermentation Technology*, **65**:199-209.

Barkocy-Gallagher, G.A., Arthur, T.M., Rivera-Betancourt, M., Nou, X., Shackelford, S.D., Wheeler, T.L. and Koohmaraie, M. (2004). Characterization of O157:H7 and other *Escherichia coli* isolates recovered from cattle hides, faeces, and carcasses. *Journal of Food Protection,* **67**(5):993-998.

Bartram, J., Catruvo, J., Exner, M., Fricker, C. and Glemacher, A. (2003). Plate counts and drinking water safety, IWA publishing Co, London, UK.

Beatson, S.A. and Walker, M.J. (2014). Tracking antibiotic resistance. *Science*, **345**:1454-1455.

Beguin, P. and Aubert, J.P. (1994). The biological degradation of cellulose. *FEMS Microbiology Review*, **13**:25-58.

Bell, C. and Kyriakides, A. (2002). *Salmonella*, Blackwell Science Ltd. pp. 30-68.
.

Benson, D.A., Karsch-Mizrachi, I. Lipman, D.J., Ostell, J. and Wheeler, D.L. (2004). GenBank. *Nucleic Acids Research,* **1**(32):23-26.

Berge, A., Adaska, J. and Sischo, W. (2004). Use of antimicrobial susceptibility patterns and pulsed-field gel electrophoresis to compare historic and contemporary isolates of multi-drug-resistant *Salmonella enterica* subsp.*enterica* serovar Newport. *Applied and Environmental Microbiology*, **70**:318-323.

Beutin, L., Krause, U., Krause, G., Miko, A., Martin, A. and Strauch, E. (2008). Evaluation of major types of shiga toxin 2e-producing *Escherichia coli* bacteria present in food, pigs and the environment as potential pathogens for humans. *Applied and Environmental Microbiology,* **74**(15):4806-4816.

Bisweswar, N., Ranjan, K.N., Sarmishtha, M., Balakrish, G., Nair, T., Shimada, A. and Asoke, C.G. (2000). Rapid method for species-specific identification of Vibrio cholerae using primers targeted to the gene outer membrane protein OmpW. *Journal of Clinical Micobiology,* 38:4145-4151.

Bitto, A.O., Kale, O.O. and Odutan, B.O. (1992). Epidemiological survey of outbreak of gastroenteritis in Rural Community of Oyo State. *West African Journal of Medicine,* **11**:34-38.

Blow, N.S., Salomon, R.N., Garrity, K., Reveillaud, I., Kopin, A., Jackson, F.R. and Watnick, P.I. (2005). *Vibrio cholerae* infection of *Drosophila melanogaster* mimics the human disease, Cholera. *Plant Science Pathogens*, **1**(1)**:**8.

Boerlin, P., McEwen, S.A., Boerlin-Petzold, F., Wilson, J.B., Johnson, R.P. and Gyles, C.L. **(**1999). Associations between virulence factors of Shiga toxinproducing *Escherichia coli* and disease in humans. *Journal of Clinical Microbiology*, **37:**497-503.

Bonardi, S., Maggi, E. and Bottarelli, A. (1999). Isolation of verocytotoxin-producing *Escherichia coli* O157:H7 from cattle at slaughter in Italy. *Veterinary Microbiology*, **67**:203–211.

Boulter, J.I., Trevors, J. and Boland, G.J. (2002). Microbial studies of compost: Bacterial identification and their potential for turf grass pathogen suppression. *World Journal of Microbiology and Biotechnology*, **18**:661-671.

Boyd, E.F. and Waldor, M.K. (1999). Alternative mechanism of cholera toxin acquisition by *Vibrio cholerae*: Generalized transduction of CTX by bacteriophage CP-T1. *Infection and Immunity,* **67**:5898-5905.

Boyen, F., Haesebrouck, F., Maes, D. and Van, I. (2008). Non-typhoidal *Salmonella* infections in pigs: A closer look at epidemiology, pathogenesis and control. *Veterinary Microbiology*, **130**:1-19.

Braden, C.R. (2006). *Salmonella enterica* serotype Enteritidis and eggs: A national epidemic in the United States. *Journal of Clinical and Infectous Diseases*, **43**:512-517.

Brambilla, E., Hippe, H., Hagelstein, A., Tindall, B. and Stackebrandt, E. (2001). 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antartica. *Extremophiles,* **5**:23-33.

Branda, S.S., Vik, S., Friedman, L. and Kolter, R. (2005). Biofilms: The matrix revisited. *Journal of Trends Microbiology*, **13**: 20-26.

Brenner, F., Villar, R., Angulo, F., Tauxe, R. and Swaminthan, B. (2000). *Salmonella* nomenclature. *Journal of Clinical Microb*iology, **38**:2465-2467.

Brenner, D.J., Krieng, N.R., Stanley, J.R. and Garrity, G. (2005). Bergey's Manual of Systematic Bacteriology, Volume 2; The Proteobacteria; 2nd edition; *Springer-Verlag*.

Brook, I. (2009). Antibiotic resistance of anaerobic bacteria. In D. Mayers, Antimicrobial drug resistance. Humana Press. Pp. 873-899.

Burtsher, C. And Wuertz, S. (2003). Evolution of the use of PCR and reverse transcriptase PCR for detection of pathogenic bacteria in biosolidis from anaerobic digestors and aerobic composters. *Applied and Environmental Microbiology*, **69**:4618-4627.

Cai, H., Lu, L., Muckle, C., Prescoot, J. and Chen, S. (2005). Development of a novel protein microarray method for serotyping *Salmonella entrica* strains. *Clinical Microbiology,* **43**:3427-3430.

Card, R. (2009). Microarrays-closing the gap between research and diagnostic tools. *Microbiologist* .**10**: 30-33.

Cardinale, E., Gross-Claude, J., Rivoal, K., Rose, V., Tall, F., Mead, G. and Salvat, G. (2005). Epidemiologycal analysis of *Salmonella enterica* spp. serovar Hadar, Brancater and Enteriditis in humans and broilers chicken in Senegal using Pulsed Field Gel Electrophoresis and antibiotic susceptibility. *Journal of Food Protection*, **60**:1312-1317.

Carraminana, J.J., Rota, C., Augustin, I. and Herrera, A.M. (2004). High prevalence of multiple resistance to antibiotics in *Salmonella* serovars isolated from poultry slaughterhouse in Spain. *Veterinary Microbiology*, **104**:133-139.

Centres of Disease Control and Prevention (CDC) (2008). Rotavirus surveillance worldwide. 2001-2008. *MMWR Morbidity and Mortality Report,* **57**(46):1255-1257.

Centres of Disease Control and Prevention (CDC) (2009). Surveillance for food disease outbreaks United States, 2006. Available http://www.cdc.gov/mmwr/preview/mmwrhtml/mm 5822a1. htm.23.

Centres of Disease Control and Prevention (CDC). (2012). Foodborne disease active surveillance network (FoodNet): FoodNet surveillance report for 2011 (final report). CDC, Atlanta, GA.

Chavasse, D.C., Shier, R.P., Murphy, O.A., Huttly, S.R.A., Cousens, S.N. and Akhtar, T. (1999). Impact of fly control on childhood diarrhoea in Pakistan: community-randomised trial. *Lancet*, **353:**22-25.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries, Part 1, 2ed. *Cambridge, UK: Cambridge University Press*. Pp 97-115.

Chen, A.C., Imachi, H., Sekiguchi, Y., Ohashi, A. and Harada, H. (2003). Archaeal community compositions at different depths (up to 30 m) of a municipal solid waste landfill in Taiwan as revealed by 16S rDNA cloning analyses. *Biotechnology Letters*, **25**:719-724.

Chen, S., Zhao, S., White, D.G., Schroeder, C.M., Lu, R., Yang, H.M., McDermott, P.F., Ayers, S. and Meng, J. (2004). Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. *Applied and Environmental Microbiology,* 70(1):1-7.

Chigor, V.N., Umoh, V.J., Smith, S.I., Igbinosa, E.O. and Okoh, A.I. (2010). Multidrug resistance and plasmid patterns of *Escherichia coli* O157:H7 and other *E. coli* isolated from diarrhoeal stools and surface water from some selected sources in Zaria, Nigeria. *International Journal of Environmental Research and Public Health,* **7**:3831-3841.

Chomvarin, C., Namwat, W., Wongwajana, S., Alam, M., Thaew-Nanngiew, K., Sichaturus, A. and Engchanil, C. (2007). Application of duplex-PCR in rapid and reliable detection of toxigenic *Vibrio cholerae* in water samples in Thailand. *Journal of General and Applied Microbiology*, **53**:229-237.

Chopra, A.K., Huang, J.H., Xu, X.J., Burden, K., Niesel, D.W., Rosenbaum, M.W., Popov, V.L. and Peterson, J.W. (1999). Role of *Salmonella* enterotoxinin overall virulence of the organism. *Microbial Pathology*, **27**:155-171.

Clinical and Laboratory Standards Institute (CLSI). (2014). Performance standards for antimicrobial susceptibility testing. Twenty-Fourth Informational Supplement: M100-S24, **34**(1).

Cohen, D., Green, M., Block, C., Dlepon, R., Ambar, R., Wasserman, S.S. and Levine, M.M. (1991). Reduction of transmission of shigellosis by control of houseflies (*Musca domestica*). *Lancet*, 1991, **337:**993-997.

Cole, J.R., Chai B., Marsh T.L., Farris, R. J., Wang, Q., Kulam, S.A., Chandra, S., McGarrell, D.M., Schmidt, T.M., Garrity, G. M. and Tiedje, J.M. (2003). "The Ribosomal Database Project (RDP-II). Previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy". *Nucleic Acids Research*, **31**:442-443.

Colwell, R.R. (2004). Infectious disease and environment: cholera as a paradigm for waterborne disease. *International Microbiology*, **7.**

Constanzo, S., Murby, J. and Bates, J. (2005). Ecosystem response to antibiotics entering the aquatic environments. Marine Pollution Bulletin, **51(**1/4):218 -223.

Countreau, S.J. (1982). Environmental management of urban solid waste. *Environmental Management of Urban Solid Waste*, p. 228. Ilus, tab. (BIRF Technical Paper, 5). The World Bank, Washington D.C.

Cowan, T. (2011). Biofilms and their management: From concept to clinical reality. *Journal of Wound Care*, **20**: 222–226.

Croxen, M.A. and Finlay, B.B. **(**2010). Molecular mechanisms of *Escherichia coli* pathogenicity. *Natural and Reviewed Microbiology*, **8:**26-38.

Dahl, C., Li-Nan, H., Shuang Z., Zhou, H. and Liang-Hu, Q. (2005). Molecular phylogenetic diversity of bacteria associated with the leachate of a closed municipal solid waste landfill. *FEMS Microbiology Letters*, **242**(2):297-303.

Darwin, K.H. and Miller, V.L. (1999). Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. *Clinical Microbiology Review*, **12**:405-428.

Deb, B.C. (1970). Cholera carrier studies in Calcuta. Bulletin of World Health Organization, **43**:376-387.

Deb, B.C., Sircar, B.K., Sengupta, P.G., De, S.P., Sen, D., Saha, M.R. and Pal, S.C. (1990). Cholera case definition. *Indian Journal of Medical Research*, **76**:814

De Beer, D. and Stoodley, P. (2006), "Microbial biofilms", in Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (Eds.). *The prokaryotes*, (pp. 904-937). New York: Springer.

Dees, P.M. and Ghiorse, W.C. (2001). Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. *FEMS Microbiol Ecology*, **35:**207-216.

DeLong, E.F. (1996). Microbial diversity in time and space. (Colwell, R. R., Simidu, U. and Ohwada, K., eds.). *TIBTECH*, **17**:125–134, Plenum Press.

Ding, H., Huang, I., Mao, X. and Zou, Q. (2011). Characterization of *stx*2 and its variants in *Escherichia coli* O157:H7 isolated from patients. *African Journal of Biotechnogy*, **10**(15):2991-2998.

DNeasy Blood and Tissue Handbook. (2006). Animal tissues (spin-column protocol): purification of Total DNA from animal tissue. Pp.28-31.

Donlan, R.M. and Costerton, J.W. (2002). "Biofilms: survival mechanisms of clinically relevant microorganisms." *Clinical Microbiology Reviews*, **15**(2):167-193.

Dubey, R.C. (2009). Multicolour Texbook of Biotechnology. 4th Edition. S. Chand and Co. India, pp. 71-80.

Duru, E.E., Agbagwa, O.E. and Umoren, F.E. (2014). Bacterial agents associated with infantile diarrhea and their antibiotics susceptibility pattern in Port Harcourt, South-South Nigeria. *Journal of Medical Sciences and Public Health*, **3**(1):1-12.

Edward, P.R. and Ewing, W.H. (2003). Identification of Enterobacteriaceae (3rd eds). Minneapolis: Burges, pp. 10-15.

Environmental Research Foundation (ERF). (2011). The Basics of Landfills: How they are constructed and why they fail. Downloaded from http://www. zerowasteamerica.org/BasicsOfLand fills.htm. Retrieved: August 31, 2011.

Epstein, E., Willson, W. D., Burge, D. C. Mullen, G. B., and Enkiri, N. K. (1976). A forced aeration system for composting wastewater sludge.Technomic Publishing Co., Inc., *Lancaster, Pennsylvania,* 487.

Elaigwu, S.E., Ajibola, V.O. and Folaranmi, F.M. (2007). Studies on the impact of municipal waste dumps on surrounding soil and air quality of two cities in Northern Nigeria. *Journal of Applied Sciences*, **7**(3):421-425.

European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA). (2011). Shiga toxin/verotoxin-producing *Escherichia coli* in humans, food and animals in the EU/EEA, with special reference to the German outbreak strain STEC O104. *European Centre for Disease Prevention and Control and European Food Safety Authority,* Stockholm, Sweden.

Ezekiel, C.N., Olarimmoye, A.O., Oyinloye, I.N.A., Olaoye, O.B. and Edum, A.O. (2011). Distribution, antibiogram and multidrug resistance in Enterobacteriaceae from commercial poultry feeds in Nigeria. *African Journal of Microbiology Research,* **5**(3):294-301.

Falase, A. (2004). Waste management practices in Nigeria. A welcome address of the opening of the basal convention Africa regional conference, Ibadan, Nigeria.

Faruque, S.M., Chowdhury, N., Kamruzzama, M., Rahman, M.H., Sack, D.A., Nair, G.B. and Mekalanos, J.J. (2003). Genetics diversity and virulence potential of environmenta *Vibrio cholerae* population in a cholera-endemic area. *Proceeding of the National Academy of Sciences,* **101**:2123-2128.

Flores-Tena, F.J., Guerrero-Barrera, A.L., Avelar-González, F.J., Ramírez-López, E.M. and Martínez-Saldaña, M.C. (2007). Pathogenic and opportunistic Gram-negative bacteriain soil, leachate and air in San Nicolás landfill at Aguascalientes, Mexico. *Revista Latinoamericanade Macrobiologia***, 49**(1-2):25-30.

Foday, P.S., Xiangbin, Y. and Quangyen, T. (2013). Environmental and health impact of solid waste disposal in developing cities: A case study of Granville Brook dumpsite, Freetown, Sierra Leone. *Journal of Environmental Protection*, **4**:665-670.

Food and Agriculture Organization (FAO). (2010). Available at http://www.fao.org/ag/agn/agns

Foster, R.C**.** (1988). Microenvironments of soil microorganisms, Biology and fertility of soils. *Springer-Verlag,* **6**:198-203.

Fotedar, R., Banerjee, U., Samantary, J.C. and Shriniwas, C. (1992). Vector potential of hospital houseflies with special reference to *Klebsiella* species**.** *Epidemiological Infections*, **109:**143-147.

Fraga, S.G., Pichel, M., Costagliola, M., Jurquiza, V., Peresuti, S., Caffer, M.I., Aulet, O., Hozbor, C., Tracanna, B.C., de Gamundi, A.V., Hernandez, D. and Ramirez, F.C. (2007). Environment and virulence factors of *Vibrio cholerae* strains isolated in Argentina. *Journal of Applied Microbiology.*

Fukushima, M., Kakinuma, K. and Kawaguchi, R. (2002). Phylogenetic analysis of *Salmonella, Shigella*, and *Escherichia coli* strains on the basis of the gyrB gene sequence. *Journal of Veterinary Research,* **15**(2):132-137.

f.Furtula, V., Jackson, C.R., Farell, E.G., Barrett, J.b., Hiott, L.M. and Chambers, P.A. (2013). Antimicrobial resistance in *Enterococcus* spp. isolated from environmental samples in an area of intensive poultry production. *International Journal of Environmental Research and Public Health,* **10**:1020-1036.

Garry, E., Ouattarra, G., Williams, P. and Pesta, M. (2009). Enumerating chromogenic agar plates using the color QCOUNT automated colony counter. *Journal of Rapid Methods and Automation in Microbiology*, **17**:46-54.

George, S.P., Ahmad, A. and Rao, M.B. (2001). A novel thermostable xylanase from Thermomonospora sp.: influence of additives on thermostability. *Journal of Bioresources and Technology,* **78**:221.

Gianfreda, L., Xu, F. and Bollag, J.M (1999.) Laccases: A useful group of oxidoreductive enzymes. *Journal of Bioremediation*, **3:**1-25

Glawe, U., Visvanathan, C. and Alamgir, M. (2005). Solid waste management in least developed Asian countries - A comparative analysis international conference on integrated solid waste management in Southeast Asian cities, Siem Reap, Cambodia 5-7 July 2005.

Glynn, M.K., Bopp, C., Dewitt, W., Dabney, P., Mokhtar. M. and Angulo, F.J. (1998). Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *New England Journal of medicine*, **388:**1333-1338.

Goldman, E. (2004). Antibiotic abuse in animal agriculture: Exacerbating drug resistance in human pathogens. *Human and Ecological Risk Assessment,* **10**:121-134.

Gordana, M., Bogdanka, A., Dragica, T., Milena, L. and Brankica, D. (2012). Antibiotic susceptibility of *Salmonella* spp: A comparison of two surveys with 5 years. *International Journal of IMAB-Annual Proceeding (Scientific Papers),* **18**(1):186-197.

Gordon, L.N. (2005). Fly transmission of Campylobacter. *Emerging Infectious Diseases*, **11:**3.

Gouveia, N. and Ruscitto do P. (2010). Health risks in Areas Close to urban Solid Waste Landfill. *Journal of Applied Sciences and Environmental Management,* **6**(1):78-83.

Greenberg, B. (1973). Flies and Disease. II. Biology and isease transmission. *Princeton University Press, Princeton, NJ.,* 1973:15.

Greenberg, E.P., Costerton, J.W. and Stewart, P.S. (1999). Bacterial biofilms: A Common Cause of Persistent Infections. *Journal of Science,* **284:**1318-1332.

Gregory, D., Keah-ying, L. and Sunny, C.J. (2013). Quantitative microbial risk assessment of pathogenic vibrios in marine recreational waters of Southern California. *Applied and Environmental Microbiology,* **79**(1):294-302.

Grimont, P. and Weill, F. (2007). Antigenic formulae of the *Salmonella* serovars. 9th ed.WHO Collaborating Center for Reference and Research on *Salmonella.*

Grisey, E., Belle, E., Dat, J., Mudry, J. and Aleya, L. (2010). Survival of pathogenic and indicator organisms in ground water and landfill leachate through coupling bacterial enumeration with tracer tests. *Desalinetia,* **261**:162-168..

Guardabassi, L., Schwarz, S. and Lloyd, D.H. (2004). Pet animals as reservoirs of antimicrobial resistance. *Journal of Antimicrobial and Chemotherapy*, **54**:321-332.

Guardabassi, L. and Courvalin, P. (2006). Model of antimicrobial action and mechanisms of bacteria resistance. In: F. Aarestrup (Eds.). Antimicrobial resistance in bacteria of animal origin . *ASM Press*. , pp. 1-18.

Guerra, B., Soto, S.M., Cal, S. and Mendoza, M.C. (2000). Antimicrobial resistance and spread of Class 1 integrons among *Salmonella* serotypes. *Antimicrobial Agents Chemotherapeutic*, **44** (in press).

Guthrie, R. (1991). Taxonomy and Grouping of the *Salmonella*, p.23-40. In *Salmonella*. CRC press.

Hagerty, D.J., Pavoni, J.L. and Heer, J.E. (1973). Solid waste management Van Nostrand Reinhold, New York.

Hakanen, A., Kotilainen, P., Helenius, H. and Sitonen, A. (2001). Reduced fluoroquinolone susceptibility in *Salmonella enterica* serotypes in travelers returning from Southeast Asia. *Emerging Infsctious Diseases,* 7: 996-1003.

Harris, R. and Paxman, J.A. (1982). A higher form of killing. Noonday Press, New York.

Hartel, P.G. (1999). The soil habitat. In: Principles and applications of soil microbiology. Eds. Sylvia, J.J., Fuhrmann, H.P.G. and Zuberer, D.A. Prentice Hall. Upper Saddle River, pp. 21-43.

Harwool, V.J., Gandhi, J.P. and Wright, A.C. (2004). Methods for isolation and confirmation of *Vibrio vulnificus* fron oysters and environmental sources: a review. *Journal of Microbiological Methods*, **59**:301-316.

Hassen, A., Belguith, K., Jedidi, N., Cherif, M. and Boudabous, A., (2002). Microbial characterization during composting of municipal solid waste. *Proceedings of International Symposium on Environmental Pollution Control and Waste Management*, Pp. 357-368.

Helmuth, R. (2000). Antibiotic resistance in *Salmonella*. In: *Salmonella* in domestic animals Wray, C and Wray, A. (Eds.). CABI publishing, **6**:89-106.

Henderson, D.A. (1999). The looming threat of bioterrorism. *Science*, **283**:1279-81.

Hendriksen, R.S., Bangtrakulnonth, A., Pulsrikarn, C., Pornreongwong, S., Hasman, H., Song, S. and Aarestrup, F.M. (2008). Antimicrobial resistance and molecular epidemiology of *Salmonella* Rissen from animals, food products and patients in Thailand and Denmark. *Foodborne Pathogens and Disease***, 5**(5):605-619.

Heuer, H., Krogerrecklenfort, E., Wellington E.M.H., Egan S, Van Elsas, J.D., Van Overbeek, Ikpeme, E., Nfongeh, J., Enyi-Idoh, K., Eja, M. and Etim, L. (2011). Antibiotic susceptibility profiles of enteric bacterial isolates from dumpsite utisols and water sources in a rural community in Cross River State, Southern Nigeria**.** *Nature and Science*, **9(**5):46-50.

Holm-Nielsen, J.B., Dahl, C.K**.** and Esbensen, K. H. (2006). Representative sampling for process analytical characterization of heterogeneous bioslurry systems. A reference study of sampling issues in PAT. *Chemometrics and Intelligent Laboratory Systems*, **83** (2):114–126.

Hoorfar, P. and Radstro, M. (2000). Automated nuclease PCR assay for identification of *Salmonella enteric. Journal of Clinical Microbiology*, 3429-3435.

Huang, L.N., Zou, H., Chen, S., Luo, S., Lan, C.Y. and Qu, L.H. (2002). Diversity and structure of the archaeal community in the leachate of a full-scale recirculating landfill as examined by direct 16S rRNA gene sequence retrieval. *FEMS Microbiology Letters*, **214**, 235-240.

Huang, L. N., Zou, H., Chen, S., Luo, S., Lan, C.Y**.** and Qu, L.H**.** (2004). Characterization of methanogenic Archaea in the leachate of a closed municipal solid waste landfill. *FEMS Microbial Ecology*, **46**:171-177.

Hugenholtz, P. and Pace, N.R. (1996). The Earth's bounty: Assessing and accessing soil microbial diversity. *Trends in Biotechnology*, **14**:190–197.

Hunter, P.R. (1997). Waterborne Disease Epidemiology and Ecology. John Wiley and Sons, UK.

Hyong, S.K., Dong-Min, K., Ganesh, P.N., Yu-mi, L., Nam-Woong, Y.,Sook, J.J., Sook-In, J., Kyung-Hwa, P., Hae-Ryoung, P., Chang, S.L. and Sun, H.L. (2008). Comparison of conventional, nested, and real-time PCR assays for rapid and

accurate detection of *Vibrio vulnificus. Journal of Clinical Microbiology*, **46**(9):2992-2998.

Ikpeme, E., Nfongeh, J., Enyi-Idoh, K., Eja, M.E. and Etim, L. (2011). Antibiotic susceptibility profiles of enteric bacterial isolates from dumpsite Utisols and water sources in a Rural Community in Cross River State, Southern Nigeria. *Nature and Science*, **9**(5):46

Interim Management Committee (2011). The report of the committee on environmental sanitation Sabon-Gari Local Government Area . *Environmental Sanitation Sabon-Gari Local Government Area.* Pp. 2-5.

Isirima, M.O., Braide, S.A., Chindah, A.C., Nwachukwu, D., Ikoro, U.J., Osuamkpe, A. and Igwe, C. (2005). Technical Reports: Institute of Pollution Studies Laboratory.Rivers StateUniversity of Science and Technology, Port Harcourt.

Jalil, K., Vadood, R. and Abolfazl, B. (2011). Direct detection of *Escherichia coli* O157 and it major virulence factor genes in animal faeces at slaughter using multiplex Polymerase Chain Reaction (PCR). *African Journal of Microbiology Research*, **5**(14):1763-1767.

Jeris, J.S., and R.W. Regan. (1973). Controlling environmental parameters for optimum composting. *Composting Science,* **14**:10-15.

Jomezadeh, N., Farajzadeh, S.A., Khosravi, A.D. and Amin, M. (2009). Detection of shiga toxin producing *E. coli* strains isolated from stool samples of patients with diarrhea in Abadan Hospitals. *Iran Journal of Biological Science*, **9**:820–824.

Kam, K.M., Luey, K.Y., Chiu, A.W., Law, C.P. and Leung, S.F. (2007). Molecular characterization of *Salmonella enterica* serotype Typhi isolates by pulse field electrophoresis in Hong Kong, 2000-2004. *Foodborne Pathogens and Diseases*, **4**(1):41-49.

Kaper, J.B., Nataro, J.P., Mobley, H.L**. (**2004). Pathogenic *Escherichia coli*. *Natural and Reviewed Microbiology*, **2:**123-140.

Karaolis, D.K., Johnson, J.A., Bailey, C.C., Boedeker, E.C., Kaper, J.B. and Reeves, P.R. (1998). A *Vibrio cholerae* pathogenicity island associated with epidemic and pandemic strains. *Procedures in Natural Acadamic Science, USA*, **95**:3134-3139.

Karami, A., Ahmadi, Z., Safiri, Z. and Pourali, F. (2010). Detection of *Salmonella* strain by rapid-cycle multiplex PCR. *Jundishapur Journal of M*icrobiology, **4**(2):91-98.

Kargar, M. and Homayoon, M. (2015). Prevalence of shiga toxins (*stx*1, *stx*2), *eae*A and *hly* genes of *Escherichia coli* O157:H7 strains among children with acute gastroenteritis in southern of Iran. *Asian Pacific Journal of Tropical Medecine*, **8**(1):24-28

Kawano, K., Okada, M., Haga, T., Maeda, K. and Goto, Y. (2008). Relationship between pathogenicity for humans and stx genotype in shiga toxin-producing *Escherichia coli* serotype O157. *European Journal of Clinical Microbiology and Infectious Diseases*, **27**(3):227-232.

Kennedy, D. (2013). Time to deal with antibiotics (Editorial). *Science*, **342**:777.

Khachatourians, G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic- resistant bacteria. *Canadian Medical Association Journal*, **159**:1129-1136.

.Khajuria, A., Yamamoto, Y. and Morioka, T. (2008). Solid waste management in Asian countries. Problems and issues. Proceedings of the 4th International Conference on Waste Management and Environment, June, 2-4, **109**:643-653.

Kimura, A.C., Pelumbo, M.S., Abbott, S., Rodrigue, Z.R. and Werner, S.B. (2005). A multi-state outebreak of *Salmonella* serotype Thompson infection from commercially distributed bread contaminated by an ill food handler. *Epidemiology of infections*, **133**:823-828.

Kobayashi, M., Sasaki, T., Saito, N., Tamura, K., Suzuki, K., Watanabe, H. and Agui, N. (1999). Houseflies: not simple mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7**.** *American Journal for Tropic and Medical Hygiene*, **61:**625-629.

Koo, H.J. and Woo, G.J. (2011). Distribution and transferability of tetracycline resistance determinantsbin *Escherichia coli* isolated from meat and meat products. *International Journal of Food Microbiology*, **28**(145):2-3.

Kotloff, K.L., Nataro, J.P., Blackwelder, W., Nasrin, D. and Farag, T. **(**2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicentre Study, GEMS): a prospective, case-control study. *Lancet* **382**(9888)**:**209–222.

Kwuaga, J.K.P. (2016), ‘‘Infectious Diseases Without Borders: A Pathway to One Health Consilience’’. *An Inaugural Lecture*. Pp. 29-30.

Lamrani, H.A., Oufdou, K. and Mezrioui, N.-e. (2010) “Determination of several potential virulence factors in non-O1 *Vibrio cholerae*, *Pseudomonas aeruginosa*, faecal coliforms and streptococci isolated from marrakesh groundwater.” *Water Science and Technology*, **61**(7)

Lan, R. and Reeves, P.R. (2002). Pandemic spread of cholera: genetic diversity and relationships with the seventh pandemic clone of *Vibrio cholerae* determined by amplified fragment length polymorphism. *Journal of Clinical Microbiology*, **40**:172-181.

Lapierre, L., San, M.B., Bravo, V. and Toro, C.S. (2006). Characterization of *tet*A B G, *aadl*A genes in tetracycline and streptomycin resistant strains of *Salmonella* spp. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, p 1059.

Lee, K., Iwata, T., Shimizu, M., Taniguchi, T., Nakadia, A., Hirota, Y. and Hayashidani, H. (2009). A novel multiplex PCR assay for *Salmonella* subspecies identification. *Journal of Applied Microbiology,* **107**:805-811.

Lemarchand, K., Berthiaume, F. Maynard, C., Harel, J., Payment, P., Masson, L. and Brousseau, R. (2005). Optimization of microbial DNA extraction and purification from raw wastewater samples for downstream pathogen detection by microarrays. *Journal of Microbiology Methods*, **65**:115-126.

Le Minor, L. and Popoff, M. (1987). Designation of *Salmonella enterica* sp. nov. as the type and only species of the genus *Salmonella. International Journal of Systematic Bacteriology*, **37**:465-468.

Leschine, S.B. (1995). Cellulose degradation in anaerobic environments. *Annual Review of Microbiology*, **49**:399-426

Levine, M.M., Kotloff, K.L., Nataro, J.P. and Muhsen, K**. (**2012). The Global Enteric Multicenter Study (GEMS): impetus, rationale, and genesis. *Journal of Clinical and Infectious Diseases,* **55**(4)**:**215-224.

Levy, S.B. (2001). "Antibiotic resistance: consequences of inaction". *Clinical Infectious Diseases*, **33**(3):124-129.

Lewis, D.L. and Gattie, D.K. (2002). Pathogen risks from applying sewage sludge to land. *Environmental science Technology Journal*, **36**:286A-293A.

Li, J., Jun, J., Rita, W.T., Foo, J.M.L., Ling, H. and Norman, W.Y.S. (2010). Antibiotic resistance and plasmid profiles of *Vibrio* isolates from cultured *Sparus sarba*. *Marine Pollution Bulletin*, **39**:245-249.

Liu, Y-N., Wang, Y., Walsh, T.R., Yi, L-X., Zhang, R. and Spencer. (2016). Emergence of plasmid mediated colistin resistance mrc-l in animals and human beings in China: a microbiological and molecular biologynstudy. *Lancet Infectious Diseases*, **16**:161-168.

Lleo, M.M., Bonato, B., Tafi, M.C., Signoretto, C., Pruzzo, C. and Canepari, P. (2005). Molecular vs culture methods for the detection of bacterial faecal indicators in groundwater for human use. *Letter*s *in Applied Mcrobiology,* **40**:289-294.

Lorentz, J., Hill, I. And Samini, B. (2000). Occupational needs stick injuries in a metropolitan police force. *American Journal of Preventive Medicine,* **18**:146-150.

Ludwig, K., Karmali. M.A., Sarkim, V., Bobrowski, C., Petric, M. and Karch, H. (2001). Antibody response to Shiga toxins *Stx*2 and *Stx*1 in children with enteropathic haemolytic-uraemic syndrome. *Journal of Clinical Microbiology*, **39**:2272-2279.

Malorny, B., Hoorfar, J., Hugas, M., Heuvelink, A., Fach, P., Ellerbroek, B.C., Dorn, C. and Helmuth, R.L. (2003). Inter- laboratory diagnostic accuracy of a *Salmonella* specific PCR base method. *International Journal of food Microbiology,* **86**:241-249.

Mandell, G.L., Bennett, J.E. and Dolan, R. (1995). Principle and practice of infectious diseases, 4th Edition. Churchill, New York. Pp 22.

Manning, S.D., Motiwala, A.S., Springman , A.C., Qi, W., Lacher, D.W., Ouellette, L.M., Mladonicky, J.M., Somsel, P., Rudrik, J.T., Dietrich, S.E., Zhang, W., Swaminathan, B., Alland, D. and Whittam, T.S**. (**2008). Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. *Protocol of Natural and Academic Science,* U. S. A. **105:**4868-4873.

Marcus, S.I., Brumell, J.H., Pfeifer, C.G. and Finlay, B.B. (2000). *Salmonella* pathogenicity islands: big virulence in small packages: a review. *Microbes and Infection,* **2**:145-156.

Marie, B., Annett, M., Patrick, F. and Lothar B. (2011). Virulence gene profiling of enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* strains: a basis for molecular risk assessment of typical and atypical EPEC strains. *BMC Microbiology,* **11**:142-149.

Marsh, T.L. (1999). Terminal restriction fragment length polymorphism (T-RFLP); an emerging method for characterizing diversity among homologous populations of amplicons. *Current Opinions in Microbiology,* **2**:323-327.

m.Masayuki, N., Eiki, Y., Akitoyo, I., Takaaki, S., Akira, T., Junko, K.A., Kazuyuki, N., Joel, M., Toshiya, H.and Hisao, K. (2012). *Salmonella* enterotoxin (Stn) regulates membrane composition and integrity. *Dis Model Mech*. **5(**4):515-521.

Mascaretti, O. (2003). Compounds that interfer with tetrahydrofolic acid biosynthesis, In Bacteria versus antibacterial agents. *ASM Press.* P. 319-326.

Matthew, A. Croxen, R.J., Law, R.S., Kristie, M.K, Marta, W.B. and Brett, F. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli. Journal of Clinical Microbiology,* **26**(4):822-880.

Mazhaheri, N.F.R., Behzadian, N.G., Zahraei, S.T. and Atash, P.N. (2005). Evaluation of *ehxA*, *stx*1 and *stx*2 virulence gene prevalence in cattle *Escherichia coli* isolates by multiplex PCR short communication. *Archives of Razi Institute,* **60**:55-66.

Mbata, C.A. (2008). Studies on the burden of solid waste biodegrading microbes and health risks on waste scavengers in Port Harcourt, Ph.D. Thesis, University of Port Harcout, Nigeria.

McKinley, V.L.and Robie, J. V. (1984). Biokinetic analyses of adaptation and succession. Microbial activity in composting municipal sewage sludge. *Applied and Environmental Biology,* **47**(5):933-941.

Meneses, Y.E. (2010). Identification and characterization of *Salmonella* serotypes isolated from pork and poultry from commercial sources. M.Sc. Thesis.

Mengistu, G., Mulugeta, G., Lema, T. and Aseffa, A. (2014). Prevalence and antimicrobial susceptibility patterns of Salmonella serovars and Shigella species. *Journal of Microbial and Biochemical Technology*. S2. 006. doi: 10.4172/1948-5948.

Messner, K., Koller, K., Wall, M.B., Akhtar, M. and Scott, G.M. (1998). Fungaltreatment of wood chips for chemicalpul ping. In: Eriksson KEL, Cavaco-Paulo A (eds) Enzyme applications in fiber processing. American ChemicalSociety, Washington DC.

Mfasunwo, O.O., Jaiyeola, G.B. and Adesugba, F.F. (2010). Impacts of a dumpsite on the neighborhood of a part of South Western Nigeria. *Research Journal of Agriculture and Biological Science,* **6**(3):204-214.

Miriagou, V., Carattoli, A. and Fanning, S. (2006). Antimicrobial resistance islands: resistance gene clusters in Salmonella chromosome and plasmids. *Microbes and infection*, **8**:1923-1930.

Miyazaki S., Sugawara, H., Gojobori, T. and Tateno, Y. (2003). DNA Data Bank of Japan (DDBJ) for genome scale research in life science. *Nucleic Acids Research,* **31**, 13-16.

Molbak, K., Olsen, J. and Wegener, H. (2006). *Salmonella* infections, Reimann, D. Cliver (eds.), foodborne infections and intoxications. Academic Press. Pp. 55-115.

Momtaz, H., Rahimi, E. and Moshkelani, S. (2012). Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Veterinarn Medicina,* **57**(4):193-197.

Monteville, T. and Matthews, K. (2008). *Salmonella* species. In food microbiology. ASM Press. Pp. 97-112.

Mooljuntee, S., Chansiripornchai, P. and Chansiripornchai, N. (2010). Prevalence of the cellular and molecular antimicrobial resistance against *E. coli* isolated from Thai broilers. *Thai Journal of Veterinary Medicine*, **40**:311–315.

Moore, M.M. and Feist, M.D. (2007). Real-time PCR method for *Salmonella* spp. targeting the *Stn* gene. *Journal of Applied Microbiology*, **102**:516-530.

Morin, N.J., Gong, Z. and Li, X.F. (2004). Reverse transcription-multiplex PCR assay for simultaneous detection of *Escherichia coli* O157:H7, *Vibrio cholerae* O1, and *Salmonella typhi. Clinical Chemistry*, **50.**

Mortimer, C., Peters, T., Gharbia, S., Logan, J. and Arnold, C. (2004). Towars the development of a DNA-sequence based approach to serotyping of *Salmonella enterica*. *BMC Microbiology*, **4**:31.

Moussa, I.M., Gassem, M.A., Al-Doss, A.A., Mahmoud, W.A.S. and Abdel, M.A.L. (2010). Using molecular techniques for rapid detection of *Salmonella* serovars in frozen chiken and chiken products collected from Riyadh, Saudi Arabia. *African Journal of Biotechnology*, **119**(5-6):139-141.

Moura, A., Henriques, I., Smalla, K. and Correia, A. (2010). Wastewater bacterial communities bring together broad-host range plasmids, integrons and a wide diversity of uncharacterized gene cassettes. *Research in Microbiology*, **161**:58-66.

Mumy, K.L. and Findlay, R.H. (2004). Convenient determination of DNA extraction efficiency using an external DNA recovery standard and quantitative-competitive PCR. *Journal of Microbiological Methods*, **57**:259-268.

Muniafu, M. and Otiato, E. (2010). Solid waste management in Nairobi, Kenya. A case for emerging economies. *The Journal of Language, Technology and Entrepreneurship in Africa*, **2**(1):342-350.

Murugkar, H.V., Rahman, H. and Dutt, P.K. (2003). Distribution of virulence genes in *Salmonella* serovars isolated from man and animals. *Indian Journal of Medical Research*, **117**:66-70.

NARMS (National Antimicrobial Resistance Monitoring System). (2006). Enteric human isolates. Final Report. Available at http://www.cdc.gov/NARMS/.

National Enteric Surveillance Program (NESP)**. (**2010). The National Microbiology Laboratory (NML) and Centre for Food-borne, Environmental and Zoonotic Infectious Diseases (CFEZID), Public Health Agency of Canada and Provincial Public Health Microbiology Laboratories, Winnipeg, Canada.

Nashwa, H.M. Mahmoud, A. and Adawy. (2009). Application of Multiplex Polymerase Chain Reaction (MPCR) for identification and characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium. *Journal of Applied Science Research*, **12**:343-2348.

Nazzal, Z., Said, H., Al-Hajiri, M. and Tamim, O. (2012). *Salmonella* food poisoning associated with macroni salad in a labourer's camp state of Qatar, 2010. *Journal of*

Community Medicine and health Education, **2**:145. http://doc.doi:10.4172/2161-0711.1000145.

NCBI Information Web Citation, (2003).<http.//www.ncbi.nlm.nih.gov/BLAST/> (January 3, 2003).

Neely, M.N. and Friedman, D.I. **(**1998). Functional and genetic analysis of regulatory regions of coliphage H-19B: location of Shiga-like toxin and lysis genes suggest a role for phage functions in toxin release. *Molecular Microbiology*, **28:**1255–1267.

Nesa, M.K., Khan, M.S. and Alam, M. (2011). Isolation, identification and characterization of *Salmonella* serovars from diarrhoeic stool samples of human. *Bangladesh Journal of Veterinary Medisine*, **9**(1):85-93.

MFasunwo, O.O., Jaiyeola, G.B. and Adesugba, F.F. (2010). Impacts of a dumpsite on the neighborhood of a part of South Western Nigeria. *Research Journal of Agriculture and Biological Science*, **6**(3):204-214.

Noah, F., Zongzhi, L., Mari, R., Rob, K., Mathew, H. And Mark, T.H. (2008). Short- term temporal variability in air-borne bacteria and fungi populations. *Applied and Environmental Microbiology*, **74**(1):200-207.

Nurmohammad, S. and Phillip, I.T. (2003). *Escherichia coli* O157:H7 Shiga toxin-encoding bacteriophages: integrations, excisions, truncations, and evolutionary implications. *American Society for Microbiology Journal of Bacteriology*.

Nwaokwe, T. (2004). National report of hazardous waste management in Nigeria. *Proceedings of the Based Convention, Africa Regional Conference* (BCARC' 04). Ibadan, Nigeria, pp 20-26.

Obi, C.L., Potgieter, N., Bessong, P.O., Igumbor, E.O., and Green, E. (2003). Prevalence of pathogenic bacteria and retroviruses in the stools of patients presenting with diarrhea from rural communities in Venda, South Africa. *South African Journal of Science*, **99**:589-591.

Obi, C.L., Ramalivhana, J., Momba, M.N.B., Onabolu, B., Igumbor, J.O., Lukoto, M., Mulaudzi, T.B., Bessong, P.O., Jansen van, R.E.L., Green, E .and Ndou, S. (2007). Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhoea and their household drinking water in rural communities in Limpopo province South Africa. *African Journal of Biotechnology*, **6**(8):1035-1047.

Obire, O., Nwaubeta, O. and Adue, S.B.N. (2002). Microbial community of a waste dump site. *Journal of Applied Sciences and Environmental Management*, **6**(1):78-83.

Odeyemi, A.T., Faweya, E.B., Agunbiade, O.R. and Ayeni, S.K. (2011). Bacteriological, mineral and radioactive contents of leachate samples from dumpsite of Ekiti State

Government Destitute Centre in Ado-Ekiti. *Archives of Applied Science Research,* **3**(94):92-108.

Odeyemi, A.T. (2012). Antibiogram status of bacterial isolates from air around dump site of Ekiti State Destitute Centre at Ilokun, Ado-Ekiti, Nigeria. *Journal of Microbiology Research,* **2**(2):12-18.

Ogbonna, D.N. and Igbenijie, M. (2006). Characteristics of microorganisms associated with waste collection sites in Port-Harcourt City, Nigeria. *Nigeria Journal of Microbiology,* **20**(3):1427-1434.

Oghenekohwo, J.E. and Akporehwe, N.J. (2015). Perspectives on waste management and community health promotion in urban cities in Nigeria. *British Journal of Education,* **3**(8):70-77.

Ogunrinola, I.O. and Adepegba, E.O. (2012). Health and Economic Implications of Waste Dumpsites in Cities: The Case of Lagos, Nigeria. *International Journal of Economic and Finance,* **4**(4):239-251.

Ogwueleka, T.C. (2009). Municipal solid waste characteristics and management in Nigeria. *Iran Journal of Environmental Health Science,* **6**(3):173-180.

Ohud, M.A., Halawani, M. and Hayam, S.A. (2012). Detection of *Salmonella* strains in clinical samples from Saudi Arabia by *invA* and *hylA* polymerase chain reaction (PCR)-base assays. *African Journal of Microbiology Research*, **6**(25):5410-5416.

Ojo, O. (2008). State of the environment in Nigeria. *Proceedings of the First National Environment for Sustainable Economic Development*. Federal Ministry of Environment, Housing and Urban Development. Transcorp, Abuja, 20th -21st April. Pp 60-61.

Okeke, I.N., Abiodun, O.A., Byarugaba, D.K., Ojo, K.K. and Opinta, J.A. (2007). Growing problem of multidrug-resistant enteric pathogens in Africa. *Emerging Infectious Deseases*, **13**(11):1640-1646.

Olatoye, I.O. (2010). The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from Beef in Ibadan Municipal, Nigeria. *African Journal of Biotechnology,* **9**(8):1196-1199.

Olesen, I., Hasman, H. and Aarestrup, F.M. (2004). Prevalence of beta-lactamases among ampicillin resistant *Escherichia coli* and *Salmonella* isolates from food animals in Denmark. *Microbial drug Resistance*, **10**:334-340

Olorunmola, F.O., Kolawole, D.O. and Lamikanra, A. (2012). Antibiotic resistance and virulence properties in *Escherichia coli* strains from case of urinary tract infections. *African Journal of Infectious Deseases*, **7**(1):1-7.

Olufunmilayo, J.W and Kelechi, H. (2016).Microorganisms associated with dump sites in Port Harcourt Metropolis, Nigeria. *Journal of Ecology and the Natural Environment*, **8**(2): 9-12.

Olukosi, O.M., Ameh, J.B., Abdullahi, I.O. and Whong, C.M.Z. (2016). Multidrug resistant *Escherichia coli* isolated from drinking water from parts of Kaduna South, Kaduna State, Nigeria. *International Journal of Applied Biological Research*, **7**(2):9-17.

Omar, A., Wright, J.B., Schultz, G., Burrell, R. and Nadworny, P. (2017). Microbial biofilms and chronic wounds. *Journal of Microorganisms*, **5**(9):1-15.

Omojowo, F.S. and Omojasola, F.P. (2013). Antibiotic resistance pattern of bacterial pathogens isolated from cow dung used to fertilize Nigerian fish ponds. *Notulae Scientia Biologicae*, **5**(1):15-19.

Onweremadu, E.U., Chukwuma, M.C. and Ogbonna, F.C. (2009). Spatial variability of mercury in soil affected by automobile wastes at Port Harcourt, Nigeria. *Advances in National and Applied Sciences*, **3**(2):260-264.

Osei, F.B. and Duker, A.A. (2008). Spatial dependency of *V. cholerae* prevalence on open space refuse dumps in Kumasi, Ghana: a spatial statistical modeling. *International Journal of Health Geography*, **7**:62.

Oshinkanlu, J.A. (1971). The first three cases of the 7th cholera pandemic in Nigeria. *Nigeria Medical Journal*. **1**:189-192.

Osorio, C.R. and Klose, K .E. (2000). A region of the transmembrane regulatory protein ToxR that Tethers the transcriptional activation domain to the cytoplasmic membrane displays wide divergence among *Vibrio* species. *Journal of Bacteriology*, **182**:526–528.

Osunwoke, J.I. and Kuforiji, O.O. (2012). Health impact of microorganisms associated with waste dump sites in a private University. *Journal of Sustainable Development and Environmental Protection***, 2**(1):59.

Oviasogie, F.E., Ajuzie, C.U. and Ighodaro, U.G. (2010). Bacterial analysis of soil from waste Dumpsite. *Archives of Applied Science Research*, **2**(5):161-167.

Oyedele, O. (2009). Solid waste management as engine for industrial development in Nigeria. *SciTopics*. Retrieved from http://www.scitopics.com/Solid_Waste_Management_as_Engine_for_Industrial_Development_in_Nigeria.html

Paice, M.G., Reid, I.D., Bourbonnais, R., Archibald, F.S. and Jurasek, L. (1993). Manganese peroxidase, produced by *Trametes versicolor* during pulp bleaching, demethylates and delignifies kraft pulp. *Applied Environmental Microbiology*, **59**:260–265.

Partanen, P., Hultman, J., Lars P., Auvinen, P. and Romantschuk, M. (2010). Bacterial diversity at different stages of the composting process. *BMC Microbiology,* **10**:94-95.

Patchanee, P., Zewde, B., Tadesse, D., Hoet, A. and Gebreyes, W. (2008). Characterization of Multidrug-Resistant *Salmonella enterica* Serovar Heildenberg Isolated from Humans and Animals. *Foodborne Pathogens and Disease*, **5**:839-849.

Pathmanathan, S.G., Cardona-Castro, N., Sanchez-Jimenez, M.M., Correa-Ochoa, M.M., Puthucheary, S.D. and Thong, K.L. (2003). Simple and rapid detection of *Salmonella* strains by PCR amplification of *hylA* gene. *Journal of Medical Microbiology*, **52**:773-776.

Paton, A.W. and Paton, J.C. (1998). Detection and characterization of shiga toxine *Escherichia coli* by using multiplex PCR assay for *stx*1, *stx*2, *eae*A, Enterohemorrhagic *E. coli hlyA*, rfb(O111) and rfb(O157). *Journal of Clinical Microbiology,* **36**(2):598-602.

Pavoni, J.L., Heer, J.E., and Hagerty, D.L. (1975). Handbook of solid waste disposal, materials and energy recovery. Van Nostrand Reinhold Company, New York

Percival, S.L., Chalmers, R.M., Embrey, M., Hunter, P.R., Sellwood, J. and Wyn-Jones, P. (2004). Microbiology of waterborne diseases. Elsevier academic press. UK.

Perez, J., Munoz-Dorado, J., De la Rubia T. and J. Martınez, J. (2002). Biodegradation and biological treatments of cellulose, hemicelluloses and lignin: an overview. *International Journal of Microbiology,* **5**:53–63.

Pierce, N.F., Greenough, W.B.I., Carpenter, C.C.J. (1971). *Vibrio cholerae* enterotoxin and its mode of action. American Society for Microbiology, **35**(1):30-35.

Pradel, N., Boukhors, K., Bertin, Y., Forestier, C., Martin, C. and Livrelli, V. (2001). Hetrogenecity of shiga toxin- produsing *Escherichia coli* strains isolated from haemolytic-uraemic symdrome patients, cattle, and foods amples in central France. *Applied and Environmental Microbiology,* **67**:2460-2468.

Prager, R., Fruth, A., Siewert, U., Strutz, U. and Tschape, H. (2009). *Escherichia coli* encoding shika toxin 2f as an emerging human pathogen. *International Journal of Medical Microbiology,* **299**(5):343-345.

Provenzano, D., Lauriano, C.M. and Klose, K.E. (2001). Characterization of the role of the ToxR-Modulated Outer Membrane Porins OmpU and OmpT in *Vibrio cholerae* virulence. *Journal of Bacteriology*, **183**(12):3652-3662.

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J. and Woodward, M.J. (2004). Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy,* **53**:208-216.

Rebollido, R., Martinez, J., Aguilera, Y., Melchor, K., Koerner, **I.** and Stegmann, R. (2008). Microbail populations during composting process of organic fraction of municipal solid waste. *Applied Ecology and Environmental Research*, **6(**3):61-67.

Relman, D.A. (1993). Universal bacterial 16S rDNA amplification and sequensing. In: Persting, D.H., Smith, T.F., Tenouer, F.C., White, T.J., eds. *Diagnostic Molecular Microbiology*, 489-495.

Reuben, R.C. and Owuna, G. (2013). Antimicrobial resistance patterns of *Escherichia coli* O157:H7 from Nigerian fermented milk samples in Nasarawa State, Nigeria. *International Journal of Pharmaceutical Science Invention*, **2**(3):38-44.

Rivera, I.N.E.K., Lipp, A., Gil, N., Choopun, A., Hug, K. and Colwell, R.R. (2003). Method of DNA extraction and application of multiplex polymerase chain reaction to detect toxic gene of *Vibrio cholerae* O1 and O139 from aquatic ecosystems. *Environmental Microbiology*, **5**:599-606.

Rmadass, K. and Palaniyandi, S. (2007). Effect of enriched municipal solid waste compost application on soil available macronutrients in the rice field. *Archives of Agronomy and Soil Science*, **53(**5):497-506. Retrieved from http:/search ebscohost. com/login.aspx? direct=true &db=s8hAN=26419316 &site=ehost_liveon September, 2010.

Rodney, M.D. (2002). Biofilms: Microbial Life on Surfaces. *Journal of Emerging Infectious Diseases*, **8**(9):881-888.

Sack, R.B., Siddique, A.K., Longini J.I.M., Nizam, A., Yunus, M. Islam, M.S., Morris, J.J.G., Ali, A., Huq, A., Nair, G.B., Qadii, F., Faruque, S.M. and Sack, D.A. (2003). A 4-year study of the epidemiology of *Vibrio cholerae* in four rural areas of Bangladesh. *Journal of Infectious Diseases*, **187**:96-101.

Sa'idu, I. (2011). Refuse dumps threaten to take over Zaria. Category: Around and about.Weekly Trust No. 767.

Salami, M.Y.I. (1972). Clinical and epidemiological aspects of cholera outbreak in metropolitan Lagos. *Nigerian Medical Journal*, **2**:1-149.

Scarpino, P.N. and Quinn, H. (2010). Dumpsite, microbial, air contamination , waste management. Department of Civil Engineering, the University of Cincinnati, Cincinnati. *Oklahoma*, Pp 45221-45271.

Scheutz, F., Teel, L.D., Beutin, L., Piérard, D., Buvens, G., Karch, H., Mellmann, A., Caprioli, A., Tozzoli, R., Morabito, S., Strockbine, N.A., Melton-Celsa, A.R., Sanchez, M., Persson, S. and O'Brien, A.D. **(**2012). Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. *Journal of Clinical Microbiology*, **50:**2951-2963.

Schwarz, S. and White, D. (2005). Phenicol resistance, pp. 124-148. In D. White, M. Alekshun, P. McDemott (eds.), Frontiers in Antimicrobial Resistance. ASM Press.

Shanmugasamy, M., Velayutham, T. and Rajeswar, J. (2011). *Inv*A gene specific PCR for detection of *Salmonella* from broilers. *Veterinary World,* **4**(12):562-564.

Shekarforoosh, S., Tahamtan, Y. and Pourbakhsh, A. (2008). Detection and frequency of stx2 gene in *E.coli* O157 and O157 strains isolated from sheep carcasses in Shreaz-Iran. *Pakistan Journal of Biomedical Sciences,* **11**(8):1085-1092.

Singh, D.V., Isac, S.R. and Colwell, R.R. (2002). Development of a hexaplex PCR assay for rapid detection of virulence and regulatory genes in *Vibrio cholerae* and *Vibrio mimicus*. *Journal of Clinical Microbiology,* **40**:4321-4324.

Sivakumar, T., Avinash, S.N., Prabhu, D., Shankar, T. and Vijayabaskar, P. (2012). Characterization of multidrug resistant patterns of *Salmonella* sp. *World Journal of Medical Sciences*, **7**(2):64-67.

Skockovả , A., Cupả kovả , Š., Karpiskovả , R. and Janstovả , B. (2012). Detection of tetracycline resistance genes in *Escherichia coli* from raw cow's milk. *Journal of Microbiology, Biotechnology and Food Science,* **1**:777-784.

Smith, R.S., Fawora, M.A., Goodluck, H.A., Nwaokorie, F.O., Aboaba, O.O. and Opere, B. (2010). Molecular typing of *Salmonella* spp isolated from food handlers and animals in Nigeria. *International Journal of Molecular Epidemiology and Genetic,* **2**(1):73-77.

Smith, S.I., Fowora, M.A., Atiba, A., Anejo-Okopi, J., Fingesi, T., Adamu, M.E., Omonigbehin, E.A., Ugo-Ijeh, M.I., Bamidele, M. and Odeigah, P. (2015). Molecular detection of some virulence genes in *Salmonella* spp isolated from food samples in Lagos, Nigeria. *Animal and Veterinary Sciences,* **3**(1):22-27.

Sood, S., Kapil, A., Das, B., Jain, Y. and Kabra, S.K. (1999). Re-emergence of chloramphenicol-sensitive *Salmonella* Typhi. *Lancet.* **353**:1241-1242.

Soto, S.M.., Rodriguez, I., Rodicio, M.R., Vila, J. and Mendoza, M.C. (2006). Detection of virulence determinants in clinical strains of *Salmonella enteric* serovar Enteritidis and mapping on Macrorestriction profiles. *Journal of medical Microbiology,* **55**:365-373.

Stanley, A.M., Andrew, S.S., Dania, A.A. and Sani, I.F. (2012). An assessment of household solid waste disposal practices in Sabon-Gari, Zaria. *ATBU Journal of Environmental Technology*, **5**(1):48-59.

Solid Waste Audit Report, Federal capital Territory, Abuja, Nigeria. (2004).

Steinberg, N. and Kolodkin-Gal, I. (2015). The matrix reloaded: Probing the extracellular matrix synchronizes bacterial communities. *Journal of Bacteriology*, **197**:2092-2103.

Stephan, A. and Mathew, U.S. (2005). Antimicrobial resistance determinants and future control. *Emerging Infectious diseases,* **11**(6):794-801.

Stigi, K.A., Macdonald, J.K., Tellez-Marfin, A.A. and Lofy, K.H**. (**2012). Laboratory practices and incidence of non-O157 shiga toxin-producing *Escherichia coli* infections. *Journal of Emerging Infectious Diseases,* **18:**477-479.

Stoesser G., Baker, W., van den Broek, A., Garcia-Pastor, M., Kanz, C., Kulikova, T., Leinonen, R., Lin, Q., Lombard, V. and Lopez, R. (2003). The EMBL nucleotide sequence database. *Nucleic Acids Research*, **31**:17-22.

Su, L.H., Chiu, C.H., Chu, C. and Ou, J.T. (2004). Antimicrobial resistance in non typhoid *Salmonella* serovars: A global challenge. *Clinical Infectious Diseases*, **77:**4273-4279.

Sukhnanand, S., Alcaine, S., Warnick, L., Su, W.-L., Hof, J., Craver, M., McDonough, P., Boor, K. and Wiedmann, M. (2005). DNA sequence-based subtyping and evolutionary analysis of selected *Salmonella enterica* serotypes. *Journal of Clinical Microbiology,* **43**:688-3698.

Sule, W.F., Adige, A.A., Abubakar, M.J. and Ojezele, M.O. (2012). Antimicrobial resistance of clinical isolates of *Salmonella typhi* in Anyigba, Kogi State, Nigeria. *Global Advanced Research Journal of Microbiology***, 1**(4):57-61.

Swartz, M.N. (2002). Human diseases caused by food-borne pathogens of animal origin. *Clinical Infections Diseases,* **34**:111-122.

Tahamtan, Y., Hayati, M. and Namavari, M.M. (2010). Prevalence and distribution of the *stx*1, stx2 *genes* in shiga toxin producing *E. coli* (STEC) isolates from cattle. *Iran Journal of Microbiology,* **2**(1):8-13.

Tagoe, D.N.A., Nyarko, H., Arthur, S.A. and Birikorang, E. (2011). A study of antibiotic susceptibility pattern of bacteria isolates in sachet drinking water sold in the Cape Coast Metropolis of Ghana. *Research Journal of Microbiology*, **6**:153-158.

Tamura, k. Dubdley, J., Nei, M. and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**:1596-1599.

Tansey, M.R., and Brock, T.D. (1978). Microbial life at high temperatures. Ecological Aspects, p. 159-216. In: D.J. Kushner (eds.), Microbial life in extreme environments. Academic Press, Inc., New York).

Thanner, S., Drissner, D. and Walsh, F. (2016). Antimicrobial resistance in agriculture. *mBIO*, **7**(2)e02227-15. Doi.10.1128/mBIO 02227-15.

The Economist (2016). When the drugs don't work: the rise of antibiotic resistance. The Economist, May, 21st-27th 2016. Pp. 16-19.

Theron, J. and Cloete, T.E. (2004). Emerging microbial detection techniques. In: Microbial waterborne pathogens. Cloete, T., Rose, I., Nel, L.H. and Ford, T. (ed), IWA publiching UK.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**:4673-4680.

Ter-Hsin, C., Yu-Chih, W., Yi-Tseng, C., Chia -Huei, Y. and Kuang-Sheng, Y. (2005). Serotypeoccurrence and antimicrobial suceptibility of *Salmonella* isolates recovered from pork carcasses in Taiwan (2000 through 2003). *Journal of Food Protection*, **69**:674-678.

Terrance, M.A., Genevieve, A.B.G., Mildred, R.B. and Koohmaraie, M. (2002). Prevalence and characterization of non-O157 shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Apply and Environmental Microbiology*, **68**:4847-4852.

Thermal Energy for Renewables (ThERRA). (2006). Proposal for the definition and calculation principle for renewable heat. (online). Available from http//www.therra.info/pdf/Definitions and Methods/Propos alDefinitionRESHeat9.pdf (accessed 21st June, 2009). Pp 120-149.

Theron, J., Cilliers, J., Du Preez, M., Brozel, V.S. and Venter, S.N. (2000). Detection of toxigenic Vibrio cholerae from environmental water samples by enrichment broth cultivation-pit-stop semi-nested PCR procedure. *Journal of Applied Microbiology*, **89**:539-546.

Threlfall, E.J. (2002). Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food and water-borne infections. *FEMS Microbiology Review*, **26**:30-32

Tiquia, S.M. (2005). Microbiological parameters as indicators of compost maturity. *Journal of Applied Microbiology*, **99**:816-828.

Tognetti, C., Mazzarino, M.J. and Laos, F. (2007). Improving the quality of municipal organic waste compost. *Bioresource Technology*, **98**:1067-1076.

Uba, S., Zairu, A., Sallau, M. S., Abba, H. and Okunola, O. J. (2013). Metals bioavailability in the leachates from dumpsites in Zaria Metropolis, Nigeria. *Journal of Toxicology and Environmental Health Sciences*, **5**(7):131-141.

Udo, S.M. (1996). Studies on the pathogenic potential and persistence of *Vibrio cholerae* in the lower Cross River Basin of Nigeria. M.Sc. Thesis, University of Calabar, Nigeria.

Udo, S.M. and Nfongeh, J.F. (2005). Studies on the prevalence of enteropathogens associated with diarrhoeal cases in Adim Community, Southeastern Nigeria. *African Journal of Environmental Pollution and Health*, **4**(2):25-30.

UNEP (2004). The use of economic instruments in environmental policy. Opportunities and challenges. United Nations Publication, ISBN: 92-807-2391-X, UNEP, Geneva.

US FDA. (2015). Guidance for industry 213, new animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food-producing animals: recommendations for drug sponsors for voluntarily aligning product use conditions with GFI# 209.

Usunwoke, J.I. and Kuforiji, O.O. (2012). Health impact of microorganisms associated with waste dumpsites in a private University. *Journal of Sustainable Development and Environmental Protection*, **2**(1):59-66.

Valentine, U. (2012). Solid waste management: a case study of Emeka, Port Harcourt, Rivers State, Nigeria.

Vally, H., Hall, G., Dyda, A., Raupach, J., Knope, K., Combs, B. and Desmarchelier, P. **(**2012). Epidemiology of Shiga toxin producing *Escherichia coli* in Australia, 2000-2010. *BMC Public Health*, **12:**63.

Van, T.T.H., Moutafis, G., Istivan, T., Tran, L.T. and Coloe, P.J. (2007). Detection of *Salmonella* spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Applied and Environmental Microbiology*, **73:**6885-6890.

Van Elsas, J.D., Semenov, A.V., Costa, R. and Trevors, J.T. **(**2011). Survival of *Escherichia coli* in the environment: fundamental and public health aspects. *ISME Journal*. **5:**173–183.

Vu, B., Chen, M., Crawford, R.J. and Ivanova, E.P. (2009), "Bacterial Extracellular Polysaccharides Involved in Biofilm Formation", *Molecules*, **14** (7): 2535-2554.

Wachukwu, C.K., Thomas, C.N. and. Kigigha, L.T. (2002). Micro organisms associated with palm weevil Larvae (*Rhynchophorus phoenicis*). *Journal of Diary and Foods Science*. **21**:44-47.

Wachukwu**,** C.K. Mbata**,** C.A. and Nyenke**,** C.U. (2010). The Health Profile and Impact Assessment of Waste Scavengers (Rag Pickers) in Port Harcourt, Nigeria. *Journal of Applied Sciences*, 10:1968-1972.

Wai- Ogosu O.A. (2004). Monitoring and evaluation of industrial waste management options in Rivers State. Paper presented at a workshop on Sustainable Environmental Practices in Rivers State organized by Rivers State Ministry of Environment, Hotel Presidential, Port Harcourt, 23-24 March, 2004.

Wattiau, P., Weijers, T., Andreoli, P., Schiliker, C., Vander Veken, H., Maas, H., Verbruggen, A., Heck, M., Wannet, W., Imberechts, H. and Vos, P. (2008). Evaluation of the Premi ®Test *Salmonella*, a commercial low- density DNA microarray system intended for routine identification and typing of *Salmonella enterica*. *Internattional Journal of Food Microbiology*, **123**:293-298.

White, J.R., Navlakha, S., Nagarajan, N., Mohammad-Reza, G., Kingsford, C. and Mihai, P**.** (2010). Alignment and clustering of phylogenetic markers - implications for microbial diversity studies. *BMC Bioinformatics*, 11:152 doi.10.1186/1471-2105-11-152.

Whittam, T.S., Wolfe, M.L., Wachsmuth, I.K., Orskov, F., Orskov, I. and Wilson, R.A. **(**1993). Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. *Infection and Immunology***, 61:**1619-1629.

Wilson, D.C. (2007). Development drivers in waste management. *Waste Management and Research,* **25**(3):198-207.

Woolhouse, M.E.J. and Ward, M.J. (2013). Sources of antimicrobial resistance. *Science*, **341**:1460-1461.

World Fact Sheet. (2001). Global report on human settlements. United Nations Human Settlements Programme (UN Habitat).

World Health Organization (1970). Principles and practice of cholera control. *Public Health Papers*, **40**:23-31.

World Health Organization (1990). *Weekly Epidemiological Record*, **47**(1):1.

World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC and P/USAID, (2003). Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World; *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Neisseria gonorrhoeae, Salmonella serotype Typhi*, *Shigella*, and *Vibrio cholerae*. WHO: pp. 103-149.

World Health Organization /United Nations Chieldren's Education Fund (2004). In: Datta, R.R., Hossain, M.S., Aktaruzzaman, M. and Fakhruddin, A.N.M. (2014). Antimicrobial Resistance of Pathogenic Bacteria Isolated from Tube Well Water

of Costal Area of Sitakunda, Chittagong, Bangladesh. *Open Journal of Water Pollution and Treatment,* **1**(1):1-9.

World Health Organization (2005). Drug-resistant *Salmonella. Fact sheet* No. 139.

World Health Organization (2007). 'Water, sanitation and hygiene: Quantifying the health impact at national and local levels in countries with incomplete water supply and sanitation coverage'.Environmental burden of disease series, no 15.

World Health Organization (2008). Cholera. Available online:http/www.who.int/mediacentre/factsheets/fs107/en/print.html (accessed 10-01-2009).

World Health Organization (2011). Solid waste management in emergencies. Fact sheet No.7

World Health Organization (2012). Water sanitation health fast facts: WHO/UNICEF joint monitoring report 2012. Accessedfrom http://www.who.int/water sanitation health/monitoring/jmp2012/ fast/en/(2013). Water quality and health strategy 2013–2020. Page6. Accessedfrom htt://www.who.int/water sanitation_health/ publications/2013/ water quality_strategy. pdf 12Jan 2014.

World Health Organization (2013). Water quality and health strategy 2013-2020. P. 6. Accessedfromhtt://www.who.int/watersanitation_health/publications/2013/water quality strategy. ptf 12 jan 2014.

Yamamoto, Y. (2002). PCR in diagnosis of infection. Detection of bacteriain cerebrospinal fluids. *Clinical and Dignostic Laboratory Immunology*, **9**(3):508-514.

Yang, Z., Kovar, J., Kim, J., Nietfeldt, J., Smith, D.R., Moxley, R.A., Olson, M.E., Fey, P.D. and Benson, A.K. **(**2004). Identification of common subpopulations of non-sorbitol-fermenting glucuronidase-negative *Escherichia coli* O157:H7 from bovine production environments and human clinical samples. *Applied and Environmental Microbiology*, **70:**6846-6854.

Yeşiller, N., Hanson, J.L**.** and Liu, W.L. (2005). Heat generation in municipal solid waste landfills. *Journal of Geotechnical and Geoenvironmental Engineering,* **131**(11):1330-1344.

Zainol, N.A., Aziz, H.A. and Yusoff, M.S. (2012). Characterization of leachate from Kuala Sepetang and Kulim landfills. A comparative study. *Energy and Environment Research*, **2**(2):45-52.

Zaria at a glance (2013). In: Uba, S., Zairu, A., Sallau, M. S., Abba, H. and Okunola, O. J. (2013). Metals bioavailability in the leachates from dumpsites in Zaria Metropolis, Nigeria. *Journal of Toxicology and Environmental Health Sciences***, 5**(7):131-141.

Zhao, S., White, D.G., Ayers, S., Friedman, S., English, L., Wagner, D., Gaines, S. and Meng, J. (2001). Identification and characterization of integron-mediated antibiotic resistant.

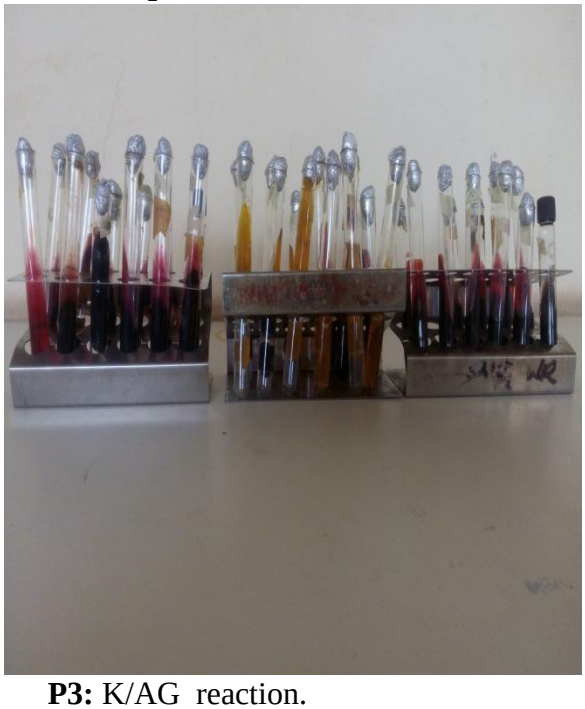
Zurburgg, C., (2002). Urban solid waste management in low-income countries of Asia. How to cope with the garbage crisis. Scientific Committee on Problems of the Environment (SCOPE). Urban solid waste management review session, Durban, South Africa.

Zymo Research. ZR Genomic DNA TM – Tissue MiniPrep. Catalog Nos. D3050 and D3051.

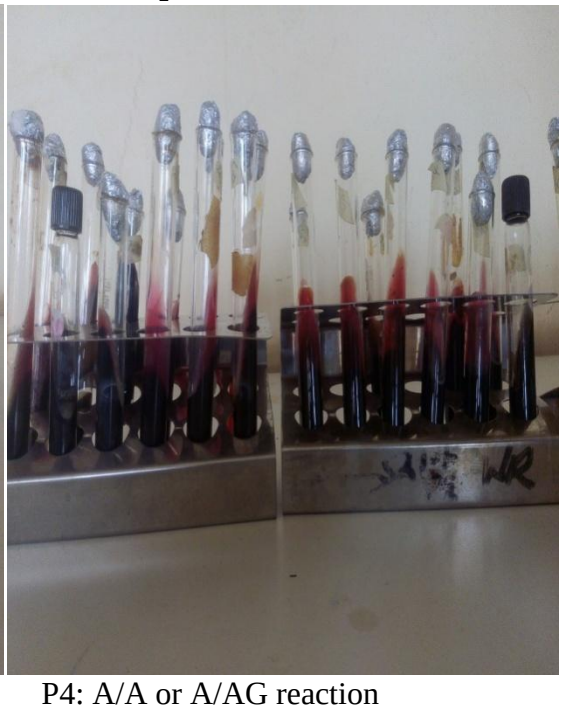
APPENDICES

APPENDIX I: Images from Triple Sugar Iron (TSI), by Isolates. P1 & P2: Formation of hydrogen sulphide determined by the blackening of the whole butt ($K/A/H_2S$ reaction)

P1: $K/A/H_2S$ reaction



P2: $K/A/H_2S$ reaction



P3: K/AG reaction.



P4: A/A or A/AG reaction



KEY:

K/AG- ALKALINE/ACID: no sugar was fermented.

A/A- ACID/ACID: glucose or lactose have been fermented or glucose and sucrose have been fermented

A/AG- ACID/ACID + Gas formation: glucose or lactose has been fermented or glucose and sucrose or glucose, lactose and sucrose have been fermented.

APPENDIX II: Images from Microgen Identification Test Kits, Presumptive *E. coli* isolates (P1) and Presumptive *Salmonella* isolates (P2)



APPENDIX III: Identification of *Salmonella* and *Escherichia coli* using Microgen™

Enterobacteriaceae GNA- ID System

Sample code	Lysine	Orn	H_2S	Glu	Mann	Xylose	ONPG	Indole	Urease	VP	Citrate	TDA	%	OC
ZC_{41} S	+	+	+	+	+	+	+	+	-	-	+	-	98.69	7762
TW_{53} S	+	+	+	+	+	+	⁻	-	+	-	+	-	97.71	7712
SG_{66} S	+	-	+	+	+	+	+	-	-	-	+	-	91.79	5742
ZC_{72} S	+	-	+	+	+	+	-	-	-	-	+	-	91.32	5702
SA_{99} S	+	+	+	+	+	-	-	-	-	-	+	-	90.87	7602
ZC_3 S	+	+	+	+	+	+	+	-	-	-	-	-	89.04	7740
TW_{87} S	+	+	+	+	+	+	+	+	+	-	+	+	83.10	7773
SG_{92} S	+	+	+	+	+	+	-	-	-	-	+	-	80.89	7702
ZC_{34} S	+	+	+	+	+	+	-	-	+	-	+	-	97.71	7712
TW_{101}S	+	-	+	+	+	+	+	-	-	-	+	-	91.79	5742
ZC_{96} S	+	+	+	+	+	-	-	-	-	-	+	-	90.87	7602
SG_{37} E	+	-	-	+	+	-	-	+	-	-	-	-	98.26	4620
ZC_{74} E	+	-	-	+	+	+	+	+	-	-	-	-	98.06	4760
TW_{77} E	+	+	-	+	+	+	+	+	-	-	-	-	97.11	6760
SA_{24} E	+	-	-	+	+	+	-	+	-	-	-	-	93.84	4720
SG_{69} E	+	-	-	+	+	-	+	+	-	-	-	-	84.38	4660
CS_{52} E	+	+	+	+	+	+	+	+	-	-	-	-	91.92	7760
ZC_{11} E	+	-	-	+	+	+	-	+	-	-	+	-	89.65	4722
TW_{73} E	+	+	-	+	+	+	+	+	-	-	-	-	97.11	6760
SA_{44} E	+	+	-	+	+	+	+	+	-	-	+	-	84.29	6762
ZC_{91} E	+	+	-	+	+	+	+	+	-	-	-	-	97.11	6760
TW_{33} E	+	-	-	+	+	+	+	+	-	-	-	-	85.00	4760
SG_{92} E	+	+	-	+	+	+	+	+	-	-	-	-	97.11	6760

GNA wells

KEY:
ZC=Zaria city, TW=Tudun-Wada, SG=Sabon-Gari, SA=Samaru, CS=Control site, ONPG=Ortho-Nitrophenyl-β-galactosidase,TDA=Triptophan deaminase, H2S=Hydrogen sulphide, VP=Voges-Proskauer, OC= Octal Code, S= *Salmonella*, E= *Escherichia coli*, %=Percentage probability,

APPENDIX IV: **Identification of the bacterial isolates by MICROGEN GNA+GNB**

test kit

	Reaction	Well number	Identified Microorganisms															
			Vibrio alginolyticus	*Escherichia coli*	*Vibrio cholerae*	*Vibrio vulnificus*	*Vibrio parahaemolytic*	*Proteus vulgaris*	*Proteus mirabilis*	*Citrbtobacter freundii*	*Klebsiella pneumonia*	*Pseudomonas aeroginosa*	*Providencia*
GN A wells	**Oxidase**		+	−	+	+	+	−	−	−	−	+	−
	Motility		+	+	+	+	+	+	+	+	−	+	+
	Nitrate		+	+	+	+	+	−	−	+	+	+	+
	Lysine	1	+	+	+	+	+	−	−	−	+	+	−
	Ornithine	2	+	−	+	+	−	−	+	−	−	−	−
	H_2S	3	−	−	−	−	−	+	+	+	−	−	−
	Glucose	4	+	+	+	+	+	+	+	+	+	+	+
	Mannitol	5	−	+	+	−	+	−	−	+	+	+	−
	Xylose	6	−	+	−	−	−	+	+	+	+	−	−
	ONPG	7	−	+	+	+	−	−	−	+	+	−	−
	Indole	8	−	+	+	+	+	+	−	+	−	−	+
	Urease	9	−	−	−	−	−	+	+	+	+	+	−
	VP	10	+	−	−	−	−	−	−	−	+	−	−
	Citrate	11	−	−	+	+	+	−	+	+	+	+	+
	TDA	12	−	−	−	+	−	+	+	−	−	−	+
	Gelatin	13	−	−	+	+	+	+	+	−	−	+	−
	Malonate	14	−	−	−	−	−	−	−	+	+	+	−
	Inositol	15	−	−	−	−	−	−	−	−	+	−	+
	Sorbitol	16	−	+	−	−	−	−	−	+	+	−	−
	Rhamnose	17	−	+	−	−	−	−	−	+	+	−	−
	Sucrose	18	−	+	+	−	−	+	−	+	+	−	+
	Lactose	19	−	+	−	+	−	−	−	+	+	−	−
GN B wells	**Arabinose**	20	−	+	−	−	−	−	−	+	+	+	−
	Adonitol	21	−	−	−	−	−	−	−	−	+	−	−
	Raffinose	22	−	−	−	−	−	−	−	+	+	−	−
	Salicin	23	−	−	−	+	−	−	−	−	+	−	−
	Arginine	24	−	−	−	−	−	−	−	+	−	+	−

KEY:

ONPG=Ortho-Nitrophenyl-β-galactosidase, TDA=Triptophandeaminase, H_2S=Hydrogen sulphide, VP=Voges-Proskauer

APPENDIX V: Isolation rate of bacteria in waste dump samples

Bacteria isolates	Number of isolates (%)
Acinetobacter baumannii	2(1.06)
Acinetobacter iwofii	2(1.06)
Citrobacter freundii	4(2.12)
Citrobcter sakazakii	3(1.59)
Enterobacter cloacae	1(0.53)
Enterobacter liquefaciens	5(2.64)
***Escherichia coli* O157:H7**	22(11.64)
Hafnia alvei	4(2.12)
Klebsiella ozaenae	8(4.23)
Klebsiella oxytoca	3 (1.59)
Klepsiella pneumonia	4(2.12)
Morganella morganii	2(1.06)
Non-O157:H7 *E.coli*	45(23.81)
Proteus mirabilis	16(8.46)
***Pseudomonas* spp**	6(3.17)
***Salmonella* Arizonae**	7(3.70)
***Salmonella* Pullorum**	5(2.64)
***Salmonella* Typhi**	3(1.59)
Vibrio alginolyticus	9(4.76)
***Vibrio cholerae* non-01**	7(3.70)
Vibrio parahaemolyticus	4(2.12)
Vibrio vulmificus	6(3.17)
TOTAL	189(100)

Total P<0.05

%- percentage=number of the organism isolated / total number of organisms isolated x 100

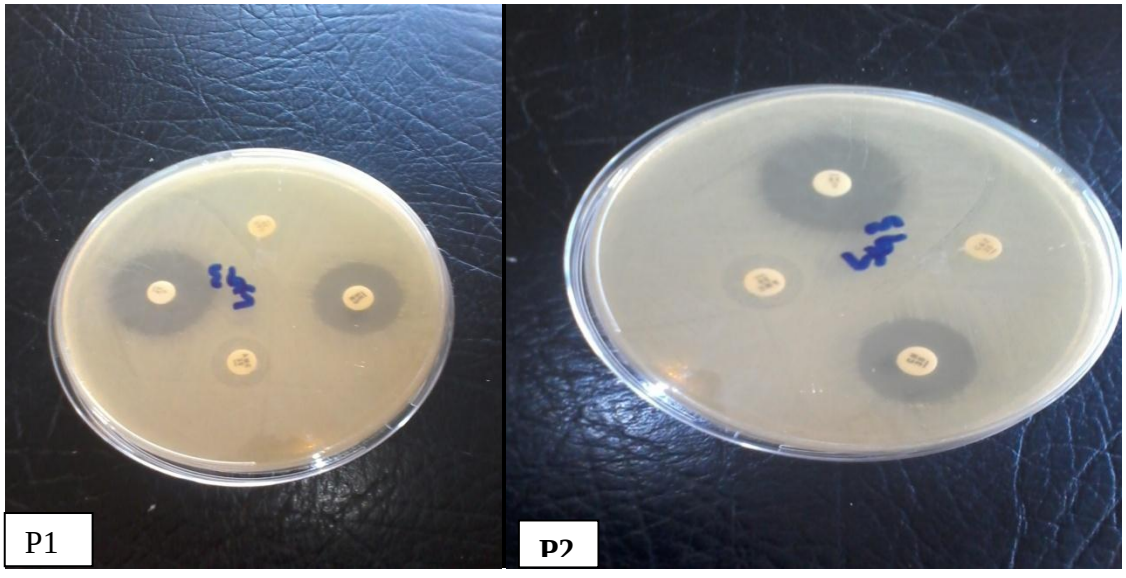
APPENDIX VI: **Zone Diameter Interpretative Standards for Bacterial Isolates**

Antibiotics	Code	Disc content (µg)	Zone diameter to the nearest mm Resistant (R)	Zone diameter to the nearest mm Intermediate (I)	Zone diameter to the nearest mm Sensitive (S)
Ampicillin	AMP	10	≤13	14 – 16	≥17
Amoxycillin-clavulanic acid	AMC	30	≤13	14 -17	≥18
Cefotaxime	CTX	30	≤22	23 – 25	≥26
Chloramphenicol	CHL	30	≤12	13 – 17	≥18
Ciprofloxacin	CIP	10	≤15 & ≤20	16-20 & 21-30	≥21 & ≥31
Gentamicin	CN	30	≤12	13 – 14	≥15
Nitrofurantoin	F	30	≤14	15 – 16	≥17
Norfloxacin	NOR	10	≤12	13 – 16	≥17
Ofloxacin	OFX	10	≤20	21 – 30	≥31
Sulfamethoxazole/ Trimethoprim	SXT	25	≤10	11 – 15	≥16
Tetracycline	TE	30	≤11	12 – 14	≥15

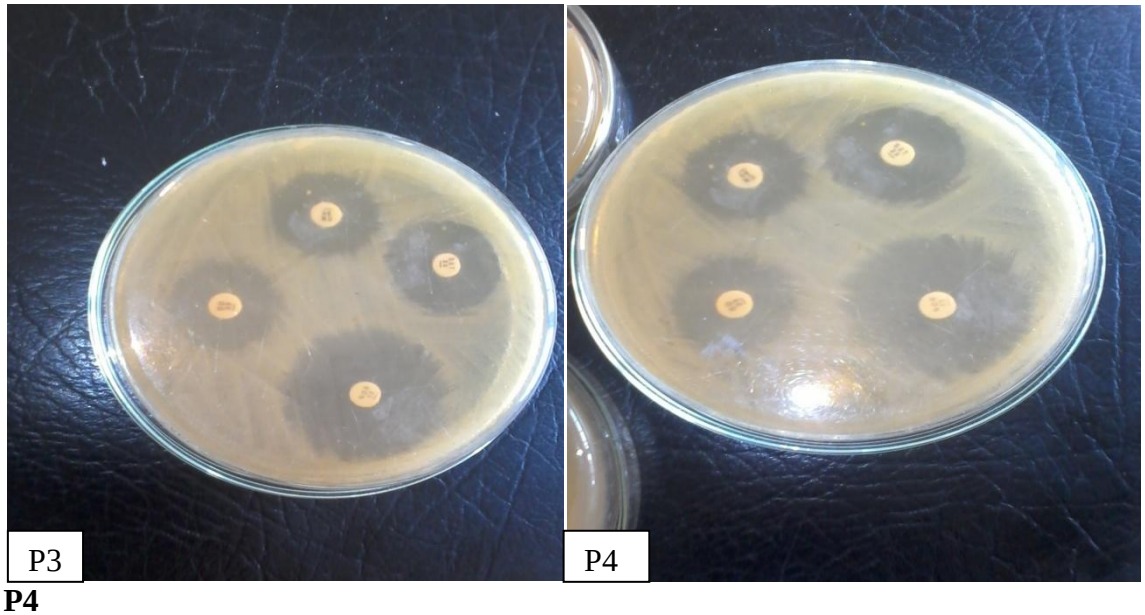
SOURCE: Clinical and Laboratory Standard Institute, CLSI, (2014)

APPENDIX VII: Images from Antibiotic resistance (P1 & P2) and subseptibility (P3 & P4) by isolates

P1: *Salmonella enterica*; **P2**: *E. coli* O157:H7. Pattern: AMP-AMC-TE



P3: *Salmonella enterica*; **P4**: *E. coli* O157:H7. Pattern: STX-CN



P4

APPENDIX VIII: Percentage Susceptibility of *Salmonella* spp, *E coli* O157:H7 and *Vibrio Cholerae* non-O1 isolated from waste dumps samples. Values given in the table are the average of triplicate

Antibiotis Tested	Disc potency (µg)	*Salmonella enterica* (N=21)			*Escherichia Coli* O157:H7(N=18)			*Vibrio cholerae* (N=7)		
		R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
AMP	10	20(95.24)	0(0.00)	1(4.76)	17(94.44)	1(5.56)	0(0.00)	7(100)	0(0.00)	0(0.00)
AMC	30	15(71.43)	4(19.05)	2(9.52)	16(88.89)	0(0.00)	2(11.11)	NT	NT	NT
CTX	30	8(38.09)	1(4.76)	12(57.14)	5(27.78)	0(0.00)	13(72.22)	0(0.00)	4(57.14)	3(42.86)
CHL	30	3(14.29)	0(0.00)	18(85.71)	0(0.00)	0(0.00)	18(100)	3(42.86)	2(28.57)	2(28.57)
CIP	10	2(9.52)	3(14.29)	16(76.19)	3(16.67)	2(11.11)	13(72.22)	NT	NT	NT
CN	30	0(0.00)	0(0.00)	21(100)	3(16.67)	0(0.00)	15(83.33)	2(28.57)	0(0.00)	5(71.43)
F	30	2(9.52)	0(0.00)	19(90.47)	4(22.22)	3(16.67)	11(61.11)	NT	NT	NT
NOR	10	NT	NT	NT	6(33.33)	2(11.11)	10(55.55)	2(28.57)	3(42.86)	2(28.57)
OFX	10	4(19.05)	4(19.05)	13(61.90)	NT	NT	NT	1(14.29)	0(0.00)	6(85.71)
SXT	25	6(28.57)	1(4.76)	14(66.67)	6(33.33)	0(0.00)	12(66.67)	7(100)	0(0.00)	(0.00)
TE	30	12(57.14)	0(0.00)	9(42.86)	16(88.89)	0(0.00)	2(11.11)	6(85.7)	0(0.00)	1(14.29)

KEY:

AMP-Ampicillin, AMC-Amoxycillin-clavulanic acid, CHL-Chloramphenicol, CTX- Cefotaxim, CIP-Ciprofloxacin, CN- Gentamicin, F-Nitrofurantoin, NOR- Norfloxacin, OFX- Ofloxacin, SXT-Sulphamethoxazole-trimethoprim, TE-Tetracycline, N- number of isolates, % percentage=Number of the organism isolated/total number of organisms isolated x 100, n(%)= Number of isolates susceptible/ intermediate/resistant and their percentage, NT-Not tested, R-Resistant, I-Intermediate susceptible, S-Susceptible.

APPENDIX IX: **Antibiotic Resistance Profile of Target Organisms' Isolated from Waste Dump Samples**

Resistance phenotype	Number of isolates resistant
AMP	2
AMP,TE	4
AMP,SXT	1
AMP, AMC,TE	3
AMP,AMC,SXT	1
AMP,SXT,TE	1
AMP,AMC,OFX	1
AMP,AMC,CTX	1
AMP, AMC,CIP,TE	2
AMP,AMC,CTX,TE	4
AMP,AMC,SXT,TE	1
AMP,AMC,CTX,SXT	2
AMP,SXT,CN,TE	2
AMP,AMC,F,TE	1
AMP,AMC,OFX.CTX	1
AMP,SXT,OFX,CHL,TE	1
AMP.SXT,NOR,CHL,TE	2
AMPAMC,CTX,F,TE	2
AMP,AMC,CTX,CHL,TE	1
AMP,AMC,SXT,NOR,CHL,TE	2
AMP,AMC,CIP,NOR,SXT,TE	3
AMP,AMC,SXT,CN,F,TE	3
AMP,AMC,NOR,CTX,F,TE	1
AMP,AMC,SXT,OFX,CIP,TE	1
AMP,AMC,SXT,OFX,CTX,,CHL,F	1
Total	**44**

KEY:

AMP-Ampicillin, AMC-Amoxycillin-clavulanic acid, CHL-Chloramphenicol, CTX- Cefotaxim, CIP-Ciprofloxacin, CN- Gentamicin, NOR- Norfloxacin, OFX-Ofloxacin, SXT-Sulphamethoxazole-trimethoprim, TE-Tetracycline.

APPENDIX X: Antibiotic Resistance Patterns and Multiple Antibiotic Resistance (MAR) Index of the Bacterial Isolates from Waste Dump Soil Samples

Isolate code	Sample location	Resistance pattern	MAR Index
TW_{92} V	Dan gama	AmpSxt	0.22
ZC_{6} E.C	Kusfa-Zaria	AmpTe	0.20
SG_{27}E.C	Rail way Enkatarko	AmpAmcCip	0.30
ZC_{62} E.C	Alhudahuda	AmpAmcCip	0.30
SA_{39}E.C	Ayin-Dogo	AmpAmcCip	0.30
ZC_{51}E.C	Limanci kona	AmpAmcCip	0.30
SG_{101} S	Rail way Enkatarko	AmpCipTe	0.30
TW_{35} S	Kwarin	AmpAmcOfx	0.30
TW_{14} V	Agolo	AmpSxtTe	0.33
SG_{40} E.C	Army children school	AmpAmcTe	0.30
ZC_{44} E.C	Kusfa-Zaria	AmpAmcTe	0.30
SA_{99} E.C	Fly over	AmpAmcTe	0.30
TW_{77} E.C	Kwarin	AmpAmcTe	0.30
ZC_{91} E.C	Dan-Gama	AmpAmcTe	0.30
SG_{34} E.C	Independent cinema	AmpAmcTe	0.30
TW_{85} S	Agolo	AmpCtxSxt	0.30
ZC_{50} S	Limanci kona	AmpCtxSxt	0.30
TW_{49} S	Kwarin	AmpAmcCtx	0.30
ZC_{72} S	Kusfa-Zaria	AmpAmcSxt	0.30
SG_{17} S	Rail way Enkatako	AmpAmcSxt	0.30
ZC_{16} S	Kusfa –Zaria	AmpAmcCtxOfx	0.40
TW_{33} S	Dan-Gama	AmpAmcCipTe	0.40
SA_{67}S	Fly over	AmpAmcCipTe	0.40
ZC_{31} S	Kusfa-Zaria	AmpAmcCipTe	0.40
ZC_{93} S	Kusfa-Zaria	AmpAmcCipTe	0.40
SA_{81} S	Ayin-Dogo	AmpAmcCipTe	0.40
SG_{36} S	Rail way Enkatarko	AmpAmcCtxChl	0.40
TW_{42} S	Agolo	AmpAmcCtxTe	0.40
ZC_{102} S	Limanci kona	AmpAmcCtxTe	0.40
SG_{84} E.C	Chikaji	AmpAmcCtxNor	0.40
SA_{5} E.C	Leather research	AmpAmcCtxNor	0.40
SG_{99} S	Independent cinema	AmpAmcSxtTe	0.40
SG_{62} V	Chikaji	AmpCnSxtTe	0.44
ZC_{29} V	Kusfa-Zaria	AmpCnSxtTe	0.44
SA_{73} S	Ayin-Dogo	AmpAmcOfxSxtTe	0.50
TW_{103} V	Agolo	AmpNorSxtTeChl	0.55
ZC_{41}V	Alhudahuda	AmpNorSxtTeChl	0.55
TW_{8} E.C	Dan-Gama	AmpAmcCtxTeF	0.50
ZC_{87} V	Limanci kona	AmpOfxSxtTeChl	0.55
TW_{19} S	Agolo	AmpAmcCtxTeChl	0.50
SG_{73}E.C	Rail way enkatarko	AmpAmcCipNorSxtTe	0.60
ZC_{23} E.C	Kusfa-Zaria	AmpAmcCtxNorTeF	0.60
TW_{74} E.C	Kwarin	AmpAmcCnNorSxtTeF	0.70
ZC_{97} S	Limanci kona	AmpAmcCtxChlOfxSxtF	0.70

KEY:

AMP-Ampicillin, AMC-Amoxycillin-clavulanic acid, Chl-Chloramphenicol, CTX- Cefotaxim, CIP Ciprofloxacin, CN- Gentamicin, F-Nitrofurantoin, NOR- Norfloxacin, OFX- Ofloxacin, SXT-Sulphamethoxazole-trimethoprim, TE-Tetracycline, ZC- Zaria city, TW-Tudun-Wada, SG- Sabon-Gari, SA- Samaru, E.C- *Escherichia coli*, S- *Salmonella*, V- *Vibrio cholerae.*