MOLECULAR CHARACTERIZATION AND EFFECTS OF NANO-PARTICLE ON RESISTANT BACTERIA ASSOCIATED WITH LIVESTOCK IN ABIA AND IMO STATES

BY

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20164996168

A DISERTATION SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,

POSTGRADUATE SCHOOL

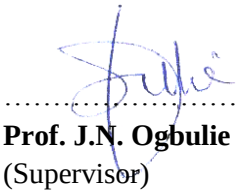
FEDERAL UNIVERSITY OF TECHNOLOGY OWERRI,

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE (Ph.D) IN MEDICAL MICROBIOLOGY

FEBRUARY, 2024

CERTIFICATION

This is to certify that this research work titled **"Molecular Characterization and Effects of Nano-Particle on Resistant Bacteria Associated with Livestock in Abia and Imo States**" was carried out by **Nwosu Ijeoma Linda (Reg. No: 20164996168)** of the Department of Microbiology, Federal University of Technology, Owerri (FUTO) under the supervision of Prof. J.N. Ogbulie, Prof. (Mrs.) C. I. Chikwendu and Dr (Mrs.) E. E. Mike Anosike in partial fulfillment for the award of a Doctor of Philosophy degree (Ph.D) of Medical Microbiology in Microbiology.



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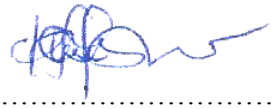

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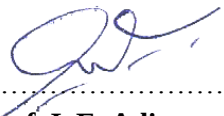

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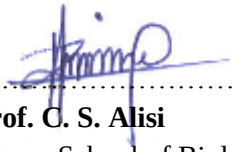

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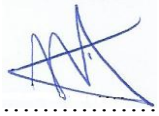

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DEDICATION

This work is dedicated to my husband and children

ACKNOWLEDGMENTS

With humility and due reverence, I thank the Almighty God for His mercies. I remain eternally grateful to Him.

I wish to express my immense gratitude to my supervisors Prof. J.N. Ogbulie, Prof (Mrs.) C. I. Chikwendu and Dr. E.E. Mike-Anosike for their untiring guidance throughout the course of the work.

I also wish to express my gratitude to, all the staff of Medical Laboratory Science Department, Abia State College of Health Aba, the Directors of Miracle and Mawa farms, all the staff of medical microbiology department of ABSUTH and Midland Diagnostic Laboratories, especially Mrs. C. Nwachukwu, Mr. O. Nwosu and Dr. (Mrs.) U. Asomuha for their assistance and encouragement during this study. I thank Prof. M. Tatfeng and his staff for the molecular characterization of the bacterial isolates. All thanks to Kingsley Nwachukwu, Uzoma, all my friends and well wishers for their encouragements, I say thank you.

Special thanks also go to the Head of Microbiology Department Prof. I.E. Adieze and all my lecturers Prof. W. Braide, Prof. C. Akujobi, Prof. E.S. Amadi, Prof. C.E. Nwanyanwu, Prof. (Mrs.) Umeh, Prof. C.O. Nweke, Dr. E.C. Chinakwe, Dr. C.C. Opurum, for reviewing this work, the Dean of Biological Sciences Prof. C.S. Alisi and his entire staff for their support. All thanks to the Dean Prof. B.O. Esonu, Associate Dean Prof. C.O. Nweke, our schedule officer Mrs. Okwuegbulam and the entire staff of postgraduate school for all their support.

I must not fail to acknowledge the following persons; My siblings, Kessy, Solo, Joe, Chidi and Ekene, words can hardly express my gratitude for all you did for me during this programme.

My course mates; Kelechi, Chioma, Ihuoma, Kingsford, Irene, Ngozi and my chauffeur, Ndubuisi, for their encouragements.

I must also acknowledge my children Ihuoma, Chizaram, Uchechukwu, Wisdom, Kamsy, Precious; my father Elder (Dr.) E.O. Adaelu and my darling husband Dr. I.O. Nwosu for encouraging and supporting me, may God bless you.

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ABSTRACT

The unregulated practice of livestock production has endangered the public health sector through the multiplication and spread of bacteria pathogens. The study investigated the molecular characterization and compared the nano-particle and antibiotic assay of bacteria associated with livestock in Abia and Imo States. Air was sampled with passive sedimentation technique; water samples were collected randomly from the water sources in the farms while hand swabs from the farmers and feeds were collected with sterile swab sticks and container respectively. Total heterotrophic bacterial count (THBC) was performed with pour plate method; total coliform count (TCC) was determined with membrane filter technique while total potential pathogenic bacteria count (TPPBC) was examined by growing them in selective media. Rectal swabs and faecal samples; raw meat and intestine samples were obtained from cow and pigs. From poultry farm, faecal sample cloacal swab and drinking water of chickens were used for experiment. Pure isolates of *E. coli* and *Klebsiellapneumoniae* were isolated from MacConkey and eosin methylene blue (EMB) agar. Antibacterial susceptibility test was performed by adopting standard method. Extended spectrum beta lactamases (ESBLs) production was screened and confirmed phenotypically using double disc synergy assay. DNA of the ESBLs producers were extracted by boiling method and quantified using nanodrop spectrophotometer while genes sequencing were performed with polymerase chain reaction (PCR). Green synthesis of silver and zinc nanoparticles (AgNPs and ZnONPs) were done using aqueous extract of the leaves of *Ocimum gratissimum* plant (scent leaves), *Vermonia amygadalina* plant (bitter leaves), *Newbouldia laevis* plant (Ogirishi leaves) and *Gongronema latifolium* plant (utazi leaves). The biosynthesis was done by dissolving 50 mM, 100 mM, 500 mM and 1000 mM of $AgNO_3$ and Zn-NO-.6H-O separately into 20 ml and 50 ml of each plant extract respectively. Thereafter, antibacterial activities of the nanoparticles were performed against livestock bacterial isolates using agar well diffusion assay. Among the four farms analyzed, THBC ranged from $6.13\pm0.6\times10^5$ to $28.43\pm0.3\times10^5$ CFU/ml; TPPBC ranged between $9.26\pm0.4\times10^5$ and $26.23\pm0.4\times10^5$ CFU/ml and TCC ranged from $9.03\pm0.3\times10^5$ to $24.06\pm0.4\times10^5$ CFU/ml. Of the four cities studied, Aba has the highest THBC ($28.43\pm0.3\times10^5$, $26.70\pm0.7\times10^5$, $26.26\pm0.5\times10^5$ CFU/ml), TPPBC ($17.47\pm0.5\times10^5$and $20.02\pm0.5\times10^5$ CFU/ml) and TCC ($24.06\pm0.4\times10^5$, $17.93\pm0.6\times10^5$ and $22.36\pm0.4\times10^5$ CFU/ml) for pig, cow and poultry farms respectively while Mbaise has the least value. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonellaenterica*, *EnterobacteraerogenesVibrio* spp and *Shigella* sp were commonly distributed in the four cities. *Escherichiacoli* (933) isolates were significantly higher followed by *Klebsiellapneumoniae* (505) while the lowest value was obtained from *Shigella* sp (3). In both pig and poultry farms, *Staphylococcusaureus* (14.6%) was quite prevalent but was isolated even more from the hands of poultry workers (53.7%). *Salmonella* spp, *Enterobacter* spp, *Vibrio* spp and *Shigella* sp were not isolated from the hands of poultry workers; *Salmonella* enterica and *Shigella* sp were not isolated cow farms. *E. coli and Klebsiellapneumoniae* were resistant to all the antibiotics between 70% to 88.6% except imipenem, meropenem and colistin where minimal effects were produced. However, colistin was the most active antibiotics amongst the three. ESBLs were produced by *Klebsiella* pneumoniae than *E. coli* in the four towns. AgNPs and ZnONPs produced significant zones of inhibition even more than control especially at highest concentrations of individual molarity. AgNPs exhibited higher antibacterial effects. This study indicated that Aba has the highest microbial load which could be attributed to the population and economic activities of the dwellers. The presence ESBLs producers in all the four towns are major concern as this could lead to spread of multidrug resistance (MDR). Appropriate personal and environmental cleanliness should be practiced by livestock farmers to reduce spread of pathogenic bacteria**.**

Keywords: Bacteria, Livestock, Multi-drug Recsitance, Nano-pacticles

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Livestock farming is currently one of the leading agricultural practices in developing countries that is economically viable and performed both in urban and rural areas (Thornton, 2010). Urban livestock is a farming activity that is practiced within the urban centers. It is an important aspect that develops the urban areas. Apart from the economic impact on cities, urban livestock also produces negative effects for instance, increased health failure, environmental contamination and spread of diseases (Asadu, Chah, Attamah & Igbokwe, 2021). Livestock farming contributes to climate change which consequently affects the distribution of bacteria and other unhealthy chemical substances (Grossi, Goglio, Vitali & Williams, 2015). Livestock farms are agents of bacterial transmission and animal-related pathogens, especially the antibiotic-resistant strains. Livestock diseases are very significant worldwide based on their effects on the environment and inhabitants. These diseases produce direct effects on human and animal health and negatively affect the economy and food supply (Thornton, 2010; Gebreyes et al., 2020).

Nigeria is geopolitically grouped into six zones including the South-East which is made up of Enugu, Anambra, Imo, Abia, and Ebonyi States. The occupation of the southeasterners is mainly trading, crop production and livestock farming (Nwanta et al., 2011). The zone has so many urban towns with growing populations such as Aba, Umuahia, Okigwe and Mbaise. A lot of urban agricultural activities take place in these towns especially rearing of sheep, goats and pig (Asadu et al., 2021). They are managed in both intensive and semi-intensive systems. Livestock farming generate animal protein for consumers and revenue to the sellers (Nwanta et al., 2011). Most livestock farmers largely venture into pig farming while 65% include poultry, and 31% indulge in goat and sheep production (Nwanta et al., 2011). These animals are sources of direct and indirect

disease spread. Microbial pathogens are transmitted via excreta, urine and flesh of animal hosts. They can also be isolated from feeds, drinking water, rain splashes, feeding troughs and hands of farmers (Alegbeleye, Singleton & Sant'Ana, 2018).

Basically, most microorganisms that colonize the body surface of animals or that get into their bodies are eliminated by either applying antibiotics as ointment on skin surfaces or ingestion through feeds, water or aerosols. Apart from therapeutic purposes, drugs can be used non-therapeutically as feed additives to enhance development of livestock (Aarestrup, 2012). The quality of feeds consumed by most animals have been improved by mixing the antibiotics with feeds in the appropriate proportion. These drugs, when consumed, eliminate the microbes that compete with the nutrients available in the provender thereby allowing the body tissues and cells have total access to the nutrients present in the digested provender (Chattopadhyay, 2014). For instance, use of tetracycline and penicillin in feeds of poultry significantly improved their egg-producing ability while chlortetracycline certainly decreased respiratory diseases associated with swine and by extension, decreased morbidity in livestock. However, frequent use of antibiotics in livestock production and consumption are not without side effects as they have led to the emergence of resistant strains (Van, Yidanaa, Smookera & Coloe, 2020). The ubiquity and survival mechanisms of these microbes within and outside of the hosts make them most successful in disease transmission among other pathogens. They are distributed through mediums such as urine, faeces and hides of livestock and from their aerosols (Klous, Huss, Heederik & Coutinho, 2016). Several strains of pathogenic bacteria such as *Escherichia coli*, *Vibrio* spp., *Shigella* spp. and *Salmonella* spp and some nonpathogenic bacteria have been isolated from air, water and soil (Ugbogu, Onyeagba, Ugbogu & Nwaugo, 2016).

Antibiotic resistance has posed serious challenge to livestock production especially in output and health of farmers due to the spread of resistant strains (Junaid et al., 2022). These resistant strains

exist due to mutation and natural selection; they are transmitted to humans through direct contact with the workers that got contaminated by the skin and faeces of animals and consumption of agricultural products (Van-Boeckel et al., 2015; Heuer, Schmitt & Smalla, 2011). Multidrug resistant enteric bacteria have been isolated successfully from faeces, drinking water and flesh of livestock as well as hand swabs of farm workers (Suzuki, Hiroki, Xia, Nishiyama, Sakamoto, Uemura, Nukazawa, Ogura, Toru Watanabe & Kobayashi, 2022); the spread of these bacteria as reported in most literature is more in animal faeces due to direct contact with farmers. These resistant bacterial strains thrive due to the secretion of β lactamases known as extended spectrum beta lactamases (ESBLs) (Widodo, Effendi, & Khairullah, 2020). Among the species of enteric bacteria isolated, *Escherichia coli* and *Klebsiella pneumoniae* are mostly predominant producers of ESBLs (Junaid et al., 2022). These Gram-negative bacteria are responsible for nosocomial infections, urinary tract infection (UTI) and pneumonia. ESBLs are plasmid-associated enzymes that inhibit most β-lactam and some cephalosporins antibiotics. Their secretions are dependent on the particular coding genes mainly $bla_{CTX\text{-}M\text{-}1}$, $bla_{CMY\text{-}2}$ and $bla_{SHV\text{-}12}$ (Lee et al., 2020). reported that ESBLs bacteria isolated from cow, pig and poultry were the reservoirs of $bla_{CTX\text{-}M}$.

Since antiquity, plant extracts are veritable tools in the treatment of infectious diseases (Shah, Rehem & Daud, 2015), since microorganisms have become resistant to conventional drugs resulting from wrong prescription by health experts and abuse by users. Due to increasing toxicity of orthodox drugs to livestock and humans, attention has moved from conventional drugs to plants. Often, as observed in most livestock farms, while farm animals consume plants directly as food sources, indirectly too, due to the bioactive and secondary metabolites in these plants, they tend to boost their immune system against any diseases. In recent times, nanotechnology has been effectively utilized in virtually all fields of human endeavors, especially in medicine (Sergeev and Shabatina, 2008). Several researches have been performed on the use of plants to synthesize

nanoparticles with metallic elements. Such elements include silver (Ag), zinc (Zn, gold (Au), titanium (Ti) and palladium (Pd) (Khan, Shariq, Asif, Siddiqui, Malan & Ahmad, 2022). There are reports that nanoparticles of different plant origin were effective in the management of inflammations, cancer and mostly used in the treatment of infectious diseases (Ghosh, Nandi & Basu, 2022). However, despite the surge of research in nanoparticles, no work has reported the antibacterial effects of silver and zinc nanoparticles of leaves of *Ocimum gratissimum* (African basil), *Vermonia amygadalina* (bitter leaf), *Newbouldia laevis* (Ogirishi leaf) and *Gongronema latifolium* (utazi leaf) especially against bacteria obtained from livestock farms within South-East region of Nigeria.

1.2 Problem Statement

Antibiotic resistance can be transferred from livestock fed with antibiotics to humans (Christy, Sampson, Edson & Anthony, 2018). Patients are harmed as a result of this process, and in some countries like Nigeria, national policies eliminating growth promotion and routine prophylactic use have not been implemented. Most bacteria, specifically Enterobacteriaceae, are common causes of both community-acquired and hospital acquired infections, including urinary tract, bloodstream, and lower respiratory tract infections. These bacteria can acquire genes encoding multiple antibiotic resistance mechanisms, including extended-spectrum lactamases (ESBLs), AmpCs, and carbapenemases; through contaminating the environment with animal feces and droppings, used as manure in plants like fruits and vegetables. The Carbapenems namely imipenem, meropenem, ertapenem, and doripenem became the antimicrobials of last resort used in treating infections due to these highly drug resistant bacteria. These antimicrobial agents became crucial in the management of life-threatening healthcare-associated and community acquired infections.

1.3 Aim of the study

The research is aimed at the Phenotypic and molecular characterization of bacteria of public health concerns associated with livestock farms in the four cities of South-East states and antibacterial activities of silver and zinc nanoparticles.

1.4 Objectives of the study are to:

1. determine the bacterial profiles of livestock farms in Aba, Umuahia, Okigwe and Mbaise
2. determine the antibiotic susceptibility and resistance patterns of bacterial isolates
3. determine the multi-drug resistance profiles of *E. coli* and *Klebsiella pneumoniae* isolated from poultry, pig and cow farms in Aba, Umuahia, Okigwe and Mbaise.
4. determine the phenotypic and genotypic expressions of ESBLs of the *E. coli* and *Klebsiella pneumoniae*
5. determine the genotypic expressions of carbapenemases of the *E. coli* and *Klebsiella pneumoniae*
6. determine the efficacy of silver and zinc nanoparticles against pathogenic bacteria isolated from the livestock farms.

1.5 Justification of study

The incessant transfer of resistant strains of Enterobacteriaceae from livestock to humans is a threat to human population due to the spread of multi-drug resistance. Due to ignorance among the livestock farmers on the standard of antibiotics recommended by WHO for treatment and growth of livestock and meat sellers, this trend tends to increase. Again, recently the four cities of the South-eastern states have experienced heightened rise in poultry, piggery and cattle farming to

cushion the effects of harsh economic conditions in the country. Through this, many families resorted to livestock farming as better alternatives without appropriate hygienic practices and government regulations. As the first time the research would be conducted in the South-east Nigeria, it will be able to estimate the bacteria loads of Enterobacteriaceae in the region and muti-drug resistant strains of *K. pneumoniae* and *E. coli* to provide the government and relevant organizations the first-hand information on antibiotic resistance and the use of nanotechnology in finding the susceptibility pattern.

1.6 Scope of study

This study coveredthe isolation, bacterial profiling of livestock farms in Aba, Umuahia, Okigwe and Mbaise, molecular characterization of bacteria, the antibiotic susceptibility and resistance patterns of bacterial isolates, multi-drug resistance profiles of *E. coli* and *Klebsiella pneumoniae* isolated from poultry, pig and cow farms in Aba, Umuahia, Okigwe and Mbaise. The phenotypic and genotypic expressions of ESBLs of the *E. coli* and *Klebsiella pneumoniae,* the genotypic expressions of carbapenemases of the *E. coli* and *Klebsiella pneumonia,* the efficacy of silver and zinc nanoparticles against pathogenic bacteria isolated from the livestock farms.

CHAPTER TWO
LITERATURE REVIEW

2.1 Livestock farming

Livestock production in developing countries provides stable food sources, jobs, and opportunities for increased income. Much of the demand for animal products will be met by local production. However, despite the growing population and demand for animal protein, consumers are becoming more concerned about the negative impacts of livestock farming on the environment, public health, and animal welfare (Ochs, Wolf, Widmar & Bir, 2018). Water and land will become increasingly competitive resources, meaning livestock producers will need to maximize production while employing their limited resources sustainably (Baldi & Gottardo, 2017). The European Union aims to be climate neutral by 2050. Moreover, societal attitudes, especially of consumers, are changing drastically which further fuels incentives for responsible research and innovation to solving pressing problems in livestock farming through circular and sustainable ways. Due to the inadequacies experienced in the traditional livestock farming system, a better and more sustainable method of livestock farming has been introduced, known as precision livestock farming (PLF). To meet the growing demand for animal protein while addressing concerns about environmental sustainability, public health, and animal welfare, farmers and animal scientists may rely increasingly on PLF technologies to digitalize livestock agriculture (Berckmans, 2017).

Precision Livestock Farming (PLF) is defined as individual animal management by continuous realtime monitoring of health welfare, production/reproduction, and environmental impact‖ (Berckmans, 2017). Precision livestock farming (PLF) technologies utilize process engineering principles to automate livestock agriculture, allowing farmers to monitor large populations of animals for health and welfare, detect issues with individual animals in a timely manner, and even anticipate issues before they occur based on previous data (Benjamin & Yik, 2019). Examples of

recent developments in PLF technologies include monitoring cattle behaviour, detecting vocalizations such as screams in pigs, monitoring coughs in multiple species to identify respiratory illness, and identifying bovine pregnancy through changes in body temperature (Neethirajan, 2017). PLF technologies can also help farmers monitor infectious diseases within livestock agriculture, improving food safety and availability (Neethirajan, Ragavan & Weng, 2018). The use of PLF technologies will ultimately improve animal health and welfare while reducing food safety issues and maximizing efficient resource use (Norton, Chen, Larsen & Berckmans, 2019).

Animal-based products have a large and increasing contribution to the human food supply (Bradford, 2001). The increasing demand is driven by the increase of both the world population and the wealth of households. Optimizing animal-based products requires optimizing the feed intake of animals, reducing the risk of diseases, and improving the animals' living environment and their general welfare state. Optimizing animal-based production systems requires, therefore, coordination among farmers, feed companies, and other actors.

Poultry is an important subsector of the livestock industry and the fastest-growing agricultural enterprise globally. It contributes significantly to the economy by providing employment opportunities in rural and semiurban areas, as well as accessible protein sources for the growing population (Rahman, Chowdhury & Parvin, 2021). Poultry production has grown from 91 million in 1,990 to 365.85 million in the fiscal year of 2020–2021, owing to the huge demand for poultry meat and eggs (Jabbar, Rahman, Talukder & Raha, 2007). The poultry industry has become the leading supplier of efficient, high-quality animal proteins to the world. Poultry meat and eggs provide several advantages relative to other sources of animal-based foods. Poultry meat compares favorably with other animal products in terms of protein content and amino acid balance, energy and micronutrients (Bohrer, 2017). In addition, poultry meat (without skin) contains a lower

amount of fat relative to mammalian meat, largely related to the greatly reduced amount of intramuscular fat in birds (Tu˚ mová & Teimouri, 2010; Pereira & Vicente, 2013).

Additionally, eggs provide an excellent amino acid profile, a good source of energy, essential fatty acids and high levels of many vitamins and minerals (Bohrer, 2017). Egg consumption is also subject to fewer religious restrictions than meat. Particularly in developing countries, the small individual size of poultry, low initial costs, and simple housing requirements require a lower initial investment relative to most other non-poultry livestock species, thus making poultry meat and eggs a common means of increasing diet quality for impoverished humans. On a larger scale, commercial meat and egg-type poultry require substantially less land per unit of protein produced than beef cattle, comparable land use compared to pork, but more than for milk production (Flachowsky, Meyer & Südekum, 2017), with a lower greenhouse gas emission than beef, milk and pork production (Zervas & Tsiplakou, 2012).

Unfortunately, as poultry production has become more concentrated and operation sizes have expanded, major environmental challenges have arisen (Rodić, Perić, Đukić-Stojčić & Vukelić, 2011). Despite their great socioeconomic benefits in terms of egg production, meat production, and employment generation, they also endanger human and animal lives by polluting water, land, and air (Muduli, Champati, Popalghat, Patel, & Sneha, 2019).

2.2 Poultry Farm and Waste Management

2.2.1 Production of other livestock feeds

Processed poultry litter is used in diets for poultry, swine, lambs, ewes, lactating cows, wintering cattle, and brood cows (Mullenix et al., 2020). Feathers contain more than 90% protein and are a good source of hydrophobic amino acids like cysteine, arginine, and threonine; feathers are also processed into feather meal for animal feed, organic fertilizer, and feed supplements (Muduli et al.,

2019). There is currently no feed company in Bangladesh that uses poultry litter as an ingredient in livestock feed.

2.2.2 Production of organic fertilizer

Application as fertilizer Poultry litter is an excellent organic fertilizer for enhancing soil fertility, root system development, and plant vigor, as well as making the plant more resistant to diseases and pest infestations (Ogbuewu et al.,2012). Direct application of poultry waste in crop fields, on the other hand, can cause soil, air, and water quality concerns that are yet to be investigated. Most of the poultry manure and litter are currently applied to agricultural land, making it the preferred and practical method in developing nations (Bolan et al., 2010). Surface and groundwater become polluted because of the potential pollutants in the manure and litter, and agricultural yields drop when there is a lot of manure and trace elements (Thyagarajan, Barathi & Sakthivadivu, 2013).

2.2.3 Composting

Composting, a bio conversion process that turns organic compounds into a soil-friendly product in a controlled manner, is one of the most successful and widely used methods for dealing with poultry waste (Thyagarajan et al., 2013). Composting has several advantages, including the slow release of nutrients, lower pathogen levels, less potential to degrade water quality, fewer weed seeds, improved soil fertility, reduced fly infestations, and foul odor (Thyagarajan et al., 2013). Composting, on the other hand, necessitates land, financial investment, labor, and management and emits harmful CH4 gas as it decomposes (Thyagarajan et al., 2013). However, vermicompost could be a better option that minimizes the environmental effect. Vermicompost is created by decomposing poultry waste and bedding materials by various worms, typically red wigglers, white worms, and other earthworms (Thyagarajan et al., 2013).

2.2.4 Production of biogas

Biogas is a gas mixture formed by the anaerobic digestion of various organic materials. It is largely composed of CH_4, carbon dioxide (CO_2), and a trace amount of hydrogen sulfide (Dobre, Nicolae & Matei, 2014).

Poultry wastes, leftover feeds, and wastewater from the premises can all be utilized to form a slurry (biomass) that can be supplied into a biogas plant for anaerobic fermentation to produce biogas (Singh, Shamsuddin & Lim, 2018). Biogas and bio-slurry replace the cost of fuel (cooking, power, or both) and fertilizer (Hossain, 2018; Arshad et al., 2018). Moreover, biogas plants reduce carbon emissions (Hossain, 2018). Bangladesh has a significant potential for producing biogas from poultry wastes as it can build at least 4 million biogas plants, which could produce 105 billion cubic feet of biogas per year, equivalent to 1.5 million tons of kerosene or 3.08 million tons of coal, and could meet the cooking and lighting needs of approximately 20% of the country‘s households (Khanam, Huque, Huda & Bashar, 2019). Approximately 91,350 home and commercial so-called biogas plants that use cattle and poultry manure as raw materials are currently available (Hossain, 2018) in some countries. Still, the energy production level from those biogas plants is unknown. Although biogas generated in anaerobic systems is useful for household and agricultural use, bio-slurry is bad for farmers and the environment (Khanam et al., 2019). Limestone is an essential ingredient of layer feeds, part of which usually passes through the faeces and accumulates in slurry. The biogas plant’s digester gradually fills up with slurryborne limestone, which also clogs the plant‘s slurry drainage system, causing producers to stop operating. On the other hand, with regular use, the biogas plant becomes so dirty and smelly that it provokes complaints of odour from neighbours.

2.2.5 Poultry farm and environmental pollution

Despite the huge benefits of poultry waste, now it is becoming an undisputable concern around the world. The constant production of poultry waste causes environmental nuisance that are hazardous to animal and human health (Gbotoso & Burt, 2013). Poultry farms have been linked to poor air quality and environmental degradation due to high atmospheric ammonia emitted from poultry litter (Sankoh, Yan & Tran, 2013). Poultry waste is contributing to global climate change by emitting greenhouse gases, such as nitrous oxide, CO_2, and CH_4, through microbial activity and changes in temperature, pH, moisture, and oxygen concentrations (Kumar & Prakash, 2020). Reportedly, these air pollutants could have a significant negative impact on both human and animal health, with a variety of illnesses like nose discomfort, breathing issues, and coughing (Sankoh, Yan & Tran, 2013). Long-term exposure to sustained air pollution leads to allergic reactions and effects life span (Chen, Ebenstein, Greenstone &Li, 2013).

Poultry litter may be sources of human and animal infections, such as zoonotic avian influenza.

Therefore, appropriate hygiene must be maintained when handling poultry manure (Chen et al., 2013). Moreover, contaminated food or water by poultry waste may contain different pathogens and water pollutants (Chen et al., 2013) that cause gastrointestinal diseases like typhoid fever, cholera, and hepatitis E infections (Cabral, 2010). Different vectors, including mosquitoes, birds, insects, and rodents, spread various diseases through waste (Ziraba, Haregu & Mberu, 2016). Poultry waste is typically discharged through a single-channel waterline that connects to ponds and rivers, causing heavy metal pollution, antibiotic residues, and microbial contamination of surface and drinking water (Zhang, 2012). Furthermore, these lead to oxygen deprivation, which could speed up the rate of toxic compound accumulation, leading to water-borne and respiratory diseases (Nwachukwu, Akinde, Udujih & Nwachukwu, 2011).

2.2.6 Challenges

One of the primary challenges facing the broiler industry, and to a lesser extent, the egg industry, is the complete removal or gradual phasing out of prophylactic or sub-therapeutic antibiotics. The primary driver for the removal of antibiotic growth promotors has been concerns over the development of antibiotic resistance in the microbiome of poultry, transference of resistant pathogens to humans, or transfer of resistance genes from poultry-associated microbes to human pathogens (Shah, Paul, Sischo, Crespo & Guard, 2017; Voss-Rech et al., 2017; Van Boeckel et al., 2019). Intensive research efforts are underway to find successful non-antibiotic strategies to maintain productivity in the absence of growth-promoting antibiotics (Rafiq et al., 2022; Rahman, Fliss & Biron, 2022), although those efforts and yet to provide solutions that are as consistently effective as antibiotics have been. In the long term, the development of effective and practical antibiotic alternatives may prove to be a benefit to the industry in terms of the reduction in antimicrobial resistance in human and poultry pathogens.

2.3 Cow and Beef Production

Beef is a high-quality source of protein that also can provide highly desirable eating experiences, and demand is increasing globally. The sustainability of beef industries requires high on-farm efficiency and productivity, and efficient value-chains that reward achievement of target-market specifications. These factors also contribute to reduced environmental and animal welfare impacts necessary for provenance and social licence to operate (Greenwood, 2021). Beef was classified into one of the five following categories: nonintact raw, intact raw, or ready-to-eat (RTE), according to the Interagency Food Safety Analytics Collaboration (IFSAC) Food Categorization Scheme, and two other categories: other beef and unspecified beef (Richardson, Bazaco, Parker, Riley & Vermerris, 2017). Beef production systems that use beef breeds should target appropriate genotypes and high productivity relative to maintenance for the breeding herd and for growing and

finishing cattle to maximize income and to limit input costs, particularly for feed, which may be 60% or more of production costs (Greenwood, 2021).

Africa has 356 million head of cattle including 44 million that were slaughtered in the production of 6.7 million tonnes of beef in 2018 (Food and Agriculture Organization of the United Nations [FAOSTAT], 2020). Most African cattle are in sub-Saharan Africa which covers an area of 22.4 million km^2 and has 700 million hectares of grasslands between the tropics of Cancer and Capricorn (Otte and Chilonda, 2002; Otte, Pica-Ciamarra & Morzaria, 2019). The countries with the largest cattle herds in Africa are Ethiopia (63 million head), Sudan (31 million), Chad (29 million), Tanzania (27 million) Nigeria (21 million) and Kenya (20 million). Uganda, Niger, South Africa, South Sudan, Mali, Madagascar and Burkina Faso each have 10–15 million head (FAOSTAT, 2020). Productivity of beef production in sub-Saharan Africa is generally poor and coupled with the growing population has resulted in low per capita consumption of beef and other meats and milk (Otte and Chilonda, 2002; Otte et al., 2019).

2.4 Antibiotics

The routine employment of antibiotics, for prevention and growth promotion purposes in livestock farming, selects for antibiotic resistance among commensal and pathogenic bacteria. Owing to the fact that most of these antibiotics are not fully metabolized but released into the environment as waste products, antibiotic resistance has an ecological impact, since these waste products still have the potential to influence the bacteria population and promote antibiotic resistance. Antibiotics encompass a wide range of chemical substances that are produced naturally, semi-synthetically, and synthetically, and are used to inhibit (bacteriostatic) bacterial growth or kill them (bactericidal) (Mili'c et al., 2013; Martinez, 2012; Gillings, 2013). They are categorized based on their effects as either bacteriostatic or bactericidal, and on their series of efficacy, as narrow or broad-spectrum antibiotics. Furthermore, the classes of drugs that are more widely used in agriculture at the global

level, which are of growing scientific concern with regards to their potential adverse effects and risk management steps, include the tetracyclines, aminoglycosides, β-lactams, lincosamides, macrolides, pleuromutilins, and sulphonamides (De Briyne, Atkinson, Borriellio & Pokludova, 2014; Baynes et al., 2016). Baynes et al., (2016) noted that these antibiotics have the same mode of actions or belong to the same general classesas those used for humans; a situation that demands the judicious use of these drugs in animal farming, as there is bound to be a degree of interaction between animals and humans. Remarkedly, the antibiotic consumption patterns in agriculture vary across regions and countries in the developing world, and even antibiotics that have been banned in other countries, including the developed countries, are still being used in most developing countries (Adebowale, Adeyemo, Awoyomi, Dada & Adebowale, 2016). However, the antibiotic consumption profiles in developing countries are greatly influenced by the gross abuse and misuse of antibiotics due to their availability over the counter, through unregulated supply chains as well as the purchase without prescriptions (Van Boeckel et al. (2015) projected that the antibiotic consumption will approximately double in the BRICS countries consisting of Brazil, Russia, India, China, and South Africa. The forecast is propelled by a shift to large-scale farms requiring the routine use of antibiotics to maintain the health of animals and productivity. The shift is caused by the progress in consumer demand for animal products.

Resistance to antibiotics is an inherent side effect associated with the overuse, abuse, or substantial use of antibiotics (Williams-Nguyen et al., 2016). The antibiotic resistance pattern varies between regions and countries corresponding to the degree of antibiotic consumption, which is guided and regulated by the antibiotic policies of a particular country (Williams-Nguyen et al., 2016). Nevertheless, China has been registered as the world's leading producer and consumer of both animals and human antibiotics. Antibiotic-related crisis is ascribed to the misuse of antibiotics that are, ultimately, discharged into the environment, the presence of antibiotic residues (parent

antibiotic or its metabolites or both found in animal derived products) in livestock products and wastes, and lastly, the lack of stringent and effective supervision and control over antibiotics production, use, and disposal (Williams-Nguyen et al., 2016). Human activities in response to industrialization drastically heightened the availability of antibiotic residues in food and the environment, and the development and distribution of antibiotic resistant bacteria along with their resistance genes, thus causing an increase in the abundance of resistant bacteria and genes (Finley et al.,2013). The antibiotic residues, and antibiotic-resistant bacteria and resistance genes are considered as environmental pollutants and responsible for a tenacious public health crisis throughout the globe (Xi et al., 2009). The health challenges linked to antibiotic-resistant microorganisms are more about restricted therapeutic remedies in most developing countries that lack access to good quality treatment, thus, accentuating infection as an important root of morbidity and mortality (Samie et al., 2012). However, the soil and water environment have been regarded as vital reservoirs and sources of antibiotic resistance (Xiao et al., 2016); more so, as they are affected by agriculture (You & Silbergeld, 2014). Not only does the administration of antibiotics in food producing animals facilitate antibiotic resistance, but it may also result in the presence of antibiotic residues (including the parent compounds or its metabolites, or both) in animal-derived products (muscles, kidney, liver, fat, milk, and egg) available for human consumption. However, these antibiotic residues have been reported to exert a huge and negative impact on public health and food safety with regards to drug toxicity, immunopathological diseases, carcinogenicity, allergic reactions, and drug sensitization, amongst others (Billah et al., 2015; Guetiya-Wadoum et al., 2016). These adverse impacts tend to be influenced by land use, contaminated water sources, national policies (that symbolize production, trade, animal health, and food security), national and international trade, animal demography, and interactions between the

human populations as well, as they are reported to vary considerably between regions and countries (WHO, 2012).

2.4.1 Impact of Antibiotics in Agriculture

The use of antibiotics is not only constrained to the clinical settings, as prescriptions involved in the therapeutic regimens for the eradication of diseases in humans. It is also employed in livestock farming, where antibiotics can be used for disease treatment of animals, and in sub-therapeutic levels in concentrated animal feed for growth promotion, improved feed conversion efficiency, and for the prevention of diseases (Hong, Al-Jassim, Ansari & Mackie, 2013; Saiful Islam, Shiraj-Um-Mahmuda & Hazzaz-Bin-Kabir, 2016). Of great concern, the uses, types, and mode of actions of the antibiotics employed in agriculture and veterinary practice are closely related or the same (that may belong to the same general classes, function and act in similar ways) to those prescribed to humans (Saiful et al., 2016). Clearly, the choice of antibiotics and the antimicrobial consumption pattern demonstrates geographical variation across the continents being influenced by the food animal species, regional production patterns and types of production system, intensive or extensive farming, purpose of farming (commercial or industrial or domestic), lack of clear legislative framework or policies on the use of antibiotics, as well as the size and socioeconomic status of the population, and the farmers in particular (Bester & Essack, 2012).

The inclusion of nonessential antibiotics in animal feed for growth promotion purposes remains largely unregulated in the underdeveloped countries (Maron, Smith & Nachman, 2013). The persistent use of these nonessential antibiotics in livestock farming can be attributed to the expansion and greater concentration of farmlands, inadequate governmental policies, and control over the use and sales of antibiotics, reduced use of infection control measures, and the unwillingness of farmers to execute delegated changes in farm practices (Kaplan, Bowman, Cordova & Kar, 2014). Developing countries continue to employ the antimicrobial agent for

growth promotion to maintain the healthy state of the animals, to increase productivity, and raise incomes for the farmers (Braykov et al., 2016). However, these are contradictory to the Swedish agricultural data, as it recorded no loss of production after the ban exercise (Cogliani, Goosens & Greko, 2011). Altogether, Van Boeckel et al. (2015) noted that on a global scale, the average antimicrobial agent consumed per annum of animal produced (per kg) varied across the animal species with values of 45 mg/kg, 148 mg/kg, and 172 mg/kg associated with cattle, chicken, and pigs, respectively. Equally, their mode of administration differs with the animal types.

In this light, Apata (2009) noted that antibiotics were added to water and feed for chicken in subtherapeutic levels for growth promotion and prophylaxis. This had a devastating effect, as even healthy birds were unnecessarily exposed to antibiotics. Moreover, as these birds compete for food sources, eventually, there exists a difference in the doses consumed between the individuals, with one receiving a higher dose than others. This introduces another differential in the selective pressure on commensals, which could lead to the selection of resistant commensals that would eventually end up in the environment (Founou & Essack, 2010). Singer, Shaw, Rhodes & Hart (2016) accorded the administration of antibiotics in animal feed or water, in which the animals are reared in groups, making it difficult to isolate only the infected animals, as well as that the isolation process could be stressful to the animals and dangerous to the veterinarian who has to administer the antibiotic process. Contrarily, Sekyere (2014), in their study, demonstrated the administration of antibiotics to pigs via the intravenous route for treatment, and in this case, shunned the exposure of healthy animals to antibiotics. However, this mode of administration might cause the accumulation of these drugs in adipose tissues, thereby posing a health risk to consumers of pork fat. In addition, Cromwell (2002) mentioned that varying quantities of antibiotics are being employed at the different stages of livestock production, especially in pig farming, that incorporates four stages viz. gestation, farrowing, weaning, and finishing. Kim et al., (2013)

emphasized the significant difference in the use of antibiotics amongst piglets, fattening pigs, and sows during therapy and growth promotion; antibiotics are employed in pig farming for treatment, metaphylaxis, prophylaxis, and growth promotion. The authors further recorded a significant difference in the use of antibiotics between the three production systems in poultry farming, including breeding poultry, broilers, and laying hens. Accordingly, these may release different masses of remnant antibiotics into the environment (Hong et al.,2013).

Generally, in the developing countries, the level and rate of antibiotic utilization in the farming sector might be influenced by the manner in which the farmers acquire (over the counters) and use these antibiotics (multidrug practices), and also, the presence of existing factors. The existing factors include a high prevalence or level of infections, profound scarcity of state management and development strategies, shortfall in husbandry zone planning, negligible hygienic practices in livestock husbandry in conjunction with the presence of an integrated agricultural system (Saiful et al., 2016; Bashahun & Odoch, 2015). Specifically, in Vietnam, there has been reported cases of frequent and uncontrolled epidemic diseases, such as the porcine reproductive and respiratory syndrome (PRRS), foot and mouth disease, and digestive tract infections and reproductive disorders in piglets and exotic sows, respectively. The disease conditions necessitate the wide use of antibiotics by producers in livestock for the prevention and therapy of diseases as one of the most likely approaches to combat diseases (Porphyre, Nguyen, Ha, Genewe & Henry, 2006). Moreover, the country practices an integrated agriculture–aquaculture farming system, whereby the aquaculture is being sustained via livestock and human wastes. This further strengthens the risk of exposure of humans, animals, and environment to available antibiotics (Kim et al.,2013). Notwithstanding, the available limited data on antimicrobial utilization in livestock farming ensues the partial reports of antimicrobial consumption and sales. This is due to the lack of surveillance systems subsidized by the government to monitor antimicrobial use and resistance, the lack of

knowledge and the reluctance of food animal producers, animal feed producers, public health and veterinary officers and veterinary pharmaceutical companies to provide such in-depth measurements (Eagar, Swan & van Vuuren, 2012). In conclusion, the information presented herein is deduced from the findings obtained by several authors who have previously conducted investigations on antibiotic use and antibiotic resistance, yet the rate of antibiotic usage and antibiotic resistance is alarming; imagine the scenario in which the real/actual data has to be presented. Seemingly, there is a need to call for cooperation or team collaboration from individuals, farmers, veterinarians, consumers, and local vendors of pharmaceutical products for the prudent use of antibiotics both in the clinical and agricultural settings across the nations or countries, in a bid to circumvent the rising antibiotic resistance of bacteria (Zhao, Dong & Wang, 2010).

2.4.2 Antibiotic resistance in Agriculture

Antibiotic use for essentially non-medical or non-therapeutic purposes in agricultural settings that are at subtherapeutic levels over an extended period is observed as a major route for the advent of antibiotic resistance and antibiotic-resistant bacteria, and resistance genes have been reported to be transferred to humans (Duro & Cook, 2014). Irrational or non-prudent use of antibiotics in foodproducing animals have resulted in antibiotic residues in animal-derived products. Therefore, antimicrobial stewardship is equally implemented to ensure prudent antibiotic use in agriculture, in order to conserve and maintain the effectiveness of available antibiotics, as well as curb the problem of antibiotic resistance and residues in food products derived from animal (Moudgil, Bedi, Moudgil, Gill & Aulakh, 2017). Sadly, stewardship interventions in developing countries have often been weakened by the attitude of poorly paid veterinarians who seek supplementary incomes from drug sales, plus the existence of inadequate regulations (Ying et al., 2017). Summarily, all stakeholders involved in the fight against antibiotic resistance must address it from the standpoint

of regulations, surveillance, research, treatment guidelines, infection control, education, and awareness (Sharma et al.,2018). The National Farmed Animal Health and Welfare Council (National Farmed Animal Health and Welfare Council, 2016) pointed out that the implementation of antimicrobial stewardship in agriculture can be approached from the following perspectives, including clinical microbiology, infection control (biosecurity), regulations, surveillance on antibiotic use and resistance, animal management, husbandry, and alternatives to antibiotics. A coordinated network of actions from veterinarians, livestock producers, pharmacists, veterinary pharmaceutical industries, and regulatory authorities are relevant to enforce prudent antibiotic use (Van Vuuren, 2014).

Following the Global Action Plan on antimicrobial resistance (WHO, 2015) and the Global principles for the containment of antibiotic resistance in animals intended for food presented by WHO (2020), nations are expected to implement measures that are in line with the key actions highlighted for the combat of antimicrobial resistance. Accordingly, Walsh & Wu (2016) expressed the interdict on the use of colistin (an antibiotic critical for the treatment of infections caused by highly resistant Gramnegative bacteria in humans) as a feed additive for animals in China. More elaborately, some European countries, including Sweden, Denmark, the United Kingdom, Netherlands, etc. introduced bans on the use of antimicrobial growth promoters (Cogliani et al., 2011), while Australia and New Zealand implemented a partial ban. Seemingly, in the United States, the restraint on antimicrobial growth promoters is voluntary. In addition, a tripartite alliance involving WHO, World Organisation of Animal Health (OIE), and Food and Agricultural Organisation of the United Nations (FAO) was formed in 2003, which led to the categorization of veterinary medicines into critically important, highly important, and important drugs for human health. This differentiation is to guide their use in animal agriculture across the globe, hence, combating antimicrobial resistance. Also, antibiotic prescription and administration

to farm animals are supervised by veterinarians, and several behavioral studies have proven that the attitude of farmers to antibiotic use is greatly influenced by veterinarians. Therefore, interventions geared toward the change of prescribing behaviour of the veterinary could go a long way to optimize antibiotic use by farmers.

However, the determinant factors, including a personal opinion regarding the contribution of veterinary medicines in antibiotic resistance, professional ethics to alleviate animal suffering, and financial dependence on clients, amongst others, have been noted to influence the prescribing behaviour of the veterinarian (Speksnijder, Jaarsma, van der Gugten, Verheij & Wagenaar, 2015). Also, Henton, Eagar, Swan & van Vuuren (2011) emphasized that the registration of over-the-counter drugs for sales is not optional in South Africa, and the drugs are distributed by the manufacturers to veterinary wholesalers, farmers' cooperatives, distributors, and feed mix companies. Also, Lee, Lee, Kang, Jeong & Lee (2015) affirmed that educational programs should be conducted for undergraduate medical and non-medical students in line with generic medicines, mechanism of antibiotic resistance, and the prudent use of antibiotics. Strategies to reduce or limit the therapeutic use of antibiotics in animals via improved animal nutrition, improved living conditions and waste management, biosecurity measures, and improvement in animals' natural immunity can result in infection prevention and control. These strategies, however, will reduce the level and type of antibiotic needed for treatment, because once the animal is exposed to infection, its immune system can fight seriously against the agent, resulting in less severe manifestations (National Farmed Animal Health and Welfare Council (2016). Moreover, reductions in antibiotic consumption can be achieved by using non-antibiotic alternatives, including prebiotics, probiotics, bacteriocins, vaccines, innate immune system potentiators, bacteriophages, and competitive exclusion cultures for non-specific and specific control of enteric pathogens in animals (Bischoff et al., 2005). Nevertheless, guided interventions, such as vaccination, antihelminthic therapy,

optimized herd management, improved biosecurity measures, prudent antibiotic use, performed as teamwork involving the farmers and veterinarians, have led to a marked curtail in antibiotic use, especially the critically important antibiotics, with a decrease of 52% and 32% of the pigs from birth to slaughter and breeding animals, respectively (Postma, Vanderhaeghen, Sarrazin, Maes & Dewulf, 2016).

2.5 Livestock farming and antibiotic resistance

According to Woolhouse, Ward, van Bunnik & Farrar (2015), antibiotic resistance in livestock farming can be looked at from four different viewpoints, i.e., the animals (cattle, pigs, poultry, sheep) and animal-derived products, farm workers, and farm environmental sites (water, soil, feeds, wastewater, sewage, lagoon, manure, and sludge after treatment). All these constitute the several compartments and different niches in the farm described as an ecosystem (Acar & Moulin, 2006). Undoubtedly, farm animals are a very important component in understanding the interplay between humans, animals, and the environment regarding bacteria, antibiotics, and antibiotic resistance gene movement (Woolhouse et al., 2015). The digestive tract of animals, like humans (farm workers), is colonized with diverse microorganisms, including commensals and resistant bacteria. Thus, it serves as the most important reservoir of microorganisms. Therefore, it can play a vital role in the dissemination and acquisition of resistant bacteria and their resistance genes (Acar & Moulin, 2006). Shobrak and Abo-Amer (2014) noted the occurrence of multidrug-resistant *E. coli* and *E. vulneris* in cloacal samples of both migrating and nonmigrating birds which served as carriers. Through the release of their faecal residues into water bodies and other environmental sources, it could enable the spread of these resistant bacterial strains and their resistance determinants even to remote areas by means of migration.

The anatomical feature of the gut varies between poultry and other mammalian animals, which in turn influences the intestinal microbiome (Pan & yu, 2014). The intestinal microbiome changes with age, the type of diet fed, antibiotics ingested, and infection with pathogens, amongst other life events (Téllez et al., 2015). It has been reported that microorganisms in the gut interact extensively with the host, diet, and the intestinal gut microbes, and exert a huge impact on the animal‘s immunity and physiology, and ultimately affects the health of the animal and its production (Kogut, Ryan & Arsenault, 2016). The continuous antibiotic exposure to animals via oral administration creates selective pressure for the development of resistance, and resistant bacteria associated with animals can then enter into the food chain through the consumption of meat (contaminated during slaughter or processing of carcass, if the gut is accidentally cut or intestine empties its contents into the thoracic and abdominal cavities when the carcass of poultry is gutted during processing) or other animal-derived products, through farm runoff water and other means. However, the greater the quantity of antibiotic used, the higher the selective pressure. Van, Moutafis, Tran & Coloe (2007) reiterated that food contaminated with antibiotic-resistant bacteria could cause amplification of resistance genes and facilitate the transfer of the antibiotic resistance determinants to other bacteria of clinical importance found in humans, and can be further transferred within humans to more pathogenic bacteria. Therefore, food or animal-derived products, including meat, milk, and eggs, may represent an active and key medium through which antibiotic resistance determinants are continually being transferred between bacteria, and from animals to humans (Wegener, 2012).

Bosco, Kaddu-Mulindwa & Asiimwe (2012) clearly documented the multidrug resistance of Salmonella isolates recovered from cattle, pigs, chickens, eggs, and animal-derived products, as well as cross-species transmission of plasmids between animal and humans in Uganda. Also, the farm environment is composed of environmental sites, such as manure, wastewater, soils, effluent,

and sewage, which serve as hotspots for antibiotic resistance pollution. More specifically, Bester and Essack (2012) indicated that animals are very exposed at a high degree to their environment, making it easier for them to be infected with bacteria harboring problematic genetic material, especially from the soil environment. In the same light, animal urine and feces containing antibiotic residues, antibiotic-resistant bacteria, and resistance genes may be released into manure, and animals might, in turn, be allowed to graze on pasture grown on soil fertilized with this raw manure. This, therefore, creates the likelihood of bringing back these xenobiotics to animals and humans (Acar & Moulin, 2006).

2.5.1 Manure

Nevertheless, manure, which has been described as a hotspot for antibiotic resistance bacteria and antibiotic resistance genes, can serve as a plausible route of transmission of these resistant bacteria and their genes into the soil and water via deliberate or accidental processes (Franklin et al., 2016). Therefore, it is advised that manure should be treated before land application via biological methods, including anaerobic digestion. However, Resende et al. (2014) in their findings noted the prevalence and persistence of potentially pathogenic bacteria which demonstrated multidrug resistance against oxacillin, ampicillin, and levofloxacin, amongst other antibiotics, both in the influent (cattle manure) and effluent (digestate) released from an anaerobic biodigester. It is suggested that the rate of survival of these bacteria depended on the temperature of the operating process in association with the duration of the fermentation process and the microbial composition. Ostensibly, Maynaud et al. (2016) further confirmed the occurrence of enteric pathogenic bacteria in the digestate obtained from the anaerobic biodigester. The authors emphasized the potential of the viable, but non-cultural state of bacteria, which might cause the regrowth of pathogens during digestate storage, prior to land spreading. Consequently, the need of post treatment of digestate via

mechanical, chemical, physical, and biological methods is very vital (Amin et al., 2017). In addition, the sanitary risk and microbiological safety of digestate should be evaluated before land application of digestate, in a bid to dodge the ecological, human, animal, and environmental health implications. Nevertheless, it is a call for concern when pathogenic bacteria are present alongside with antibiotic-resistant bacteria in untreated or treated animal manure.

Alternatively, the very common and economic approach to manage manure generated from livestock farming is by application on nearby agricultural fields. However, raw manure can be flushed by heavy rainfall or runoffs from the surface of manure-amended soils into nearby water bodies used by humans for sanitation and domestic purposes (Polard & Mora, 2017). Moreover, due to the constraints on available freshwater resources in developing countries, wastewater serves as a vital source of water and nutrients for irrigation of agricultural fields, in a bid to circumvent the problem of food insecurity in these countries (Jimenez, 2006). However, wastewater may infiltrate into groundwater, causing pollution and contamination with toxic chemicals, antibiotics, and organic matter. In addition, owing to the lack of or inefficient wastewater treatment plants, wastewater or improperly treated wastewater is mostly released into surface water bodies that act as reservoirs for domestic and industrial wastes, causing pollution (Edokpayi, Odiyo & Durowoju, 2017).

2.5.2 Soil

The soil is an ecosystem and a natural resource with unique biodiversity, taking into consideration abundance, quantities of species, and functions of organisms (Edokpayi et al., 2017; Comerford et al.,2013). With respect to total biomass, microorganisms are considered as the principal part of the soil community, and are basically responsible for decomposition of organic matter, degradation of toxic compounds, and nutrient transformation (Edokpayi et al., 2017). Interestingly, the soil is

composed of microorganisms that produce antibiotic by so doing; it can equally serve as a reservoir of antibioticresistant bacteria and resistance genes (Sethi, Kumar & Gupta, 2013; Nesme & Simonet, 2015). The soil serves as a hub to establish connections between the air, water, rocks, and organisms, and it is involved in many different functions termed ecosystem services in the natural world (Monier et al., 2011). Thus, it can be described as quite a large reservoir of antibiotic resistance determinants, since it includes the antibiotic resistance determinants found in all plants, fungi, soil bacteria, small animals, and protists (Monier et al., 2011). In addition, Riesenfeld, Goodman & Handelsman (2004), indicated that uncultured soil bacteria are a possible reservoir of antibiotic resistance genes with greater diversity as compared to previous findings, and such diversity can be ascertained and fathomed using culture-independent methods. Furthermore, vegetables grown in unfertilized soil were equally shown to harbour antibiotic-resistant bacteria and resistance determinants that naturally occur in soils (Marti et al., 2013).

The abundance and the mobility of antibiotic-resistant bacteria and resistance determinants in the soil can be greatly influenced by the application of manure (containing antibiotic residues, antibioticresistant bacteria, and their resistance genes on mobile elements) during fertilization of the soil, the use of wastewater (black or grey water) for the irrigation of agricultural lands, and the use of antibiotics to treat crop diseases (Oluyege, Oluwaniyi & Ijasan, 2015). More specifically, when soils are treated or amended, antibiotics and their degraded metabolites, as well as antibiotic-resistant bacteria and their corresponding resistance genes, are introduced into the soil environment. Therefore, transfer of antibiotic resistance genes becomes inevitably fast, owing to the rapid growth of bacteria and horizontal gene transfer (HGT) (Normark & Normark, 2002). However, the persistence and the rate of dissipation of the antibiotic resistance genes is dependent on the vertical gene transfer/horizontal gene transfer (VGT/HGT) of antibiotic resistance gene (ARG), the transport and viability of the bacteria harbouring the genes, whether the free DNA

obtained from cell lysis will be degraded, adsorbed to soil or organic matter, or acquired by new cells, as well as the transportation of the extracellular ARG (Chee-Sanford et al., 2009). However, the introduction into the soil environment via manure fertilization has been reported to cause an alteration in phylogenetic structure, amplification in resistance level, and disturbance in ecological function (e.g., nutrient cycling) in the microenvironment (Ding & He, 2010).

The soil is regarded as a reservoir of antibiotic-resistance genes since most antibiotics are derived from soil microorganisms that are intrinsically resistant to the antibiotics produced. In addition, water potentially contaminated with faecal microorganisms and organic fertilisers used on food crops may disseminate drug-resistant bacteria in the soil. Water is a major way of dissemination of bacteria between different environmental compartments. Large amounts of antibiotics are released into municipal wastewater due to incomplete metabolism in human beings or due to the disposal of unused antibiotics. Some available data show that antibiotic-resistant bacteria and antibiotic-resistant genes can be detected in wastewater samples and that the conditions in wastewater treatment plants (WWTPs) are favourable for the proliferation of resistant bacteria. In the last decade, several studies have reported high concentrations of tetracycline and sulphonamide-resistant bacteria and sulphonamide-resistant genes in WWTPs (Bouki, Venieri & Diamadopoulos, 2013; Novo, Andre, Viana, Nunes & Manaia, 2013).

2.5.3 Water

Microorganisms (specifically, bacteria) do not live in isolation (Finley et al., 2013), but are found in milieu/medium (humans, air, water, plants, and soil) known as their habitat (aquatic ecosystem), which offers them with the appropriate nutritional and growth requirements necessary for survival. Consequently, water represents one of the most important habitats for bacteria on the planet earth, and serves as a main natural route for the dissemination of microorganisms between different

environmental compartments and/or aquatic ecosystems, humans, and other animals (Vaz-Moreira, Nunes & Manaia, 2014). According to Taylor, Verner-Jeffreys & Baker-Austin (2011), the aquatic environment is considered as a fundamental setting for environmental release, transformation, mixing, and persistence of antibiotic residues, antibiotic-resistant bacteria, and antibiotic resistance genes.

The water (aquatic) environment can be subdivided into marine and fresh water based on salinity, average temperature, depth, and nutrient content (Aryal, Karki & Pandey, 2015). More elaborately, the microbial aquatic environment includes surface and ground waters, drinking water, tap water, and wastewater. These waters have dynamic and distinct bacterial composition patterns influenced by temporal and spatial unevenness in physicochemical and biotic factors, including environmental stresses and nutrient composition (Ibekwe, Ma & Murinda, 2016)). Nevertheless, some known waterborne bacteria include *E. coli*, Vibrio, Shigella, and Salmonella species (Cabral, 2010). The aquatic environment has been reported to be the origin and reservoir of antibiotic-resistant bacteria and resistance genes (Cabral, 2010).

Microbial communities respond to drastic changes in the ecosystem functioning, species composition and abundance, due to pollution (Igbinosa & Odjadjare, 2015), resulting in hypoxia, eutrophication, bioaccumulation and dissemination of pathogens (Igbinosa & Odjadjare, 2015). Consequently, the abundance of antibiotic residues and pool of antibiotic-resistant bacteria and their resistance genes in aquatic ecosystems can be influenced or altered or amplified by the discharge of wastewaters from industrial and municipal wastewater treatment plants, runoff from manure-fertilized agricultural land, leakage from septic tanks and broken sewage pipes, and feces from wildlife, into water bodies (Oluyege, Oluwaniyi & Ijasan, 2015).

2.5.3.1 Drinking water

Most of the population in rural settings and urban settlements rely on untreated groundwater as a source of drinking water, due to the scarcity of fresh surface waters (Oluyege et al., 2015). Untreated groundwater is considered safe for drinking, because it originates from the ground. Therefore, it is described as a natural water habitat protected from human intervention. Apparently, its microbiota reflects the natural population of the habitat (Lundborgg & Tamhankar, 2017). Nevertheless, depending on the location/environment, groundwater becomes vulnerable to contamination with antibiotic residues, antibiotic resistant bacteria, and resistance genes from surface runoff of animal feces deposited on the ground in concentrated animal feeding operations, seepage of liquid/solid manure from storage sites (lagoons), leachate from landfill sites, spillage from broken sewage pipes, and leakage from septic tanks (Lundborgg & Tamhankar, 2017).

2.5.3.2 Waste water

Wastewater constitutes - black water and grey water. It describes all water with adversely affected quality due to influences from human activities (Lundborgg & Tamhankar, 2017). Wastewater embodies water from livestock and poultry farms, aquaculture farms, and municipality that is sometimes released into the environment without treatment or used for irrigation by farmers involved in urban agriculture, since it contains significant amounts of micronutrients and organic matter, and provides water (Lundborgg & Tamhankar, 2017). Due to the unavailability of good quality water worldwide, farmers resort to employing wastewater for irrigation, thereby promoting plant growth, water conservation, nutrient recycling, and reductions in inorganic fertilizer applied to the soil, and polluted water being discharged into the surroundings (Lundborgg & Tamhankar, 2017). Owing to its numerous sources of water collection, wastewater contains diverse elements, inorganic minerals, antibiotic residues, antibioticresistant bacteria and resistance genes, human and

animal feces and urine, etc (Lundborgg & Tamhankar, 2017). The sewage or wastewater treatment plant receives the wastewater and partially treats or treats it, before discharge of its effluents into the environment. Therefore, the wastewater treatment plant serves as a link between human activities and the environment, and serves as a potential reservoir and release channel of antibiotic-resistant bacteria and resistance genes, giving a perfect opportunity for the transfer of antibiotic resistance genes (Adebowale et al., 2016). The biological processes at the wastewater treatment plant might cause a reduction in the volume of antibiotics to varying degrees (Lundborg & Tamhankar, 2017).

2.6 Antimicrobial resistance

Globalization and enhanced mobility, a growing human population, close contact with animals and their environment, intensive farming, pollution, ecosystem degradation, and climate changes have led to the emergence of new pathogens and the spread of antimicrobial resistance (AMR). Throughout history, bacterial infections have been a major cause of human and animal diseases. The discovery and use of antibiotics enabled their effective management from a clinical point of view, but at the same time resulted in increased AMR. If no measures are undertaken to mitigate the current rates of emergence and spread of AMR, it is estimated that AMR will result in a financial burden of 100 trillion dollars at the global level and cause over 10 million deaths per year by 2050 (Brogan & Mossialos, 2016). Antimicrobial resistance leads to failure of clinical antimicrobial therapy and has raised urgent global public health concerns Humans can acquire antimicrobial resistance from drugs through the food chain or the environment (contaminated water, air, soil, or manure). While antimicrobials have been regular supplements in animal feed that maintain health and improve the productivity of livestock, their over-use in feeding forage has led to a rise in antibacterial resistance (Brogan & Mossialos, 2016).

Antibiotic resistance is a natural phenomenon that occurs when microorganisms are exposed to antibiotic drugs. Under the selective pressure of antibiotics, susceptible bacteria are killed or inhibited, while bacteria that are naturally (or intrinsically) resistant or that have acquired antibiotic-resistant traits have a greater chance to survive and multiply. Not only the overuse of antibiotics but also the inappropriate use (inappropriate choices, inadequate dosing, poor adherence to treatment guidelines) contribute to the increase of antibiotic resistance (Prestinaci, Pezzotti & Pantosti, 2015).

An increasing number of pathogenic organisms are resistant to one or more antimicrobial drugs. As a consequence, some common infections have become extremely difficult and, in some cases, nearly impossible to treat. Pneumonia, which was readily treatable after the introduction of penicillin, now more often requires second and third-line antibiotics. Cystitis, one of the most common bacterial infections in women, which was easily treatable using oral medication, now needs quite always more complex antibiotic treatments that impose additional costs on the patients and the health system (Kaye, Engemann, Fraimow & Abrutyn, 2004; Chen, Chopra & Kaye, 2009).

2.6.1 Antimicrobial resistance in livestock

As part of the Global action plan on AMR led by the Tripartite (FAO, OIE and WHO), the individual organisation strategies and national action plans, a key pillar in addressing AMR is surveillance. In human medicine, the WHO's Global Antimicrobial Resistance Surveillance System collects AMR data in selected indicator bacteria to estimate the global burden of AMR (WHO, 2020). However, in animals, no such global system exists. In Europe, AMR data in zoonotic and indicator bacteria from food animals and their products are collected annually by countries in selected food animal species and age groups depending on the year. In the latest report for 2017– 2018, some promising trends were noted in food animals: a) decreased prevalence of

extended spectrum beta-lactamase-/AmpCproducing *Escherichia coli*; b) a significant increase in the proportion of fully susceptible *E. coli* (approximately 25% in some Member States); c) resistance to colistin was uncommon; and d) carbapenemase-producing *E. coli* were not detected in poultry (WHO, 2020). In North America, similar monitoring of AMR in animals has been implemented. However, in LMICs, only 10% of countries reported monitoring AMR in animals (WHO, 2020). In the absence of national or regional AMR data, Van Boekel et al., (2015) reviewed point prevalence surveys to provide a snapshot of AMR levels in animals and animal food products in LMICs in four bacterial species: *E. coli*, *Campylobacter* spp., non-typhoidal *Salmonella* spp. and *Staphylococcus aureus* between 2000 and 2018. Some of the key observations from this study were: a) geographic variation in the number of studies performed (fewer studies conducted in Africa compared to Asia); b) overall increase in AMR levels over time in different livestock commodities; and c) geographic difference in AMR levels and patterns of resistance appear to be associated to regional antimicrobial consumption patterns.

2.6.2 Ways to reduce antimicrobial resistance

As discussed earlier, the introduction of antibiotics has increased AMR in livestock farms. Several strategies have been mapped out to reduce the spread of AMR.

2.6.2.1 The use of new technology

A few different methods, such as nanotechnology, anaerobic digestion, and biochar composting have been developed to minimize antimicrobial resistant bacteria (ARB) and antimicrobial-resistant genes (ARGs) in recent years (Goulas, Belhadi & Descamps, (2020).). Yu et al., (2017) collected water samples (including feedlots, fishponds, and wastewater treatment plants) and performed the removal test of ARGs for water by graphene oxide (GO) nanosheet. The results showed that GO nanosheet could nonspecifically bind to the ARGs (such as tetA, ermB, ampC, and sul2) by π-stacking interactions, and approximately 80% of ARGs can be removed from water

by GO. The removal efficacy of GO nanosheet for ARGs was reduced by <40% after 5 regeneration cycles, indicating excellent stability and reusability of GO nanosheet. GO nanosheet may be a desirable candidate for the efficient treatment of ARGs in animal wastewater or other water (Yu et al., 2017). The effects of anaerobic digestion on the removal of ciprofloxacin, sulfamethoxazole, and enrofloxacin and their ARGs in a dairy farm (Yu et al., 2017). The result showed that degradation of sulfamethoxazole, ciprofloxacin, and enrofloxacin was 100, 92, and 84%, respectively; removal efficiency of their resistance genes was 78.3% (ciprofloxacin), 50.3% (enrofloxacin), and 37% (sulfamethoxazole), respectively. It was noted that anaerobic digestion can be a promising practice for minimizing antibiotic residues and ARGs in animal waste (Yu et al., 2017).

2.6.3 Reduction of pathogens in livestock farms

2.6.3.1 Vaccination

Vaccination programs are routinely used to prevent bacterial and viral infections in livestock. However, many of the pathogens carried by livestock that contaminate water do not cause performance-limiting disease. Consequently, there is little financial incentive for pharmaceutical companies to produce, or for producers to purchase vaccines that limit the occurrence of many intestinal pathogens. Efforts to develop vaccines against microbes that reside in the intestinal tract have proven to be notoriously difficult because eliciting a specific immune response in this environment presents a significant challenge. Increasing concerns over food safety have led to the development of commercial vaccines against *E. coli* O157:H7, Salmonella, and Leptospira, but uptake of these vaccines has been limited by a lack of financial return to the producer. Introduction of financial incentives for producers to implement sound food safety practices could overcome this adoption barrier (McAllister & Toppt, 2012).

2.6.3.2 Biosecurity

Biosecurity measures have been used to limit the spread of pathogens in livestock production since the beginning of confined livestock production. These measures are most amenable to poultry and swine operations and less suitable for extensive systems used in ruminant production. In these systems, livestock imports are closely monitored for pathogens and often subject to quarantine, or they must be certified as disease free before entrance into the herd or flock. Feed must be monitored for pathogen contamination, and biosecurity programs must be accompanied by vector control measures that limit the introduction of pathogens by insects or rodents. Compared with free-range production, biosecure production of poultry and swine has been shown to reduce pathogen loads of bacteria such as Campylobacter, likely as a result of differences in vector contact. However, even with biosecurity measures, absolute elimination of pathogens is not ensured, necessitating the multiple-barrier approach (McAllister & Toppt, 2012).

2.6.3.3 Use of competitive approach and probiotics

Considerable effort has been devoted to the development of microbial probiotics that exclude or reduce microbial pathogens within the digestive tract of livestock. This approach has been broadly used to limit the occurrence of Salmonella and Campylobacter in poultry, enterotoxigenic *E. coli* in swine, and *E. coli* O157:H7 in cattle. However, the impact of this approach on many of the pathogens is unknown. Lactobacillus, Bifidobacterium, Propionibacterium, Enterococcus, and Bacillus are bacteria that are commonly used as probiotics for livestock. Several of these bacteria produce antimicrobial peptides known as bacteriocins that are toxic to bacterial pathogens. Others may compete with pathogens for nutrients within the intestinal tract or block adhesion sites on the intestinal epithelium, thereby limiting the persistence of pathogens in the intestinal tract. However,

there is little evidence that probiotics have a detrimental effect on pathogens that enter manure in a viable state, still necessitating the proper manure handling practice (McAllister & Toppt, 2012).

2.6.3.4 Use of antimicrobials

Several antimicrobials have been shown to reduce the occurrence of pathogens in livestock, such as Salmonella in poultry and swine and *E. coli* O157:H7 in beef cattle. However, there is a growing concern over the subtherapeutic use of antimicrobials in livestock because it may contribute to the development of resistance to antimicrobials used to treat infection in humans. As a result, it is unlikely that the subtherapeutic use of antimicrobials will be viable for pathogen control in livestock over the long term. Other forms of biological control, such as the use of pathogen-specific bacteriophages, may gain popularity in a post-antimicrobial era. Bacteriophages have the advantage that they specifically target pathogens within the microbial community while leaving commensal bacteria unharmed. Broadspectrum antimicrobials often remove both pathogenic and commensal bacteria from the intestinal tract, thereby leaving livestock more susceptible to posttreatment infection by pathogens (McAllister & Toppt, 2012).

2.7 Extended-spectrum beta-lactamases (ESBLs)

Extended-spectrum beta-lactamases (ESBL) are enzymes responsible for the hydrolysis of oxyiminobeta-lactam antibiotics, which are important therapeutic agents for the treatment of serious human and animal infections. ESBL were first described in 1983 in Enterobacteriaceae (new taxonomy Enterobacterales) and since then, with the research of the scientific community, it has been observed that ESBL-producing Enterobacteriaceae (E-ESBL) are a real threat to human health, being responsible for 1700 deaths in the USA due to therapeutic failure in severe infections in 2013 (Friese et al., 2013). The presence of E-ESBL in several ecological niches, as commensals

in humans and animals and as environmental contaminants, is reported worldwide in the production of animals due to their direct connection with the food chain (Laubeet al., 2013). The ESBL-producing bacteria in livestock have their existence in sick and healthy cattle, pig and poultry farms (Friese et al., 2013; Laubeet al., 2013). ESBLs have become widespread throughout the world and are now found in a significant percentage of *Escherichia coli* and *Klebsiella pneumoniae* strains in some countries. They have also been found in other Enterobacteriaceae strains and *Pseudomonas aeruginosa*. The ESBL are enzymes that are classified into several types, being CTX-M, SHV and TEM the most prevalent around the world (Paterson & Bonomo, 2005). However, there are other ESBL such as OXA, PER, VEB, BES, GES, SFO, TLA, and IBC (Paterson & Bonomo, 2005).

2.7.1 CTX-M

CTX-M are enzymes with environmental origins which are currently the most widespread type of ESBL and are commonly associated with E-ESBL reports (Canton, Gonzalez-Alba & Galan, 2012). Variants such as CTX-M-15, responsible for infectious outbreaks around the world, are associated with a clone responsible for extraintestinal *Escherichia coli* (*E. coli*) infections resistant to antibiotics, the ST131 (Paterson & Bonomo, 2005).

2.7.1.1 Brief history of CTX-M

The first description of an E-ESBL in cattle was in Japan, where a CTXM-2 *E. coli* producer was detected in cattle faeces from an important region close to the centre of the country (Shiraki, Shibata, Doi & Arakawa, 2004). The CTX-M-1, CTX-M-14 and CTX-M-15 are the most prevalent with CTX-M-1 reported in 20 countries, most frequently in Europe (n = 14). It has been found in Germany, Denmark, Spain, Finland, France, Hungary, Portugal, Netherlands, United Kingdom,

Czech Republic, Slovakia, Sweden, Switzerland and Turkey (Moosavian & Ahmadkhosravy, 2016; Wang, Huang, Surendraiah, Wang, Komal, Zhuge, Chern, Kryszuk, King & Wormser, 2013). CTX-M15 and CTX-M-14 are the most important CTX-M enzymes due to their large diffusion and relation to outbreaks and severe extraintestinal infections (Canton etal., 2012; Matsumura et al., 2015). CTX-M15 was first described in 2001 in E-ESBL isolate in a hospital in New Delhi, India, and today is the most widespread ESBL in the various niches and the most important of all, due to its high relation to important, for human health, E-ESBL clones (Canton et al., 2012). CTX-M-15 has been reported in all continents (Europe, North America, South America, Asia, Africa, Oceania and Antarctica with reports in all major ecological niches (humans, animals, and environment), these ESBL producers of CTX-M15 are an excellent example of the public health threat that involves circulation of resistant Enterobacteriaceae and resistance genes among the different ecological niches (Chen et al., 2014; Dia et al., 2016; Liao et al., 2017). The virulent and multi-resistant CTX-M-15-producing *E. coli* O25bST131 clone is certainly one of the most well adapted circulant clones among E-ESBL, which is responsible for outbreaks and deaths around the world and is not related only to infectious processes, but is also reported in human intestinal colonization (elderly, adults and children) and animals (terrestrial and aquatic) and environmental contamination (Badran, Qamer Din & Shehabi, 2016; Brahmi, Dunyach-Remy, Touati & Lavigne, 2015; Dolejska et al., 2011; Ewers et al., 2010).

2.7.2 SHV

The SHV-1 -lactamase is most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance in this species (Tzouveleks & Bonomo, 1999). In many strains of *K. pneumoniae, bla*SHV-1 or a related gene is integrated into the bacterial chromosome (Livermore, 1995). Although it has been hypothesized that the gene encoding SHV-1

may exist as part of a transposable element, it has never been proven (Jacoby & Sultan, 1991). Unlike the TEM-type lactamases, there are relatively few derivatives of SHV-1. Furthermore, the changes that have been observed in *bla*SHV to give rise to the SHV variants occur in fewer positions within the structural gene. The majority of SHV variants possessing an ESBL phenotype are characterized by the substitution of serine for glycine at position 238. A number of variants related to SHV-5 also have a substitution of lysine for glutamate at position 240. It is interesting that both the Gly238Ser and Glu240Lys amino acid substitutions mirror those seen in TEM-type ESBLs. The serine residue at position 238 is critical for the efficient hydrolysis of ceftazidime, and the lysine residue is critical for the efficient hydrolysis of cefotaxime (Huletsky, Knox & Levesque, 1993).

2.7.2.1 Brief history of SHV

The encoded enzyme SHV−1 (sulfhydryl reagent variable) proved its activity against penicillin and first generation cephalosporins (Matthew, Hedges & Smith, 1979) and was confirmed part of the conjugative plasmid p453 (Barthélémy, Peduzzi & Labia, 1988). Briefly, 1891 SHV allelic variants have been described, having developed resistance to 3rd generation cephalosporin (Tzouvelekis & Bonomo, 1999), monobactam and carbapenems (Poirel et al., 2003). Only a small proportion is biochemically and/or genetically characterized. SHV β-lactamases can be divided into three subgroups on the basis of molecular characteristics or functional properties: (i) subgroup 2b (n = 37), able to hydrolyze penicillin and early cephalosporins (cephaloridine and cephalothin) and strongly inhibited by clavulanic acid and tazobactam; (ii) subgroup 2br (n = 7), broad-spectrum β-lactamases that acquired resistance to clavulanic acid; and (iii) subgroup 2be (n = 46), comprises ESBLs that can also hydrolyze one or more oxyimino β-lactams (cefotaxime, ceftazidime, and aztreonam). Among the majority of unclassified variants, subgroup 2b and the

few 2br variants are scattered all over the tree. Subgroup 2be showed clustering of most of the ESBL variants (including SHV-2a, SHV-5, and SHV-12), together with few non-classified enzymes (SHV−29, SHV−152, SHV−153, SHV−160, and SHV−165). It has been proposed that SHV β-lactamases descended from an unidentified ancestor holding an extended spectrum phenotype (2be) and that subgroup 2b derived from it (Hall & Barlow, 2004).

Most of SHV ESBLs (25 out of 46) are unique cases, with only one report so far. Seventeen variants are exclusively found in clinical *K. pneumoniae*: *bla*SHV-6, *bla*SHV-13, *bla*SHV-16, *bla*SHV-18, *bla*SHV-23, *bla*SHV-45, *bla*SHV-64, *bla*SHV-66, *bla*SHV-86, *bla*SHV-90, *bla*SHV-91, *bla*SHV-98, *bla*SHV-99, *bla*SHV-100, *bla*SHV−104, *bla*SHV-105, and *bla*SHV-134. These variants have been described worldwide (Brazil, Portugal, Algeria, USA, Tunisia, Netherlands, France, South Africa, Colombia, and China) and are mostly associated to plasmids. Four variants have been described only in clinical *E. coli*: (i) *bla*SHV-15, described together with *bla*CMY-2 in a strain imported from India into the United Kingdom (ii) *bla*SHV-24, identified in Japan on a transferable 150 Kb plasmid conferring high-level resistance to ceftazidime but not cefotaxime and cefazolin (Hall & Barlow, 2004) emergence of SHV-24 might have been driven by the extensive use of ceftazidime in Japan, enabling bacterial survival in high concentrations of this drug.

2.7.2.2 The activities of SHV extended beta-lactamases

Extended-spectrum SHV β-lactamases belong to functional group 2be, while very recently they were assigned to subclass A1 of serine β-lactamases, clustering with TEM and CTX-M enzymes among other clinically relevant β-lactamases (Bush, 2013; Philippon, Slama, Dény & Labia, 2016). SHV ESBLs consist of two subdomains: an α/β that includes an antiparallel five-stranded β-sheet flanked by α-helices, and an allα-helical subdomain (Philipponet al., 2016). Similar to TEM β-lactamases (Philippon et al., 2016) the active site is located within the cleft created by the

subdomains and it contains the Ser70 residue that mediates the nucleophilic attack on the carbonyl group of the β-lactam ring. In the vicinity of this serine residue, several conserved structural and functional amino acid motifs have been identified.

These include the Ser70XXLys (—SXXK motif, with X representing variable amino acids), the Ser130AspAsn (—SDN motif), the Glu166XXLysAsn (—EXXLN motif), and the Lys234Thr/SerGly (—KTG motif) (Bush, 2013). Each SHV ESBL has one (SHV-2, SHV-6, SHV-8, SHV-24, SHV-27, SHV-38, SHV-41, SHV-57, SHV-98, SHV-99, SHV-102, and SHV-104) to six (SHV-128) amino acid substitutions when compared to SHV-1, indicating that even a single amino acid substitution is enough to convey an extended-spectrum phenotype (Bush, 2013). Therefore, we can speculate that other SHV ESBLs may still evolve from a parental SHV β-lactamase due to single spectrum-extending substitutions, although the majority of them have possibly emerged through a stepwise acquisition of several mutations (substitutions, deletions and/or insertions) from preexisting extended-spectrum SHV variants.

2.7.3 OXA

The OXA-type enzymes are another growing family of ESBLs. It entirely differs from TEM and SHV enzymes and shows only 20% sequence similarity with the other members. This OXA enzyme confers resistance to ampicillin and cephalothin antibiotics. Further, it is characterized by their high hydrolytic activity against oxacillin and cloxacillin, and it is poorly inhibited by clavulanic acid. The OXA βlactamases family was originally created as a phenotypic rather than a genotypic. Eventually, most of the ESBLs have been found in *E. coli*, *K. pneumoniae*, and other Enterobacteriaceae (Rasmussen & Bush, 1997).

2.8 Detection of ESBLs producers

2.8.1 Screening test

This method is based on testing the organisms for resistance to an indicator cephalosporin which are hydrolyzed by TEM, SHV, and CTX-M types (such as cefpodoxime), however, cefotaxime, ceftriaxone, and ceftazidime are also used (Drieux, Brossier, Sougakoff & Jarlier, 2008).

2.8.2 Phenotypic confirmatory tests:

This test works based on synergy between the indicator cephalosporin and clavulanic acid, however it needs to be demonstrated (ESBLs are inhibited by clavulanic acid). Moreover, in reference laboratory there is also genotypic confirmatory test which works based on testing for genes encoding ESBLs by molecular analysis, primarily polymerase chain reaction amplification of specific sequences. Eventually, this is usually reserved for epidemiological purposes as it identifies the particular genotype of ESBL, and it is less specific and sensitive than phenotypic confirmatory test (Drieux et al., 2008).

2.8.2.1 Cephalosporin/clavulanate combination disks

Significantly, the CLSI advocates use of cefotaxime (30µg) or ceftazidime (30µg) disks with or without clavulanate (10µg) for phenotypic confirmation of the presence of ESBLs in *Klebsiella* and *E. coli*, *P. mirabilis* and *Salmonella* species. Besides, the CLSI recommends that the disk test to be performed with confluent growth on Mueller-Hinton agar. Specifically, a difference of ≥5mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disks is taken to be a phenotypic confirmation of ESBL production (Drieux et al., 2008).

2.8.2.2 Broth micro-dilution

Phenotypic confirmatory testing can also be performed by broth micro-dilution assays using ceftazidime (0.25-128µg/mL), ceftazidime plus clavulanic acid (0.25/4-128/4µg/mL), cefotaxime (0.25-64µg/mL), or cefotaxime plus clavulanic acid (0.25/4-64/4µg/mL) (Bradford, 2001). Practically, broth micro-dilution is performed using standard methods. Specifically, phenotypic confirmation is considered as ≥3 twofold serial-dilution decreases in minimum inhibitory concentration (MIC) of either cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone (Wayne, 2009). For Enterobacter spp. *Citrobacter freundii*, Morganella, Providentia and Serratia spp., it is better to use cefepime or cefpirome which are fourth generation or extended spectrum cephalosporin in the confirmatory tests as they are less prone to attack by the chromosomal AmpC βlactamases, which may be induced by clavulanate in these species (Queenan, Foleno, Gownley, Wira & Bush, 2004).

2.9 Detection of ESBLs producers

2.9.1 Disk-Diffusion methods

The Clinical and Laboratory Standards Institute (CLSI) has proposed disk-diffusion methods for screening in order to detect ESBL production by *Klebsiella pneumoniae, K. oxytoca, E. coli* and *Proteus mirabilis*. Additionally, the laboratories using disk-diffusion methods for antibiotic susceptibility testing are able to screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production by using cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone disks (Drieux et al., 2008). Notably, isolate showing resistance or diminished susceptibility to any of the above antibiotic disks, indicating suspicion for ESBL production, and phenotypic confirmatory tests should be used to ascertain the diagnosis.

2.9.2 Screening by dilution antimicrobial susceptibility tests

The CLSI has proposed dilution methods for screening for ESBL production by *Klebsiella pneumoniae* and *K. oxytoca*, *E. coli* and *P. mirabilis* by using ceftazidime, aztreonam, cefotaxime or ceftriaxone at a screening concentration of 1 µg/mL or cefpodoxime at a concentration of 1 µg/mL for *P. mirabilis*; or 4µg/mL for the others. Growth at or above this screening antibiotic concentration is suspicious of ESBL production and is an indication for the organism to be tested by a phenotypic confirmatory test (Drieux et al., 2008).

2.10 Nanoparticles

Nanoparticles are the units measured in nanometers (1 nm = 10-9 m or 10-7cm). They have several applications due its submicroscopic particle size. They are highly mobile in the free state and possess high surface area. Nanoparticles based technologies are focused on efficiency and sustainability. They are having wide scope in areas like industrial catalysis, pharmaceuticals, semiconductors, batteries etc. Fullerens, Liposomes, metal nanoparticles, nanodroplets and dendrimers are some examples of nanoparticles (Cao, 2004). Modern science is actively exploring naturally available medicinal plants to find out potential applications. Research shows that plants based synthesized nanoparticles like Gold (AuNPs), Silver (AgNPs), Copper (CuNPs), Zinc (ZnNPs), Platinum (PtNPs), Iron (FeNPs), Nickel (NiNPs), Cobalt (CoNPs) show anticancer, antibacterial, antioxidant, anti-inflammatory and antiviral properties. Characterization of these metal ion nanoparticles is achieved through Analytical Chemistry Instrumental techniques which involves microscopic and spectroscopic studies (Mudshinge, Deore, Patil & Bhalgat, 2011).

Nanoparticles are considered as one of the most promising modalities in the field of medicine. They are believed to provide multiple advantages like improved permeability for hydrophilic drugs, extended half-life in plasma, targeted delivery, and increased therapeutic-index (Mudshinge

et al., 2011). There is an enormous amount of active research going on right now that is aimed towards exploring the benefits of nanoparticles in a wide range of therapeutic applications. For example, nanoparticles are being developed to deliver small molecule drugs, imaging molecules, cells, and genes to specific sites e.g., cancer cells (Amreddy et al., 2018). Nanoparticles can be used as diagnostic tools e.g., carbon nanotubes to detect cancer cells in blood (Sanginario, Miccoli & Demarchi, 2017) or gold nanorods to detect kidney damage (Marom et al., 2012), antibacterial therapy (e.g., quantum dots to treat bacterial infections (Courtney et al., 2017), bioimaging tools (e.g., luminescent and magnetic nanoparticles for OI and MRI, respectively (Lacroix, Delpech, Nayral, Lachaize & Chaudret, 2013) and vaccines (e.g., against respiratory viruses (Al-Halifa, Gauthier, Arpin, Bourgault & Archambault, 2019). Recent advances in gene therapy have further reinvigorated the interest in nanomedicine since nanoparticles are being considered as an attractive alternative to viral vectors for gene delivery (Al-Halifa et al., 2013).

2.10.1 Synthesis of Nanoparticles

The development in the synthesis of metal oxide nanoparticles is amazing given its wide range of applications. Focused efforts have been made since 1980 to develop synthetic processes for nanoparticles with desired features (Hemlataet al., 2020). The two different methods for generating nanomaterials are top-down and bottom-up approaches (Ifijen & Maliki, 2022). A considerable amount of material can be transformed into nanosized particles using the top-down method. A sophisticated, expensive, and highly energy-intensive apparatus is required to maintain certain variables like pressure, temperature, and environment (inert and non-flammable). Despite using expensive setup and equipment, top-down techniques have been discovered to produce nanomaterial with surface blemishes and non-uniform shapes, which limit its usefulness. As a result, the bottom-up method is most frequently used to generate metallic nanoparticles. The

bottom-up approach involves the combination of atomic or molecular species to produce nanostructures (Ifijen & Maliki, 2022).

2.11 Green synthesis

The phenomenon of green synthesis technology permits and promotes sustainable environmentfriendly, economically viable, and biologically secure synthesis of NPs from microbes and plants (Neupane et al., 2022; Bhardwaj, Singh, Kumar, Kumar & Budhwar, 2020). The ability of organisms to survive in condtions with high metal concentrations has advanced with time (Bisen, 1996). These organisms could not only have the capacity to amend the chemical composition of the detrimental metallic compounds and metals but also reduce or eliminate their toxic effects (Sharma & Bisen, 1992; Sharma, Singh, Shukla, Ahmad & Bisen, 2001). Both extracellular and intracellular NPs synthesis occurs in biological systems. The broader field of nanobiotechnology is comprised of eukaryotic and prokaryotic originated biological entities including plants, fungi, yeast, viruses, bacteria, actinomycetes, cyanobacteria, and algae (Zhang, Ma, Gu, Huang & Zhang, 2020). There are primarily two main methods for producing metal oxide nanoparticles (MeONPs): the topdown method, which uses energy from biological, physical, and chemical sources to break down larger structures into smaller components, and another one the bottom-up method, which initiates at the atomic level and generates large nanostructures through a variety of chemical, physical, or biological reactions (Bahrulolum et al.,2021). When using this strategy, the most common techniques used to fashion nanostructured carriers (NC) are chemical and biological (Das et al., 2017).

These natural biogenic MeONPs could be classified into two broader categories based on their synthesis through biosorption wherein the organisms have a direct binding affinity towards the metal ions derived from soil or aqueous sample. Firstly, the metal ions are physically attached to

the cell walls of certain fungi, bacteria, and plants produce peptides that can assemble into stable nanoparticulate structures (Yong, Rowson, Farr, Harris & Macaskie, 2002). Secondly, bio-reduction is another form of biogenic synthesis wherein chemical reduction employs biological methods and produces a stable form of metal ions through dissimilatory metal reduction. The enzyme is oxidized while the metal ion is reduced resulting in ineffective production of MeONPs followed by their safe removal from the polluted sample (Deplanche, Caldelari, Mikheenko, Sargen & Macaskie, 2010).

The methodologies of green synthesis mediated through biological precursors depend on various reaction parameters such as solvent concentration, temperature variability, pressure, and a wide range of pH. For the synthesis of MeONPs, plant diversity has been taken under the consideration as per their effective characteristics for a broader range of phytochemicals in their crude solvent extracts, specifically flavones, amides, terpenoids, carboxylic acids, phenols, and ascorbic acids which are capable of reducing metal salts into MeONPs (Doble, Rollins & Kumar, 2010; Singh et al., 2018). The dried biomass of the plants is used as a bio-reducing agent and a precursor in the general process that uses plants to make metallic NPs (Korde et al., 2020). Thus, green approaches avoid the use of costly chemicals, utilize less energy, and produce products and byproducts that are environmentally friendly (Korde et al., 2020). Green synthesis of MeONPs is emerging to be far superior to the chemical synthesis of NPs.

2.12 Zinc nanoparticles

Zinc oxide nanoparticles (ZnONPs) have been the subject of widespread study as a result of their important physical and chemical features. Several researchers have used their nanoparticles as photocatalytic and antimicrobial agents due to their high surface-to-volume ratio, small size, antibiotic activity, and semiconducting features (Ifijen & Maliki., 2022). Due to the difficulty in

compiling the list of different methods of synthesis, the best and most inexpensive with high-yield techniques for producing ZnO nanoparticles have been outlined.

2.12.1 Green synthesized ZnO NPs

A novel antimicrobial material was fabricated by loading green synthesized zinc oxide nanoparticles (ZnONPs) in an electronspun polyacrylic acid (PAA) and polyallylamine hydrochloride (PAH) polymeric fibers for wound healing application (Bandeira et al., 2021). ZnO NPs exhibited high antimicrobial activity, covering a large variety of microorganisms that comprise, drug-resistant bacteria and are less cytotoxic against L929 fibroblast cells of the mouse. ZnO NPs were greensynthesized from Ilex paraguariensis leaf extract. The antimicrobial activity of ZnO NPs against *S. aureus* (Gram-positive) was higher than the *E. coli* (Gram-negative) and the minimum inhibitory concentration (MIC) for *S. aureus* was 35 g mL− 1, however, *E. coli* exhibited higher resistance, even at 100 g mL−1 concentration with a 70% viability. The resazurin viability assay demonstrated that PAA/PAH/ZnONPs have the potential to inhibit both bacterial strains, since no dye reduction was observed. Besides the antimicrobial property of ZnONPs, they also mimic the morphology (extracellular matrix) of the skin tissue, thereby, assisting in cell attachment, growth and better wound healing (Bandeira et al., 2021).

Despite the consistent dispersion of ZnO, particle agglomerates of varying sizes were observed and this drawback (particle agglomeration) needs to be addressed for successful usage in the future (Bandeira et al., 2021). Prosopis cineraria (PC) leaf extract was used to biofabricate zinc oxide (ZnPC) and iron oxide (FePC) NPs, by the co-precipitation method. The anti-inflammatory activity of the asprepared NPs was confirmed through the proteinase inhibition and albumin denaturation methods. By applying ZnPC and FePC regularly, the healing process had been hastened because of their antiinflammatory effect, through reduction of proinflammatory cytokine

levels. The 1,1-diphenyl-2picrylhydrazyl (DPPH) method, confirmed the antioxidant properties and the free radical inhibiting efficiency of ZnPC and FePC in a concentration-dependent manner. The antioxidant effect of the biofabricated NPs, contributed to angiogenesis, collagenation, fibroblast proliferation, granulation tissue formation and maturation. ZnPC and FePC ointments were exposed to the dermis, at 0.10 and 0.16 μg/cm^2 /hr of skin permeation rates for the zinc and iron contents that were observed, which entailed a very low penetration of the metal ions and non-toxicity. The wound-healing effect of the nanoparticles prepared, was supported by hydroxyproline content, enzymatic antioxidant profile and inflammatory markers. Topical application of ZnPC ointment demonstrated a quicker tissue injury healing than the application of FePC ointment. Swift wound healing property of the prepared nanoparticles, might be attributed to the synergistic effect of polyphenolic compounds of the PC leaves and metal oxides. This study proved the possibility of utilizing ZnPC and FePC-based nano-ointment for dermal total thickness wound healing topical application. The ZnPC-treated wound healed completely on 15th day when FePC showed a wound closure rate of 95.47 ± 1.25% in Wistar rats. Thus, the as-prepared ZnPC and FePC NPs can, effectively, be involved in wound healing (Yadav, Yadav & Verma, 2021).

ZnO NPs fabricated and stabilized with leaf extract of Aloe barbadensis act as an effective absorbent material for removing two carcinogenic dyes, viz: Malachite green (cationic azo dye) and Congo red (anionic azo dye). At the maximum quantity of 70 mg/mL of as-prepared ZnO NPs, an adsorption efficiency of 90.7% was obtained within 90 min for the Malachite green dye and 92.30% of adsorption efficiency was reported for the Congo red dye at 80 mg/mL maximum concentration of ZnO-NPs within 120 min. The study of the different parameters, such as: the amount of adsorbent, time and pH, through the batch process, showed that ZnO-NPs can be used effectively, as an adsorbent for both the azo dyes. The as-synthesized ZnO-NPs exhibited a potent antibacterial activity against four bacterial strains, viz., of *Bacillus subtilis*, *Bacillus licheniformis*

(Gram-positive bacteria), *Escherichia coli* and *Klebsiella pneumoniae* (Gram-negative bacteria) and antifungal activity against two fungal strains, of *Candida albicans* and *Aspergillus niger*. The antimicrobial efficiency increases due to the accumulation of the as-prepared ZnO-NPs and the reactive oxygen species (ROS) production (Yadav et al., 2021).

ZnO-NP/ silica gel dressing (ZnO-NP/SG-30 ppm), significantly reduced mouse skin wounds within 11 days of the study when compared to the ZnO-NP/SG-15 ppm and the control sample of ZnO-NPs. ZnO-NP/SG-30 ppm demonstrated a recovering percentage of 95%, which is considerably, much more significant than the control sample (61%). The dressing also enhanced apoptosis, bacterial clearance, reepithelialization and stem cell activation, during the healing process of wounds in mice. It showed a controlled degradation, blood clotting and swelling than the control samples. Wound healing and antimicrobial properties of the as-prepared dressings on the skin's surface, enhanced its practical application (Batool, Khurshid, Qureshi & Daoush, 2021). ZnO NPs were green synthesized with Aloe Vera extract and were surface-modified with polydopamine (PDA) by a one-step and straight approaches (Tavakoli, Kharaziha & Nemati, 2021). The as-prepared ZnO nanoparticles had rod-shaped morphology with a large surface area for bacterial contact and a uniform PDA layer (average thickness of 23 ± 2 nm). The PDA did not have any significant effect on the morphology of nanomaterials. PDA@ZnO NPs can effectively create blood clots due to their negative surface charge. In addition, BCI was halved after the surface modification of ZnO. PDA@ZnO NPs were non-toxic to human cells and they also promoted cell survival more than the ZnO NPs. PDA@ZnO NPs can thus, be employed for wound repairing applications (Tavakoli et al., 2021). The wound healing process, which includes four stages, viz: hemostasis, inflammation, proliferation and remodeling in general (Bagheri, Validi, Gholipour, Makvandi & Sharifi, 2021).

2.12.2 Antimicrobial activities of zinc nanoparticles

ZnO nanoparticles stand out among all nanoparticles in terms of their potent ability to stop bacterial growth (Bagheriet al., 2022). Moreover, the ZnO NPs have been widely employed in the manufacture of pharmaceuticals and the treatment of skin ailments. Similar to this, silver nanoparticles were also utilized as inorganic antibacterial agents and added to a variety of medical equipment, dental composite materials, and textile products to inhibit bacterial development for an extended period of time. In order to prevent bacteria from growing in the wounded skin, a considerable amount of silver is used in topical preparations and saturates bandages (Sathappan et al.,2021). Increased ROS production, which might be achieved by preventing charge carrier recombination, could boost ZnO NPs' antibacterial activity. It is most likely accomplished by delivering charge carriers to the nanoparticles' surfaces via defect levels in the forbidden gap, where they can interact with oxygen and water molecules to produce more ROS (Sathappan et al., 2021).

Due to the creation of defects, such as oxygen and zinc vacancies in doped ZnO NPs, it has been demonstrated that doping ZnO with transition metals and rare earth elements, particularly silver and selenium, enhances its antibacterial properties (Carofiglio, Barui, Cauda & Laurenti, 2020). Notably, these selenium or silver-doped batches of ZnO nanoparticles have smaller band gaps than batches of ZnO nanoparticles that have not been doped. The antibacterial characteristics of various selenium-doped and silver-doped nanostructures have subsequently been studied by a number of scientists (Carofiglio et al., 2020). Different nanoparticles have different antibacterial mechanisms. The antibacterial mechanism is not entirely understood for all types of nanoparticles. While some postulated processes are related to the physical makeup of the nanoparticles (e.g., the nanoparticle's ability to abrasively damage membranes), others are related to the accelerated release of antibacterial metal ions from nanoparticle surfaces (Seil & Webster, 2012). As the

particle size lowers, the specific surface area of a dosage of nanoparticles grows, allowing for increased material interaction with the environment (Seil & Webster, 2012). Therefore, an increase in the surface-to-volume ratio boosts the antibacterial action of materials that are naturally antibacterial, such as zinc oxide nanoparticles that have been doped with silver and selenium (Seil & Webster, 2012). As a result, a nanoparticle made of an inherently antibacterial substance may exhibit antibacterial activity through a variety of mechanisms, including the release of antibacterial metal ions from the particle surface and the nanoparticle's antibacterial physical characteristics, such as cell wall penetration or membrane damage (Seil & Webster, 2012). By comparing the results from additional tests, it is feasible to pinpoint the characteristics of nanoparticles that are most crucial for developing the ideal antibacterial particle.

The mechanochemically generated selenium-doped ZnO nanoparticles were examined for their antibacterial properties in a study (Dutta, Nenavathu & Talukdar, 2014). By using the TBARS assay, the formation of ROS (reactive oxygen species) in culture conditions was linked to selenium (Se) doped ZnO nanoparticles. Doping of zinc nanoparticles with Se affected their antimicrobial activities. The batches of selenium-doped ZnO nanoparticles at 0.45 mg/mL only showed a 51% inhibition of *E. coli* growth as compared to 0.45 mg/mL of pristine ZnO nanoparticles, which completely inhibited growth. This demonstrates that Se-doped ZnO NPs had weaker antibacterial activity than unmodified ZnO NPs. The decreased antibacterial activity of selenium-doped ZnO nanoparticles was attributed to two opposing factors, such as the generation of ROS for growth inhibition, which was countered by the maintenance of *E. coli* growth due to the availability of Se micronutrients in culture media, as confirmed by inductively coupled plasma mass spectrometer measurement. The higher ROS production by selenium-doped ZnO nanoparticles was attributed to the formation of oxygen vacancies, which was corroborated by the green emission peak seen at 565 nm. The green method of synthesis of selenium doped zinc oxide

nano-antibiotic (Se-ZnO–NAB) from *Curcuma longa* extract has been shown to be effective in preventing the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) (Majid et al., 2020). The toxicity potential of the developed Se-ZnO–NAB was thoroughly assessed in an animal model. When compared to medicines, the antibacterial activity against MRSA exhibited noticeably low minimum inhibitory concentration at 6.2 g mL^{-1}.

It was also discovered that transition metal doping improves the specific annihilation of cancerous cell when compared with normal human cell (Ifijen & Maliki, 2022). The main machinery behind the toxic nature of the nanoparticles is the production of reactive oxygen species (ROS). Surface structure and particle size of nanoparticles are also found to be an affecting factor in the preferential killing of cancerous cells.

2.13 Silver Nanoparticles

Addition of nanoparticles to Ag_2O reduced its nanoscale dimensions, while improving their antibacterial activities (Marouzi, Sabouri & Darroudi, 2021). AgNP's size ranges between 35 and 40 nm and due to their antibacterial qualities, are widely employed in consumer items and protect against harmful microbes (Marouzi et al., 2021). The application of AgNPs in pharmaceuticals and medicine has led to several advancements in research. The outstanding applications of AgNPs are anti-microbial and delivery of specified drugs. Ag NPs have a beneficial impact on seed germination. AgNPs are used as nano fertilizers and are used to treat various plant diseases. Ag NPs accelerate the shelf life of crops and have also been reported to increase the chlorophyll yield (Marouzi et al., 2021). The Ag NPs concentration can enormously affect the root and shoot length in various plants. It can also affect the proteins and carbohydrate content in some plants. Ag NPs possess anti-bacterial and antifungal properties. They bind to the bacteria's cell wall and cytoplasmic membrane via electrostatic interaction and thus act as bactericidal agents. They also inhibit protein synthesis by denaturing the ribosomes present in the cytoplasm. Ag NPs possess a

substantial contribution to strengthening the human immune system, especially in wound healing with antitumorigenic properties.

2.13.1 Green synthesized AgNPs

In the green process, bioactive AgNPs have been synthesized by using secondary metabolites, with active functional groups, such as alkaloids, flavonoids, saponins, steroids, tannins and other nutritional compounds which serve as nucleating and stabilizing agents (Mamatha et al., 2021). Lately, AgNPs with *Coriolus versicolor* (CV-AgNPs) and *Boletus edulis* (BE-AgNPs), were synthesized by coupling AgNPs with extracts from *C. versicolor* and *B. edulis* mushrooms via the microwave-assisted green synthesis technology. The average diameter of CV-AgNPs and BE-AgNPs is -86.0 ± 3.8 and -87.7 ± 0.8 nm, respectively. These NPs have negative zeta potential on their surfaces, which improves their stability. These nanoparticles exhibit promising antimicrobial activity against gram-positive bacterial strains (*Staphylococcus aureus* and *Enterococcus faecalis*) and gram-negative bacterial strains (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). Although both these nanoparticles exhibited significant inhibitory effects in fungal strains of *Candida utilis*, they were ineffective against *Candida albicans*. CV-AgNPs and BE-AgNPs demonstrated anti-proliferative activity in MCF-7, HUH-7 and HT- 29 cancer cell lines, in a dose and time-dependent manner. From the MTT cell proliferation assay results, BE-AgNPs were found to possess higher anti-proliferative activity than CV-AgNPs in all three cell lines at 48 h. CV-AgNPs and BE-AgNPs could cause the migration of L929 cell lines (murine fibroblast cells) and effectively heal wounds at low concentrations. When L292 cells were treated with2.50 μg/mL and 1.25 μg/mL of CV-AgNPs and BE-AgNPs, migration of fibroblast cells was more significant than the control at 24 and 48 h. These nanoparticles have antibacterial, antifungal, anticancer and wound-healing properties (Mamatha et al., 2021).

AgNPs were green synthesized by using the non-heterocystous, filamentous *Cyanobacterium phormidium* sp. AgNPs improved the activity of chloramphenicol against methicillin-resistant *Staphylococcus aureus* (MRSA) strain. The AgNPs showed topical effectiveness in different wound types, such as: incision, excision and burn. There was no bleeding, microbial contamination or pus formation in the NPs-treated wounds. Wound healing potency of AgNPs were confirmed by an increase in the rate of wound closure, hydroxyproline content and a reduction in the period of epithelialization. The escalation of inflammatory cytokines and enzymatic antioxidant levels, assisted the wound repairing effect of the as-prepared AgNPs. The antimicrobial AgNPs had wound-healing abilities that were helpful for advanced medical applications (Marouzi et al., 2021). Aqueous extract of *Azadirachta indica* (AI) leaves, yielded fabricated spherical-shaped AI-AgNPs with 33 nm particle diameter (Chinnasamy, Chandrasekharan, Koh & Bhatnagar, 2021).

Leaf extract of AI contained terpenoids, terpenes, phenolic compounds and flavonoids. Futhermore, When AI extract is added to $AgNO_3$, the colour of the solution changes from transparent to brown, indicating the formation of AIAgNPs. Toxicity study confirmed the normal egg-laying capacity and the eclosion of the F1 generation of *Drosophila* which were administered 100 mg/mL of AI-AgNPs. 500 mg/mL of the as-prepared NPs, affirmed the excellent radical scavenging effect in 2,2-diphenyl-1picryhydrazil (DPPH) radical (65.17%) and {2,2′ -Azinobis-(3-ethylbensothiazoline-6-sulfonic-acid)} (ABTS) radical (66.20%) scavenging assays. AI-AgNPs demonstrated antibacterial activity against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with a zone of inhibition of: 17.7, 18.7, 10.3 and 17.7 mm, respectively. The reason for higher bacterial suppression by AI-AgNPs than AI-extract alone, was the significant damage, caused to the bacterial cell wall, the disintegration of the cell membrane and the intercellular content outflow by AI-AgNPS (Chinnasamy et al., 2021).

AgNPs were bio-fabricated with leaf extract of Parkia biglandulosa (commonly referred to, as the badminton ball tree). Leaf extract from *P. biglandulosa* (Pb) serves as a reducing and a capping agent for Ag+ reduction. As observed from the transmission electron microscopy results, the AgNPs fabricated by using P. biglandulosa leaf extract had an average diameter of -15 nm and were spherical. AgNPs were also chemically synthesized (cAgNPs) with a reducing agent (sodium borohydride) and were used for comparison with biosynthesized Pb-AgNPs. Pb-AgNPs exhibited the most antibacterial activity at a concentration of 0.02 M with 12 mm of the zone of inhibition (ZIC) against *Bacillus cereus*. In contrast, cAgNPs exhibited mild antibacterial activity with 6.5 mm of ZIC. Alamar blue reduction assay confirmed the biocompatibility of PbAgNPs (99.82%) with human skin fibroblast cells and was more significant than the native nanoparticles (98.29%). The biosynthesis approach was single-step, rapid, facile as well as safe, and served as an effective alternative to the conventional physical and chemical methods (John, Shaji, Vealyudhannair, Nidhin & Krishnamoorthy, 2021).

Echinophora platyloba DC extract was used to prepare AgNPs and an eco-friendly method was employed to conjugate Chloroxine (COX), an antibacterial drug with AgNPs, forming COX-AgNPs. COX changed the shape of the AgNPs into flower-like. The biofabricated AgNPs synthesized in this study, were relatively smaller (19.77 ± 1.06 nm) than other AgNPs found in the existing literature. The as-synthesized AgNPs, possessed remarkable antibacterial and antifungal properties against several microorganisms. AgNPs, Chloroxine, COX-AgNPs and *Echinophora platyloba* DC extracts, exhibited antibacterial activity against bacteria *E. coli* and *S. aureus*. However, the efficiency of COX-AgNPs was higher than the rest, since it inhibited 100% bacterial growth at ≥ 0.25 μg/mL of concentration. S. aureus was more sensitive than *E. coli* in all of these cases. AgNPs, Chloroxine, COX-AgNPs and *Echinophora platyloba* DC extracts exhibited antifungal activity against Aspergillus niger, Candida albicans and Trichophyton rubrumare more

effectively. Chloroxine and COX-AgNPs inhibited 100% fungal growth at a concentration of ≥0.125 μg/mL (John et al., 2021).

The conjugation of nanoparticles with antibiotics and antibacterial drugs augmented biological activity w.r.t. to the free antibacterial and antibiotic molecules. Two ointments were taken to study wound healing ability ointment 1: Calendula flower oil-Vaseline and ointment 2: Calendula flower oilVaseline having COX-AgNPs. Ointments 1 and 2 exhibited wound contraction percentages of 95.60 ±0.33% and 99.50 ± 0.28%, respectively, on the 20th day. COX-AgNPs acts as a suitable platelet activator. The efficacy of the AgNPs was improved by conjugating the nanoparticles with chloroxine, whereby, COX-AgNPs had the potential to accelerate wound repair, coupled with its antimicrobial activity (Shahabadi, Zendehcheshm, Khademi, Rashidi & Chehri, 2021).

2.13.2 Antimicrobial activities of AgNP

The small size of AgNPs facilitates it to act as an effective bactericidal agent (Shahabadi et al., 2021). The surface area of Ag NPs enhances anti-microbial impacts. The bactericidal reports have stated that thiol (SH) links of important compounds can be connected to Ag+ ions, causing them to be desalinated (Gupta & Silver, 1998). Anti-infective agents contain microbial strains that are a key source of concern in the public health system (Kyriacou, Brownlow & Xu, 2004). Differentiating between Gram-positive and Gramnegative bacteria is aided by distinct peptidoglycan layers. Between the exoplasmic face and the cytoplasmic face, gram-negative microorganisms have a thin peptidoglycan layer of 1–5 nm. In contrast, Gram-positive organisms have a thick peptidoglycan layer of 30 nm and no exoplasmic face (Singh, Singh, Prasad & Gambhir, 2008). The continuous release of AgNPs may be the reason for killing microbes, although the exact mechanism remains unknown (Bapat et al., 2018). The electrostatic interaction facilitates the adherence of AgNPs to the cell wall and permeability of the cytoplasmic membrane enhancing their affinity towards sulfur proteins, thereby disrupting the bacterial envelope

(Khorrami, Zarrabi, Khaleghi, Danaei & Mozafari, 2018). Silver ions inhibit protein synthesis by denaturing the ribosomes in the cytoplasm (Darroudi, 2014). The dissolution efficiency depends on intrinsic AgNPs and surrounding media, whose exposure plays a significant role in the anti-bacterial effect (Schmalz, Hickel, van Landuyt & Reichl, 2018). Ag NPs are used in purification by forming composites where they have been used as material media for the transportation of antibiotics and as casting material (Li, Leung, Yao, Song & Newton, 2021). The stability of Ag NPs in the reaction medium regulates the progression of microbial growth (Singh et al., 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Materials used in the study include the following

3.1.1 Media

The media used in this study are as follows; Peptone water (PW), Nutrient agar, Blood agar, Salmonella-Shigella agar, MacConkey agar, Thiosulphate citrate bile salt sucrose, Mannitol salt agar. Voges-Proskauer, Citrate agar, Luuria Bertani (LB).

3.1.2 Reagents/Chemicals

The chemicals include; Methyl red, Kovac Reagent, Hydrogen peroxide. Acetone, Alcohol, Safranin, Lugo's iodine. Crystal violet, Immersion oil, Silver nitrate, Zinc nitrate hexahydrate.

3.1.3 Glass wares and other materials

The glass waresand other materials used include; Conical flask, Beaker, Petri dishes, Test tubes, micropipettes

3.1.4 Equipment

Bijou bottles, petri dishes, Erlenmeyer flask, industrial Electric blender, test tubes, wire loop, water bath, centrifuge, magnetic stirrer, hot air oven, incubator, centrifuge, magnetic stirrer, hot air oven, incubator, bijou bottles, petri dishes, Erlenmeyer flask, industrial blender, test tubes, wire loop, water bath X-Ray Diffractogram spectroscopy, Scanning electron microscopy (SEM), Energy dispersive Xray spectroscopy, Fourier transform infrared radioscopy machine (EDX).

3.1.5 Plants

The plants used for the green synthesis are as listed below;

Ocimum gratissimum (scent leaf)

Vermonia amygadalina (bitter leaf)

Gongronema latifolium (utazi leaf)

Newbouldia laevis (Ogirishi leaf)

Plant 3.1: Scent leaf
Botanical name: Ocimum gratissimum. Common names: Igbo name: nchawu, Youruba name: efinrin, Hausa name: daidoya

Plant 3.2: Bitter leaf. Botanical name: Vermonia amygdalina, Igbo name: Onugbu, Youruba name: ewuro, Hausa name: chusar doki

Plant 3.3: Ogirishi leaf. Botanical name: newbouldia laevis, Igbo name: Ogirishi, Youruba name: eko akoko, Hausa name: aduruku

Plant 3.4: Utazi leaf. Botanical name: Gongronema latifolium, Igbo name: Utazi, Youruba name: arokeke, Hausa name:

3.2 Study Area

The study was conducted in the four most populated cities in Nigeria's South-East region, namely Aba, Umuahia (Abia State) and Okigwe, Mbaise (Imo State). The selection of the cities was based on random sampling where livestock activities are predominant. The samples were collected from urban areas where livestock farming is actually practised. These four cities stretch from latitude 4º50' to 7º20' N and longitude 6º51' to 8º20'E. These cities in the South-East region have common boundary with Enugu State in the North, in the East it is bounded by Cross River and Akwa Ibom States, in the West by Delta State and River Niger (Kalu & Zakiora, 2019). The zone has diverse ecological variations and land mass of 22,525 km^2 (Madu, 2006). Its annual rainfall is between March and October while the dry season starts from November and ends in February (Kalu & Zakiora, 2019).

3.3 Study Period

The study was carried out from December 2019 to April, 2022. A total of 600 samples (air, water, soil, feeds) were collected from pig, poultry and cow farms including hand swabs of their keepers.

3.4 Sample Collection/Sampling

The feed of pigs were collected from an abattoir at Aba River Abattoir, Ogbor hill Aba, Abia State while that of poultry were sampled from MAWA and Miracle poultry farms. In Umuahia, pig and poultry feeds were sampled from Ubakala Abattoir and Orie Ugba markets, respectively. The feeds of pig were collected from Umuchieze Ihiteafoukwu Ahiazu Mbaise. For Okigwe, the feeds were collected from Gariki abattoir. Air, water and soil samples were collected from these farms in the four cities of the two states using the scientific standards while hand swabs of the livestock workers were also collected with sterile swab sticks.

3.4.1 Pig, Poultry and Cow Samples

Rectal swab and raw meat of cow and pig were sampled while cloacal swab, faecal sample and drinking water of poultry were obtained from the farms. A total of 110 rectal swabs each from both cow and pig were collected and 100 samples of raw meat each were also obtained. In the poultry farm, a total of 270 samples were obtained from faecal swab (90), cloacal swab (90) and drinking water (90). The raw meat samples (20 g each) were collected, kept in separate sterile plastic bags and labelled appropriately. Rectal swabs were taken by inserting a sterile swab stick into the rectum (1.5 inches) of cow and pig by gently rotating it for 30 s. In chickens, the swab was inserted into the cloacal opening by mildly swabbing the mucosal region until little pellets of faeces stain the swab stick. All the swab samples were collected and returned to their containers separately containing 5 ml of peptone water (PW) labelled and packaged in sterile plastic bags and transferred to Midland Laboratories Limited, Aba for processing.

3.4.2 Meat Sample Preparation

Twenty grams of raw meat samples were weighed and suspended into a beaker containing 220 ml of 0.1% Peptone water (PW) and properly mixed by shaking for 5 min and allowed to incubate for 24 h at 37^{o}C. Similarly, the swab samples obtained from rectum of pigs and cows and cloaca of chickens homogenized in 5 ml PW were kept in the incubator for 24 h at 37^{o}C immediately after returning from the sample collection site. After the 24-hour incubation, an aliquot from each suspension was transferred onto already prepared nutrient agar plates using spread plate techniques. After 5 min, the plates were kept in the incubator and observed for bacterial growth after 24 h incubation at 37^{o}C.

3.5 Air Quality Sampling

Passive air sampling was performed using settle plate technique. Freshly prepared nutrient agar (NA), blood agar (BA), Salmonella-Shigella agar (SSA), mannitol salt agar (MSA), MacConkey (MCA) and thiosulphate citrate bile salt sucrose (TCBS) plates were allowed to solidify and dry. The plates were exposed at the height of 1.5 m above the ground for 60 min at various locations in the poultry farm, cow abattoir and pig farms. The samples were sealed, labeled appropriately and then put inside sterile polythene bags and transported to Midland Laboratory Limited, Aba and incubated at 30^{o}C for 24 h for bacterial growth. The experiment was repeated in triplicate and expressed as CFU/plate/hour.

3.6 Water quality sampling and Serial dilution

Water samples were collected from drinking troughs in all the pig and poultry farms in Aba, Umuahia, Okigwe and Mbaise except for cow abattoir, and kept in 2.5 litres plastic containers and transported to the laboratory for analysis (Ugbogu et al., 2016). Ten-fold serial dilution of the water samples were performed according to method described by Harley and Prescott (2002) with slight modification. Adopting aseptic technique, 10 ml of water sample from the stock solution was transferred to sterile test tubes containing 90 ml of distilled water. This was tagged first dilution (10^{-1}), the tube was covered with cotton wool and shaken vigorously. From this tube, another 10 ml was taken and transferred to the second tube containing 90 ml of distilled water with pipette and shaken vigorously also. This procedure was repeated till the tenth tube where 10 ml after shaking the tube was discarded.

The diluted water samples (10^{-4}) were further analysed for total heterotrophic bacterial count (THBC), total coliform count (TCC), and total potential pathogenic bacterial count (TPPBC).

3.7 Soil Quality Sampling and serial dilution

Twenty grammes (20g) of soil samples were collected in sterile polythene bags using soil auger at 5 cm deep (Bhat, Shankar, Shkha, Yunus & Shukla, 2011). The soil samples were collected from different locations in the pig farm, poultry farm and cow farm. Soil samples were placed on ice in a cooler box immediately after collection and transported to the laboratory for analysis. Ten-fold serial dilution (10^{-1}-10^{-10}) was performed according to the method described by Adhikari, Barnes, Schiewer & White (2007) with slight modifications. Ten grammes of soil was suspended into a tube (10^{-1}) containing 90 ml of distilled water and mixture shaken properly. From the first tube, 10 ml was taken and transferred to the second tube containing 90 ml of distilled water with pipette and shaken vigorously. This procedure was repeated till the tenth tube where 10 ml after shaking the tube was discarded. The diluted water sample (10^{-4}) was further analysed for total heterotrophic bacterial count (THBC), total coliform count (TCC), Total potential pathogenic bacterial count (TPPC).

3.8 Hand swab sampling and serial dilution

Hand swab samples were collected with sterile swab sticks from the hands of the livestock farmers and properly labeled. In the laboratory, 5 ml of normal saline was transferred into the swab sticks and allowed to stand for 10 min. Thereafter, ten-fold serial dilution (10^{-1}-10^{-4}) was performed with the normal saline inoculated with swab sticks. One ml from the swab container was transferred with the aid of pasture pipette into 9 ml of distilled water in the first tube labelled 10^{-1}. The solution was properly mixed by continuously pipetting it in the tube, up and down a few times. The process was repeated until the fourth tube where 1 ml was discarded. The diluted water sample (10^{-4}) was further analysed for total heterotrophic bacterial count (THBC), total coliform count (TCC), Total potential pathogenic bacterial count (TPPC).

3.9 Feed

Total heterotrophic bacterial count, total potential pathogenic bacterial count and total coliform counts were performed according to the method described by Onyeagba (2015) and Adhikari et al. (2007) with slight modifications.

3.10 Bacterial Enumeration

3.10.1 Total Heterotrophic Bacteria Count

After ten-fold serial dilution, 1 ml from 10^{-4} was pipetted onto nutrient agar (NA) plates in triplicates. The discrete colonies in each NA plate were counted and recorded in CFU/ml for water, soil, hand swabs and feed samples and CFU/plate/hour for air sample. Plates with colonies between the ranges of 30-300 were counted. The heterotrophic bacterial count was recorded as total heterotrophic bacterial count (THBC).

3.10.2 Total Coliform Bacterial Count

3.10.2.1 Water

TCC was performed using membrane filter technique according to method described by Harley and Prescott (2002) with slight modification. After serial dilution, 100 ml of the water sample from 10^{-4} dilution was transferred onto a membrane filter with pore size of 0.45 µm. After filtration, the absorbent paper was laid carefully on the MacConkey agar plate with sterile tweezers. The plates were incubated at 30ºC for 24 h. After incubation, total coliform bacterial colonies were enumerated with the help of a magnifying glass.

3.10.2.2 Soil

After serial dilution, 50 ml filtrate from 10^{-4} was transferred onto a membrane filter (0.45 µm). The absorbent paper after filtration was transferred onto MacConkey agar plate and incubated for 24 h at 30ºC. For confirmation, inoculums from the MacConkey agar plate were inoculated in

tubes containing 10 ml of lactose bile broth. The mixture was incubated for 24 h at 30^{o}C for fermentation to occur.

3.10.2.3 Hand swab

A ten-fold serial dilution (10^{-1}- 10^{-4}) was conducted, thereafter 1 ml from the 10^{-4} dilution was inoculated onto MAC plate using spread plate technique for 24 h at 37^{o}C.

3.10.3 Total potential pathogenic bacteria

TPPB was enumerated with selective media selected for potential pathogenic bacteria. SSA for*Salmonella* sp. and *Shigella* sp.; TCBS for *Vibrio cholera* and *Vibrio parahaemolyticus*; EMB for *Escherichia coli* and *Enterobacter aerogenes*; MSA for *Staphylococcus aureus*; Blood agar for *Streptococcus pyogenes* and MCA for *Pseudomonas aeruginosa*. For air samples, each plate was exposed to air for 60 min; aliquots from water, soil and feeds were seeded onto the media plates while hand swabs were inoculated onto the plates. After incubation for 24 h at 30^{o}C, the bacterial species were identified based on their colony appearances (Lama, Bates, Covington, Allen & Antunes, 2013). The colonies on each plate were counted with magnifying glass.

3.11 Morphology

3.11.1 Gram staining

A smear of the individual colonies was made on a grease free slide and allowed to air dry. The underneath of the slide was heated by passing through a smokeless flame 3 times to heat fix the slide. The slide was placed on a staining rack and flooded with crystal violet for 1min. It was washed off with slow running water, flooded again with lugols iodine for 1min and washed off. The slide was decolourized using acetone and immediately washed off with water. The slide was

counter stained using safranin for 1min, washed off with water and blot dried with Whatman No 1 filter paper. A drop off oil immersion was added to the slide and viewed under x100 objective lens.

3.11.2 Motility

This was done as described by Cheesbrough (2006). Each test organism was inoculated into the medium using stab inoculation method and incubated at 37^{o}C for 24 hours. Motile bacteria swam and gave a diffused speeding growth that was easily recognized by the naked eye.

3.12 Biochemical test

3.12.1 Oxidase test

The method described by Cheesbrough (2010) was used. A drop of oxidase reagent (1%N-tetra methyl-P-phenylene diamine) was added to a filter paper in a Petri dish. A smear of the bacterial colony was made. A change in purple colour within 10 s indicated a positive result.

3.12.2 Catalase test

Using a slide method, a 24 h culture of the isolate was placed on a clean slide and a drop of hydrogen peroxide was added. A positive result showed the presence of bubble (Cheesbrough, 2010).

3.12.3 Coagulase test

A 24 h culture was placed on a clean slide which was divided into two sections (one served as control) and a drop of normal saline was used on each of the smear until the formation of a homogenous suspension. A drop of human plasma was added to one of the suspensions and stirred for 5 s. Clumping of the suspension signifies a positive test (Cheesbrough, 2006).

3.12.4 Indole test

Peptone water containing tryptophan was used to inoculate the 24 h bacterial isolate and incubated at 37^{o}C for 48 hours. A 0.5 ml of KOVAC reagent (P-dimethyl amino benzaldehyde) was added to the culture and mixed vigorously and was allowed to stand for 30 minutes. The formation of a middle ring red colour indicated positive result.

3.12.5 Methyl red test

An aliquot of the isolates was introduced into already prepared glucose phosphate broths and were incubated at 37^{o}C for 48 h. After wards, 5 drops of methyl red solution were added. A red colouration indicated a positive result (acidic pH) while a yellow colouration denotes a negative result (Cheesbrough, 2010).

3.12.6 Citrate test

Heavy inoculums of the isolate were inoculated onto a prepared Simmon's citrate medium using a sterile loop. The set up was incubated for 48 h at 37^{o}C. A positive result was indicated by a change in colour of the medium from light green to blue (Cheesbrough, 2010).

3.12.7 Voges-Proskauer test

Heavy inoculums of the test organism were inoculated into Voges-Proskauer medium contained in different test tubes. These tubes were incubated for 48 h at 37^{o}C. Then, 0.5 ml of alpha nephtol was added, followed by 0.5 ml of 40% KOH. Upon agitation, it was allowed to stand for 30 min. A red colour signified a positive result (Cheesbrough, 2010).

3.12.8 Urease test

Urease test was prepared according to the manufacturer's instructions. The isolates were inoculated onto the urease agar and incubated for 24 h at 37^{o}C. A positive result was indicated by a change in colour from yellow to pink due to ammonia production (Cheesbrough, 2010).

3.12.9 Hydrogen sulphide test

Peptone water containing 0.01% cysteine and lead acetate paper inserted as filter was used to prepare a broth of the isolate. The set up was incubated for 6 days. A control set up was an uninoculated broth culture. 0.5 ml of 2N HCl was then added to the culture. The blackening of the lead acetate paper denoted a positive result (Cheesbrough, 2010).

3.13 Antibiotic susceptibility test of isolates

The antibacterial susceptibility of each of the isolate was determined using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2016). The antibiotics, amoxycillin-clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30µg), gentamicin (10 µg), cefpodoxime (30 µg), Ciprofloxacin (5 µg), amikacin (30 µg), Colistin (10µg), cefepime (30µg), Cloxacillin (10µg), meropenem (10 µg), imipenem (10 µg) and aztreonam(10µg) (Oxoid, UK) were used for the study.

Each of the isolated colonies from the overnight culture was suspended in peptone broth to obtain inoculum density equivalent to 0.5 MacFarland standard (10^6 cfu/ml). An aliquot of each organism was inoculated onto Muller-Hinton agar (MHA) using sterile cotton tipped swabs. The suspension was evenly spread onto the surface of the MHA (Oxoid, UK) and left to stand for 15 min for the surface moisture to dry. Using a pair of sterile forceps, the antibacterial discs under study were dropped onto the agar surface afterwards, the plates were incubated at 37^{o}C for 24 h.

After overnight incubation, the diameter of the zone of inhibition around each antibiotic disc was measured using a ruler and recorded. The zone sizes obtained were compared with the zone diameter chart supplied by the CLSI (M100-S26, 2018). Isolates were interpreted as resistance (R), sensitive (S) and intermediate (I) according to CLSI standard. Isolates showing resistance to three or more classes of antibiotics were considered multidrug resistant (MDR).

3.14 Screening for extended spectrum beta lactamases (ESBLs) production

The isolates were screened for ESBLs by placing the antibiotics ceftazidime (30 μg), ceftriaxone (30 μg) and cefotaxime (30 μg) on the MHA according to Kirby-Baur procedure. The isolates that displayed zone of inhibition of ≤22 mm with ceftazidime (30 μg) and ≤ 27 mm with cefotaxime (30 μg) and ceftriaxone were positive for ESBL production. They isolates were chosen and subjected to ESBLs confirmatory test (CLSI, 2016).

3.15 Phenotypic confirmation for ESBLs production

ESBLs confirmatory test was performed using Double Disc Synergy (DDS) test according to CLSI guidelines. Amoxycillin/clavulanic acid (20/10 μg) (Augmentin), ceftazidime (30 μg), ceftriaxone (30 μg) and cefotaxime (30 μg) discs were used for the experiment. Amoxycillin/clavulanic acid (20/10 μg) was placed at the center of the MHA plate inoculated with bacterial suspension of 0.5 McFarland turbidity standard. Ceftazidime (30 μg), ceftriaxone (30 μg) and cefotaxime (30 μg) discs were placed center to center from the centrally placed augmentin disc at the distance of 30 mm apart in the same plate. The plate was incubated for 24 h at 37°C. Isolates resistant to the disc in uncombined states (≤ 27 mm) but with increased zone of inhibition (> 5 mm) for the combined form with clavulanic acid was confirmed ESBLs producing isolates (Drieux et al., 2008).

3.16 Genotypic Detection

3.16.1 DNA extraction

Five milliliters of an overnight broth culture of the bacterial isolates in Luria Bertani (LB) were spun at 14000 rpm for 3 min. The cells were re-suspended in 500 µl of normal saline and heated at 95^0C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000 rpm. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at -20^{o}C for other downstream reactions.

3.16.2 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microlitres of the extracted DNA was loaded onto the lower base, the upper pedestal was brought down to contact the extracted DNA on the lower base. The DNA concentration was measured by clicking on the measure button.

3.16.3 16S rRNA Amplification

The 16S rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3’ and 1492R: 5'-CGGTTACCTTGTTACGACTT-3’ primers on an ABI 9700 applied biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DTNPs, MgCl), the primers at a concentration of 0.5 µM and the extracted DNA as template.

The PCR conditions were as follows: initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 52ºC for 30 s; extension, 72ºC for 30 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 130V for 30 min and visualized on a blue light transilluminator for a 1500bp amplicons.

3.16.4 Sequencing

Sequencing was done using the BigDye® terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10 µl, the components included 0.25 µl BigDye® terminator v1.1/v3.1, 2.25 µl of 5 x BigDye sequencing buffer, 10 µM Primer PCR primer, and 2-10 ng PCR template per 100 bp. The sequencing conditions were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4 min.

3.16.5 Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor 1969).

3.17Extended Spectrum Beta-Lactamases detection

3.17.1 Amplification of SHVgenes

SHV genes from the isolates were amplified using the SHV F: 5' CGCCTGTGTATTATCTCCCT-3’ and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3' primers on an ABI 9700 Applied

Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 56ºC for 40 s; extension, 72ºC for 50 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 120V for 25 min and visualized on a UV transilluminator for a 281bp product size.

3.17.2 Amplification of TEMgenes

TEM genes from the isolates were amplified using the TEMF: 5‘-ATGAGTATTCAACATTTCCGTG-3’ and TEMR: 5‘-TTACCAATGCTTAATCAGTGAG-3’ primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 55ºC for 30 s; extension, 72ºC for 30 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 120V for 25 min and visualized on a UV transilluminator for a 400bp product size.

3.17.3 Amplification of CTX-Mgenes

CTX-M genes from the isolates were amplified using the CTX-MF: 5'-CGCTTTGCGATGTGCAG-3’ and CTX-MR: 5'-ACCGCGATATCGTTGGT-3’ primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted

DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 52ºC for 30 s; extension, 72ºC for 30 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 120V for 25 min and visualized on a UV transilluminator for a 550bp product size.

3.17.4 Amplification of KPCgenes

KPC genes from the isolates were amplified using the KPCF: 5‘-GCTCAGGCGCAACTGTAAG-3’ and KPCR: 5‘-AGCACAGCGGCAGCAAGAAAG-3’ primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 µM and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 50ºC for 30 s; extension, 72ºC for 30 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 130V for 25 min and visualized on a blue transilluminator for a 150 bp product size.

3.17.5 Amplification of NDMgenes

NDM genes from the isolates were amplified using the NDMF: 5′-GGTTTGGCGATCTGGTTTTC-3′ and NDMR: 5′-CGGAATGGCTCATCACGATC-3′ primers on an ABI 9700 applied biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 µM and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 55ºC for 30 s; extension, 72ºC for 30 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 130V for 25 min and visualized on a blue transilluminator for a 621 bp product size.

3.17.6 Quinolone genes amplification

3.17.6.1 QnrB Amplification

QnrB genes from the isolates were amplified using the QnrB: 5′-GATCGTGAAAGCCAGAAAGG-3′ and QnrB: 5′-CGATGCCTGGTAGTTGTCC-3′ primers on an ABI 9700 applied biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 µM and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 50ºC for 30 s; extension, 72ºC for 30 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 130V for 25 min and visualized on a blue transilluminator for a 450 bp product size.

3.18 Plant Collections

The leaves of *Ocimum gratissimum* (scent leaf), *Vermonia amygadalina* (bitter leaf) and *Gongronema latifolium* (utazi leaf) were collected from a garden in Abayi, Osisioma LGA of Abia State while *Newbouldia laevis* (Ogirishi leaf) leaves were collected from the back of School of Science building, Federal University of Technology Owerri, Nigeria, for this study. The plant taxonomist from the Department of Agriculture, Federal University of Technology Owerri, Nigeria, identified the species based on morphological characteristics. All the leaves were collected in the morning hours and they were washed to remove impurities and dried under shade at room temperature for 14 days.

3.19 Plant preparation

The dried leaves of the four plant species were blended with electric grinder and stored in air tight container and preserved in the refrigerator at 4ºC for future use. Twenty grammes (20 g) of each blended leaf powder was dissolved in 200 ml of distilled water. The extract was obtained by

heating for 1 hour in a water bath at 60°C. The extract was filtered through Whatman No.1 filter paper and centrifuged for 20 minutes at 4000 revolution per minute (rpm). Without additional purification, the supernatants were carefully collected by transferring it to a new sterile container (Adelere et al., 2017).

3.20 Biosynthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles were prepared from 50 mM, 100 mM, 500 mM and 1000 mM of silver nitrate ($AgNO_3$) solutions. The protocol used for the preparation of AgNPs was described by Aziz et al. (2014) with slight modifications. 20 ml of each plant extract was added to 100 ml of 50mM, 100mM, 500 mM and 1000 mM of $AgNO_3$ in a container. It was incubated at 30^{o}C for 2 h under static conditions. A change in colour of the solution indicated the formation of AgNPs (Ajayi et al., 2018).

3.21 Biosynthesis of Zinc oxide Nanoparticles (ZnONPs)

Zinc oxide nanoparticles were synthesized according to the method described by Ajayiet al. (2018) with slight modifications. 50mM, 100mM, 500 mM and 1000 mM of zinc nitrate hexahydrate Zn+NO+.6H+O (Sigma-Aldrich) was added to 50 ml extract and kept on a magnetic stirrer at 60^{o}C for 2 h. Once the reaction was completed, the mixture was allowed to cool down at 25^{o}C, centrifuged (HERMLE Z326K) at 10,000 rpm for 10 min. The supernatant was discarded and the remaining pellet was washed thrice with distilled water, poured into a clean Petri plate, and oven-dried at 90^{o}C. The dried material was then ground into fine powder in pestle and mortar and calcinated for 2 h at 500^{o}C to remove any impurities. The annealed powder was stored in an airtight sterile container (Ajayi et al., 2018).

3.22 Standardization of isolates

The isolates were standardized using McFarland standard 1.5x 10^8 cfu/ml. A McFarland Standard is a barium chloride and sulfuric acid chemical solution. A fine precipitate of barium sulfate is formed as a result of the chemical interaction between these two substances. The turbidity of a McFarland Standard is visually comparable to a bacterial suspension of known concentration after shaking thoroughly. To make McFarland turbidity standards, different amounts of 1% Sulfuric acid and 1% Barium chloride are mixed to generate solutions with appropriate optical densities. McFarland standards of differing degrees of turbidity that represent variable bacterial density or cell count can be generated by altering the volume of these two chemical reagents. The optical density of the 0.5 McFarland turbidity standard is comparable to the density of a bacterial suspension containing 1.5 x 10^8 colony forming units (CFU/ml) (CLSI, 2016). The 0.5 McFarland standard is made by dissolving 1 percent BaCl (0.05ml) in 1% H_2SO_4 (9.95 ml), yielding 1.5x 108 cfu/ml in cell density. For antimicrobial susceptibility testing, the 0.5 McFarland standard is the most commonly used standard.

The test organism was placed in fresh broth and allowed for 12 to 18 hours. The cloudy soup was then placed in well-labeled tubes and spun, after which it was rinsed twice (2x) with sterile water. The organism is then visually evaluated to the McFarland standard by adding water until it meets the McFarland standard.

3.23 Preparation of nanoparticle stock

In preparation of the stock solution of nanoparticle of 10,000 µg/ml, 0.2 grams of the green synthesized nanoparticle was dissolved in 20 ml of sterile water (solvent).

3.24 Antibacterial test for the Silver and zinc oxide nanoparticles

The antibacterial activity of the synthesized AgNPs was evaluated against bacteria that was gotten from lifestock that showed antibiotic resistance. Nutrient agar was used for the antibiotic susceptibility testing. The organisms were grown overnight in peptone water and incubated at 37ºC for 18 h after which they are standardized. The 18h old cultures were used to seed the plates of Nutrient agar with the aid of sterile wire loop. The seeded plates were allowed to stand for 3-5 hours and then three (3) holes of 6mm was bored on the plate using cork hole borer. Then 20 µl of the prepared reconstituted solution was then placed on each plate accordingly and the plates were incubated at 37^{O}C for 16-24 hours after which the zone of inhibition was computed. The agar well diffusion test was performed in triplicate

3.25 Antibacterial activity of nanoparticles

The culture of suspensions of each bacterial isolate were adjusted and compared with 0.5 McFarland turbidity standard tube. One milliliter from the container with each bacterial suspension (*E. coli*, *Shigella* sp, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Staphylococcusaureus*, *Salmonella* sp) was transferred onto Mueller-Hinton agar (MHA) and swabbed uniformly on the agar plate surface with sterile swab sticks. It was allowed for 10 min for drying. Thereafter, five wells were made aseptically on each agar plate with sterile cork borer of 6 mm. About 20 µl of each AgNPs solution was transferred with micropipette into wells 1, 2 and 3 each with 2500, 5000 and 10,000 µg/ml concentrations respectively for 50 mM, 100 mM, 500 mM and 1000 mM while the remaining 4 and 5 were for positive control (colistin) and negative control (distilled water). The experiment was repeated in triplicates, and the plates were incubated for 24 h at 37ºC. The AgNPs of *O. gratissimum*, *V. amygadalina*, *N. laevis* and *G. latifolium* were used for the experiment. The experiment was repeated also with ZnONPs.

3.26 Statistical analysis

The results were expressed as mean± SD using GraphPad prism graphical statistical package version 5. The student t-test at $p<0.05$ was applied to assess the difference between the mean for variables in triplicates and two-way analysis of variance (ANOVA) for more than two variables followed by Bonferreni post hoc test.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 THBC, TPPBC and TCC of air, water soil, hand swabs and feed samples of pig farm in Aba, Umuahia, Okigwe and Mbaise.

The total heterotrophic bacteria count (THBC) from the pig farms varied between $6.13\pm0.6\times10^5$ and $28.43\pm0.3\times10^5$ CFU/ml while total potential pathogenic bacteria count (TPPBC) ranged from $9.83\pm1.0\times10^5$ to $26.23\pm0.4\times10^5$ CFU/ml. The value obtained for total coliform bacteria count (TCC) was between $12.73\pm0.5\times10^5$ and $24.06\pm0.4\times10^5$ CFU/ml. Of the four cities, Aba ($28.43\pm0.3\times10^5$, $24.06\pm0.4\times10^5$, $26.23\pm0.4\times10^5$ CFU/ml) had the highest values. THBC and TCC were higher in soil samples while hand swabs of workers had higher TPPBC than other samples. Air samples had the least counts of bacteria. The THBC, TPPBC and TCC of samples obtained from air, water, soil, feeds and hand swabs of farmers in pig farm are shown in Apendix 1 (Figure 4.1, 4.2, 4.3, 4.4 & 4.5)

4.1.2 THBC, TPPBC and TCC of air, water soil, hand swabs and feed samples of cow abattoir in Aba, Umuahia, Okigwe and Mbaise

THBC of air, soil and hand swabs from the cow ranged from $10.50\pm0.6\times10^5$ to $26.70\pm0.7\times10^5$ CFU/ml; TPPBC had values between $9.26\pm0.5\times10^5$ and $17.47\pm0.5\times10^5$ CFU/ml. The values of TCC were between $9.03\pm0.6\times10^5$ and $18.33\pm0.5\times10^5$ CFU/ml. In Aba and Mbaise, the highest value for THBC and TPPBC ($26.70\pm0.7\times10^5$ and $17.47\pm0.5\times10^5$ CFU/ml) and TCC ($18.33\pm0.5\times10^5$ CFU/ml) respectively were obtained. Soil samples had the highest bacterial count (Apendix 2 Figure 4.6, 4.7, 4.8 & 4.9).

4.1.3 THBC, TPPBC and TCC of air, water soil, hand swabs and feed samples of poultry farm in Aba, Umuahia, Okigwe and Mbaise

The THBC varied between $9.53\pm0.8\times10^5$ and $26.26\pm0.5\times10^5$ CFU/ml; TPPBC was within the range of $9.86\pm0.4\times10^5$ to $20.20\pm0\times10^5$ CFU/ml while the values $9.97\pm0.8\times10^5$ to $22.36\pm0.4\times10^5$ CFU/ml were for TCC. The highest counts of THBC ($26.26\pm0.5\times10^5$ CFU/ml) were obtained in Aba and Mbaise. TPPBC and TCC were more only in Aba ($20.20\pm0.5\times10^5$ and $22.36\pm0.4\times10^5$ CFU/ml) respectively. The hand swabs of workers produced the highest count for TPPBC while highest amount of THBC and TCC appeared more in soil samples. Result for the enumeration of bacteria in poultry farm is shown in Table 4.3.

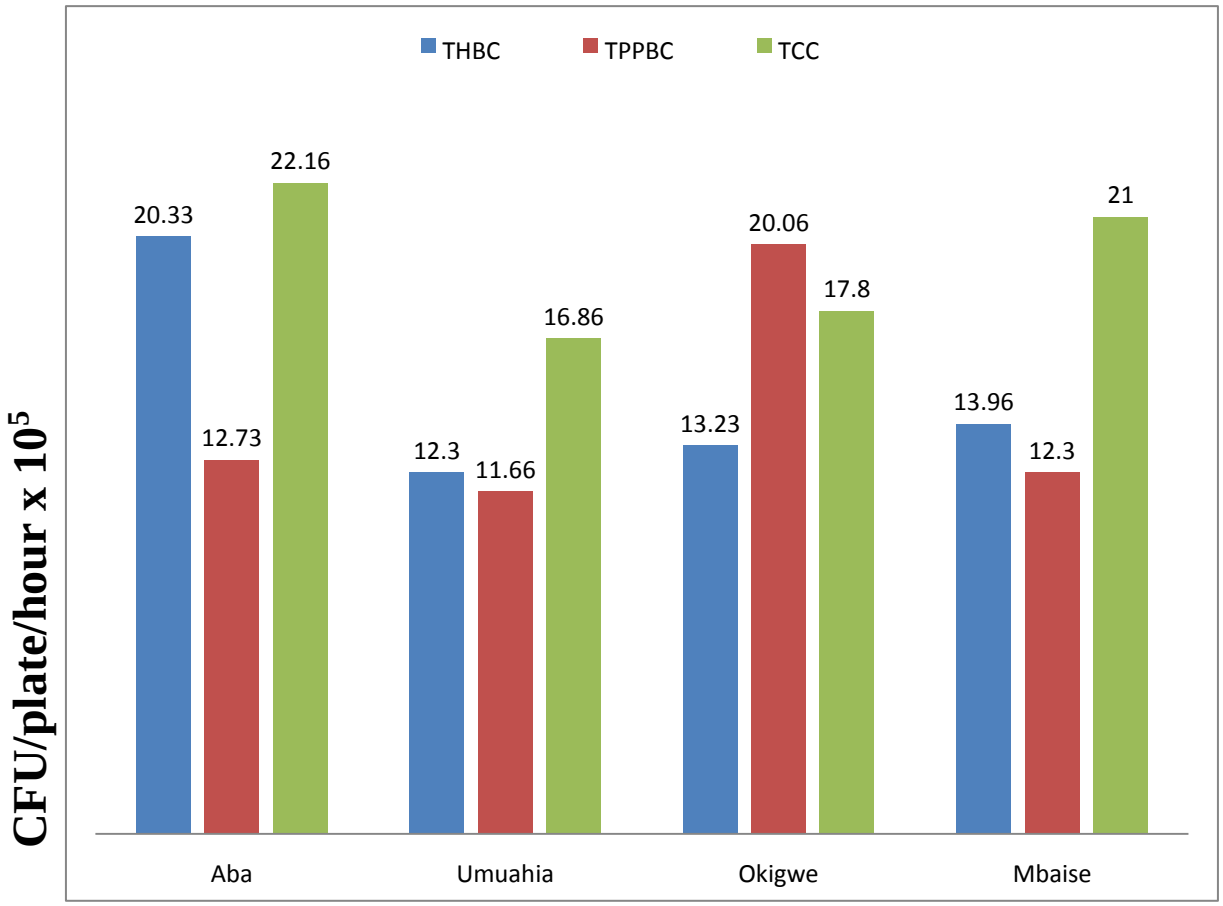

Figure 4.1: THBC, TPPBC and TCC of Air (CFU/Plate/hour) Samples of Pig Farm in Aba, Umuahia, Okigwe and Mbaise

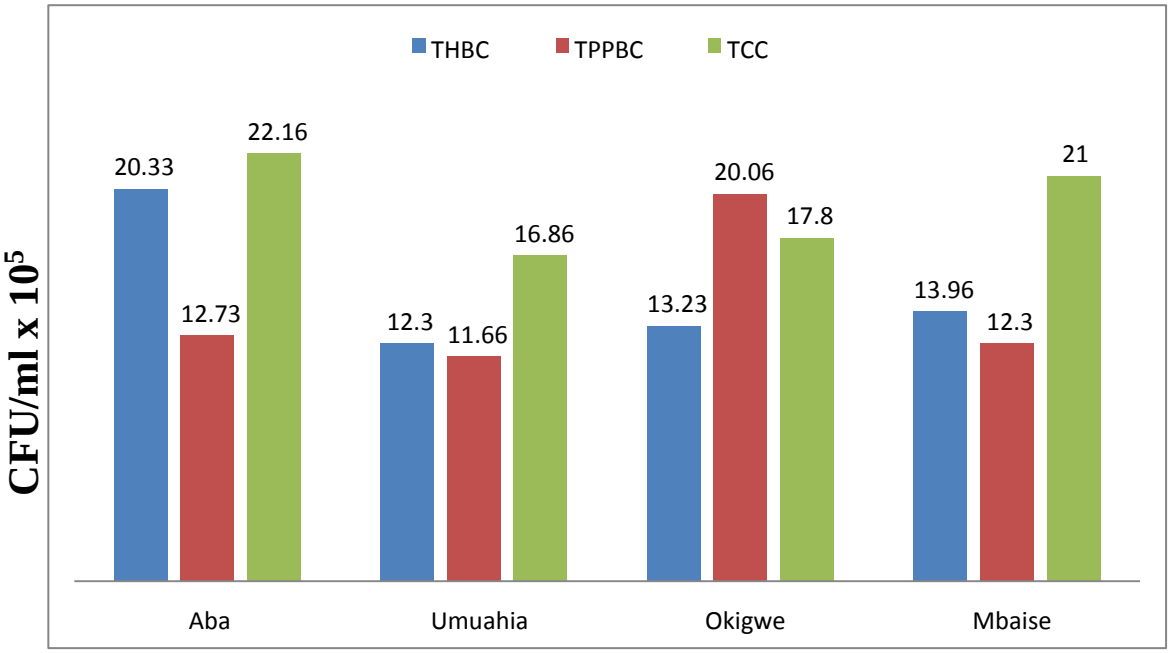

Figure 4.2: THBC, TPPBC and TCC of Water (CFU/ml) Samples of Pig Farm in Aba, Umuahia, Okigwe and Mbaise

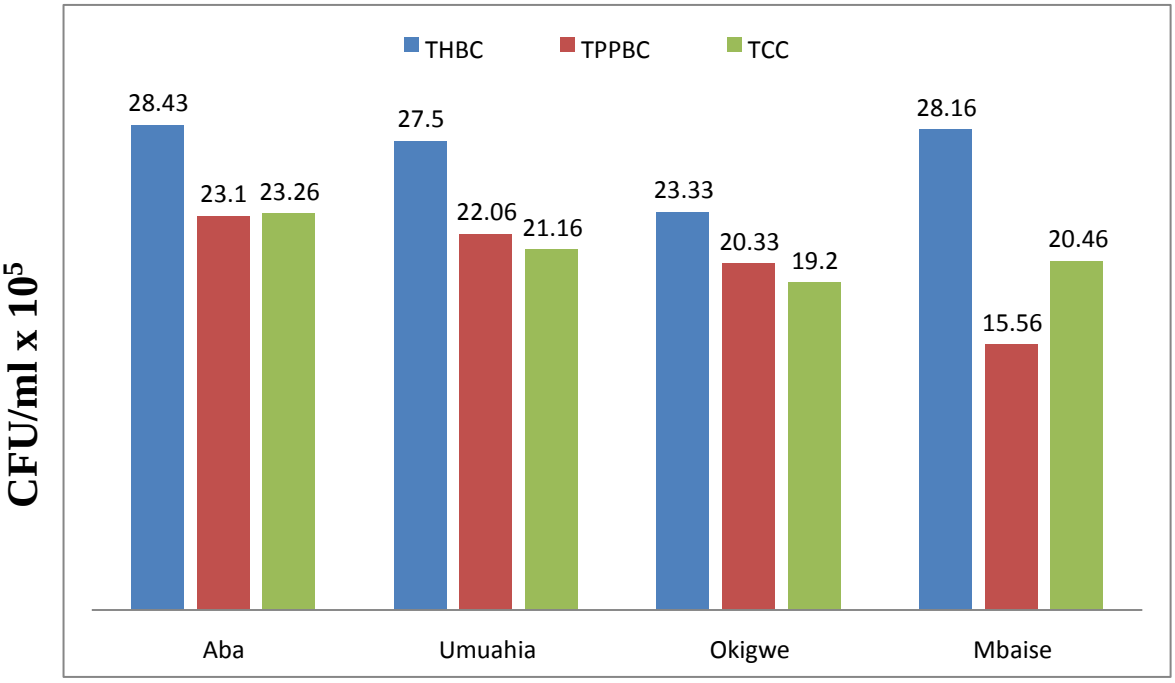

Figure 4.3: THBC, TPPBC and TCC of soil (CFU/ml), samples of pig farm in Aba, Umuahia, Okigwe and Mbaise.

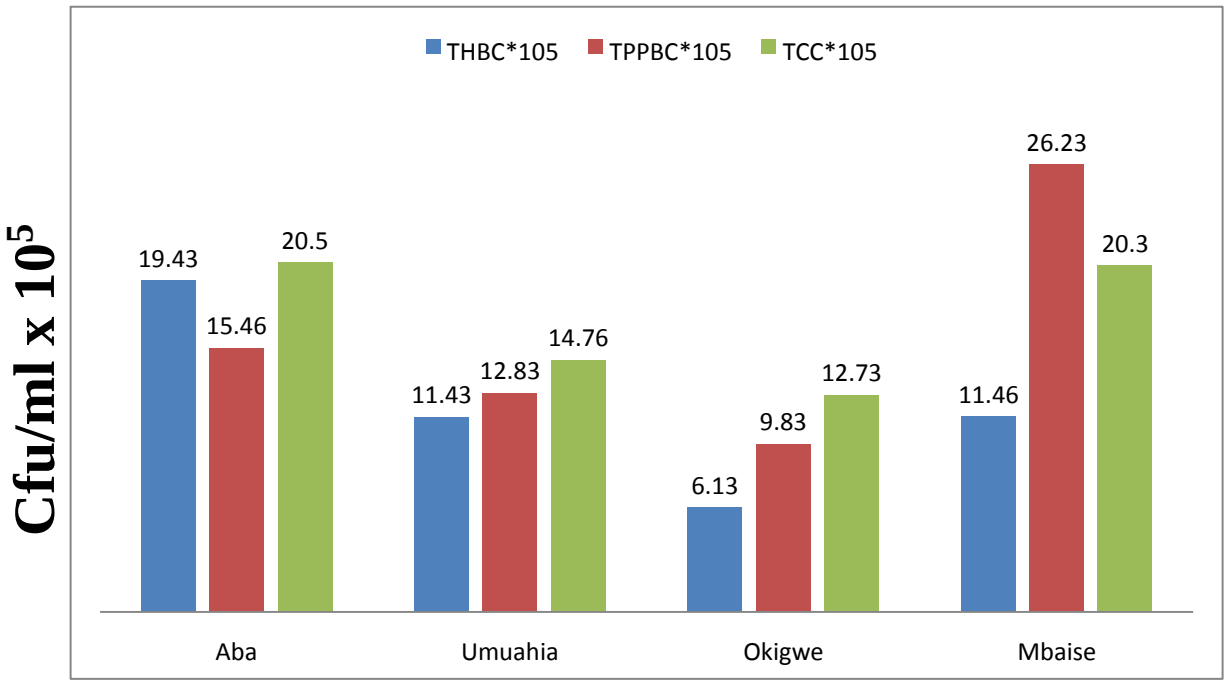

Figure 4.4: THBC, TPPBC and TCC of Hand Swab (CFU/ml) Samples of Pig Farm in Aba, Umuahia, Okigwe and Mbaise

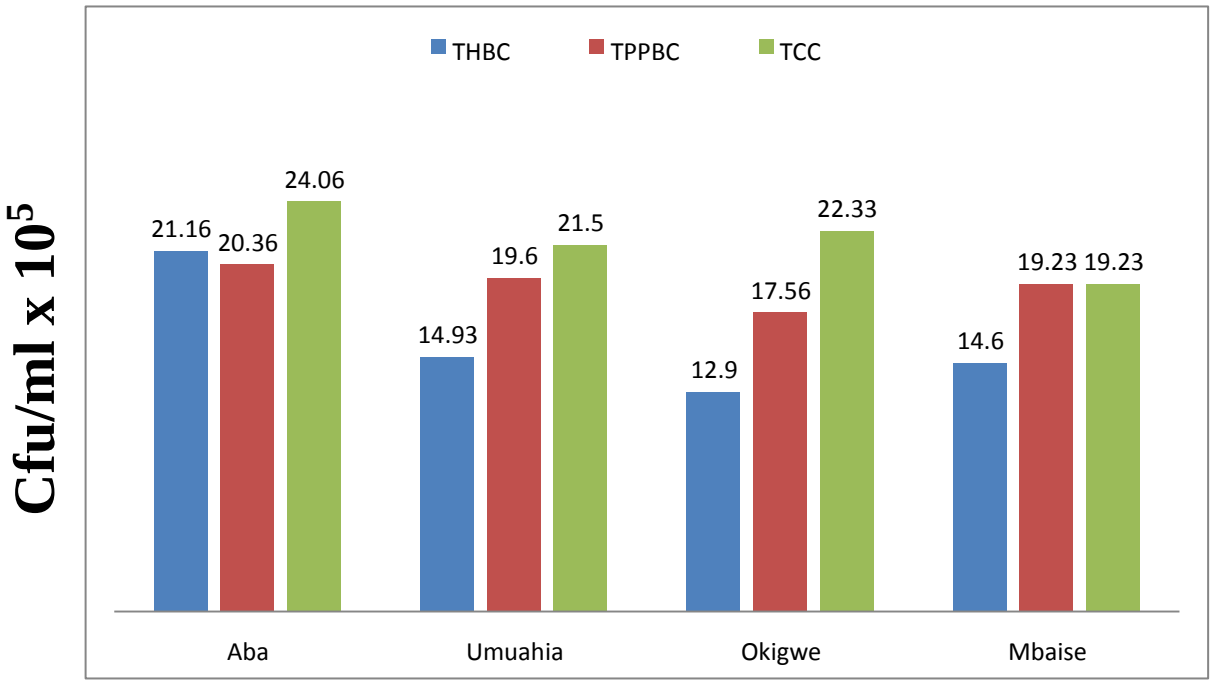

Figure 4.5: THBC, TPPBC and TCC of Feed (CFU/ml) Samples of Pig Farm in Aba, Umuahia, Okigwe and Mbaise

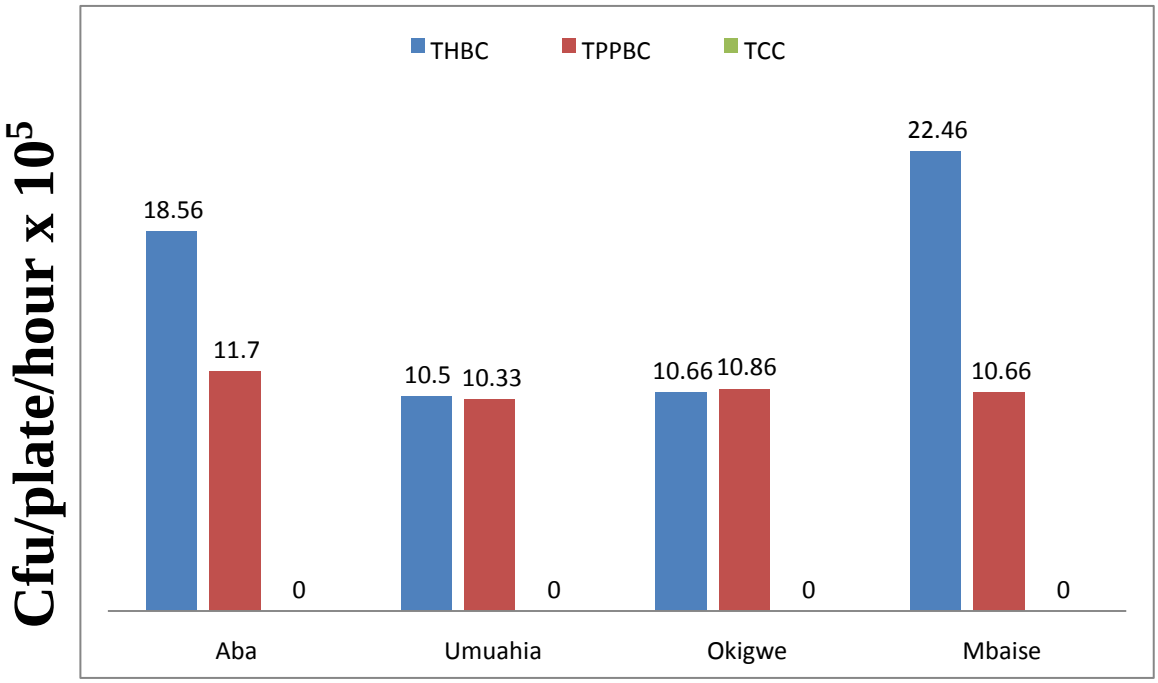

Figure 4.6: THBC, TPPBC and TCC of Air (CFU/Plate/hour) Samples of Cow Abattoir in Aba, Umuahia, Okigwe and Mbaise

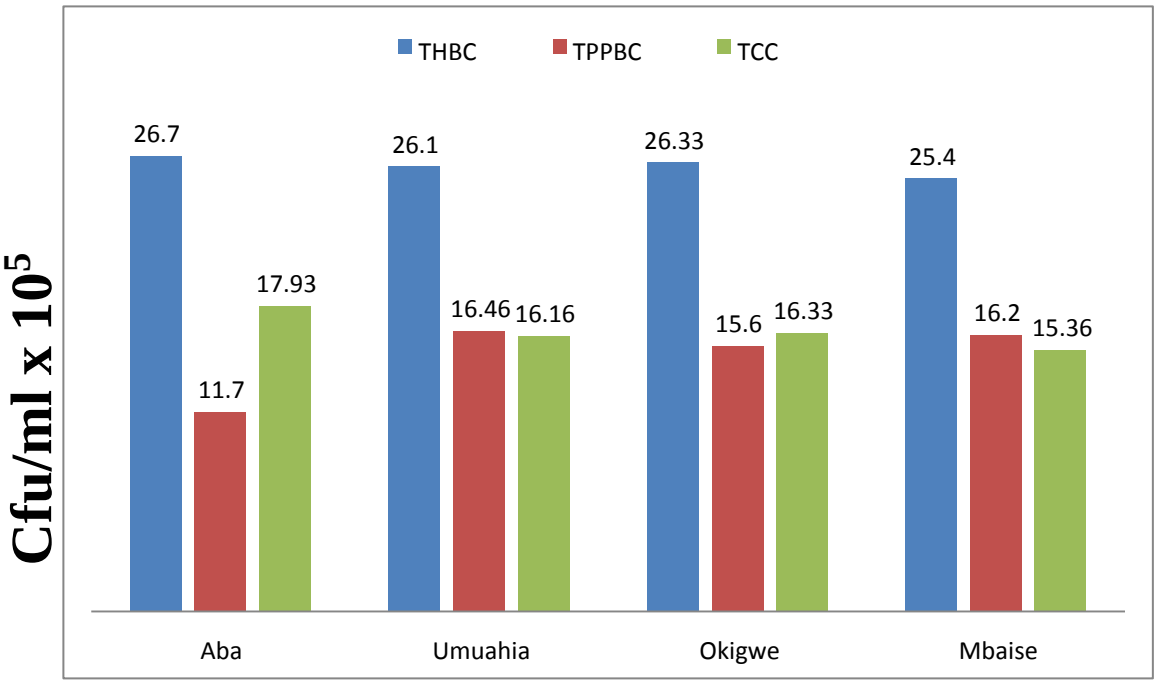

Figure 4.7: THBC, TPPBC and TCC of soil (CFU/ml) samples of cow abattoir in Aba, Umuahia, Okigwe and Mbaise

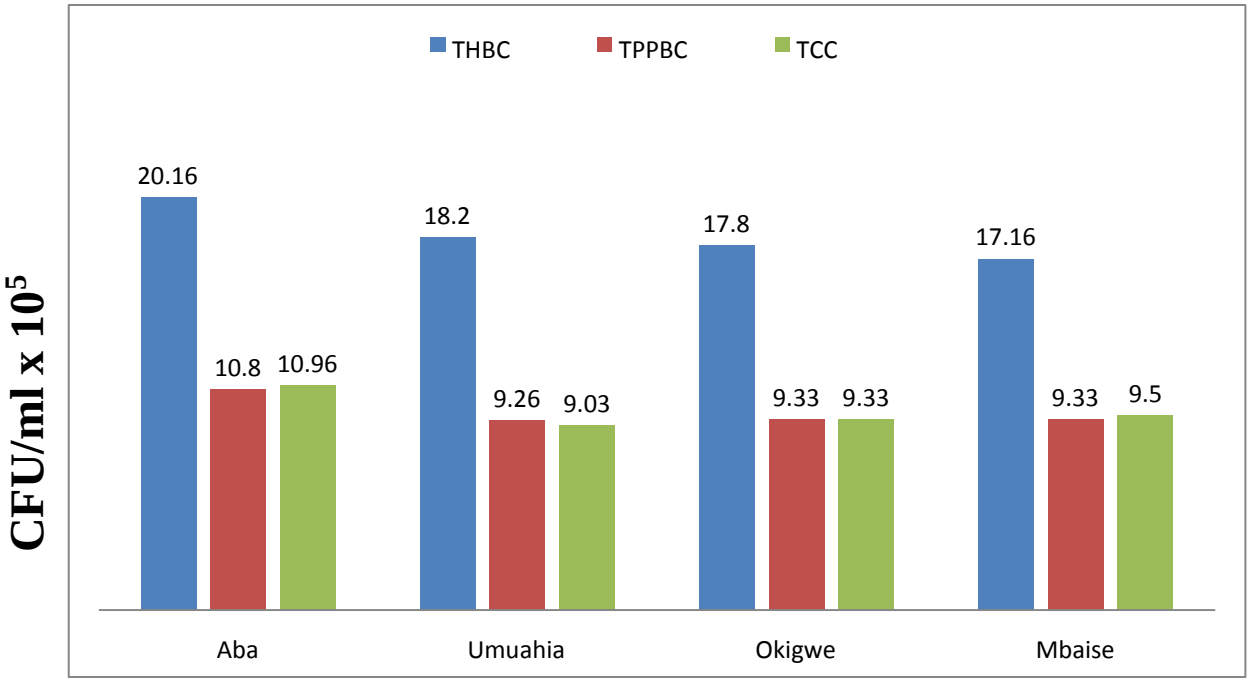

Figure 4.8: THBC, TPPBC and TCC of hand swab (CFU/ml) samples of cow abattoir in Aba, Umuahia, Okigwe and Mbaise

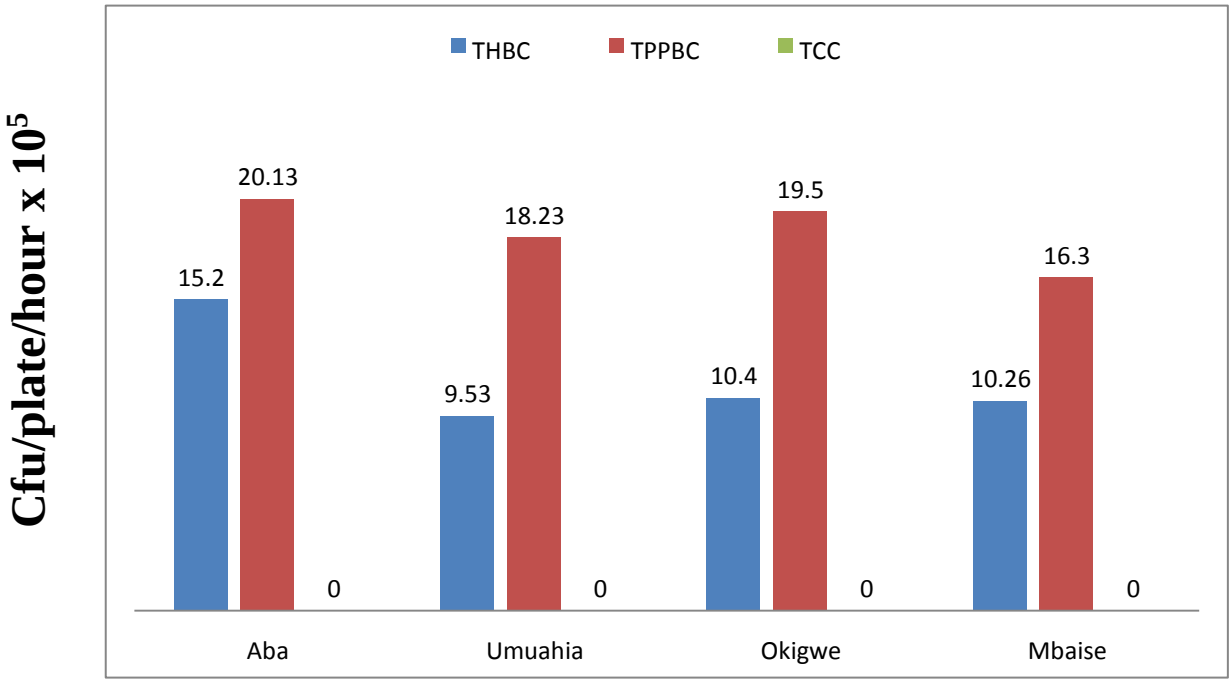

Figure 4.9: THBC, TPPBC and TCC of air (CFU/plate/hour), samples of poultry farm in Aba, Umuahia, Okigwe and Mbaise

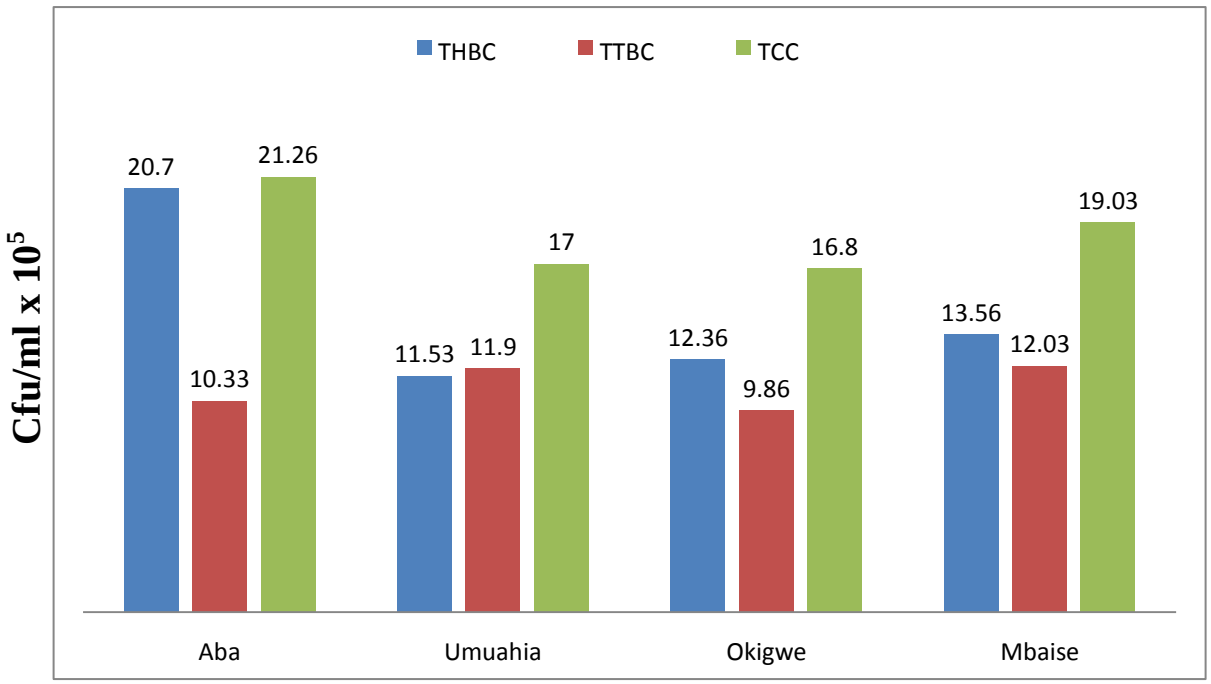

Figure 4.10: THBC, TPPBC and TCC of water (CFU/ml) samples of poultry farm in Aba, Umuahia, Okigwe and Mbaise

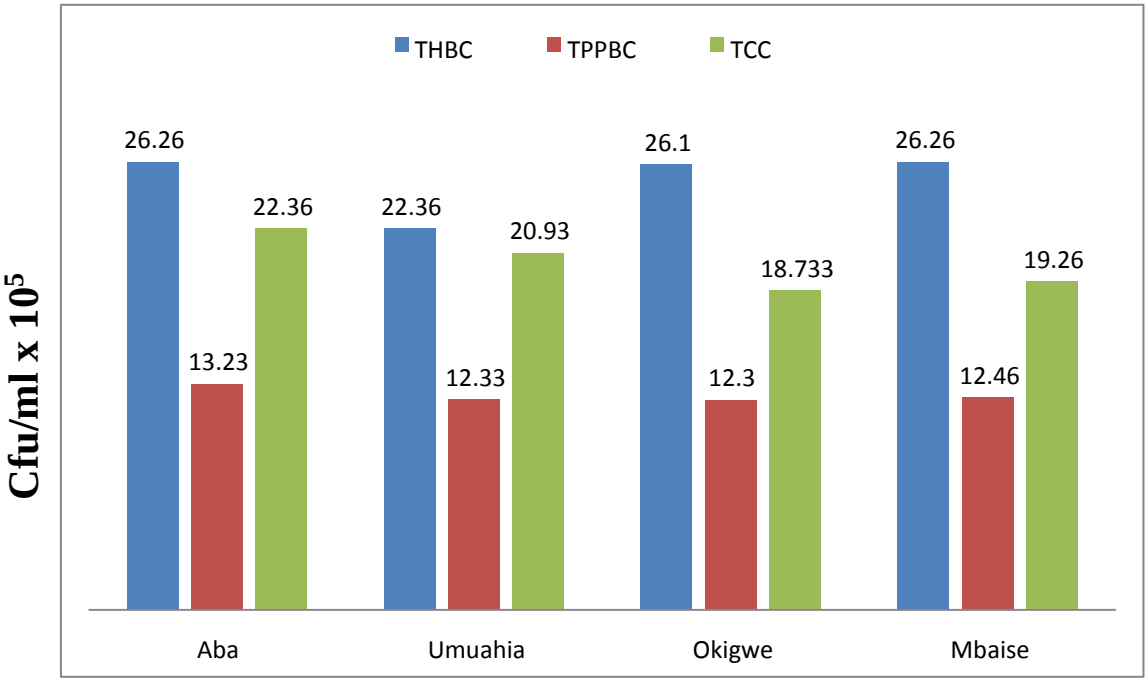

Figure 4.11: THBC, TPPBC and TCC of soil (CFU/ml) samples of poultry farm in Aba, Umuahia, Okigwe and Mbaise

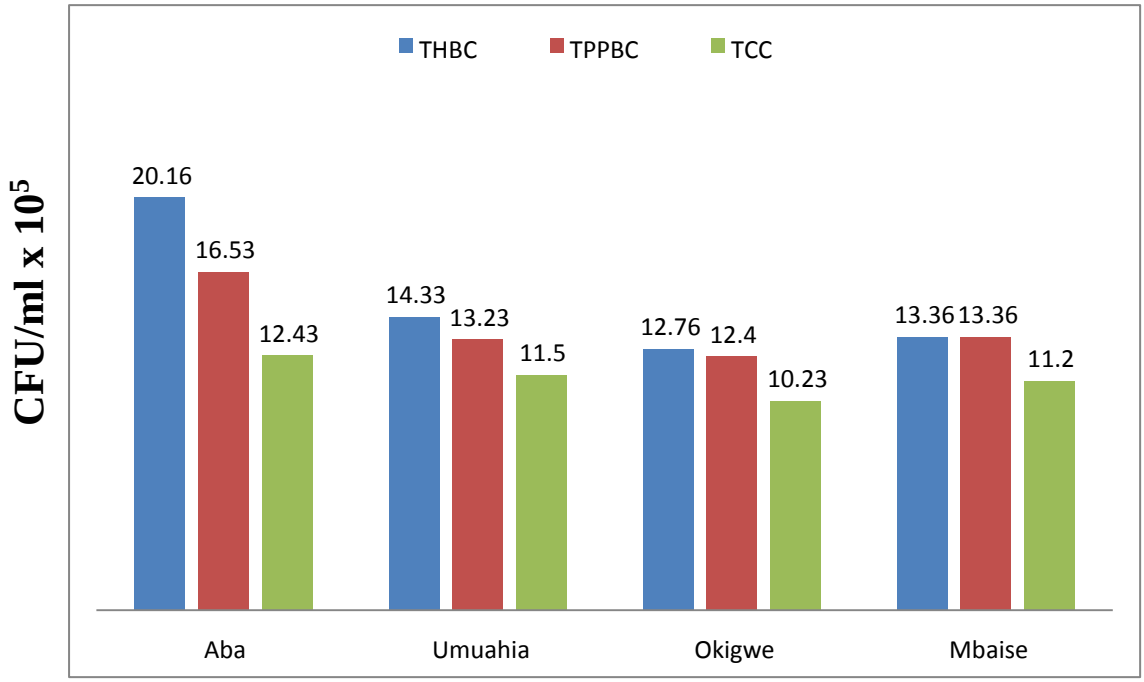

Figure 4.12: THBC, TPPBC and TCC of hand swab (CFU/ml) samples of poultry farm in Aba, Umuahia, Okigwe and Mbaise

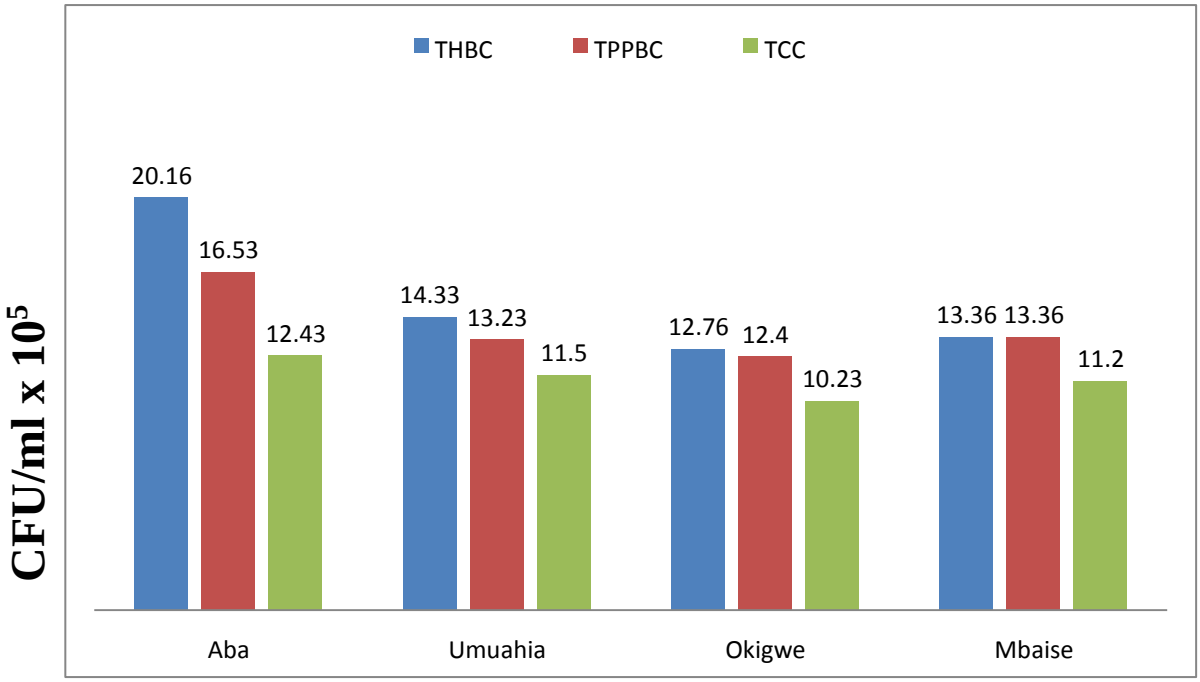

Figure 4.13: THBC, TPPBC and TCC of feed (CFU/ml) samples of poultry farm in Aba, Umuahia, Okigwe and Mbaise

4.1.4 Prevalence of bacteria isolated from livestock farms and workers in Aba, Umuahia Mbaise and Okigwe

The total number of *Escherichia coli* (933) isolates were significantly higher followed by *Klebsiella pneumoniae* (505) while the lowest value was obtained from *Shigella* sp (3). From the four cities studied, the percentage occurrence of *E. coli* was more compared with other bacterial isolates. Moreover, *Klebsiella pneumoniae*, were more prevalent in poultry farms while *E. coli* occurred more in cow farms. In both pig and poultry farms, *Staphylococcus aureus* (14.6%; 18/123) was quite prevalent but was isolated even more from the hands of poultry workers (53.7%; 66/123). *Salmonella enterica*, *Enterobacter* aerogenes, *Vibrio* sp and *Shigella* sp were not isolated from the hands of poultry workers as well as the farm excluding *Salmonella* sp (80%; 12/15). *Salmonellaenterica* and *Shigella* sp were not isolated cow farms. The prevalence of bacteria isolates from livestock and workers in Aba, Umuahia, Mbaise and Okigwe is presented in Figure 4.4.

4.1.5 Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* isolated from livestock farms and workers in Aba

In Aba, only *E. coli*, *Klebsiella pneumoniae* and *Salmonella enterica* were considered due to their importance and high prevalence when compared with other isolates, therefore, the number of *E. coli* (218) isolated was more in the four farms while *Salmonella pneumoniae* was the lowest (5). *Klebsiella pneumoniae* were slightly higher in cow (35.5%; 65/183) and pig (36.6%; 67/183) farms respectively than other isolates. *Salmonella pneumoniae* were not isolated from the farms except only in poultry farms (100%; 5/5). The Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* isolated from livestock farm and workers in Aba is presented in Table 4.5.

4.1.6 Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* isolated from livestock farms in Umuahia.

In Umuahia, *E. coli* was the most prevalent (202) compared to *Klebsiella pneumoniae* (45) and *Salmonella enterica* (1). From the three farms, *E. coli* was significantly higher in cow (33.2%; 67/202) and poultry (25.7%; 52/202) farms, while *Klebsiella pneumoniae* were appreciably more in pig farms (35.6%; 16/45) than cow (31.4%; 14/45) and poultry (20.0%; 9/45) farms. *Salmonella*

enterica were not isolated in all the farms except in poultry farms where only 1(100%) was isolated (Table 4.6).

4.1.7 Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* isolated from livestock farms and farmers in Mbaise

In Mbaise, *E. coli* was the highest bacterial pathogen isolated (185). It was more prevalent in pig farms (35.1%; 65/185), followed by cow farms (33.5%; 62/185). *Salmonella enterica* were more in poultry farms (26.2%; 22/84). Apart from poultry farms (100%; 2/2) where *Salmonella enterica* were prevalent, they were not isolated in cow and pig farms (Table 4.7).

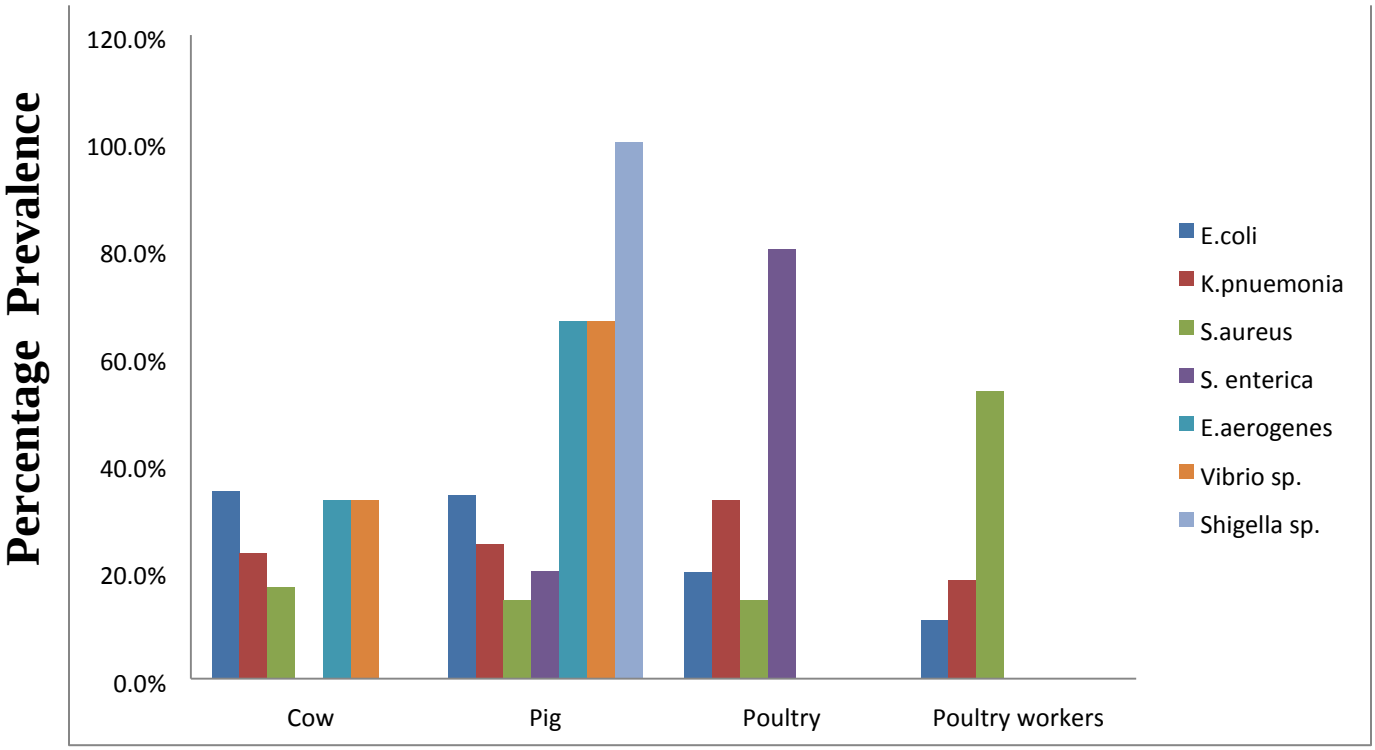

Figure 4.4: Prevalence of bacteria isolated from livestock farms and workers in Aba, Umuahia Mbaise and Okigwe

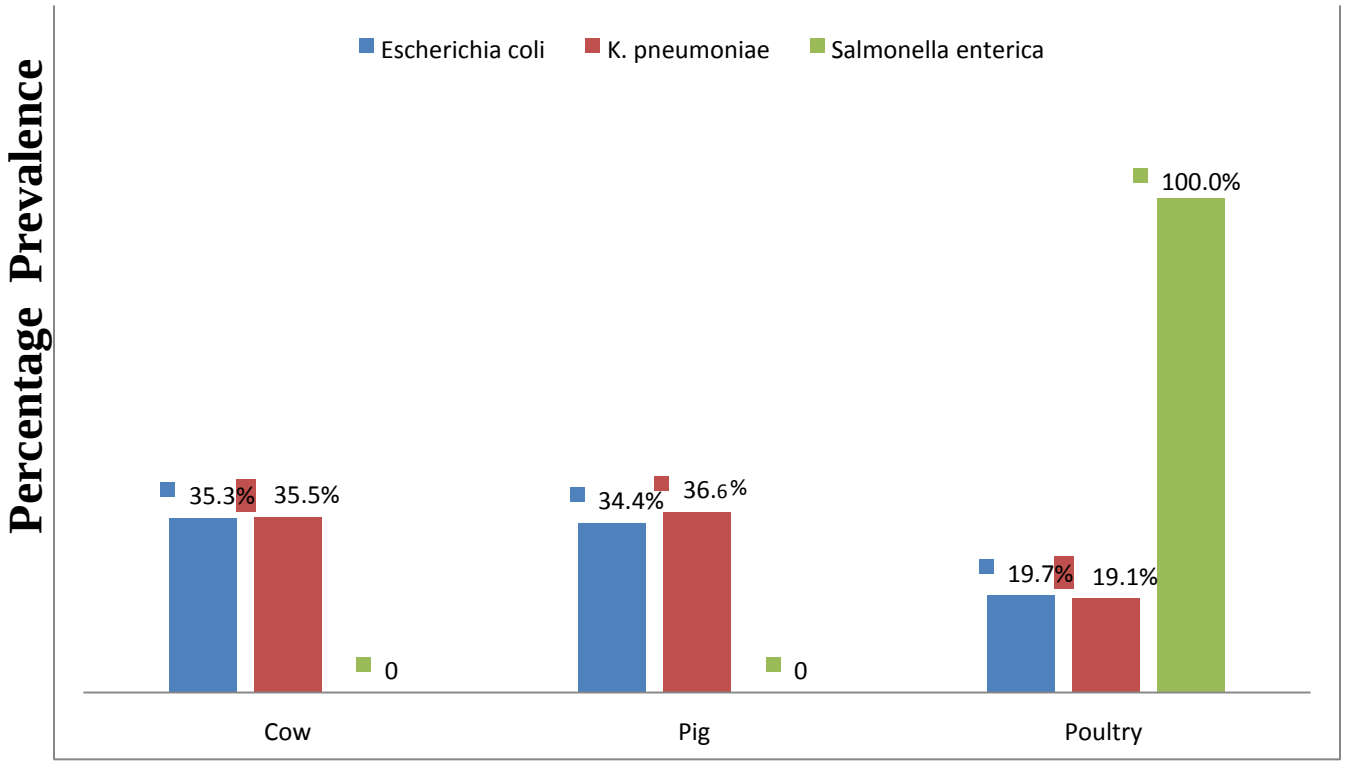

Figure 4.5: Prevalence of *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enterica* isolated from livestock farms and workers in Aba

4.1.8 Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* isolated from livestock farms and workers in Okigwe

E. coli maintained the trend of being the highest isolated bacterial pathogens even in Okigwe where 175 numbers were isolated. *Klebsiella pneumoniae* were significantly prevalent in cow (35.7%; 15/42) and pig (35.7%; 15/42) farms. However, *E. coli* occurred more in poultry farms (20.4%; 19/175) than the other isolates. *Salmonella enterica* were not isolated in all the three farms. The prevalence of *Escherichia coli, Klebsiella* pneumoniae, and *Salmonella* enterica isolated from livestock farms and workers in Okigwe is presented in Table 4.8.

4.1.9 Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* isolated from all the poultry workers in Aba, Umuahia, Mbaise and Okigwe

The poultry workers whose hands were assessed for bacterial contaminants, it was observed that *E. coli* has the highest occurrence in Umuahia (28.8%; 21/73) and Okigwe (15.1%; 11/73). *Klebsiella pneumoniae* were mostly isolated in Aba (40.0%; 16/40) and Mbaise (25.0%; 10/40). In all the four cities, *Salmonella enterica* were not isolated (Table 4.9).

4.1.10 Antibiotic Resistance Profile of *Escherichia coli* from Cow sample in Aba

110 rectal swabs and 100 raw meat (beef) samples of cow were assessed of which 43 and 34 *E. coli* isolates were obtained as well as 35 and 30 *Klebsiella pneumoniae* respectively. From rectal swab, the highest resistance level of *E. coli* was recorded to cefotaxime (74.4%), Amoxicillin with clavulanic acid (74.4%) followed by cefpodoxime (72.1%), ceftazidime (69.8%) and cefotaxime (69.8%) while from raw meat, cefotaxime (76.5%), Amoxicillin with clavulanic acid (76.5%) followed by ceftriaxone (73.5%) and ceftazidime (70.6%) had the highest resistance level of *E. coli*. The least resistance was observed for amikacin (18.6%) and meropenem (25.6%) (from rectal swab) and colistin (11.8) and amikacin (23.5) (from raw meat). In *Klebsiella pneumoniae*, the highest resistance was observed against Amoxicillin with clavulanic acid (88.6%) and cefotaxime (88.6%) followed by cefpodoxime (85.7%) from rectal swab samples while from raw meat samples, *Klebsiella pneumoniae* were resistant to Amoxicillin with clavulanic acid (86.7%) and cefotaxime (86.7%) followed by cefpodoxime (76.7%) produced the highest resistance level. The lowest resistance level was observed against colistin and amikacin with 8.6% and 13.3%; 22.9% and 20.0% for rectal swab and raw meat respectively (Tables 4.4 and 4.5).

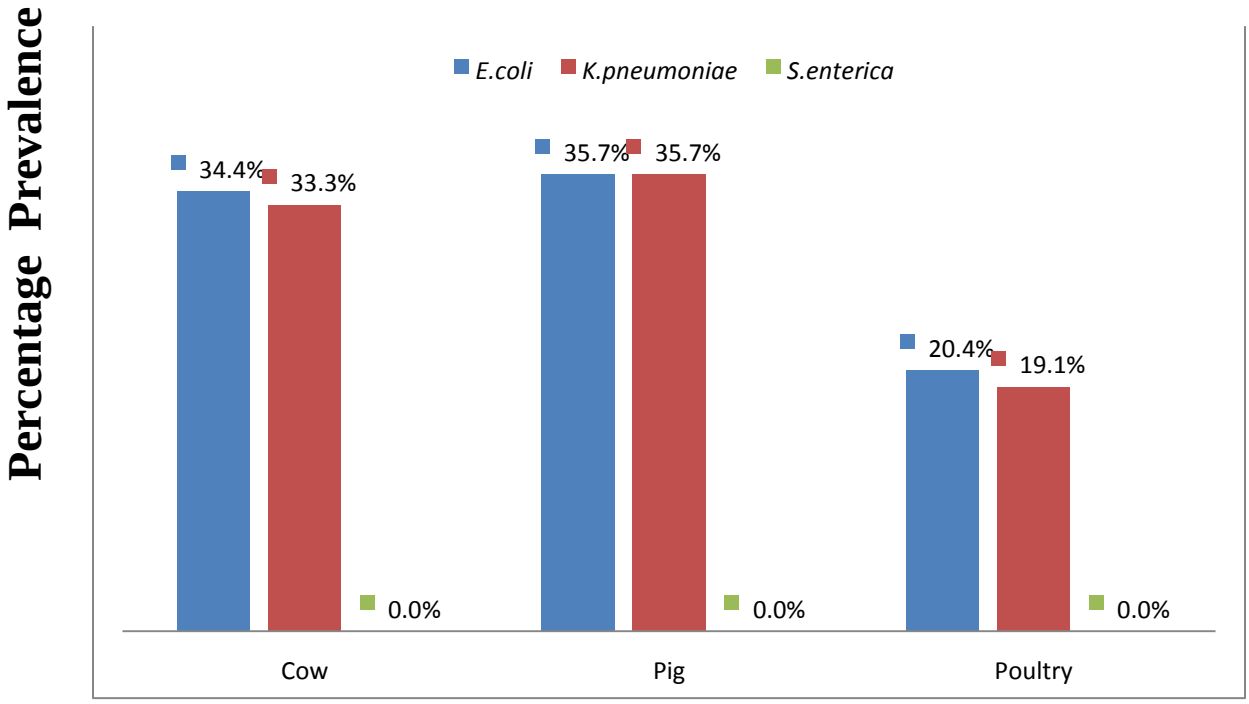

Figure 4.8: Prevalence of *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enterica* Isolated from livestock farms and workers in Okigwe

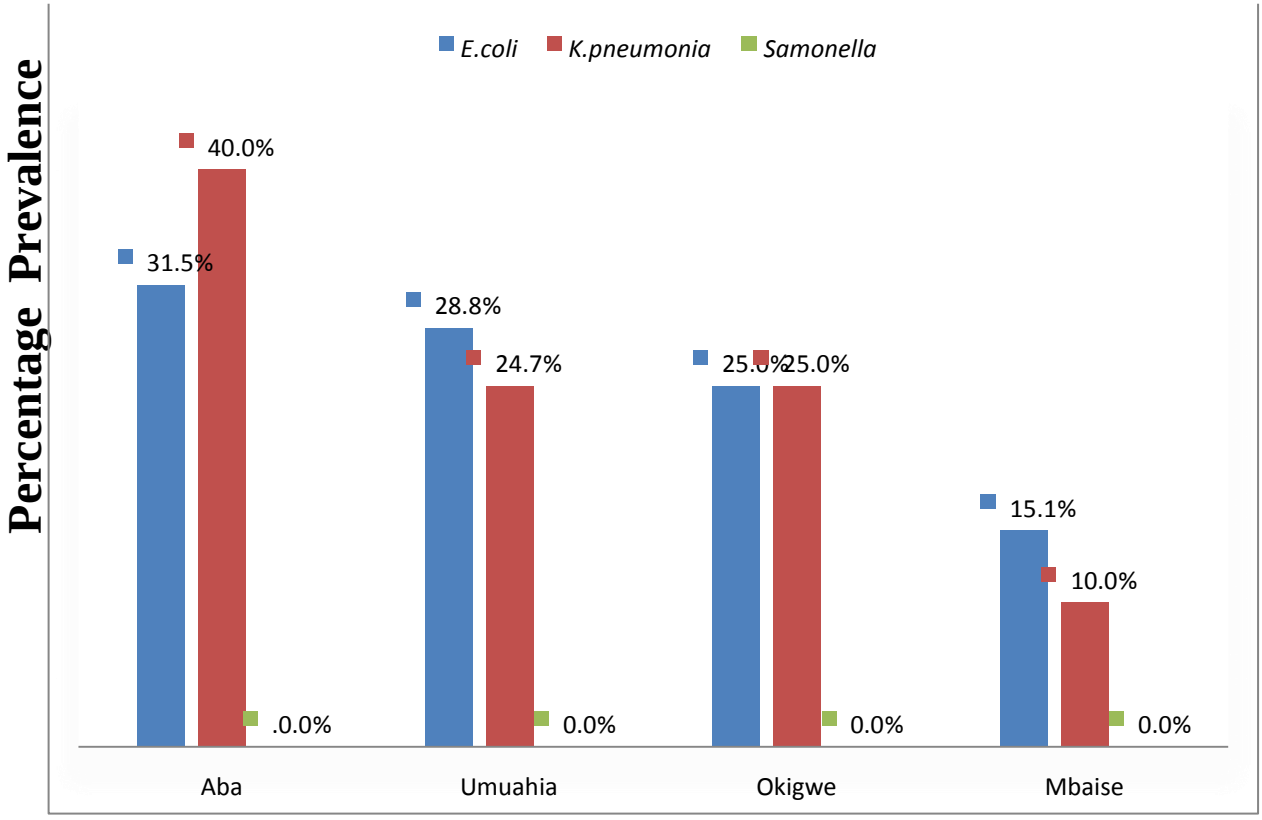

Figure 4.9: Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* isolated from all the poultry workers in Aba, Umuahia, Mbaise and Okigwe

Table 4.4: Antibiotic Resistance Profile of *Escherichia coli* from Cow sample in Aba

Antibiotic	**Feacal sample and rectal swab (n=110)** *Escherichia coli* (n=43) No (%)	**Raw meat (Beef) and intestine (n=100)** *Escherichia coli* (n=34) No (%)	**Total** *Escherichia coli* (n=77) No (%)
CN	13(30.2%)	13(38.2%)	26(33)
CIP	12(27.9%)	10(29.4%)	22(28.6)
AK	8(18.6%)	8(23.5%)	16(20.8)
OB	20(46.5%)	15(44.1%)	35(45.5)
AMC	32(74.4%)	26(76.5%)	58(75.3)
CPD	31(72.1%)	23(67.6%)	54(70.1)
CFP	30(69.8%)	23(67.6%)	53(68.8)
CTX	32(74.4%)	26(76.5%)	58(75.3)
CAZ	30(69.8%)	24(70.6%)	54(70.1)
CTR	30(69.8%)	25(73.5%)	55(71.4)
AZT	25(58.1%)	19(55.9%)	44(57.1)
IMP	12(27.9%)	10(29.4%)	22(28.6)
MER	11(25.6%)	10(29.4%)	21(27.3)
COL	18(9.3%)	4(11.8%)	8(10.4)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

4.1.11 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from cow sample from Aba

110 rectal swabs and 100 raw meat (beef) samples of cow were assessed of which 35 and 30 *Klebsiella pneumoniae* respectively. In *Klebsiella pneumoniae*, the highest resistance was observed against Amoxicillin with clavulanic acid (88.6%) and cefotaxime (88.6%) followed by cefpodoxime (85.7%) from rectal swab samples while from raw meat samples, *Klebsiella pneumoniae* were resistant to Amoxicillin with clavulanic acid (86.7%) and cefotaxime (86.7%) followed by cefpodoxime (76.7%) produced the highest resistance level. The lowest resistance level was observed against colistin and amikacin with 8.6% and 13.3%; 22.9% and 20.0% for rectal swab and raw meat respectively (Table 4.5).

4.1.12 Antibiotic Resistance Profile of *E. coli* from poultry sample in Aba

Of the 50 *E. coli* isolates in faecal swab (FS), 75% (highest) were resistant to AMC (70%) followed by CAZ (68%) while the lowest resistance was against colistin (20%). The values of *E. coli* isolates obtained from cloacal swab (CS) and drinking water (DW) were 20 and 40 respectively. The highest resistance of *E. coli* in CS was against AMC (75%) followed by CAZ (70%) while for DW, the highest was against AMC (72.5%). The lowest resistance of *E. coli* in CS and DW were observed for colistin (15% and 17.5%) respectively. In the study, a total of 20 (FS), 50 (CS) and 30 (DW) isolates of *K. pneumoniae* were isolated. Antibiotic resistant pattern of *E. coli* and K. pneumoniae obtained from faecal samples, cloacal swabs and drinking water of poultry were presented in Table 4.6 and4.7.

4.1.13 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from poultry sample from Aba

Highest resistance of *K. pneumoniae* was observed against AMC (70%) and CTR (70%) in FS while the lowest resistance was observed against colistin (10%). In CS samples, *K. pneumoniae* isolated produced the highest resistance against AMC (70%); while in DW, the isolate has highest resistance for AMC (70%) and CTR (70%). The lowest resistance of *K. pneumoniae* was observed for colistin (8% and 16.7%) in CS and DW samples respectively. Antibiotic resistant pattern of *E. coli* and *K. pneumoniae* obtained from faecal samples, cloacal swabs and drinking water of poultry were presented in Table 4.6 and 4.7.

.

Table 4.5: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from cow sample from Aba

Antibiotic	**Feacal sample and Rectal swab (110)** *K. pneumoniae* (n=35) No (%)	**Raw meat (Beef) and Intestine (n=100)** *K. pneumoniae* (n=30) No (%)	**Total** *K. pneumoniae* (n=65) No (%)
CN	15(42.9)	13(43.3)	28(43.1)
CIP	11(31.4)	9(30.0)	20(30.8)
AK	8(22.9)	6(20.0)	14(21.5)
OB	16(45.7)	14(46.7)	30(46.2)
AMC	31(88.6)	26(86.7)	57(87.7)
CPD	30(85.7)	23(76.7)	53(81.5)
CFP	25(71.4)	22(73.3)	47(72.3)
CTX	31(88.6)	26(86.7)	57(87.7)
CAZ	25(71.4))	22(73.3)	47(72.3)
CTR	25(71.4)	22(73.3)	47(72.3)
AZT	23(65.7)	19(63.3)	42(64.6)
IMP	11(31.4)	10(33.3)	21(32.3)
MER	12(34.3)	11(36.7)	23(35.4)
COL	3(8.6)	4(13.3)	7(10.8)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL=

Colistin**.**

Table 4.6: Antibiotic Resistance Profile of *E. coli* from poultry sample in Aba

	Faeces (n=90)	**Cloacal swab (n=90)**	**Drinking water(n=90)**	**Total**
Antibiotics	*E coli* (n=50) No (%)	*E coli* (n=20) No (%)	*E coli* (n=40) No (%)	*E coli* (n=110) No (%)
CN	27(54)	11(55)	21(52.5)	59(64.9)
CIP	23(46)	9(45)	18(45)	50(55)
AK	26(52)	10(50)	20(50)	56(61.6)
OB	29(58)	12(60)	24(62.5)	65(71.5)
AMC	35(70)	15(75)	29(72.5)	79(86.9)
CPD	32(64)	13(65)	25(62.5)	70(77)
CFP	31(62)	13(65)	24(60)	68(74.8)
CTX	29(58)	12(60)	21(52.5)	62(68.2)
CAZ	34(68)	14(70)	23(57.5)	71(78.1)
CTR	31(62)	13(65)	20(50)	64(70.4)
AZT	24(48)	9(45)	17(42.5)	50(55)
IMP	23(46)	8(40)	16(40)	47(51.7)
MER	21(42)	7(35)	16(40)	44(48.4)
COL	10(20)	3(15)	7(17.5)	20(22)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL=Colistin.

Table 4.7: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from poultry sample from Aba

	Faeces (n=90)	**Cloacal swab (n=90)**	**Drinking water (n=90)**	**Total**
Antibiotics	*K.* pneumoniae (n=20) No (%)	*K. pneumoniae*. (n=50) No (%)	*K. pneumoniae* (n=30) No (%)	*K. pneumoniae* (n=100) No (%)
CN	10(50)	28(56)	14(46.7)	52(52)
CIP	12(60)	29(58)	17(56.7)	58(58)
AK	9(45)	20(40)	13(43.3)	42(42)
OB	10(50)	25(50)	14(46.7)	49(49)
AMC	14(70)	35(70)	21(70)	70(70)
CPD	13(65)	31(62)	19(63.3)	63(63)
CFP	12(60)	30(60)	18(60)	60(60)
CTX	11(55)	29(58)	17(56.7)	57(57)
CAZ	10(50)	25(50)	13(43.3)	48(48)
CTR	14(70)	34(68)	21(70)	69(69)
AZT	12(60)	27(54)	15(50)	54(54)
IMP	9(45)	23(46)	12(40)	44(44)
MER	8(40)	21(42)	11(36.7)	40(40)
COL	2(10)	4(8)	5(16.7)	11(11)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

4.1.14 Antibiotic Resistance Profile of *E. coli* from poultry sample in Aba

A total of 40 isolates of *E. coli* and 38 isolates of *K. pneumoniae* were obtained from 110 samples of rectal swabs of pig. Similar trend occurred for 100 samples of raw meat (pork), where 35 and 67 isolates of *E. coli* and *K. pneumoniae* respectively were obtained. Amongst the antibiotics used for the study, *E. coli* and *K. pneumoniae* produced highest resistance for ceftazidime (77.5%) and AMC (81.6%) respectively from rectal swab followed by CTX (75%) and CTR (78.9%). The lowest resistance of *E. coli* and *K. pneumoniae* was against colistin (5.0% and 7.9%) respectively (Tables 4.8).

4.1.15 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Pig samples in Aba

From the 100 samples of raw meet (pork) assessed, only 35 *E. coli* and 67 *K. pneumoniae* isolates were obtained. *E. coli* produced highest resistance against AMC (77.1%), CTX (77.1%) and CAZ (77.1%) and *K. pneumoniae* highest resistance level was for AMC (82.8%) followed by CPD (72.4%). Lowest resistance was observed in colistin (5.7%) and (6.9%) for *E. coli* and *K. pneumoniae* respectively followed by Amikacin (17.2%) for *K. pneumoniae* (Tables 4.15).

4.1.16 Antibiotic Resistance Profile of *Escherichia coli* from Cow samples in Umuahia

A total of 36 isolates of E. *coli* were isolated from combined faecal sample and rectal swab (FS-RS) and only 31 isolates were obtained from raw meat and intestine (RM-IS). From faecal sample, 75.0% of *E. coli* produced the highest resistance against CTX followed by AMC (72.2%) while 77.4% of the *E. coli* were highly resistance against CTX and AMC, followed by CFP (74.2%) and CAZ (74.2%). In FS-RS; RM-IS, the lowest resistance was observed for colistin (8.3%) and (9.7%) respectively. The antibiotic resistance profile of *E. coli* from cow in Umuahia is presented in Table 4.16.

4.1.17 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Cow sample in Umuahia

A total of 8 and 6 *Klebsiella pneumoniae* isolates were obtained from FS-RS and RM-IS respectively. In the FS-RS of cow, the highest resistance was produced against CTX (87.5%) and AMC (87.5%) followed by CPD (75.0%), CFP (75.0%) and CAZ (75.0%). From RM-IS samples, 83.3% of *Klebsiella pneumoniae* obtained were resistant to AMC and CTX. No *Klebsiella pneumoniae* obtained from FS-RS and RM-IS grew in the presence of COL and also for MER except for FS-RS samples tested against them (Table 4.17).

Table 4.8: Antibiotic Resistance Profile of *Escherichia coli* from Pig sample in Aba

Antibiotic	**Feacal and sample Rectal swab (110)** *E. coli* (n=40) No (%)	**Raw meat (Pork) and Intestine (n=100)** *E. coli* (n=35) No (%)	**Total** *E. coli* (n=75) No (%)
CN	19(47.5)	17(48.6)	36(48)
CIP	12(30)	11(31.4)	23(30.7)
AK	11(27.5)	10(28.6)	21(28)
OB	17(42.5)	16(45.7)	33(44)
AMC	29(72.5)	27(77.1)	56(74.7)
CPD	25(65.5)	21(60)	46(61.3)
CFP	28(70)	25(71.4)	53(70.7)
CTX	30(75)	27(77.1)	57(76)
CAZ	31(77.5)	27(77.1)	58(77.3)
CTR	23(57.5)	21(60)	44(58.7)
AZT	26(65)	24(68.6)	50(66.7)
IMP	5(37.5)	12(34.3)	27(36)
MER	7(35)	12(34.3)	26(34.7)
COL	2(5)	2(5.7)	4(5.3)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.9: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Pig samples in Aba

Faecal sample and Rectal swab (n=110)		**Raw meat(Pork) Total and Intestine (n=100)**	
Antibiotic	*K. pneumoniae* (n=38) No (%)	*K. pneumoniae* (n=29) No (%)	*K.* pneumoniae (n=67) No (%)
CN	17 (44.7)	17(58.6)	34(50.7)
CIP	11(28.9)	8(27.6)	19(28.4)
AK	7(18.4)	5(17.2)	12(17.9)
OB	17(44.7)	14(48.3)	31(46.3)
AMC	31(81.6)	24(82.8)	55(82.1)
CPD	29(76.3)	21(72.4)	50(74.6)
CFP	27(71.1)	20(69.0)	47(70.1)
CTX	27(71.1)	20(69.0)	47(70.1)
CAZ	26(68.4)	18(62.1)	44(65.7)
CTR	30(78.9)	17(58.6)	47(70.1)
AZT	24 (63.4)	12(55.2)	40(59.7)
IMP	13(34.2)	9(31.0)	22(32.8)
MER	12(31.6)	7(24.1)	19(28.4)
COL	3(7.9)	2(6.9)	5(7.5)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL=Colistin**.**

Table 4.16: Antibiotic Resistance Profile of *Escherichia coli* from Cow samples in Umuahia

	Faecal sampleand Rectal swab (n=100)	**Raw meat (Beef) and Intestine (n=100)**	**Total**
Antibiotic	*Escherichia coli* (n=36) No (%)	*Escherichia coli* (n=31) No (%)	*Escherichia coli* (n=67) No (%)
CN	10(27.8)	9(29)	19(28.4)
CIP	9(25.0)	8(25.8)	17(25.4)
AK	7(19.4)	5(16.1)	12(17.9)
OB	17(47.2)	15(48.4)	32(47.8)
AMC	26(72.2)	24(77.4)	50(74.6)
CPD	24(66.7)	22(71.0)	46(68.7)
CFP	25(69.4)	23(74.2)	48(71.6)
CTX	27(75.0)	24(77.4)	51(76.1)
CAZ	24(66.7)	23(74.2)	47(70.1)
CTR	25(69.4)	21(67.7)	46(68.7)
AZT	22(61.1)	20(64.5)	42(62.7)
IMP	10(27.8)	9(29.0)	19(28.4)
MER	10(27.8)	9(29.0)	19(28.4)
COL	3(8.3)	3(9.7)	6(9.0)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.17: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Cow sample in Umuahia

	Faecal sample and Rectal swab (100)	**Raw meat (pig) and Intestine (n=100)**	**Total**
Antibiotics	*K. pneumoniae* (n=8) No (%)	*K. pneumoniae* (n=6) No (%)	*K. pneumoniae* (n=14) No (%)
CN	3(37.5)	2(33.3)	5(35.7)
CIP	2(25.0)	1(16.7)	3(21.4)
AK	1(12.5)	0(0.0)	1(7.1)
OB	5(62.5)	3(50.0)	8(57.1)
AMC	7(87.5)	5(83.3)	12(85.7)
CPD	6(75.0)	4(66.7)	10(71.4)
CFP	6(75.0)	4(66.7)	10(71.4)
CTX	7(87.5)	5(83.3)	12(85.7)
CAZ	6(75.0)	4(66.7)	10(71.4)
CTR	5(62.5)	4(66.7)	9(64.3)
AZT	4(50.0)	3(50.0)	7(50.0)
IMP	2(25.0)	0(0.0)	2(14.3)
MER	2(25.0)	0(0.0)	2(14.3)
COL	0(0.0)	0(0.0)	0(0.0)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

4.1.18 Antibiotic Resistance Profile of *E coli* from poultry sample in Umuahia

From the study, 90 samples each were obtained from FS, CS and DW. Twenty-two isolates of *E. coli* were obtained from FS and out of the total CS assessed, only 20 E. coli were isolated; 10 only were obtained from samples of DW. Briefly, 81.8% of *E. coli* were resistant to CTR, 77.3% of the isolates resisted the activities of CPD and AZT. From cloacal swab, the isolates were resistant to AMC (85.0%), CTR (85.0%), CPD (80.0%) and AZT (80%); the *E. coli* isolated from drinking water produced resistance against AMC (82.7%), CPD (80.0%), CTR (80.0%) and AZT (80.0%). In all the samples, *E. coli* produced lowest resistance against colistin (18.2%), (15%) and (10%) respectively (Tables 4.18).

4.1.19 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from poultry sample from Umuahia

Fourteen *K. pneumoniae* were isolated from FS, 10 isolates were obtained from cloacal swab and 12 isolates were obtained from drinking water. From FS, the highest resistance of *K. pneumoniae* was obtained against CTR (85.7%), followed by AMC (78.6%) and CFP (71.4%). In cloacal swab, the isolates were resistant against CTR (80%) while 80.6% produced the highest resistance in drinking water sample against CTR (80.6%) (Tables 4.19).

4.1.20 Antibiotic Resistance Profile of *Escherichia coli* from Pig sample in Umuahia

A total of 33 of *E. coli* isolates were obtained from 100 faecal samples of pig and rectal swabs (FSRS). A total of 100 samples of raw meat and intestine samples (RM-IS), where 29 of *E. coli* were isolated. Amongst the antibiotics used for the study, *E. coli* had the highest resistance for AMC (87.9%) from FS-RS and RM-IS respectively followed by CTR (78.8%) for FS-RS and CTX (79.3%) and CFP (79.3%). The lowest resistance of *E. coli* was against colistin. From FS-RS samples, 3.0% of *E. coli*were resistant. From RM-IS however, only 10.3% of *E. coli*were resistant (Tables 4.20).

4.1.21 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Pig sample in Umuahia

A total of 9 isolates of *Klebsiella pneumoniae* were obtained from 100 faecal samples of pig and rectal swabs (FS-RS). From a total of 100 samples of raw meat and intestine samples (RM-IS), 7 isolates of *Klebsiella pneumoniae* were isolated. Amongst the antibiotics used for the study, *Klebsiella pneumoniae* produced highest resistance for AMC (87.5%). The lowest resistance of *Klebsiella pneumoniae* was against colistin (12.5%) (Tables 4.21).

Table 4.18: Antibiotic Resistance Profile of *E coli* from poultry sample in Umuahia

	Faeces (n=90)	**Cloacal swab (n=90)**	**Drinking water (n=90)**	**Total**
Antibiotics	*E coli* (n=22) No (%)	*E coli* (n=20) No (%)	*E coli* (n=10) No (%)	*E coli* (n=52) No (%)
CN	12(54.5)	11(55)	5(50)	28(53.8)
CIP	9(40.9)	8(40)	4(40)	21(40.4)
AK	10(45.5)	9(45)	5(50)	24(46.2)
OB	12(54.5)	11(55)	5(50)	28(53.8)
AMC	18(81.8)	17(85)	8(80)	43(82.7)
CPD	17(77.3)	16(80)	8(80)	41(78.8)
CFP	16(72.7)	15(75)	7(70)	38(73.1)
CTX	14(63.6)	13(65)	6(60)	33(63.5)
CAZ	16(72.7)	15(75)	7(70)	38(73.1)
CTR	18(81.8)	17(85)	8(80)	43(82.7)
AZT	17(77.3)	16(80)	8(80)	41(78.8)
IMP	7(31.8)	8(40)	4(40)	19(36.5)
MER	8(36.4)	6(30)	3(30)	17(32.7)
COL	4(18.2)	3(15)	1(10)	8(15.4)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.19: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from poultry sample from Umuahia

	Faeces (90%)	**Cloacal swab (90%)**	**Drinking water (90%)**	**Total**
Antibiotics	*K. pneumoniae* (n=14) No (%)	*K. pneumoniae* (n=10) No (%)	*K. pneumoniae* (n=12) No (%)	*E. coli* (n=36) No (%)
CN	7(50)	5(50)	6(50)	18(50)
CIP	5(35.7)	3(30)	4(33.3)	12(33.3)
AK	5(35.7)	3(30)	4(33.3)	12(33.3)
OB	6(42.9)	6(60)	7(58.3)	19(52.8)
AMC	11(78.6)	7(70)	8(66.7)	26(72.2)
CPD	9(64.3)	6(60)	7(58.3)	22(61.1)
CFP	10(71.4)	7(70)	8(66.7)	25(69.4)
CTX	8(57.1)	5(50)	6(50)	19(52.8)
CAZ	9(64.3)	6(60)	7(58.3)	22(61.1)
CTR	12(85.7)	8(80)	9(75)	29(80.6)
AZT	8(57.1)	5(50)	6(50)	19(52.8)
IMP	4(28.6)	2(20)	3(25)	9(25)
MER	3(21.4)	2(20)	3(25)	8(22.2)
COL	2(14.3)	1(10)	2(16.7)	5(13.9)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.20: Antibiotic Resistance Profile of *Escherichia coli* from Pig sample in Umuahia

	Faecal sample and Rectal swab (100)	**Raw meat (pork) and Intestine (100)**	**Total**
Antibiotics	*E. coli* (n=33) No (%)	*E. coli* (n=29) No (%)	*E. coli (n=62)* No (%)
CN	15(45.5)	12(41.4)	27(43.5)
CIP	11(30.0)	15(51.7)	26(41.9)
AK	9(27.3)	13(44.8)	22(35.5)
OB	15(45.5)	17(58.6)	32(51.6)
AMC	29(87.9)	26(89.7)	55(88.7)
CPD	24(72.7)	22(75.9)	46(74.2)
CFP	26(78.8)	23(79.3)	49(79.0)
CTX	24(72.7)	23(79.3)	47(75.8)
CAZ	20(60.6)	18(62.1)	38(61.3)
CTR	26(78.8)	20(69)	46(74.2)
AZT	23(69.7)	17(58.6)	40(64.5)
IMP	11(33.3)	9(31.0)	20(32.3)
MER	13(39.4)	10(34.5)	23(37.1)
COL	1(3)	3(10.3)	4(6.5)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.21: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Pig sample in Umuahia

Antibiotic	**Faecal sample and Rectal swab (n=100)** *K. pneumoniae* (n=9) No (%)	**Raw meat (pork) and Intestine (n=100)** *K. pneumoniae* (n=7) No (%)	**Total** *K. pneumoniae* (n=16) No (%)
CN	3(33.3)	2(28.6)	5(31.3)
CIP	3(33.3)	3(42.9)	6(37.5)
AK	4(44.4)	3(42.9)	7(43.8)
OB	5(55.6)	5(71.4)	10(62.5)
AMC	8(88.9)	6(85.7)	14(87.5)
CPD	7(77.8)	4(57.1)	11(68.8)
CFP	6(66.7)	5(71.4))	11(68.8)
CTX	7(77.8)	6(71.4)	13(62.5)
CAZ	7(77.8)	4(57.1)	11(68.8
CTR	5(55.6)	4(57.1)	9(56.3)
AZT	4(44.4)	3(42.9)	7(43.8)
IMP	2(22.2)	2(28.6)	4(25.0)
MER	2(22.2)	3(42.9)	5(31.3)
COL	1(11.1)	1(14.3)	2(12.5)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

4.1.22 Antibiotic Resistance Profile of *Escherichia coli* from Cow sample in Mbaise

A total of 40 *E. coli* isolates were isolated from 100 faecal sample and rectal swab (FS-RS). From 100 raw meat and intestine (RM-IS), only 22 isolates were obtained. In FS-RS, the highest resistance of *E. coli* was produced against AMC (80%); followed by CTX (70%) and CPD (70%). The *E. coli* isolated from RM-IS, 86.4% were highly resistant against AMC, followed by CTX (81.8%) and CAZ (77.3%). The lowest resistance of *E. coli* isolated from FS-RS and RM-IS samples were obtained for colistin (10%) and (9.1%) respectively (Tables 4.22).

4.1.23 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from cow sample in Mbaise

A total of 12 isolates of *Klebsiella pneumoniae* were obtained from 100 samples each of both FS-RS and RM-IS. In the FS-RS samples, the highest resistance was produced against AMC (87.5%) and CTX (87.5%) followed by CPD (75.0%), CFP (75.0%) and CAZ (75.0%). From RM-IS samples, 83.3% of *Klebsiella pneumoniae* obtained were resistant to AMC and CTX. No *Klebsiella pneumoniae* obtained from FS-RS and RM-IS grew in the presence of COL except and MER except for FS-RS (Tables 4.23).

4.1.24 Antibiotic Resistance Profile of *E. coli* from poultry sample from Mbaise

A total of 90 samples each of FS, CS and DW, out of which 17, 21 and 12 isolates of *E. coli* were obtained respectively. Briefly, from 17 *E. coli* isolates obtained from FS, however, only 70% were resistant to AMC and AZT while the lowest resistance was against colistin (14.3%). From CS, only 71.4% of *E. coli* isolates produced highest resistance against AMC and AZT while from DW, only 75% produced highest resistance against AMC and AZT. The lowest resistance of *E. coli* in FS, CS and DW were observed for COL (11.8%, 14.3% and 16.7%) respectively (Table 4.24).

4.1.25 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from poultry sample in Mbaise

In the study, a total of 14 (FS), 12 (CS) and 16 (DW) isolates of *Klebsiella pneumoniae* were obtained. Highest resistance of *Klebsiella pneumoniae* was observed against CPD (78.6%) in FS while the lowest resistance was observed against COL (14.3%). In CS samples, *Klebsiella pneumoniae* isolated produced the highest resistance against CPD (83.3%); while in DW, the isolate had the highest resistance for CPD (75%). The lowest resistance of *Klebsiella pneumoniae* was observed for COL (14.3%, 8.3% and 12.5%) in FS, CS and DW samples respectively (Table 4.25).

Table 4.22: Antibiotic Resistance Profile of *Escherichia coli* from Cow sample in Mbaise

Antibiotics	**Faecal sample and Rectal swab (n=100)** *Escherichia coli* (n=40) No (%)	**Raw meat (Beef) and Intestine (n=100)** *Escherichia coli* (n=22) No (%)	**Total** *Escherichia coli* (n=62) No (%)
CN	15(37.5)	7(31.8)	22(35.5)
CIP	12(30.0)	5(22.7)	17(27.4)
AK	8(20.0)	4(18.2)	12(19.4)
OB	18(45.0)	9(40.9)	27(43.5)
AMC	32(80.0)	19(86.4)	51(82.3)
CPD	28(70.0)	16(72.7)	44(71.0)
CFP	26(65.0)	15(68.2)	41(66.1)
CTX	28(70.0)	18(81.8)	46(74.2)
CAZ	26(65.0)	17(77.3)	43(69.4)
CTR	25(62.5)	15(68.2)	40(64.5)
AZT	23(57.5)	14(63.6)	37(59.7)
IMP	12(30.0)	8(36.4)	20(32.3)
MER	11(27.5)	7(31.8)	18(29.0)
COL	4(10)	2(9.1)	6(9.7)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.23: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from cow sample in Mbaise

	Faecal sample and Rectal swab (n=100)	**Raw meat (Beef) and Intestine (n=100)**	**Total**
Antibiotics	*K. pneumoniae* (n=12) No (%)	*K. pneumoniae* (n=12) No (%)	*K. pneumoniae* (n=24) No (%)
CN	4(37.5)	2(33.3)	5(35.7)
CIP	3(25.0)	1(16.7)	3(21.4)
AK	2(12.5)	0(0.0)	1(7.1)
OB	6(62.5)	3(50.0)	8(57.1)
AMC	10(87.5)	5(83.3)	12(85.7)
CPD	6(75.0)	4(66.7)	10(71.4)
CFP	7(75.0)	4(66.7)	10(71.4)
CTX	9(87.5)	5(83.3)	12(85.7)
CAZ	6(75.0)	4(66.7)	10(71.4)
CTR	5(62.5)	4(66.7)	9(64.3)
AZT	4(50.0)	3(50.0)	7(50.0)
IMP	2(25.0)	0(0.0)	2(14.3)
MER	2(25.0)	0(0.0)	2(14.3)
COL	0(0.0)	0(0.0)	0(0.0)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.24: Antibiotic Resistance Profile of *E coli.* from poultry sample from Mbaise

	Faeces (n=90)	**Cloacal swab (n=90)**	**Drinking water(n=90)**	**Total**
Antibiotic	*E coli* (n=17) No (%)	*E coli* (n=21) No (%)	*E coli* (n=12) No (%)	*E coli* (n=50) No (%)
CN	8(47.1)	10(47.6)	6(50)	24(48)
CIP	6(35.3)	8(38.1)	5(41.7)	19(38)
AK	5(29.4)	7(33.3)	4(33.3)	12(32)
OB	7(41.2)	9(42.9)	5(41.7)	36(42)
AMC	12(70.6)	15(71.4)	9(75)	36(72)
CPD	11(64.7)	13(61.9)	8(66.7)	32(64)
CFP	11(64.7)	14(66.7)	8(66.7)	33(66)
CTX	9(52.9)	11(52.4)	7(58.3)	27(54)
CAZ	7(41.2)	9(42.9)	5(41.7)	21(42)
CTR	9(52.9)	10(47.6)	6(50)	25(50)
AZT	12(70.6)	15(71.4)	9(75)	36(72)
IMP	6(35.3)	7(33.3)	4(33.3)	17(34)
MER	5(29.4)	6(28.6)	3(25)	14(28)
COL	2(11.8)	3(14.3)	2(16.7)	7(14)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.25: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from poultry sample in Mbaise

	Faeces (n=90)	**Cloacal swab (n=90)**	**Drinking water (n=90)**	**Total**
Antibiotics	*K. pneumoniae* (n=14) No (%)	*Klebsiella* spp. (n=12) No (%)	*Klebsiella* spp. (n=16) No (%)	*Klebsiella* spp. (n=42) No (%)
CN	6(42.9)	5(41.7)	7(43.8)	18(42.9)
CIP	5(35.7)	4(33.3)	6(37.5)	15(35.7)
AK	4(28.6)	3(25)	5(31.3)	12(28.6)
OB	5(35.7)	4(33.3)	6(37.5)	15(35.7)
AMC	9(64.3)	8(66.7)	10(62.5)	27(64.3)
CPD	11(78.6)	10(83.3)	12(75)	33(78.6)
CFP	9(64.3)	8(66.7)	10(62.5)	27(64.3)
CTX	8(57.1)	7(58.3)	9(56.3)	24(57.1)
CAZ	7(50)	6(50)	8(50)	21(50)
CTR	6(42.9)	5(41.7)	7(43.8)	18(42.9)
AZT	9(64.3)	8(66.7)	10(62.5)	27(64.3)
IMP	4(28.6)	3(25)	5(31.3)	12(28.6)
MER	3(21.4)	2(16.7)	4(25)	9(21.4)
COL	2(14.3)	1(8.3)	2(12.5)	5(11.9)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

4.1.26 Antibiotic Resistance Profile of *Escherichia coli* from Pig sample in Mbaise

A total of 100 samples each of pig faecal sample and rectal swabs (FS-RS) and raw meat and intestine samples (RM-IS) were obtained. Out of which 26 isolates of *E. coli* and 39 isolates of *Klebsiella* spp were obtained. *E. coli* produced highest resistance for CTX (88.5%) followed by AMC (80.8%), CAZ (76.9%), CTR (76.9%) and AZT (76.9%) respectively from FS-RS. However, from RM-IS, 79.5% produced highest resistance against AMC followed by AZT (76.9%), CTX (74.4%) and CFP (71.8%). The lowest resistance was against COL (15.4%) and (15.4%) for FS-RS and RM-IS respectively (Tables 4.26).

4.1.27 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Pig sample in Mbaise

Klebsiella pneumoniae produced highest resistance for AMC (91.7%) and CTX (91.7%) followed by CFP (83.3%), CAZ (75.0%) and CTR (75.0%) from FS-RS samples. From RM-IS samples, 93.8% *Klebsiella pneumoniae* produced the highest resistance against CTX (93.8%) followed by CFP (75.0%). The lowest resistance of *Klebsiella pneumoniae* was against COL (16.7%) and (18.8%) respectively for FS-RS and RM-IS samples (Tables 4.27).

4.1.28 Antibiotic Resistance Profile of *Escherichia coli* from Cow sample in Okigwe

A total of 19 *E. coli* isolates were isolated from 100 faecal sample and rectal swab (FS-RS). From 100 raw meat and intestine (RM-IS), only 13 isolates were obtained. In FS-RS, the highest resistance of *E. coli* was produced against CTX (75%); followed by AMC (72.2%). The *E. coli* isolated from RM-IS, 77.4% were highly resistant against AMC followed by CAZ (74.2%) and CFP (74.2%). The lowest resistance of *E. coli* isolated from FS-RS and RM-IS samples were obtained for COL (8.3%) and (9.7%) respectively (Tables 4.28).

4.1.29 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from cow sample in Okigwe

A total of 8 and 7 isolates of *Klebsiellapneumoniae* were obtained from 100 samples each of both FSRS and RM-IS. In the FS-RS samples, the highest resistance was produced against CTR (87.5%) followed by OB (75.0%), CTX (75.0%) and AZT (75.0%). From RM-IS samples, 85.7% of *Klebsiella pneumoniae* obtained were resistant to CTR followed by OB (71.4%), CTX (71.4%) and AZT (71.4%). *Klebsiella pneumoniae* produced lowest resistance for COL (12.5%) and (14.3%) respectively obtained from FS-RS and RM-IS (Tables 4.29).

Table 4.26: Antibiotic Resistance Profile of *Escherichia coli* from Pig sample in Mbaise

Antibiotics	**Faecal sample and Rectal swab (n=100)** *E. coli* (n=26) No (%)	**Raw meat (pork) and Intestine (n=100)** *E. coli* (n=39) No (%)	**Total** *E. coli* (n=65) No (%)
CN	13(50)	20(51.3)	33(50.8)
CIP	10(38.5)	17(43.6)	27(41.5)
AK	9(34.6)	14(35.9)	23(35.4)
OB	15(57.7)	22(56.4)	37(56.9)
AMC	21(80.8)	31(79.5)	52(80.0)
CPD	19(73.1)	23(59.0)	42(64.6)
CFP	15(57.7)	28(71.8)	43(66.2)
CTX	23(88.5)	29(74.4)	52(80.0)
CAZ	20(76.9)	19(48.7)	39(60.0)
CTR	20(76.9)	27(69.2)	47(72.3)
AZT	20(76.9)	30(76.9)	50(76.9)
IMP	9(34.6)	12(30.8)	21(32.3)
MER	7(26.9)	13(33.3)	20(30.8)
COL	4(15.4)	6(15.4)	10(15.4)

Key:CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB= Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.27: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Pig sample in Mbaise

Antibiotic	**Faecal sample and Rectal swab (n=100)** *K. pneumoniae* (n=12) No (%)	**Raw meat (pork) and Intestine (n=100)** *K. pneumoniae* (n=16) No (%)	**Total** *K. pneumoniae* (n=28) No (%)
CN	6(50.0)	8(50.0)	14(50.0)
CIP	5(41.7)	7(43.8)	12(42.9)
AK	5(41.7)	7(43.8)	12(42.9)
OB	6(50.0)	10(62.5)	16(57.1)
AMC	11(91.7)	13(68.8)	24(85.7)
CPD	7(58.3)	11(68.8)	18(64.3)
CFP	10(83.3)	12(75.0)	22(78.6)
CTX	11(91.7)	15(93.8)	26(92.9)
CAZ	9(75.0)	10(62.5)	19(67.9)
CTR	9(75.0)	9(56.3)	15(53.6)
AZT	4(33.3)	7(43.8)	11(39.3)
IMP	3(25)	7(31.3)	8(28.6)
MER	4(33.3)	6(37.5)	10(35.7)
COL	2(16.7)	3(18.8)	5(17.9)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB= Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.28: Antibiotic Resistance Profile of *Escherichia coli* from Cow sample in Okigwe

Antibiotics	**Faecal sample and Rectal swab (n=100)** *Escherichia coli* (n=19) No (%)	**Raw meat (Beef) and Intestine (n=100)** *Escherichia coli* (n=13) No (%)	**Total** *Escherichia coli* (n=32) No (%)
CN	5(26.3)	9(29)	19(28.4)
CIP	4(21.1)	8(25.8)	17(25.4)
AK	7(19.4)	5(16.1)	12(17.9)
OB	17(47.2)	15(48.4)	32(47.8)
AMC	26(72.2)	24(77.4)	50(74.6)
CPD	24(66.7)	22(71.0)	46(68.7)
CFP	25(69.4)	23(74.2)	48(71.6)
CTX	27(75.0)	24(77.4)	51(76.1)
CAZ	24(66.7)	23(74.2)	47(70.1)
CTR	25(69.4)	21(67.7)	46(68.7)
AZT	22(61.1)	20(64.5)	42(62.7)
IMP	10(27.8)	9(29.0)	19(28.4)
MER	10(27.8)	9(29.0)	19(28.4)
COL	3(8.3)	3(9.7)	6(9.0)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.29: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from cow sample in Okigwe

Antibiotics	**Faecal sample and Rectal swab (n=100)** *K. pneumoniae* (n=8) No (%)	**Raw meat (Pork) and Intestine (n=100)** *K. pneumoniae* (n=7) No (%)	**Total** *K.* pneumoniae (n=15) No (%)
CN	4(50)	3(42.9)	7(46.7)
CIP	5(62.5)	4(57.1)	9(60)
AK	4(50)	3(42.9)	7(46.7)
OB	6(75)	5(71.4)	11(73.3)
AMC	4(50)	3(42.9)	7(46.7)
CPD	5(62.5)	4(57.1)	9(60)
CFP	3(37.5)	3(42.9)	6(40)
CTX	6(75)	5(71.4)	11(73.3)
CAZ	4(50)	3(42.9)	7(46.7)
CTR	7(87.5)	6(85.7)	13(86.7)
AZT	6(75)	5(71.4)	11(73.3)
IMP	3(37.5)	3(42.9)	6(40)
MER	4(50)	4(57.1)	8(53.3)
COL	1(12.5)	1(14.3)	2(13.3)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

4.1.30 Antibiotic Resistance Profile of *E. coli* from poultry sample in Okigwe

A total of 90 samples each of FS, CS and DW, out of which 18, 15 and 10 isolates of *E. coli* were obtained respectively. Briefly, from 18 *E. coli* isolates obtained from FS, however, 88.9 % were resistant to AMC followed by AZT (83.3%) followed by CFP (77.8%) and CTX (72.2%) while the lowest resistance was against COL (16.7%). From CS, 86.7% of *E. coli* isolates produced highest resistance against AMC and AZT followed by CFP (73.3%) and CTX (73.3%) while from DW, only 75% produced highest resistance against AMC and AZT. The lowest resistance of *E. coli* in CS and DW were observed for COL (13.3% and 10%) respectively (Table 4.30).

4.1.31 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from poultry sample in Okigwe

In the study, a total of 11 (FS), 10 (CS) and 9 (DW) isolates of *Klebsiella pneumoniae* were obtained. Highest resistance of *Klebsiella pneumoniae* was observed against AMC (81.8%) and AZT (81.8%) in FS while the lowest resistance was observed against COL (9.1%). In CS samples, *Klebsiella pneumoniae* isolated produced the highest resistance against AMC (80%) and AZT (80%); while in DW, the isolate had the highest resistance for AMC (77.8%) and AZT (77.8%). The lowest resistance of *Klebsiella pneumoniae* was observed for COL (10% and 11.1%) in CS and DW samples respectively. The antibiotic resistance profile of *E. coli* and K*lebsiella pneumoniae* obtained from faecal samples, cloacal swabs and drinking water of poultry were presented in Table 4.31.

4.1.32 Antibiotic Resistance Profile of *Escherichia coli* from Pig sample in Okigwe

A total of 100 samples each of pig faecal sample and rectal swabs (FS-RS) and raw meat and intestine samples (RM-IS) were obtained. Out of which 12 isolates of *E. coli* and 19 isolates of *Klebsiella pneumoniae* were obtained. *E. coli* produced highest resistance for CAZ (83.3%) followed by AK (75.0%) and CPD (75.0%) respectively from FS-RS. However, from RM-IS, *E. coli* produced 94.7% resistance against AK and CPD followed by AZT (89.5%) and CAZ (84.2%). The lowest resistance was against COL (25.0%) and (12.5%) for FS-RS and RM-IS respectively (Tables 4.32).

4.1.33 Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Pig sample in Okigwe

Klebsiella pneumoniae produced highest resistance for CTR (87.5%) from FS-RS samples. From RMIS samples, 85.7% *Klebsiella pneumoniae* produced the highest resistance against CTX. The lowest resistance of *Klebsiella pneumoniae* was against COL (12.5%) and (14.3%) respectively for FS-RS and RM-IS samples (Tables 4.33).

Table 4.30: Antibiotic Resistance Profile of *E. coli* from poultry sample in Okigwe

	Faeces (90)	**Cloacal swab (90)**	**Drinking water (n=90)**	**Total**
Antibiotics	*E coli* (n=18) No (%)	*E coli* (n=15) No (%)	*E coli* (n=10) No (%)	*E coli.* (n=38) No (%)
CN	11(61.1)	10(66.7)	6(60)	27(71.1)
CIP	8(44.4)	7(46.7)	4(40)	19(50)
AK	7(38.9)	6(40)	4(40)	17(44.7)
OB	10(55.6)	9(60)	6(60)	25(65.8)
AMC	16(88.9)	13(86.7)	8(80)	37(97.4)
CPD	12(66.7)	10(66.7)	6(60)	28(73.7)
CFP	14(77.8)	11(73.3)	7(70)	32(84.2)
CTX	13(72.2)	11(73.3)	7(70)	31(81.6)
CAZ	10(55.6)	9(60)	8(60)	25(65.8)
CTR	12(66.7)	10(66.7)	6(60)	28(73.7)
AZT	15(83.3)	13(86.7)	8(80)	36(94.7)
IMP	6(33.3)	5(33.3)	3(30)	14(36.8)
MER	5(27.8)	4(26.7)	2(20)	11(28.9)
COL	3(16.7)	2(13.3)	1(10)	6(15.8)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.31: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from poultry sample in Okigwe

	Faeces (n=90)	**Cloacal swab (n=90)**	**Drinking water (n=90)**	**Total**
Antibiotics	*K. pneumoniae* (n=11) No (%)	*K. pneumoniae* (n=10) No (%)	*K. pneumoniae* (n=9) No (%)	*K. pneumoniae* (n=30) No (%)
CN	5(45.5)	4(40)	3(33.3)	12(40)
CIP	4(36.4)	3(30)	2(22.2)	9(30)
AK	3(27.3)	2(20)	2(22.2)	7(23.3)
OB	6(54.5)	5(50)	4(44.4)	15(50)
AMC	9(81.8)	8(80)	7(77.8)	24(80)
CPD	7(63.6)	6(60)	5(55.6)	18(60)
CFP	8(72.7)	7(70)	6(66.7)	21(70)
CTX	6(54.5)	5(50)	4(44.4)	15(50)
CAZ	8(72.7)	7(70)	6(66.7)	21(70)
CTR	7(63.6)	6(60)	5(55.6)	18(60)
AZT	9(81.8)	8(80)	7(77.8)	24(80)
IMP	4(36.4)	3(30)	2(22.2)	9(30)
MER	3(27.3)	2(20)	2(22.2)	7(23.3)
COL	1(9.1)	1(10)	1(11.1)	3(10)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.32: Antibiotic Resistance Profile of *Escherichia coli* from Pig sample in Okigwe

Antibiotics	**Faecal sample and Rectal swab (n=100)** *E. coli* (n=12)	**Raw meat (Pork) and Intestine (n=100)** *E. coli* (n=19)	**Total** *E. coli* (n=31)
CN	6(50)	12(63.2)	18(58.1)
CIP	6(50)	15(78.9)	21(67.7)
AK	9(75)	18(94.7)	27(87.1)
OB	6(50)	10(52.6)	16(51.6)
AMC	5(41.7)	11(57.9)	16(51.6)
CPD	9(75)	18(94.7)	27(87.1)
CFP	7(58.3)	14(73.7)	21(67.7)
CTX	6(50)	11(57.9)	17(54.8)
CAZ	10(83.3)	16(84.2)	26(83.9)
CTR	7(58.3)	14(73.7)	21(67.7)
AZT	9(75)	17(89.5)	26(83.9)
IMP	5(41.7)	7(36.8)	7(38.7)
MER	4(33.3)	5(26.3)	9(29)
COL	3(25)	2(10.5)	5(16.1)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

Table 4.33: Antibiotic Resistance Profile of *Klebsiella pneumoniae* from Pig sample in Okigwe

Antibiotics	**Faecal sample and Rectal swab (n=100)** *K. pneumoniae* (n=8)	**Raw meat (Pork) and Intestine (n=100)** *K. pneumoniae* (n=7)	**Total** *K. pneumoniae* (n=15)
CN	4(50)	3(42.9)	7(46.7)
CIP	5(62.5)	4(57.1)	9(60)
AK	4(50)	3(42.9)	7(46.7)
OB	6(75)	5(71.4)	11(73.3)
AMC	4(50)	3(42.9)	7(46.7)
CPD	5(62.5)	4(57.1)	9(60)
CFP	3(37.5)	3(42.9)	6(40)
CTX	6(75)	5(71.4)	11(73.3)
CAZ	4(50)	3(42.9)	7(46.7)
CTR	7(87.5)	6(85.7)	13(86.7)
AZT	6(75)	5(71.4)	11(73.3)
IMP	3(37.5)	3(42.9)	6(40)
MER	4(50)	4(57.1)	8(53.3)
COL	1(12.5)	1(14.3)	2(13.3)

Key: CN= Gentamicin; CIP= Ciprofloxacin; AK=Amikacin; OB = Cloxacillin; AMC= Amoxicillinclavulanic acid; CPD= Cefpodoxime; CRO= Ceftriaxone; CTX= Cefotaxime; CAZ= Ceftazidime; CFP= Cefepime; AZT= Aztreonam; MER= Meropenem; IMP= Imipenem; COL= Colistin.

4.1.34 Distribution of ESBLs in *Escherichiacoli* and *Klebsiellapneumoniae* obtained from cow abattoir.

Among all the farms in all the selected states, the highest ESBL production was seen in poultry (47.3%; 54/114) followed by pig (30.5%; 69/226) and lowest in cow (24.3%; 55/226) farms. Between the two bacterial isolates, the highest ESBL was observed more in *K. pneumoniae* (50.0%; 92/184) than *E. coli* (31.0%; 86/277). The distribution of ESBLs producers at the three farm sites is presented in Figures 1, 2 and 3

4.1.35The genotypic characterization of the bacterial isolates

Out of the 32 sample-coded bacterial isolates phenotypically identified, 6 isolates were genotypically confirmed each to be *E. coli* and *K. pneumoniae*; 3 were *E. cloaca* and 2 were *S. enterica*. Only 1 isolate was confirmed *P. mirabilis*. The non-identified strains were 14 in number (Table 4.34). The obtained 16s rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolate W1 showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Proteus, Klebsiella, E coli* and *Salmonella* sp and revealed a closely relatedness to *Proteus mirabilis, Klebsiella pneumoniae, Enterobacter cloacae* and *Salmonella enterica* (Figure 4).

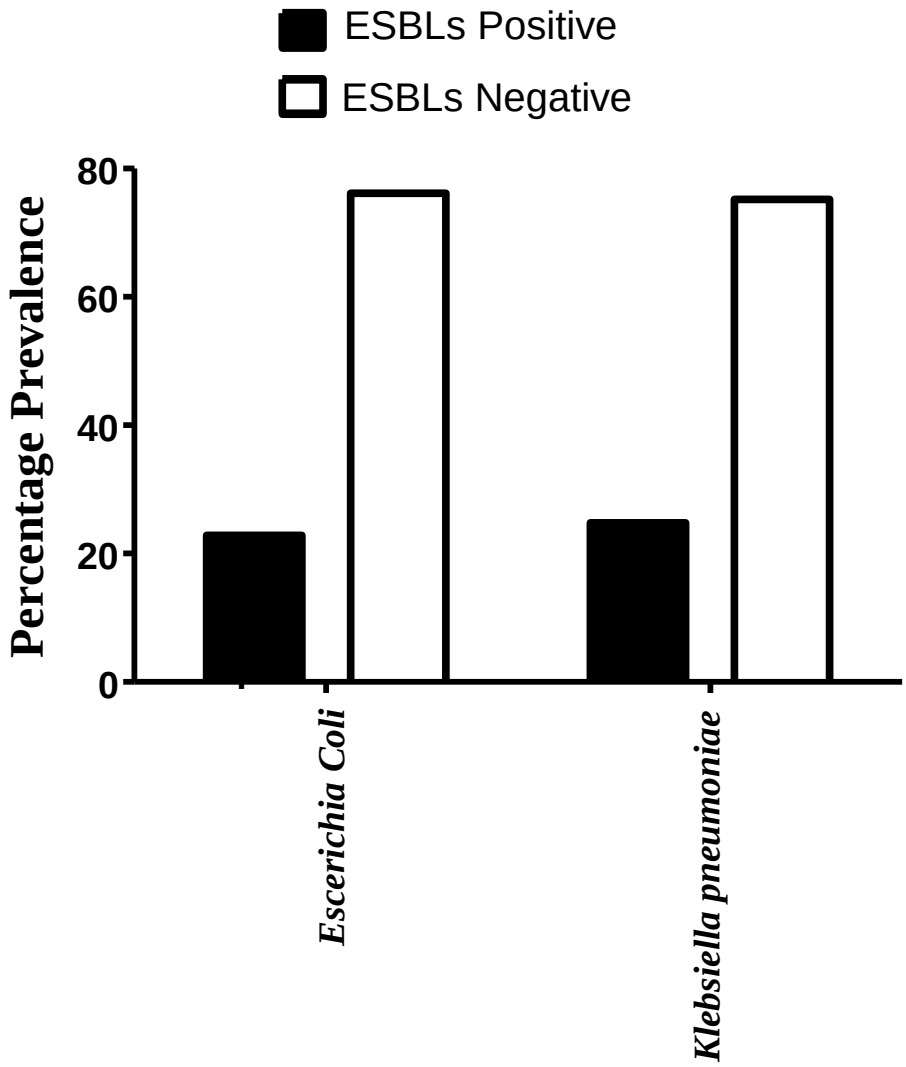

Figure 4.10: Distribution of ESBLs in *Escherichiacoli* and *Klebsiellapneumoniae* obtained from cow abattoir.

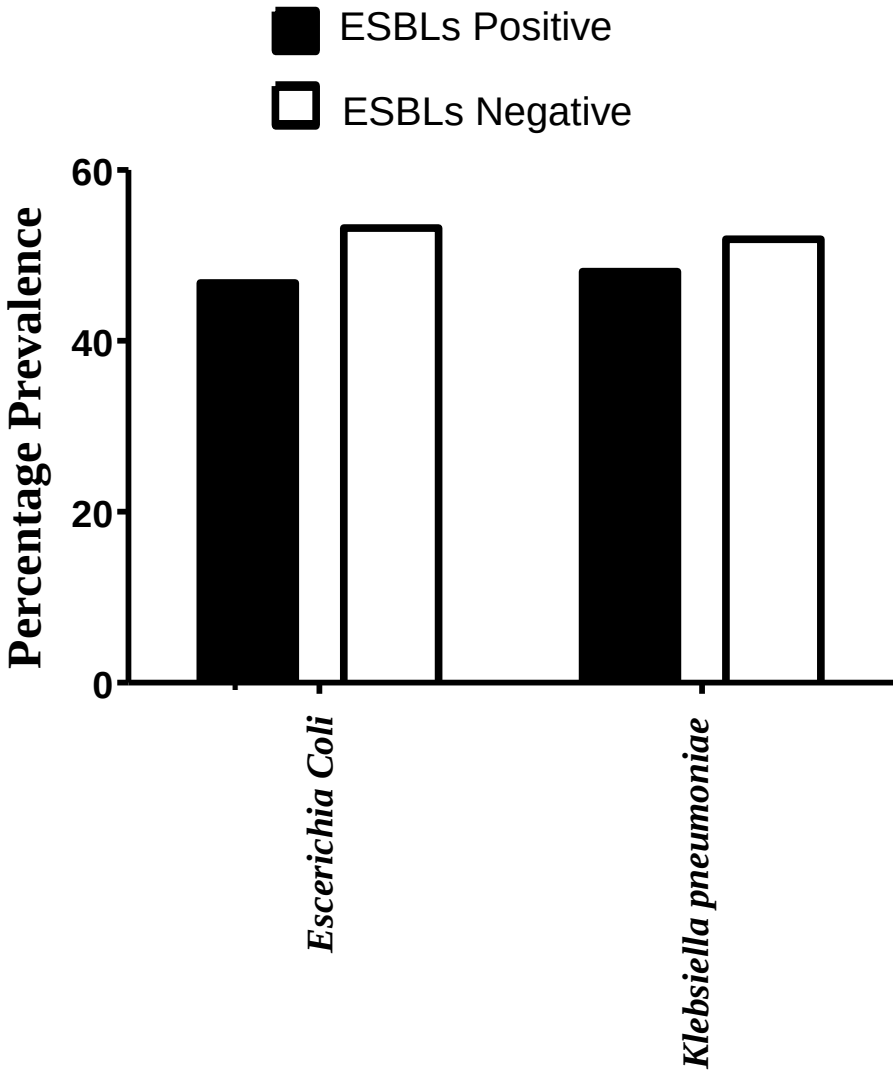

Figure 4.11: Distribution of ESBLs in *Escherichiacoli* and *Klebsiellapneumoniae* obtained from poultry farms

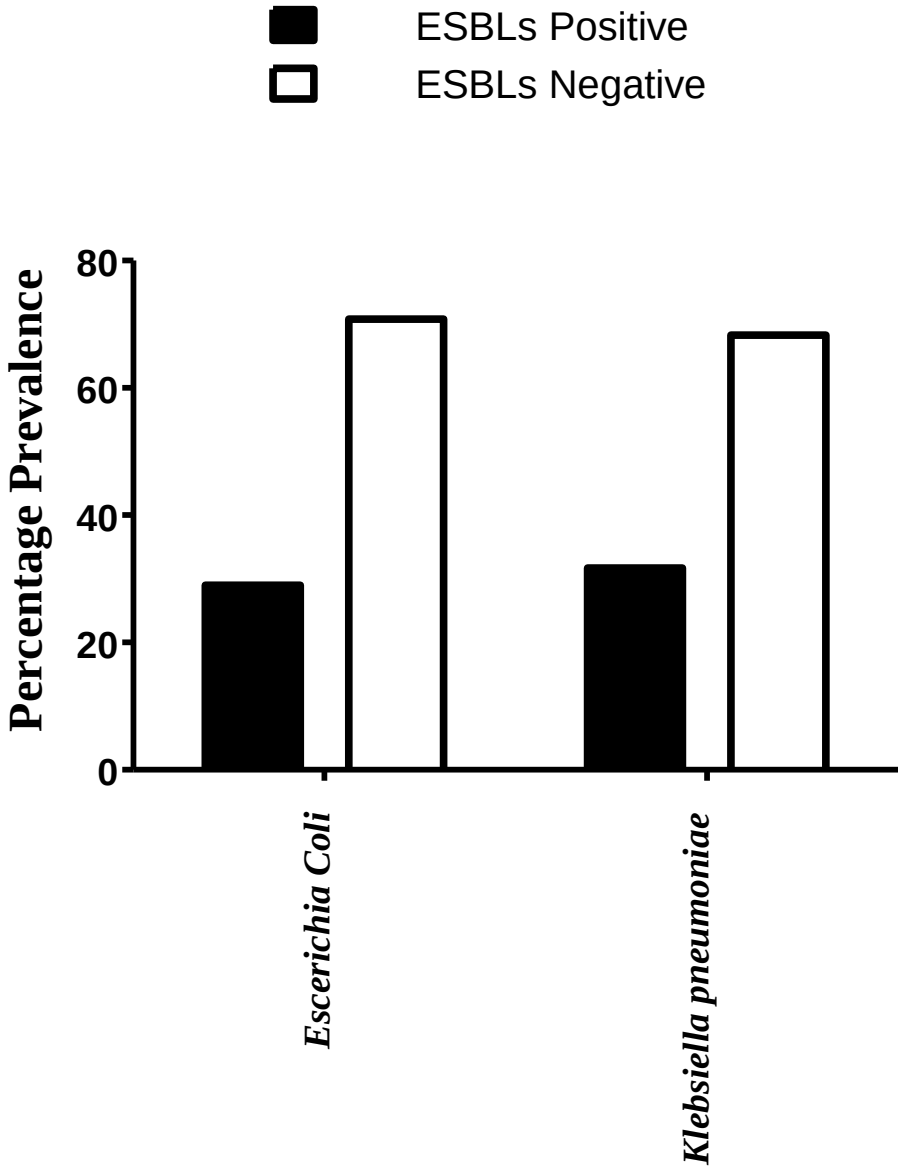

Figure 4.12: Distribution of ESBLs in *Escherichiacoli* and *Klebsiellapneumoniae* obtained from pig farms

Table 4.34: The genotypic characterization of the bacterial isolates

S/N	Sample code	Lab code	CTX-M	QnrA	TEM	NDM	KPC	QnrB	SHV	16 RAN gene	Phenotypic characterization	Genotypic characterization
1	E2	N1	-	-	-	-	+	-	-	+	*Escherichiacoli*	*Escherichiacoli*
2	E5	N2	-	-	-	-	+	-	-	+	*Escherichiacoli*	ND
3	E7	N3	-	-	-	-	-	-	-	+	*Escherichiacoli*	ND
4	E8	N4	-	-	-	-	-	-	-	+	*Escherichiacoli*	*Escherichiacoli*
5	E9	N5	-	-	-	-	-	-	-	+	*Escherichiacoli*	ND
6	E11	N6	-	-	+	-	+	-	-	+	*Escherichiacoli*	*Escherichiacoli*
7	E17	N7	+	-	-	-	+	-	-	+	*Escherichiacoli*	ND
8	E18	N8	+	-	+	-	+	-	-	+	*Escherichiacoli*	*Escherichiacoli*
9	E19	N9	+	-	+	-	-	-	-	+	*Escherichiacoli*	ND
10	E21	N10	-	-	-	+	+	+	-	+	*Escherichiacoli*	*Escherichiacoli*
11	E27	N11	-	-	+	-	+	-	-	+	*Escherichiacoli*	ND
12	E28	N12	-	-	-	-	-	-	-	+	*Escherichiacoli*	*Enterobacter cloacae*
13	E30	N13	-	-	-	-	-	-	-	+	*Escherichiacoli*	*Enterobacter cloacae*
14	E32	N14	-	-	-	-	-	-	-	+	*Escherichiacoli*	ND
15	K1	N15	-	-	+	-	-	-	-	+	*Klebsiella pneumoniae*	*Klebsiella pneumonia*
16	K3	N16	-	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	*Klebsiella pneumonia*
17	K4	N17	+	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	ND
18	K10	N18	+	-	-	-	+	-	-	+	*Klebsiella pneumoniae*	ND
19	K12	N19	+	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	*Klebsiella pneumonia*
20	K13	N20	+	-	-	-	+	-	-	+	*Klebsiella pneumoniae*	ND
21	K16	N21	+	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	*Klebsiella pneumonia*
22	K20	N22	+	-	-	-	+	-	-	+	*Klebsiella pneumoniae*	ND
23	K22	N23	-	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	*Klebsiella pneumonia*

24	K23	N24	-	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	ND
25	K24	N25	-	+	-	-	+	-	-	+	*Klebsiella pneumoniae*	*Klebsiella pneumonia*
26	K25	N26	-	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	*Klebsiella pneumonia*
27	K26	N27	-	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	ND
28	S6	N28	-	-	-	-	+	-	-	+	*Salmonella enterica*	*Salmonella enterica*
29	S1	N29	-	-	-	-	-	-	-	+	*Salmonella enterica*	*Salmonella enterica*
30	S15	N30	-	-	-	-	-	-	-	+	*Salmonella enterica*	*Escherichiacoli*
31	S29	N31	-	-	-	-	-	-	-	+	*Salmonella enterica*	*Enterobacter cloacae*
32	S31	N32	-	-	-	-	-	-	-	+	*Salmonella enterica*	*Proteusmirabilis*

Key: ND =Not Determined

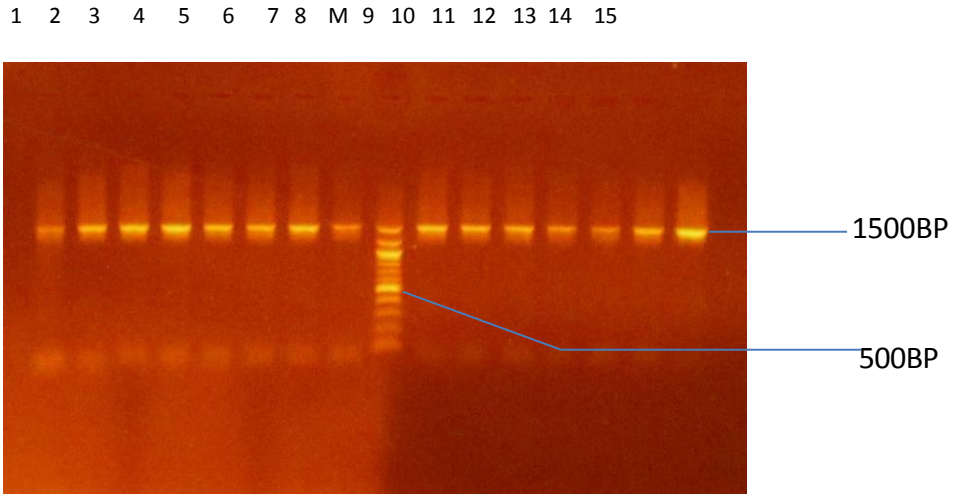

Figure 4.13: Agarose gel electrophoresis of some selected bacterial isolates. Lane 1 – 15 represents 16SrRNA gene bands (1500bp). Lane M represents the 100bp Molecular ladder.

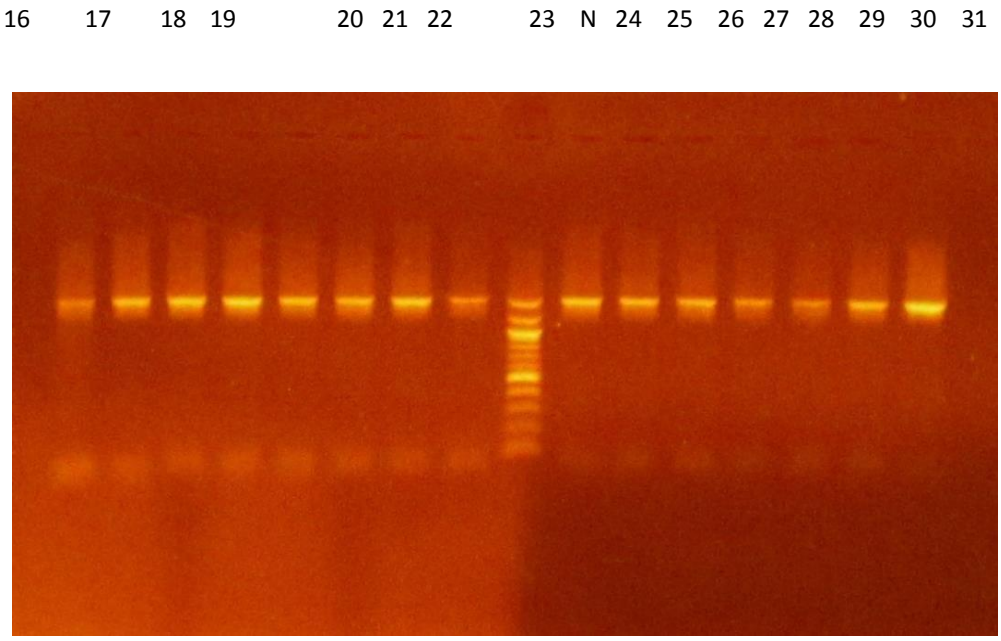

Figure 4.14: Agarose gel electrophoresis of some selected bacterial isolates. Lane 16 – 30 represents 16SrRNA gene bands (1500bp). Lane M represents the 100bp Molecular ladder.

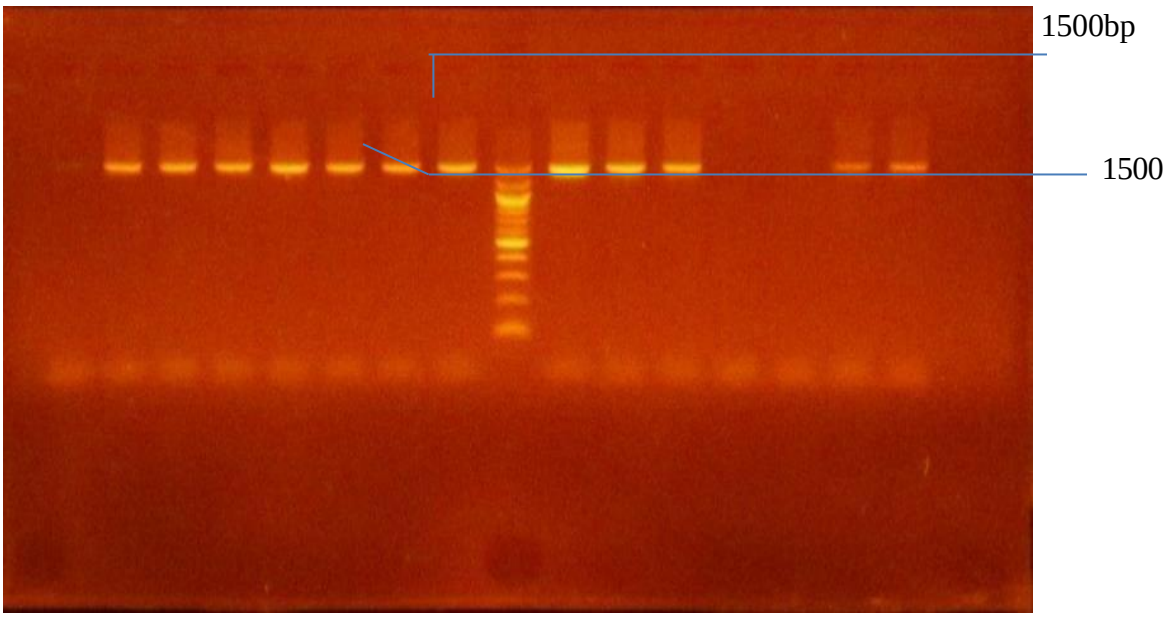

Figure 4.15: Agarose gel electrophoresis of some selected bacterial isolates: Lane 16,17,18,19,20,21,22,23,24,25,26,29and30represents16SrRNAgenebands(1500bp).LaneN represents the 100bp Molecular ladder

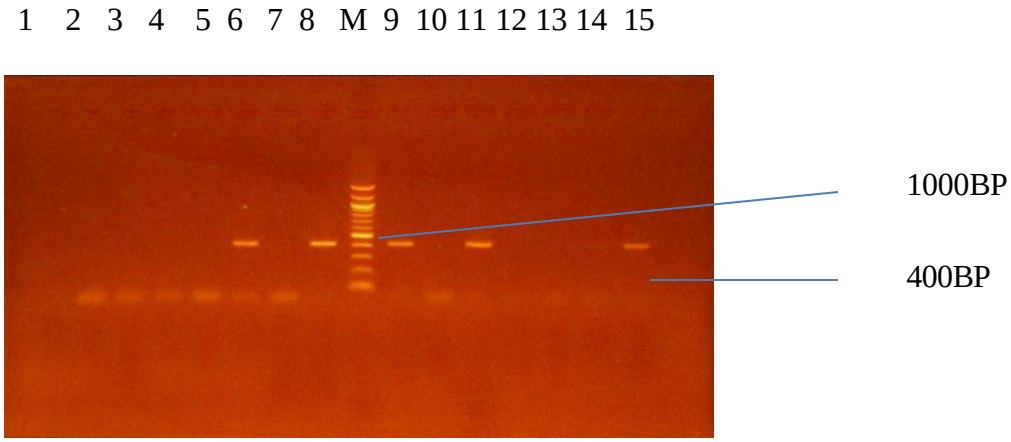

Figure 4.16: Showing Agarose gel electrophoresis of BlaTEM gene of some selected bacterial isolates. Lane 6, 8, 9, 11 and 15 represents the BlaTEM gene bands (400bp). Lane M represents the 100bp Molecular ladder of 1500bp.

1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15 16

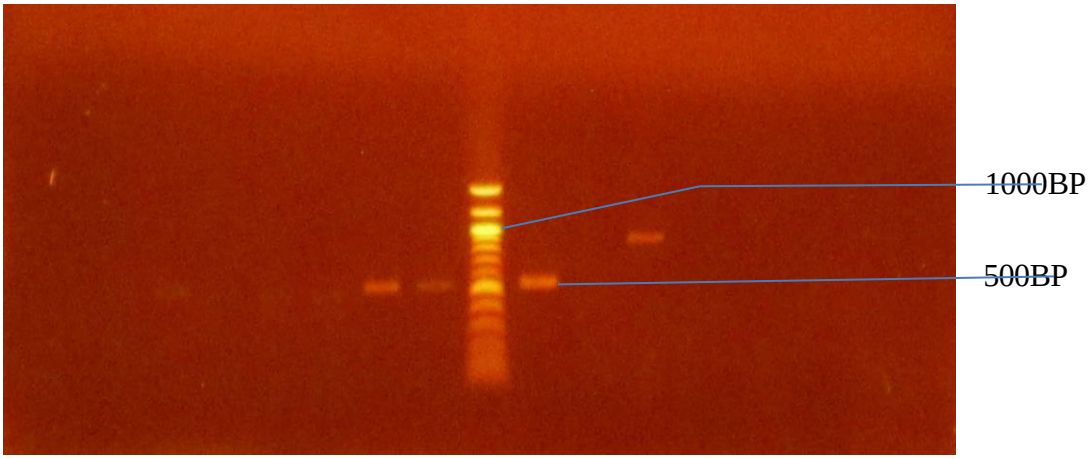

Figure 4.17: Showing agarose gel electrophoresis of some selected bacterial isolates. Lane 3, 5- 9represent CTX-Mgenebands (500bp). While 11 is a spurious amplification. LaneM represents the 100bp Molecular ladder.

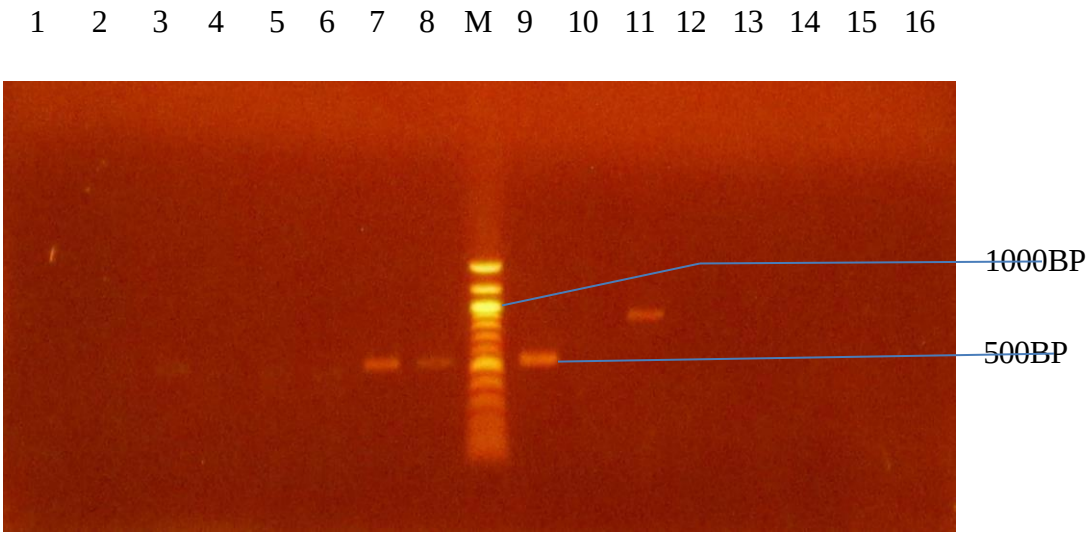

Figure4.18:Showingagarosegelelectrophoresisofsomeselected bacterialisolates.Lane9and10 represent NDM gene bands (550bp). Lane M represents the 100bp Molecular ladder.

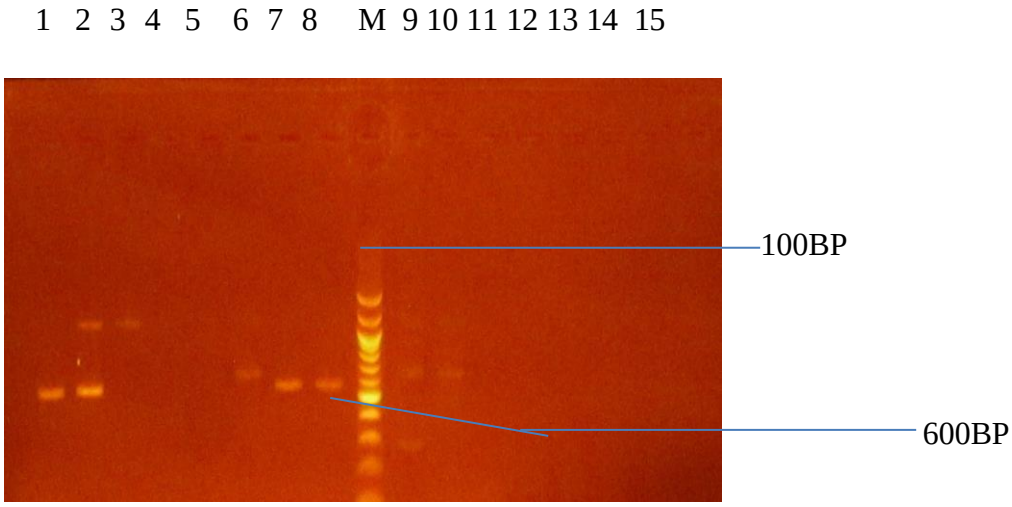

Figure 4.19: Agarose gel electrophoresis of KPC gene of some selected bacterial isolates. Lane 1, 2, 7 and 8 represent the KPC gene band (600bp). Lane M represents the 100bp Molecular ladder of 1500bp.

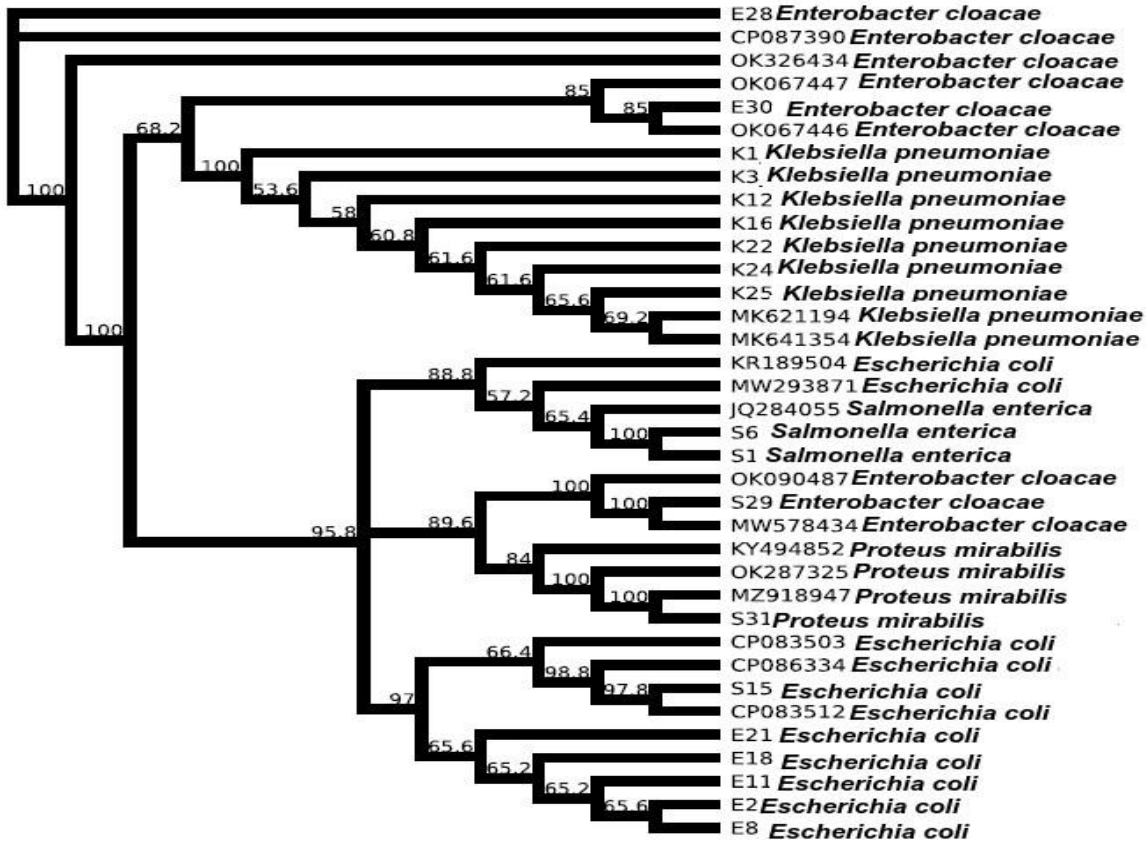

Figure 4.20: Evolutionary distances between the bacterial isolates

4.1.36 Antibacterial activity of ZnOPs of scent leaf at different concentrations and molarities

ZnONP of scent leaf aqueous extract had no antibacterial activities against *Escherichia coli, Vibrio parahaemolyticus, Vibrio cholerae, Staphylococcus aureus* and *Salmonella enterica* at all the concentrations. At 50 mM and 100 mM, the nanoparticles had no effects on the bacteria used in the study, however, as the molarity increases (0.5 M and 1 M) with corresponding concentrations, the nanoparticles produced zones of inhibitions between 7.0 and 7.5 mm against *Shigella* sp, *Klebsiella pneumoniae* and *Enterobacter aerogenes*. The highest zone of inhibition (7.5 mm) was produced at 7500 µg/ml against *K. pneumoniae*. The Antibacterial activity of ZnONPs of scent leaf aqueous extract against bacteria at different molarities and concentrations is presented in Table 4.35

4.1.37 Antibacterial activity of ZnONPs of bitter leaf aqueous extract against bacteria at different molarities and concentrations

ZnONP of bitter leaf aqueous extract had no antibacterial activities against *Vibrio parahaemolyticus* and *Salmonella enterica* at all the concentrations. At 50 mM and 100 mM, the nanoparticles had no effects on the bacteria in all the concentrations except at 7500 µg/ml where the nanoparticles produced zones of inhibitions between 7.0 and 7.5 mm against *Escherichia coli, Shigella* sp, *Klebsiella pneumoniae, Vibrio parahaemolyticus* and *Enterobacter aerogenes*. The highest zone of inhibition (8.0 mm) was produced at 7500 µg/ml of 50 mM slightly above the control (7.3 mm) against *S. aureus* (Table 4.36).

4.1.38 Antibacterial activity of ZnONPs of ogirishi leafat different concentrations and molarities

The 7500 µg/ml concentration of 1 M of the ZnONP of Ogirishi leaf produced the highest zone of inhibition against *Vibrio parahaemolyticus* (10.4 mm). The lowest zone of inhibition was recorded against *Enterobacter aerogenes* (7.0 mm) at the 5000 µg/ml of 0.5 M. At all the concentrations, *K. pneumoniae, S. aureus* and *S. enterica* were resistant to the ZnONP of Ogirishi. At 2500 µg/ml of 50 mM, all the bacterial isolates were resistant to the ZnONP. The antibacterial activity of ZnONPs of *Newbouldia laevis* (ogirishi leaf) at different concentrations and molarities are presented in Table 4.37

4.1.39 Antibacterial activity of ZnONPs of utazi leafat different concentrations and molarities

At 7500 µg/ml concentrations of 100 mM and 1 M, the ZnONP of utazi leaf produced the highest zone of inhibition against *K. pneumoniae* (8.9 mm) and *E. aerogenes* (8.9 mm). The lowest zone of inhibition of 7.0 mm was recorded at 7500 µg/ml of 50 mM and 500 mM against *Shigella* sp, *Enterobacter aerogenes, E. coli, K. pneumoniae and V. parahaemolyticus*. At all the concentrations, *V. cholera* and *S. aureus* were

resistant to the ZnONP of utazi. At 2500 and 500 µg/ml of 50 mM, all the bacterial isolates were resistant to the ZnONP (Table 4.38)

4.1.40 Antibacterial activity of AgNPs of scent leafat different concentrations and molarities

At 7500 µg/ml concentrations of 1 M, the AgNP of scent leaf produced the highest zone of inhibition against *K. pneumoniae* (23.0 mm) followed by *V. parahaemolyticus* (16.0 mm). The lowest zone of inhibition of 7.0 mm was recorded at 7500 µg/ml of 50 mM and 2500 of 1 M against *K. pneumoniae* and *V. parahaemolyticus* respectively. At 2500 and 500 µg/ml of 50 mM, all the bacterial isolates were resistant to the AgNP (Table 4.39).

4.1.41 Antibacterial activity of AgNPs of bitter leaf at different concentrations and molarities

The 7500 µg/ml concentration of 0.5 M of the AgNP of bitter leaf produced the highest zone of inhibition against *K. pneumoniae* (21.0 mm). The lowest zone of inhibition was recorded against *E. coli*, *Shigella* sp, *Enterobacter aerogenes* and *V. parahaemolyticus* (7.0 mm) at the 5000 µg/ml of 0.5 M and 2500 µg/ml of 0.5 M. At 2500 µg/ml of 50 mM, all the bacterial isolates were resistant to the AgNP. The antibacterial activity of AgNPs of bitter leafat different concentrations and molarities is presented in Table 4.40.

4.1.42 Antibacterial activity of AgNPs of ogirishi leafat different concentrations and molarities

The AgNP of ogirishi produced the highest inhibition (24.0 mm) against *E. coli* and *K. pneumoniae* at 7500 µg/ml of 1 M followed by *S. enterica* (20.0 mm) while the lowest inhibition was against *S. aureus* and *E. coli* at 2500 µg/ml and 5000 µg/ml of 0.5 M respectively. At 2500 µg/ml of 50 mM, all the bacterial isolates were resistant to the AgNP of ogirishi. *V. cholerae* was not inhibited by the nanoparticle at all the concentrations and molarities (Table 4.41).

4.1.43 Antibacterial activity of AgNPs of utazi leafat different concentrations and molarities.

The AgNP of utazi produced the highest inhibition (26.0 mm) against *E. coli* and *K. pneumoniae* at 7500 µg/ml of 1 M. The lowest inhibition was against *E. coli* and E. aerogenes at 7500 µg/ml of 50 mM and 7500 µg/ml of 100 mM respectively. At 2500 and 500 µg/ml of 50 mM, all the bacterial isolates were not susceptible to the AgNP of utazi. The antibacterial activity of AgNPs of utazi leafat different concentrations and molarities is presented in (Table 4.42).

Table4.35: Antibacterial activity of ZnOPs of scentleaf at different concentrations and molarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholerae*	*S. aureus*	*S. enterica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	-	-	-	-	-	-	-	-
100mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	-	7.0	7.1	7.0	-	-	-	-
0.5M	2500		-	-	-	-	-	-	-
	5000		7.0	-	-	-	-	-	-
	7500		7.2	7.2	7.1	-	-	-	-
1M	2500		-	-	-	-	-	-	-
	5000		7.0	7.1	7.0	-	-	-	-
	7500		7.3	7.5	7.3	-	-	-	-
	Control	10.0	7.3	7.4	8.1	8.2	8.0	8.1	8.0

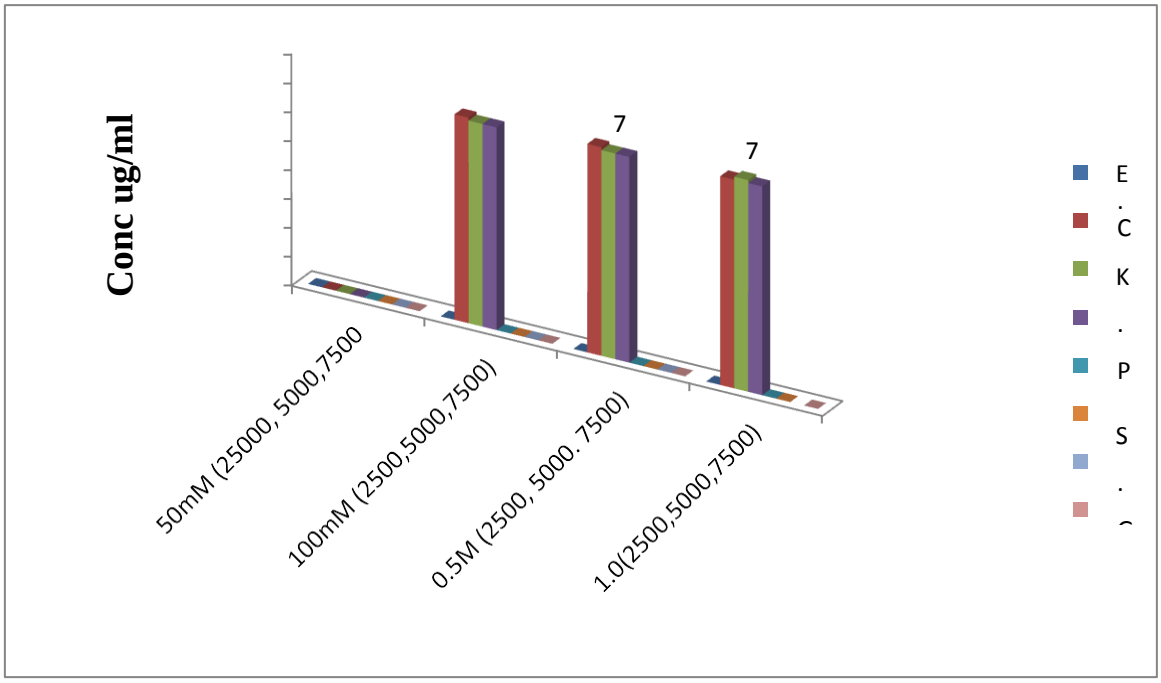

Figure 4.21: Anti bacterial activity of ZnOPs of scent leaf at different concentrations and molarities

Table 4.36: Antibacterial activity of ZnONPs of bitter leaf aqueous extract against bacteria at different molarities and concentrations.

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholerae*	*S. aureus*	*S. entrica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	-	-	-	7.3	-	-	8.0	-
100mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	7.0	7.1	7.7	7.5	7.3	-	-	-
0.5M	2500	-	-	-	-	-	-	-	-
	5000	7.0	-	-	-	-	-	7.1	-
	7500	7.4	-	-	7.1	-	-	7.5	-
1M	2500	-	-	-	-	-	-	-	-
	5000	7.1	-	-	-	-	-	7.3	-
	7500	7.6	-	-	-	-	-	7.8	-
	Control	7.0	15.0	7.0	13	7.0		7.3	7.0

Table 4.37: Antibacterial activity of ZnONPs of ogirishi leafat different concentrations andmolarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholerae*	*S. aureus*	*S. enterica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	7.1	-	-	-
	7500	8.0	-	-	-	7.3	-	-	-
100mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	-	-	-	-	-	-	-	-
0.5M	2500	-	-	-	-	-	-	-	-
	5000	7.8	-	-	7.0	-	-	-	-
	7500	8.9	-	-	7.3	-	8.7	-	-
1M	2500	-	-	-	-	7.3	-	-	-
	5000	7.1	7.1	-	-	8.0	-	-	-
	7500	7.6	7.7	-	-	10.4	-	-	-
	Control	7.3	7.3	10.0	8.0	7.0		14.0	7.0

Table 4.38: Antibacterial activity of ZnONPs of utazi leaf at different concentrations and molarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholerae*	*S. aureus*	*S.* sp
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	-	7.0	-	7.0	-	-	-	7.3
100mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	-	-	7.8	8.9	-	-	-	7.8
0.5M	2500	-	-	-	-	-	-	-	-
	5000	7.0	-	7.0	-	7.0	-	-	-
	7500	7.1	-	8.1	7.1	7.3	-	-	7.6
1M	2500	-	-	7.0	-	-	-	-	-
	5000	7.1	-	7.3	7.5	7.1	-	-	-
	7500	7.4	-	8.9	8.1	7.5	-	-	-
	Control	7.2	11.0	7.0	7.2	7.5		7.9	7.7

Table 4.39: Antibacterial activity of AgNPs of scent leaf at different concentrations and molarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholera*	*S. aureus*	*S. enterica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	7.3	7.3	-	7.4	-	-	-	10.0
100mM	2500	-	-	-	-	-	-	-	-
	5000	7.1	-	-	8.0	-	-	7.3	8.0
	7500	7.5	7.5	7.4	10.0	-	-	7.8	12.0
0.5M	2500	-	-	-	-	7.5	-	-	7.1
	5000	-	7.3	-	7.3	8.0	-	-	8.5
	7500	-	10.0	7.0	7.7	10.0	7.1	7.3	8.9
1M	2500	-	-	9.0	-	7.0	-	-	-
	5000	7.1	8.4	13.0	8.4	11.0	-	7.1	9.0
	7500	8.0	13.0	23.0	13.0	16.0	7.5	7.6	13.0
	Control	10	7.3	7.4	7.1	8.2	7.3	8.1	8.0

Table 4.40: Antibacterial activity of AgNPs of bitter leaf at different concentrations and molarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholerae*	*S. aureus*	*S. enterica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	7.4	-	-	-	8.3
	7500	-	7.2	-	8.5	7.0	-		10.0
100mM	2500	-	-	-	8.0	-	-	-	7.4
	5000	-	7.0	-	13.0	7.0	-	-	8.0
	7500		9.5	-	15.0	7.6	-	-	12.0
0.5M	2500	7.0	7.0	10.0	7.0	7.0	-	-	14.0
	5000	11.0	10.0	15.0	13.0	9.0	-	-	15.0
	7500	15.0	16.0	21.0	16.0	14.0	7.1	7.1	19.0
1M	2500	-	-	7.1	-	-	-	-	-
	5000	7.1	-	7.4	-	-	7.1	-	-
	7500	8.0	7.3	7.7	7.3	7.2	7.3	7.3	7.1
	Control	7.0	15.0	7.0	13	7.0	7.4	7.3	7.0

Table 4.41: Antibacterial activity of AgNPs of ogirishi leaf at different concentrations and molarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumo niae* Zones of inhibiti on	*E. aerogenes*	*V. parah aemol yticus*	*V. choler ae*	*S. aureus*	*S. enterica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	7.3	-	-	-	-
	7500	-	-	7.3	12.0	7.0	-	-	7.2
100mM	2500	-	-	-	7.0	-	-	-	-
	5000	-	-	-	11.0	-	-	-	-
	7500	17.0	-	7.7	15.0	7.3	-	-	9.5
0.5M	2500	-	7.7	7.5	7.3	8.0	-	7.0	8.0
	5000	7.0	8.0	18.0	12.0	10.0	-	11.0	13.0
	7500	12.3	16.0	20.0	19.0	15.5		13.0	15.0
1M	2500	14.0	7.0	12.0	-	10.0	-	8.0	12.0
	5000	17.0	11.0	20.0	11.0	12.0	-	11.0	15.0
	7500	24,0	18.0	24.0	13,0	18.0	-	19.0	20.0
	Control	7.3	7.3	10.0	8.0	7.0	-	14.0	7.0

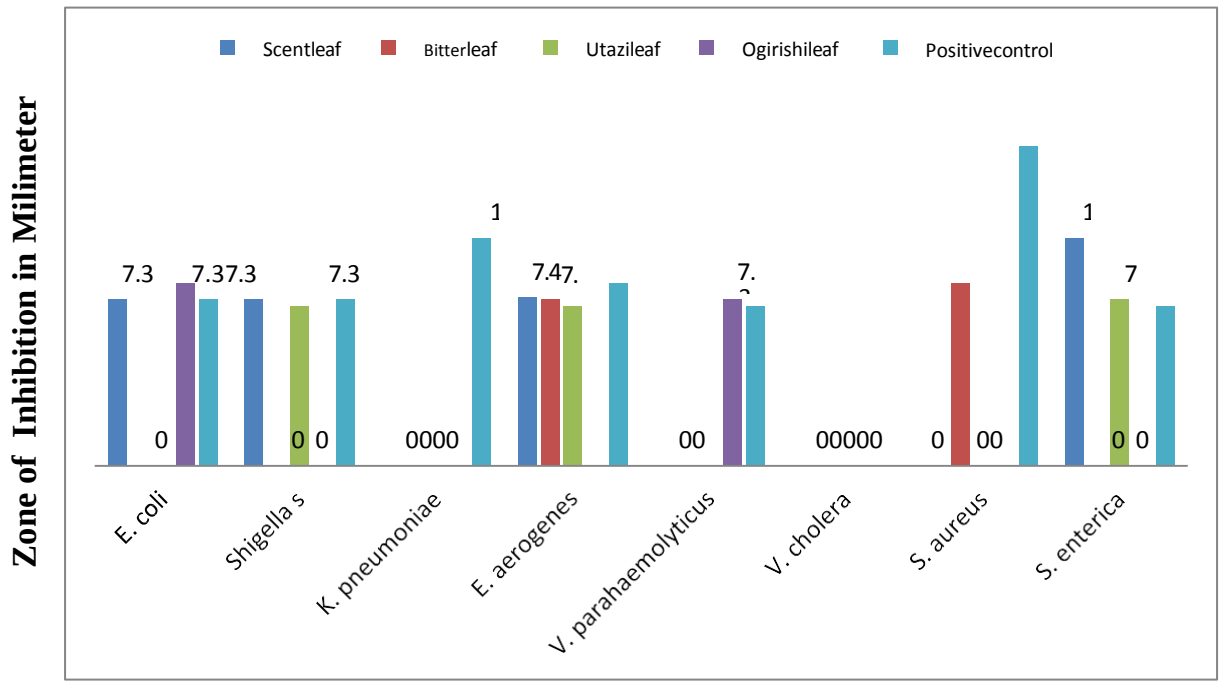

Figure 4.22: Comparison of the Antibiotic Activities ZnONPs Synthesized from four different aqueous leaf extract against some bacteria isolates 0.5M

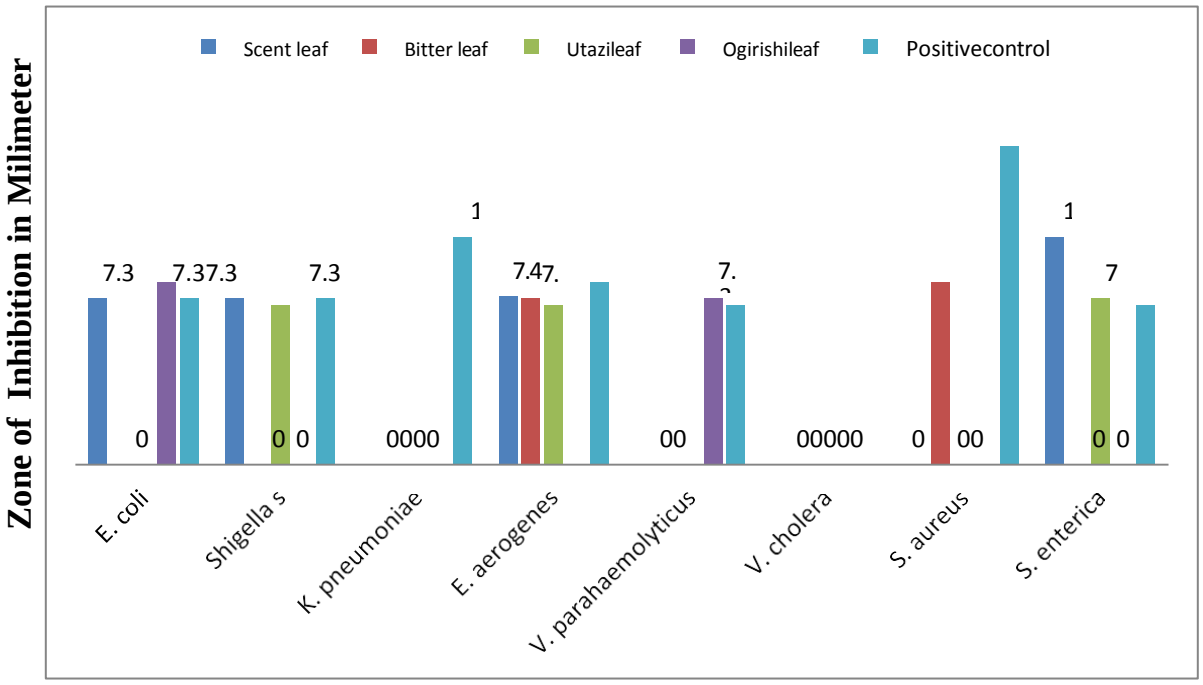

Figure 4.23: Comparison of the Antibiotic Activities ZnONPs Synthesized from four different aqueous leaf extract against some bacteria isolates 50mM

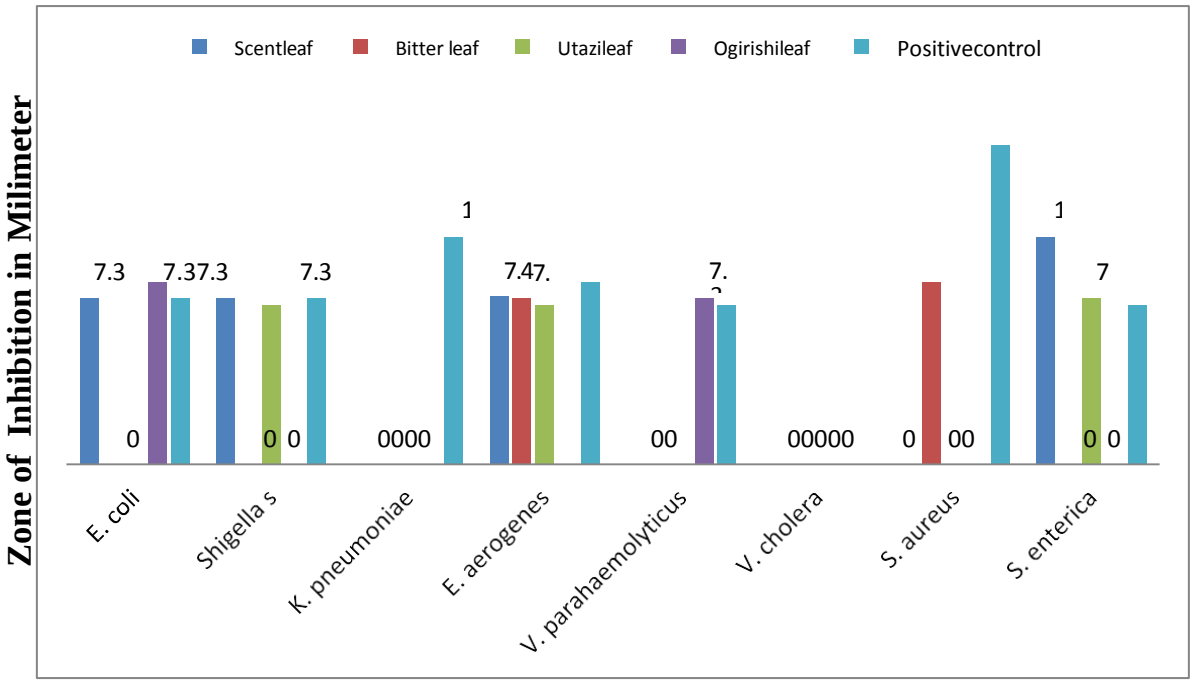

Figure 4.24: Comparison of the Antibiotic Activities ZnONPs Synthesized from four different aqueous leaf extract against some bacteria isolates 100mM

Table 4.42: Antibacterial activity of AgNPs of scent leaf at different concentrations and molarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigell a* sp	*K. pneumo niae*	*E. aeroge nes*	*V. parah aemol yticus*	*V. choler a*	*S. aureus*	*S. enter ica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	7.3	7.3	-	7.4	-	-	-	10.0
100mM	2500	-	-	-	-	-	-	-	-
	5000	7.1	-	-	8.0	-	-	7.3	8.0
	7500	7.5	7.5	7.4	10.0	-	-	7.8	12.0
0.5M	2500	-	-	-	-	7.5	-	-	7.1
	5000	-	7.3	-	7.3	8.0	-	-	8.5
	7500	-	10.0	7.0	7.7	10.0	7.1	7.3	8.9
1M	2500	-	-	9.0	-	7.0	-	-	-
	5000	7.1	8.4	13.0	8.4	11.0	-	7.1	9.0
	7500	8.0	13.0	23.0	13.0	16.0	7.5	7.6	13.0
	Control	10	7.3	7.4	7.1	8.2	7.3	8.1	8.0

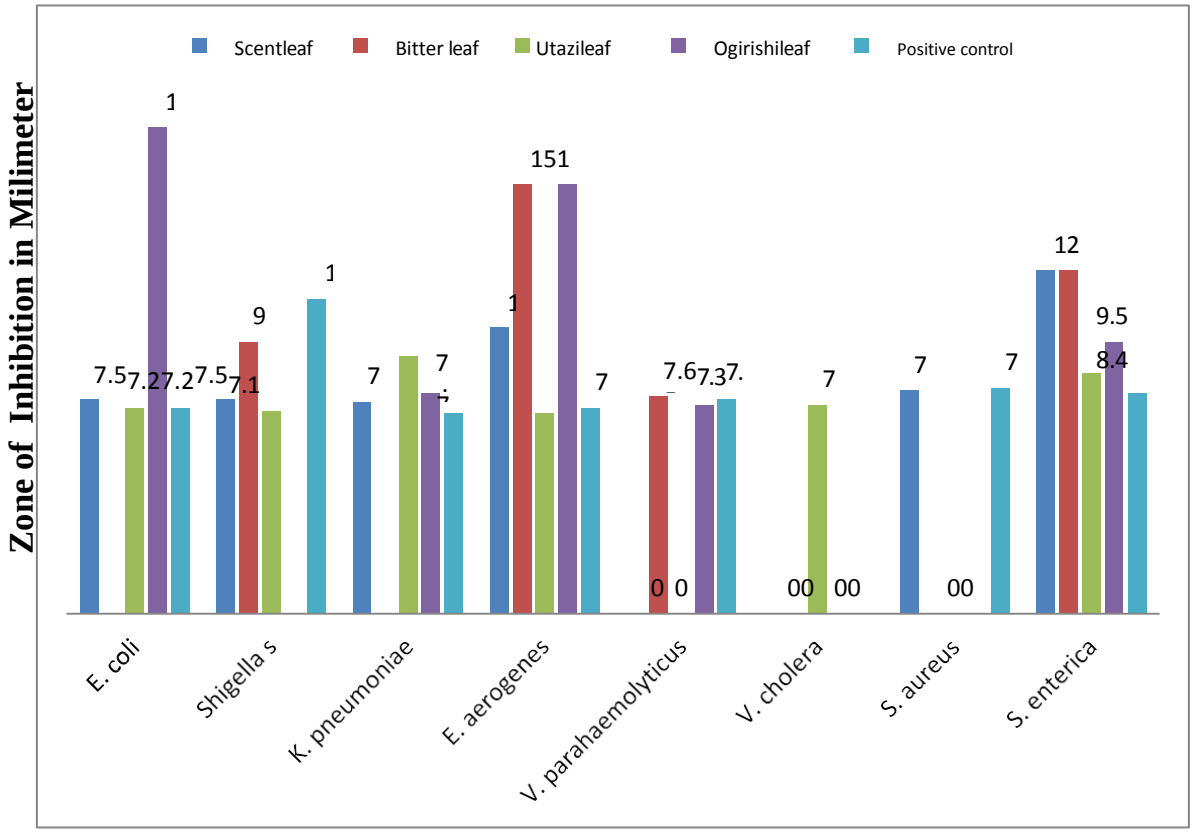

Figure 4.25: Comparison of the Antibiotic Activities AgNPs Synthesized from four different aqueous leaf extract against some bacteria isolates 0.5M

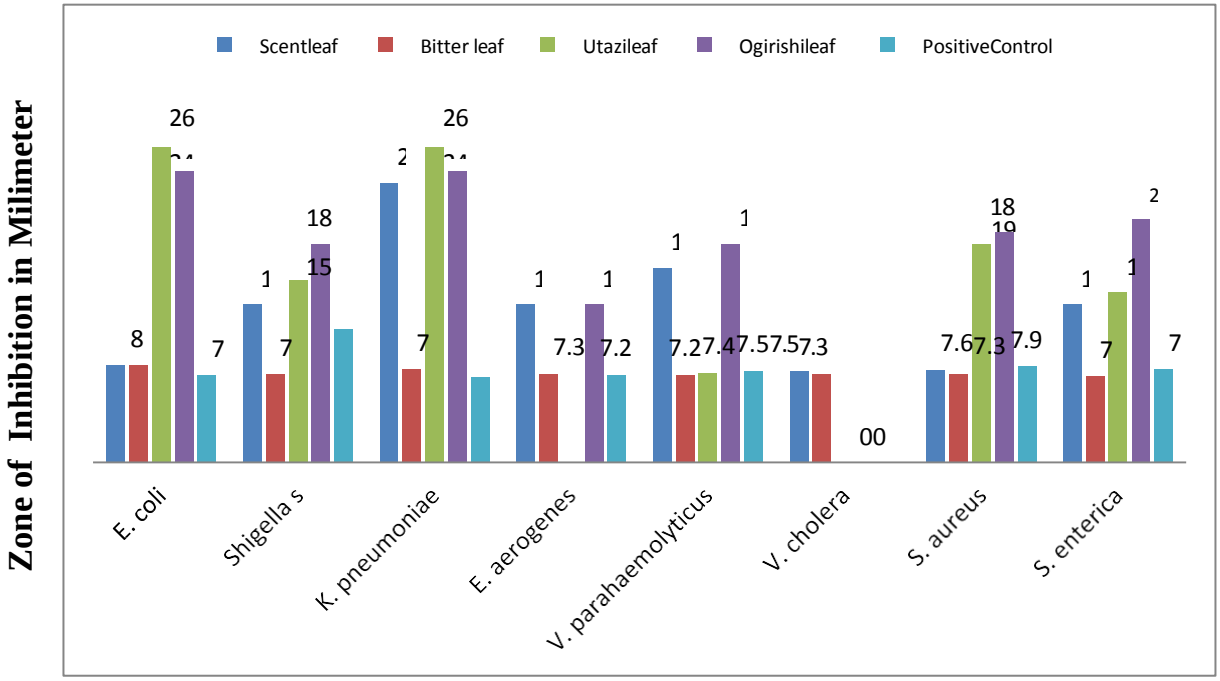

Figure 4.26: Comparison of the Antibiotic Activities AgNPs Synthesized from four different aqueous leaf extract against some bacteria isolates 1M

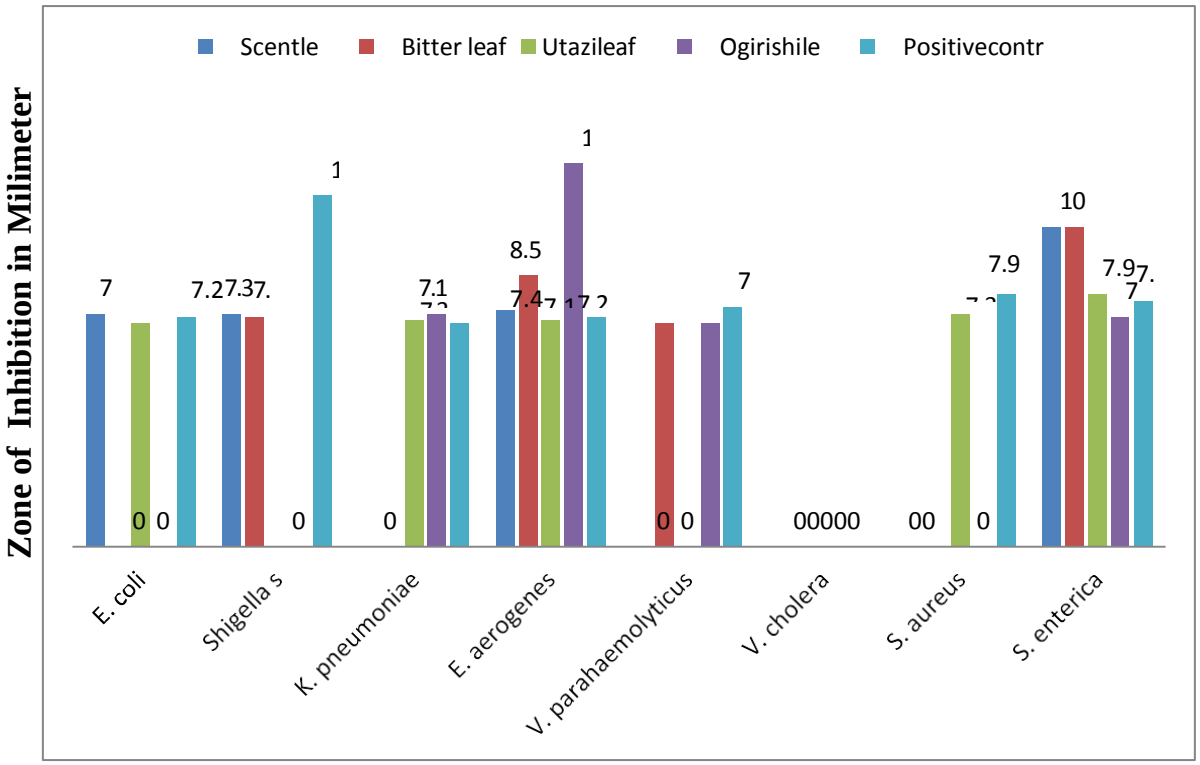

Figure 4.27: Comparison of the Antibiotic Activities AgNPs Synthesized from four different aqueous leaf extract against some bacteria isolates 50mM

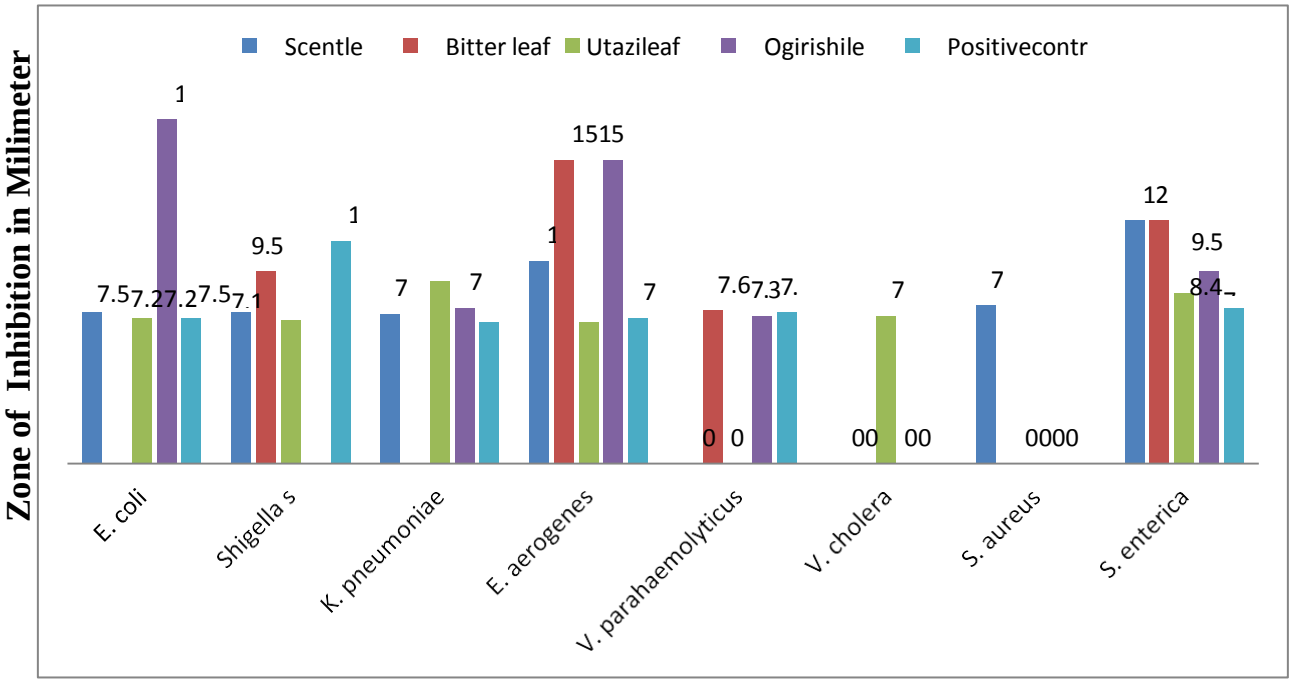

Figure 4.28: Comparison of the Antibiotic Activities AgNPs Synthesized from four different aqueous leaf extract against some bacteria isolates 100mM

Table 4.43: Antibacterial activity of AgNPs of bitter leaf at different concentrations and molarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholerae*	*S. aureus*	*S. enterica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	7.4	-	-	-	8.3
	7500	-	7.2	-	8.5	7.0	-		10.0
100mM	2500	-	-	-	8.0	-	-	-	7.4
	5000	-	7.0	-	13.0	7.0	-	-	8.0
	7500		9.5	-	15.0	7.6	-	-	12.0
0.5M	2500	7.0	7.0	10.0	7.0	7.0	-	-	14.0
	5000	11.0	10.0	15.0	13.0	9.0	-	-	15.0
	7500	15.0	16.0	21.0	16.0	14.0	7.1	7.1	19.0
1M	2500	-	-	7.1	-	-	-	-	-
	5000	7.1	-	7.4	-	-	7.1	-	-
	7500	8.0	7.3	7.7	7.3	7.2	7.3	7.3	7.1
	Control	7.0	15.0	7.0	13	7.0	7.4	7.3	7.0

Table 4.44: Antibacterial activity of AgNPs of ogirishi leaf at different concentrations and molarities

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholerae*	*S. aureus*	*S. enterica*
				Zones of inhibition					
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	7.3	-	-	-	-
	7500	-	-	7.3	12.0	7.0	-	-	7.2
100mM	2500	-	-	-	7.0	-	-	-	-
	5000	-	-	-	11.0	-	-	-	-
	7500	17.0	-	7.7	15.0	7.3	-	-	9.5
0.5M	2500	-	7.7	7.5	7.3	8.0	-	7.0	8.0
	5000	7.0	8.0	18.0	12.0	10.0	-	11.0	13.0
	7500	12.3	16.0	20.0	19.0	15.5		13.0	15.0
1M	2500	14.0	7.0	12.0	-	10.0	-	8.0	12.0
	5000	17.0	11.0	20.0	11.0	12.0	-	11.0	15.0
	7500	24,0	18.0	24.0	13,0	18.0	-	19.0	20.0
	Control	7.3	7.3	10.0	8.0	7.0	-	14.0	7.0

Table 4.45: Antibacterial activity of AgNPs of utazi leaf at different concentrations and molarities.

Molarity	Conc (µg/ml)	*E. coli*	*Shigell a* sp	*K. pneumo niae*	*E. aeroge nes*	*V. parah aemol yticus*	*V. cholerae*	*S. aureus*	*S.enteric a*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	7.0	-	7.1		-	-	7.3	7.9
100mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	7.5	-	-	-	-	7.1
	7500	7.2	7.1	9.0	7.0	-	7.3	-	8.4
0.5M	2500	-	8.0	8.0	-	7.1	-	-	8.0
	5000	10.0	9.0	15.0	8.0	8.0	-	8.0	13.0
	7500	12.0	12.0	19.0	11.0	9.3	7.3	13.0	17.0
1M	2500	18.0	7.0	13.0	-	-	-	-	-
	5000	23.0	13.8	22.0	-	7.3	-	15.0	9.4
	7500	26.0	15.0	26.0	-	7.4	-	18.0	14.0
	Control	7.2	11.0	7.0	7.2	7.5	-	7.9	7.7

Table 4.46: Comparison of the Antibiotc Activities ZnONPs Synthesized from four different aqueous leaf extract against some bacteria isolates 1M

Bacteria	Scent leaf	Bitter leaf	Utazileaf	Ogrishleaf	+ Control	-Control
E.coli	7.3	7.6	7.4	7.6	7.3	-
*Shigella*s	7.5	-	-	7.7	7.3	-
K. pneumoniae	7.3	-	8.9	-	10.0	-
E. aerogenes	-	-	8.1	-	8.0	-
V.parahaemolyticus	-	-	7.5	10.4	7.0	-
V.cholerae	-	-	-	-	-	-
S. aureus	-	7.8	-	-	14.0	-
S. enterica	-	-	-	-	7.0	-

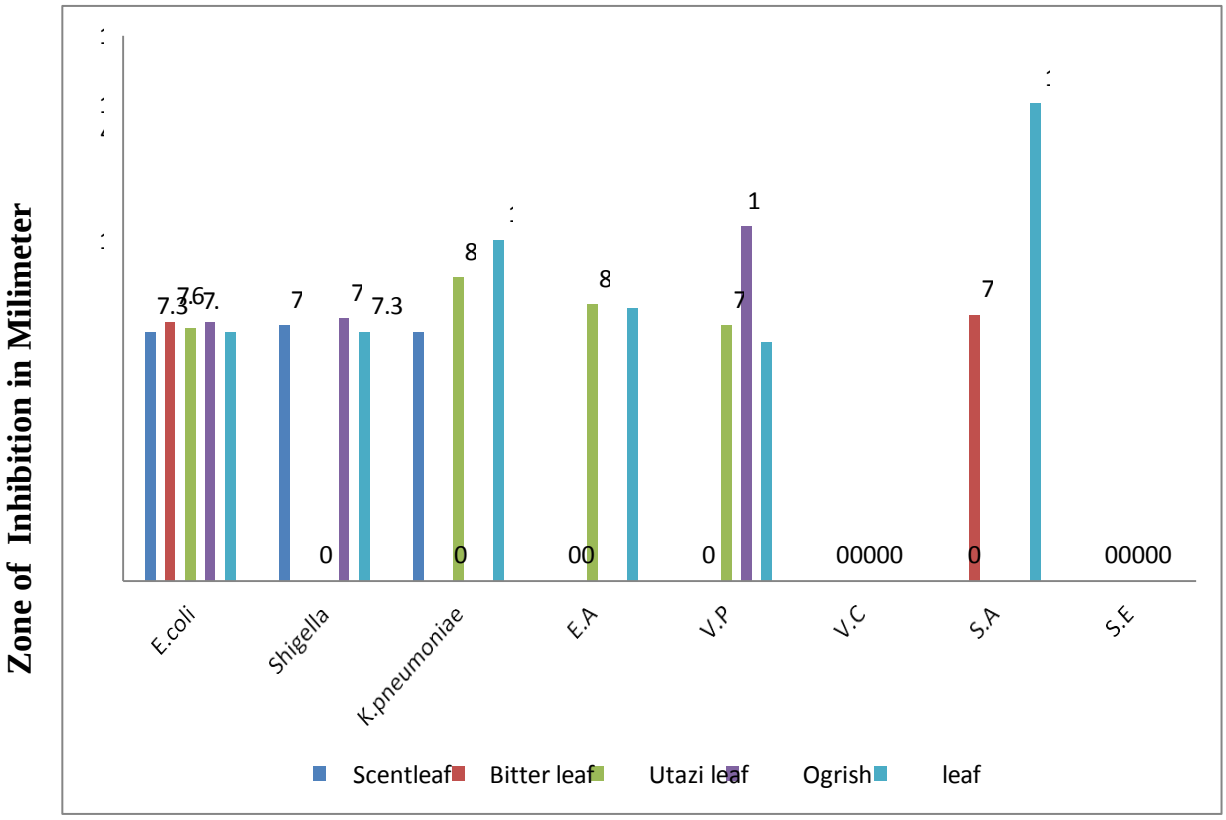

Figure 4.29: Comparison of the Antibiotic Activities of ZnONPs Synthesized from four (4) different aqueous leaf Extract against some resistant bacteria isolate from Livestock

Table 4.47: Antibacterial activity of AgNPs of utazi leaf at different concentrations and molarities.

Molarity	Conc (µg/ml)	*E. coli*	*Shigella* sp	*K. pneumoniae*	*E. aerogenes*	*V. parahaemolyticus*	*V. cholerae*	*S. aureus*	*S. enterica*
50mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	-	-	-	-	-	-
	7500	7.0	-	7.1		-	-	7.3	7.9
100mM	2500	-	-	-	-	-	-	-	-
	5000	-	-	7.5	-	-	-	-	7.1
	7500	7.2	7.1	9.0	7.0	-	7.3	-	8.4
0.5M	2500	-	8.0	8.0	-	7.1	-	-	8.0
	5000	10.0	9.0	15.0	8.0	8.0	-	8.0	13.0
	7500	12.0	12.0	19.0	11.0	9.3	7.3	13.0	17.0
1M	2500	18.0	7.0	13.0	-	-	-	-	-
	5000	23.0	13.8	22.0	-	7.3	-	15.0	9.4
	7500	26.0	15.0	26.0	-	7.4	-	18.0	14.0
	Control	7.2	11.0	7.0	7.2	7.5	-	7.9	7.7

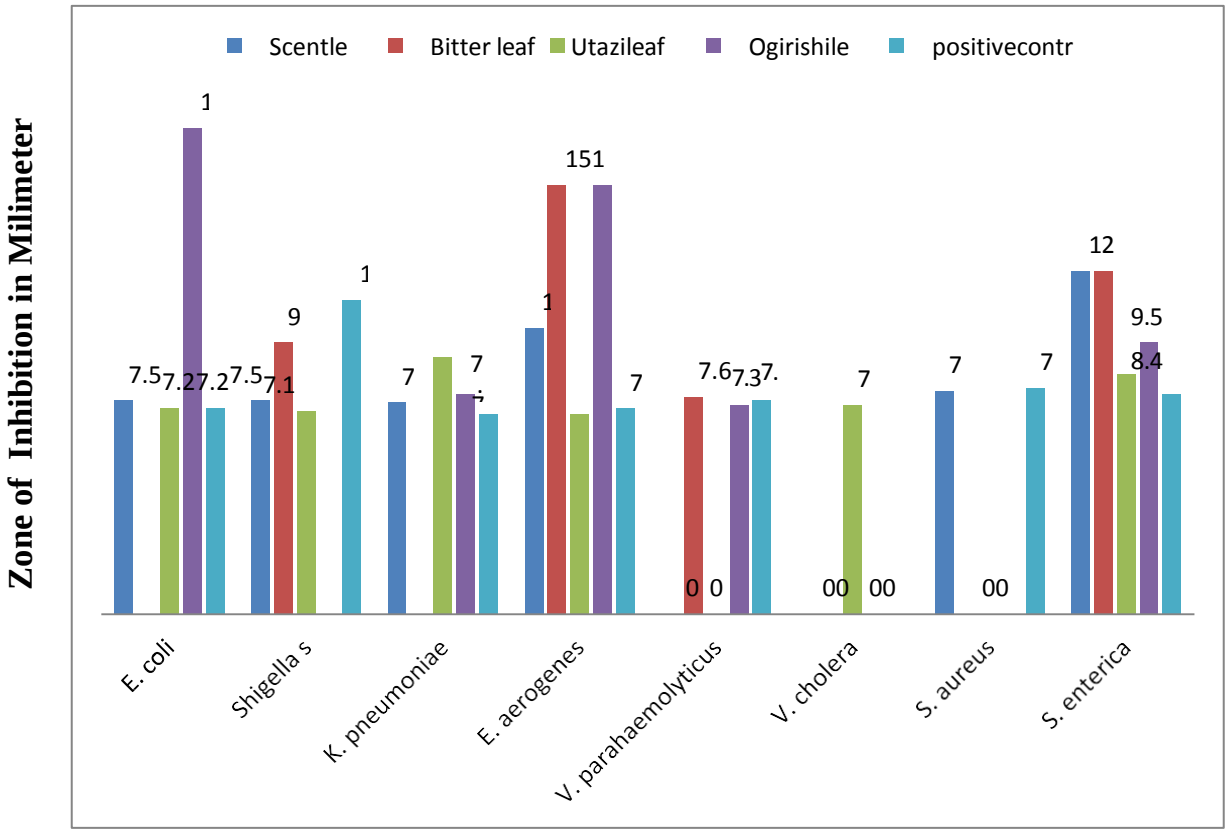

Figure 4.30: Comparison of the Antibiotic Activities AgNPs Synthesized from four different aqueous leaf extract against some bacteria isolates 0.5M

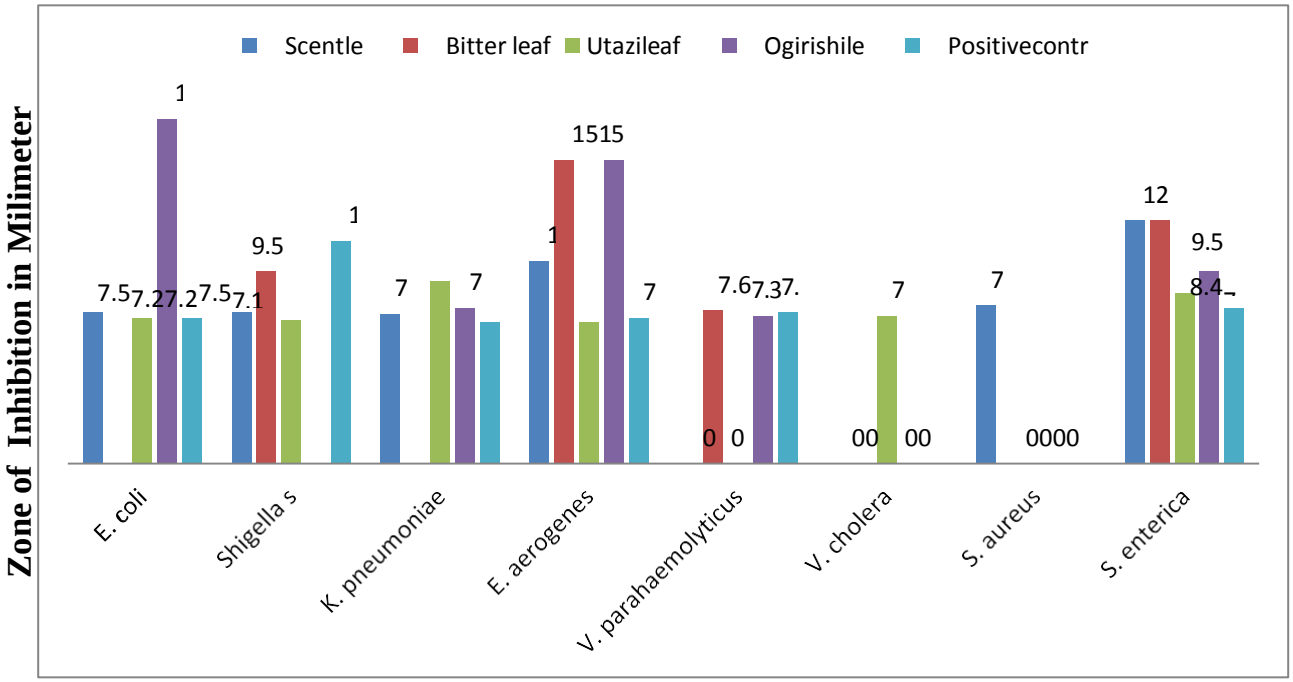

Figure 4.31: Comparison of the Antibiotic Activities AgNPs Synthesized from four different aqueous leaf extract against some bacteria isolates 100mM

4.1.44 Characterization

4.1.44.1 Fourier-Transform Infrared (FTIR) Analysis

The Infrared (IR) spectral for the silver nanoparticles is presented in Figures 4.32 – 4.35, while the data and assignment are presented in Table 4.48.

A recorded spectrum gives the position of bands related to the strength and nature of bonds, and specific functional groups, providing the information concerning molecular structures and interactions The IR spectral for the Ag nanoparticles is presented in Figures 4.32 – 4.35, while the data and assignment are presented in Table 4.48.

Table 4.48 shows the IR spectra for the different plant leaves synthesize Ag nanoparticles. The peaks at > 3734.8 cm^{-1} are related to Free OH species with co-adsorbed H_2O on Ag surfaces while O-H stretching was observed at 3649.1 cm^{-1}, 3734.8 cm^{-1}, 3321.1 cm^{-1} and 3377.0 cm^{-1}. The characteristics bands at 3283.8 cm^{-1}, 3302.4 cm^{-1} and 3216.7 cm^{-1} were attributed to the O-H stretch with H bonded. Alkane C-H stretch vibrations were observed at 2847.7 and 2914.8 cm^{-1} for Ag nanoparticles from bitter leaf plant extracts. Carboxylic Acid O-H Stretchband was observed in Ogirishi leaf plant extract Ag nanoparticle at 2109.0 cm^{-1}. At the characteristics band ranging from 2128.3, 2105.9 to 2176.8 cm^{-1} were attributed to the stretching mode of C=C conjugated & C≡C Alkyne stretch Carboxylic Acid O-H Stretch. The characteristics peak at around 2310.9 cm^{-1} is due to the Alkyne stretch Carboxylic Acid O-H, allene C=C=C was observed in 1908.4 cm^{-1}, 1923.3 cm^{-1} and 1986.7 cm^{-1} for both ogirishi and scent leaf but absent in utazi and bitter leaf Ag nanoparticles. 1416.4 $cm^{-1}$and 1464.9 cm^{-1} were only obsereved only in bitter leaf Ag nanoparticles indicating functional group of alkane bending -C-H. Bands of 1315.8 cm^{-1}, 1341.8 cm^{-1}, 1341.8 $cm^{-1}$and 1338.1 cm^{-1} were attributed to amine C-N stretch. Ether C-O stretch at band 1043.7 $cm^{-1}$for Ag nanoparticles synthesized using bitter leaf was observed. Bands at 715.6 cm^{-1}, 864.7 cm^{-1} to 879.7 cm^{-1}which indicated alkene =C-H bending for both ogirishi and scent leaf but absent in utazi and bitter leaf Ag nanoparticles

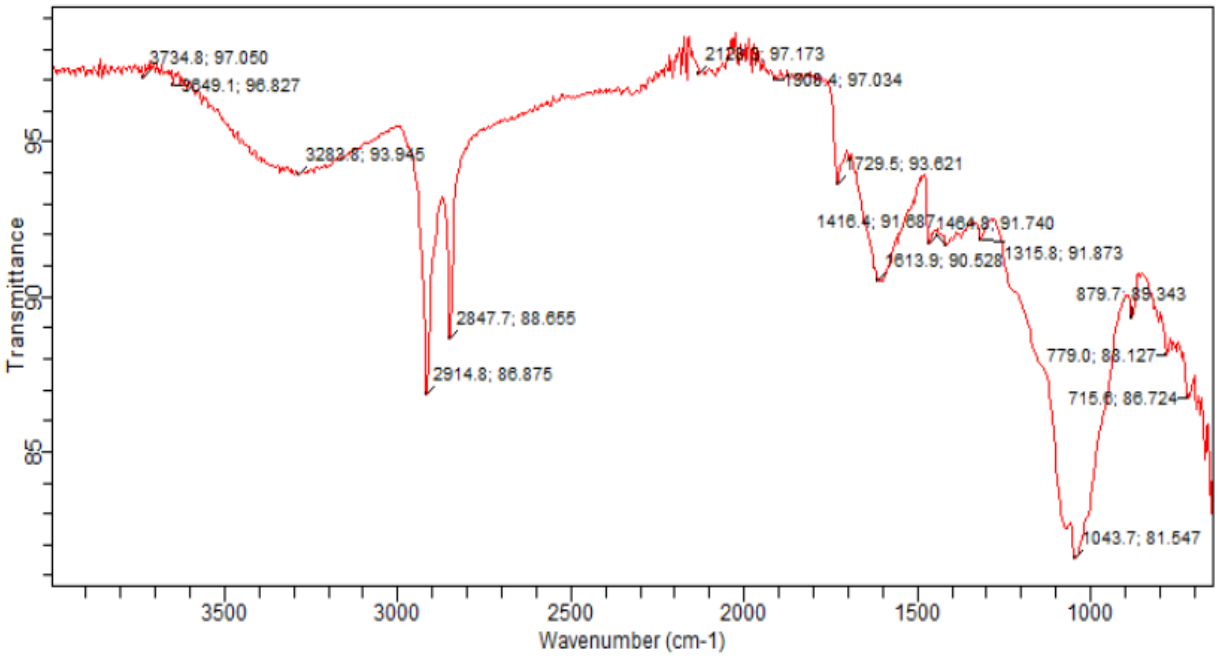

Figure 4.32: IR spectra for silver nanoparticle from scent leaf

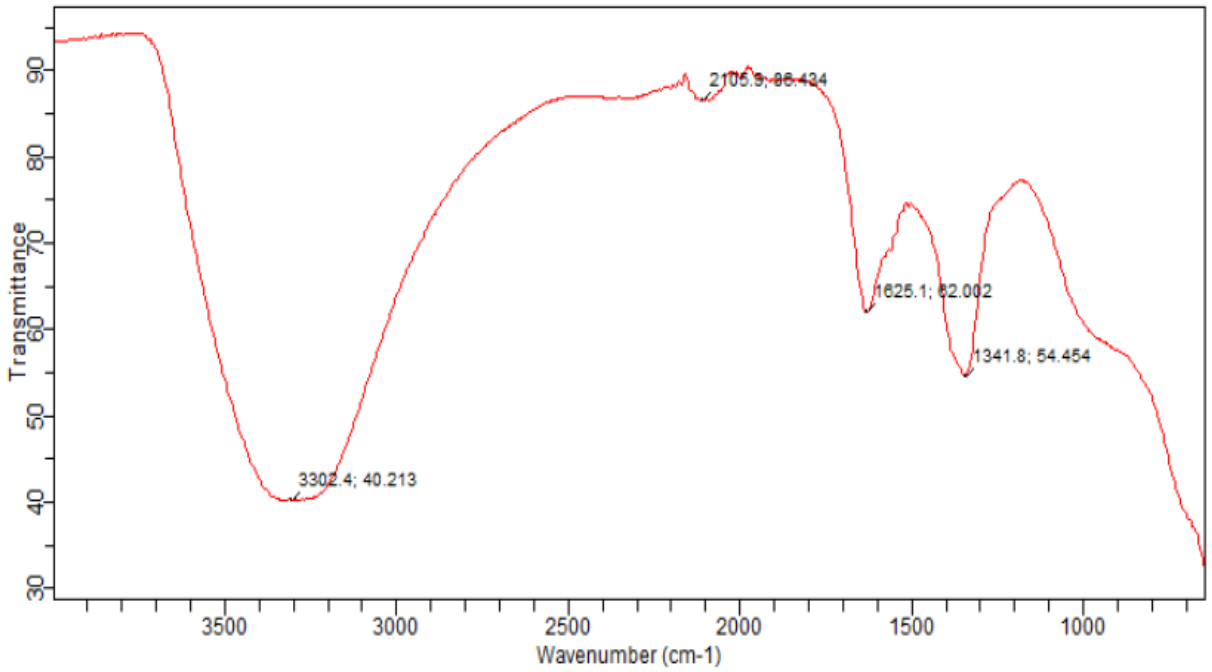

Figure 4.33: IR spectra for silver nanoparticle from Bitter leaf

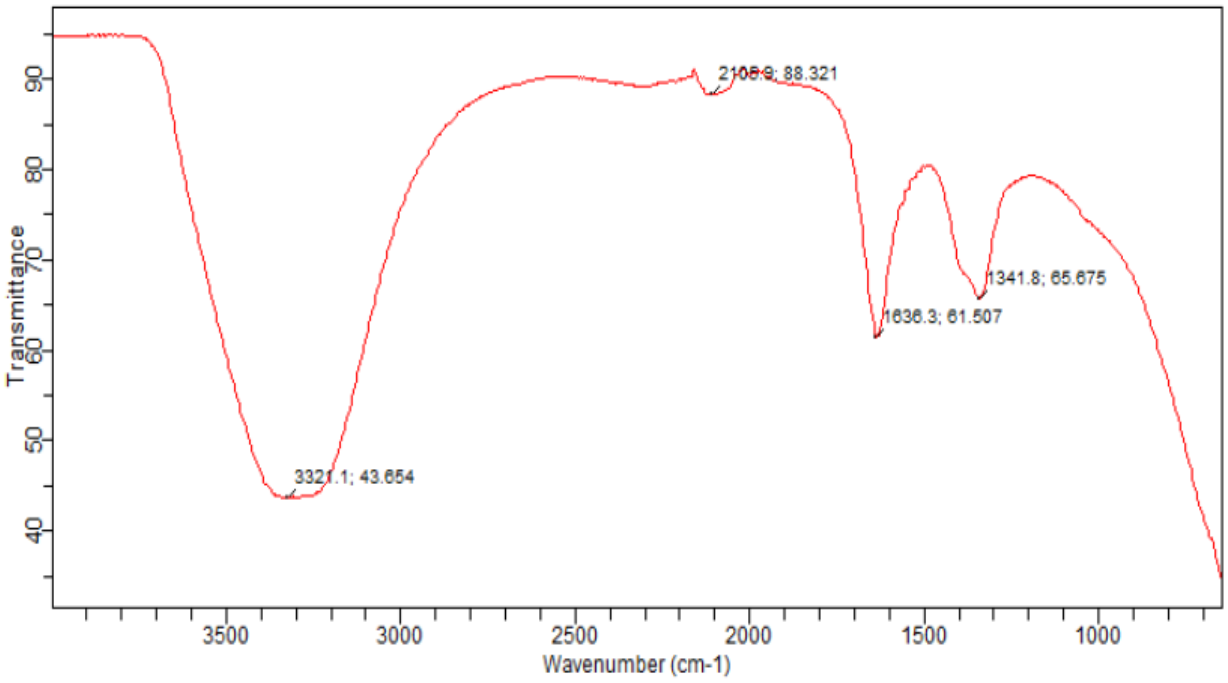

Figure 4.34: IR spectra for silver nanoparticle from Ogirishi leaf

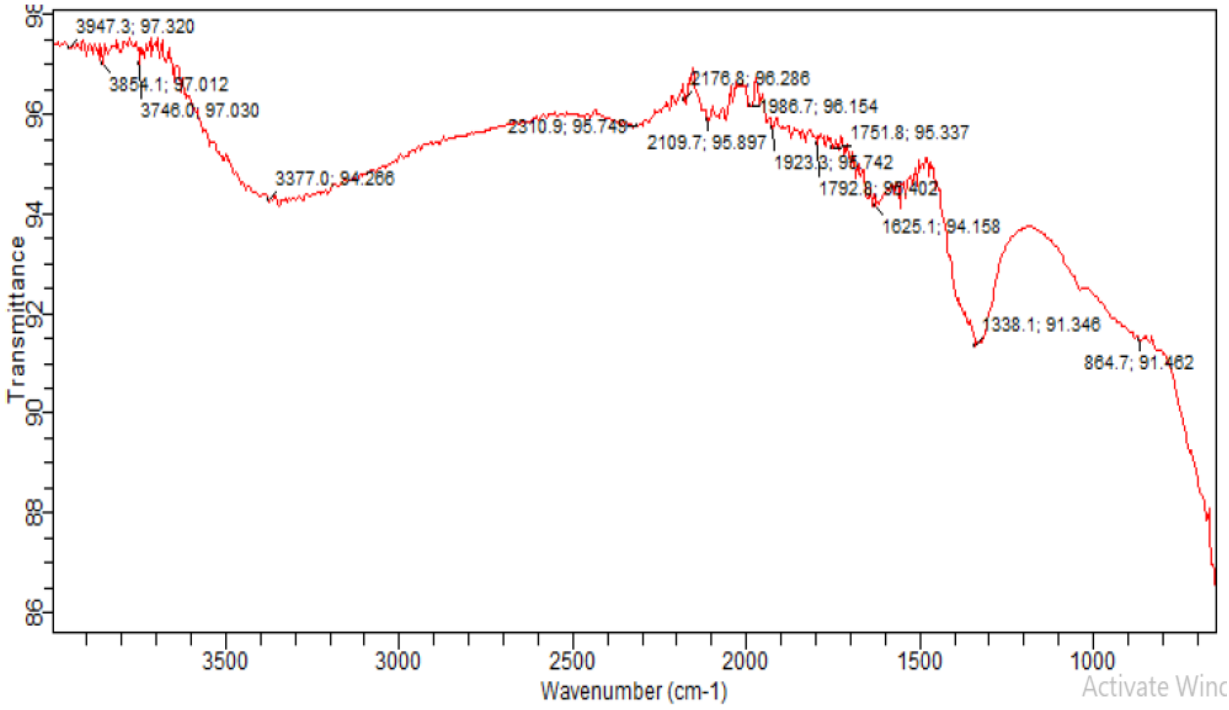

Figure 4.35: IR spectra for silver nanoparticle from Utazi leaf

Table 4.48: IR spectral data for the different silver nanoparticle

Assignment	PLANT LEAF			
	Bitter leaf	**Scent leaf**	**Utazi leaf**	**Ogrishi leaf**
alkene =C-H bending	715.6-879.7	-	-	864.7
ether C-O stretch	1043.7	-	-	-
amine C-N stretch	1315.8	1341.8	1341.8	1338.1
alkane bending -C-H	1416.4 1464.9	-	-	-
carbonyl C=O stretch	1729.5	-	-	1729.8 1751.8
C=C unsaturated compoundAlkene stretch	1613.9	1625.1	1636.3	1625.1
Allene C=C=C	1908.4	-	-	1923.3 1986.7
C=C conjugated & C≡C Alkyne stretch	2128.3	2105.9	2105.9	2109.7, 2176.8
Carboxylic Acid O-H Stretch				2310.9
aliphatic methelene Alkane C-H stretch	2914.8, 2847.7	-	-	-
O-H stretch with H bonded	3283.8	3302.4	3216.7	-
O-H stretching	3649.1 3734.8		3321.1	3377.0
Free OH	-	-	-	3746.0, 3947.3 3854.1

4.1.44.2 Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX) Analysis

The surface morphologies of biosynthesized Ag nanoparticles were studied by using SEM, and the results are presented in Figures 4.36 to 4.39. For more insight into the features of the biosynthesized Ag Nanoparticles, the analysis of the sample was performed using EDS techniques. The weight concentration of Ag in the different Ag nanoparticles were 55.0%, 60.20%, 50.2% and 68.2% for scent leaf, bitter leaf, ogirishi and utazi aqueous leaf extract Ag nanoparticles respectively.

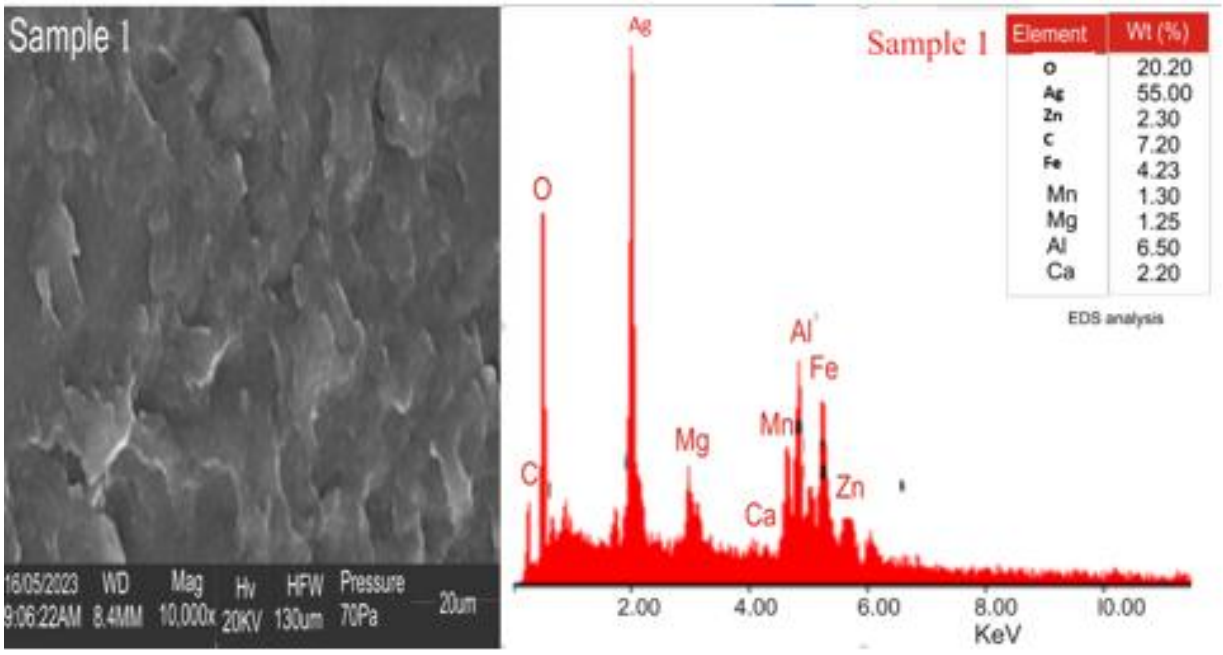

Figure 4.36: SEM-EDX for Ag nanoparticle from scent leaf extract

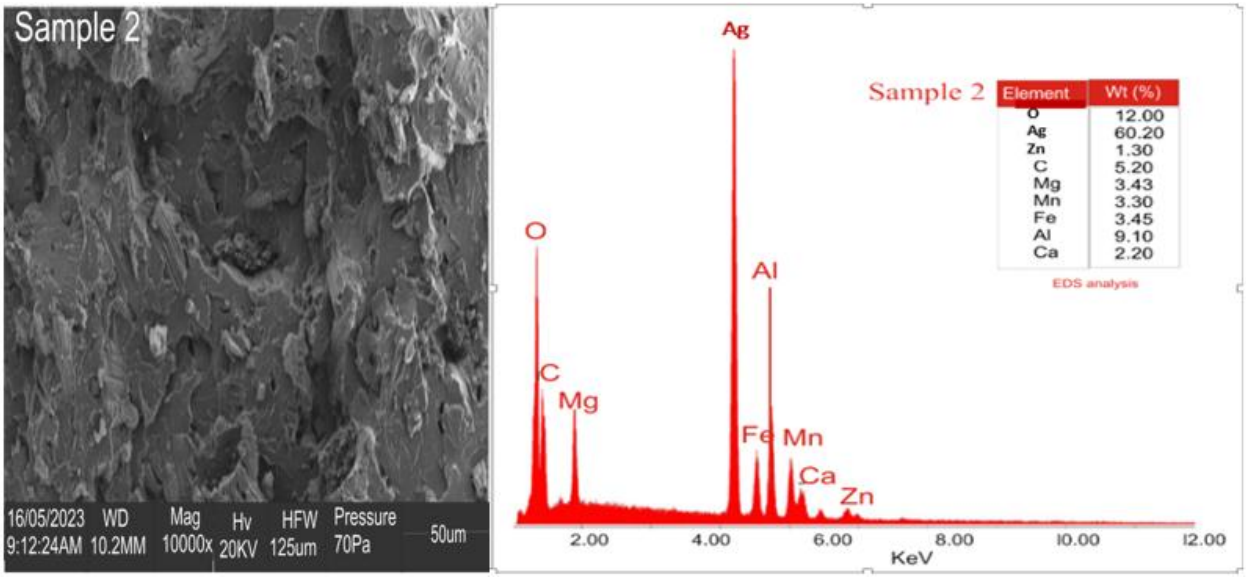

Figure 4.37: SEM-EDX for Ag nanoparticle from Bitter leaf extract

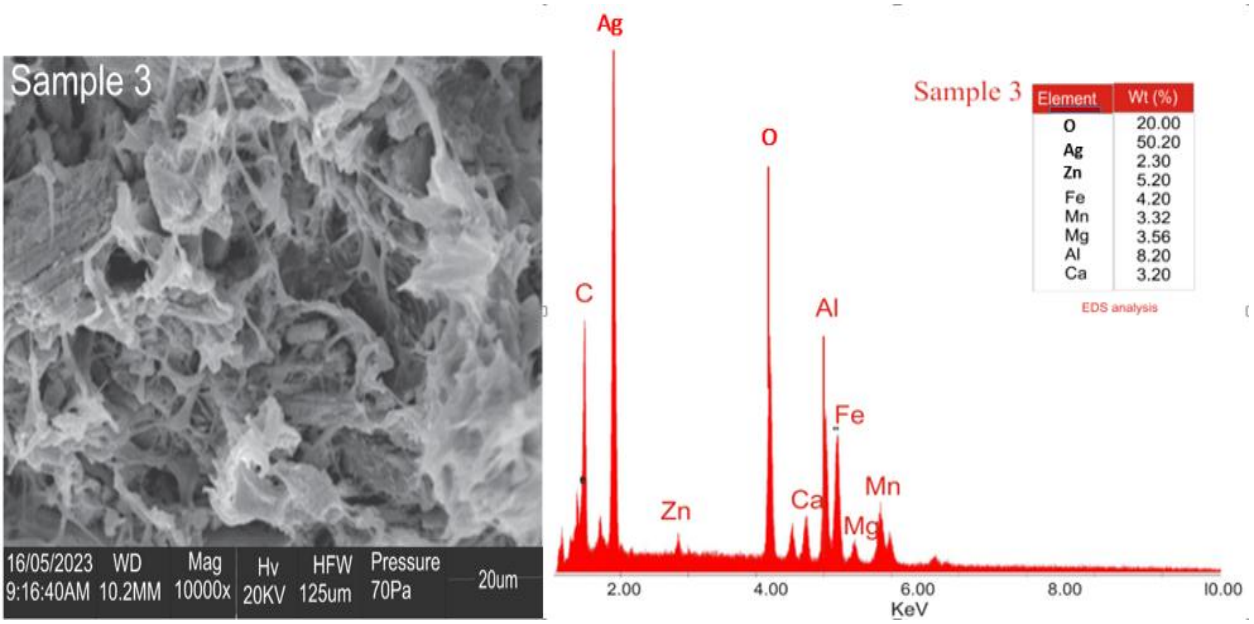

Figure 4.38: SEM-EDX for Ag nanoparticle from Ogirishi leaf extract

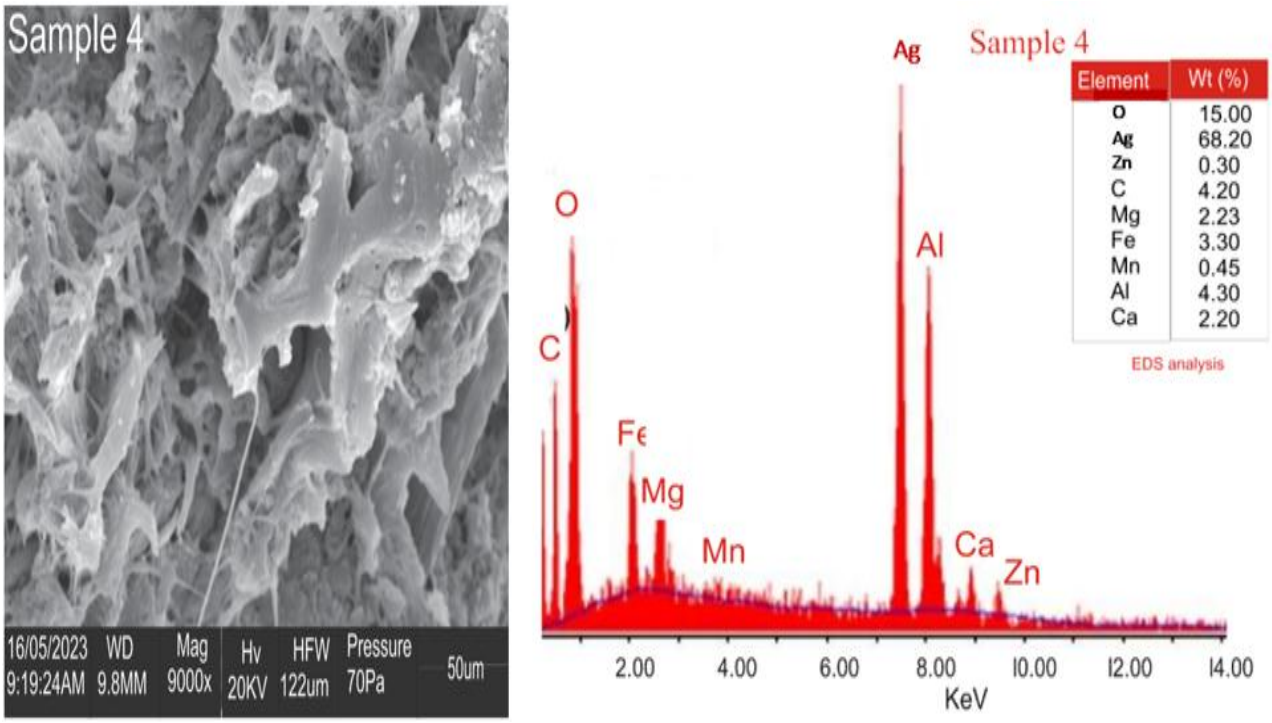

Figure 4.39: SEM-EDX for Ag nanoparticle from utazi leaf extract

4.2 Discussion

This work focuses on the use of aqueous plant extract in silver nanoparticle synthesis process to yield safer and higher output of silver nanoparticles and the effect of silver nanoparticles on *Escherichia coli*, *Shigella* spp, *Klebisella* species, *Pseudomonas* species, *Vibro p*, *Vibro c*, *Staphylococcus aureus* and *Salmonella* spp. The use of green plant extract as a capping agent and reducing agent has been gaining more grounds, it has been reported that phenolic species existing in plant extracts have revealed high antibacterial and antioxidant properties, which are important characteristics for bioapplications (Ayandiran et al., 2018).

Bacterial counts in livestock farms

Livestock farming is a major contributor to small and medium scale enterprises and has been advocated for at both the state and federal levels. The spread of bacteria in livestock farms has been a major challenge in public health sector particularly in the South-East region. Total heterotrophic bacteria (THB) are the most multifaceted group of microorganisms with different nutritional and survival requirements. Some are primary colonizers while others are secondary invaders directly deriving their nutrients from the primary colonizers. The compositions of these microbes differ based on the location and nutrient available (Serves, Sora & Ciferrri, 1996). According to World Health Organization (WHO, 2018), the standard bacterial count stipulated for drinking water is approximately 100 CFU/ml. Infectious diseases result when that quantity is exceeded. From this study, THBC was very significant ($p<0.05$) in pig farm (Figure 4.1, 4.2, 4.3, 4.4, 4.5 and appendix 4.1) cow farm (Figure 4.6, 4.7, 4.8, and appendix 4.2) and poultry farm (Figure 4.9, 4.10, 4.11, 4.12, 4.13 and appendix 4.3) ($28.43\pm0.5\times10^5$; $26.70\pm0.7\times10^5$ and $26.26\pm0.5\times10^5$ CFU/ml) respectively. The high values obtained could be attributed to water and soil pollution and improper disposal of waste generated from homes and industries (Ali, Tanko & Rilwanu, 2021).

These wastes serve as breeding sites for bacteria (Agodo, Ajiji, Anyanwu & Ajide, 2016). During rainfall, splashes of water from these wastes percolate through underground water and affect the streams while the surrounding air becomes polluted.

THB, total coliform (TC) as well as the hand swabs of livestock keepers were assessed from air, water, soil and feeds. THBC and TCC were appreciably higher in soil samples followed by water. The least values were obtained in air, feeds and hands of keepers. Soil and water support the multiplication of these microbes due to availability of nutrients. High values of THBC and TCC in soil samples is as a result of their ability to tap the available nutrients in soil sediments more than water (Adhikari, Barnes, Schiewer, & White, 2007). According to Adhikari et al., (2007) the availability of nutrient and water retaining capacity of soil bacteria tend to increase their survival rate of bacteria. Air particles do not have enough resources to sustain microbes; the chemical preservatives used in producing feeds inhibit the survival of bacteria (Soriano, 2020). The total potential pathogenic bacteria count (TPPBC) was seen to increase among hand swabs ($26.23\pm0.4\times10^5$ and $20.20\pm0.5\times10^5$ CFU/ml) of pig and poultry keepers respectively while soil sample ($17.47\pm0.5\times10^5$ CFU/ml) had the highest TPPBC for cow farm. These increased values could be as a result of improper handling of the excreta, feeds and drinking water of the animals (McAllister & Toppt, 2012) and due to frequent close contact of livestock farmers with their livestock in our area (Klous et al., 2016). Most pathogenic bacteria which colonize the skins and excreta of host animals can be transmitted as zoonotic pathogens to humans (Klous et al., 2016). The results indicate that samples from Aba had the highest isolated THB, TC and TPPB. The increase in THBC, TCC and TPPBC in Aba can be attributed to the location of the pig and cow farms and the activities being performed. Aba River, where the research was conducted is a tributary of Imo River and all abattoir activities take place there. The river runs through two local

government areas in the State, and serve as sites for washing slaughtered animals and burning of hides (Ngozi & Humphrey, 2019).

Bacterial prevalence

Generally, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Enterobacter aerogenes*, *Vibrio* sp. and *Shigella* sp. were distributed in all the farms of the four cities with *E. coli* and *Shigella* sp. showing the highest and least prevalence, respectively (Table 4.4). The bacterial species isolated from the farms in the four cities were in agreement with Adogo etal. (2016). Out of the 933 *E. coli* isolated, cow abattoir (35.0%) had the highest percentage followed by pig farms (34.1%) while the poultry farm had the least (19.9%). *E. coli* was among the most isolated bacteria from dirty water resulting from indiscriminate disposal of farm waste including droppings and carcasses of dead animals. Poor sanitary conditions of the pig farm especially drinking water influenced the level of contaminants; improper and infrequent cleaning of piggery (Asambe, Sackey & Tekdek, 2018) also led to the heightened values of *E. coli*. The increased *E. coli* isolates in cow abattoir could be attributed to soil contamination (Mills, Lee, Evans, Garabed & Lee, 2021). The unhygienic environment where the cows are slaughtered, and prepared for delivery to different selling points and markets could be attributed to the microbial contamination. The unhygienic surrounding could be as a result of faecal contaminants from the animals themselves. This alligned with findings of Mills et al. (2021) which reported different strains of *E. coli* obtained from soil samples in the cow pen alongside *Salmonella* spp and *K. pneumoniae*. K. pneumoniae, another Gram-negative bacterium followed after *E. coli*. From the entire farms, a total of 505 isolates of *K. pneumoniae* were identified with poultry farm having the highest percentage. *S. enterica* was not isolated in cow abattoirs and from the poultry workers. The presence of *S. enterica* in poultry farms but absent in the poultry workers is a great concern. Probably, it could be as a result of constant hand washing by the workers after feeding the

chickens and cleaning the pen. Furthermore, from the poultry farms and poultry workers, *E. aerogenes*, *Vibrio* sp and *Shigella* sp were not isolated. Besides, *Shigella* sp too was not isolated from cow abattoir. The prevalence of *E. coli*, *K. pneumoniae* and *S. enterica* from all the farms in Aba, Umuahia, Mbaise and Okigwe as presented from Tables 4.5 to 4.9 indicated that only *E. coli* and *K. pneumoniae* had the highest prevalence.

Antibiotic resistance

Antibiotic resistance has been identified as one of the major threats to public health; it is a drawback in the treatment of diseases. It is not only incurred from different parts of the host's body including animals, but also, exists in the environment. The majority of the tested antibiotics in different classes (monobactams, cephalosporins and fluoroquinolones) in Tables 4.10 and 4.33 were less potent against the bacteria studied; hence, these bacteria demonstrated high resistance between approximately 70% and 89%. The frequent use of antibiotics in livestock has led to an increase in antibiotic resistance (Van Boeckel et al., 2015). According to Landers, Cohen, Wittum & Larson (2012), Chattopadhyay (2014) and Manyi-Loh, Mamphweli, Meyer & Okoh (2018) the emergence of antibiotic-resistant bacteria was attributed to the incessant use of antibiotics in feed production to enhance the quick development of livestock for economic purposes and the desire for the sick animals to get cured quickly. From this work, the increased multi-drug resistance observed can be a result of the mismanagement of the drugs (Manyi-Lou et al., 2018).

Therefore, through the consumption of the animals' flesh and their unhygienic handling when they are sick, MDR then can be transmitted from the livestock to humans (Landers et al., 2012).

Furthermore, from the Tables 4.10 to 4.33, *K. pneumoniae* and *E. coli* produced the highest resistance against Amoxicillin-clavulanic acid (AMC), CPD, CTR, CTX and CAZ. These drugs are used to treat different diseases among farm animals and over time, the bacterial pathogens

could develop multidrug resistance due to frequent use (Acar and Moulin, 2006), horizontal gene transfer as well as location (Davies, 2013). It was observed in pig farms and cow abattoirs, the bacterial isolates from faeces and rectal swabs, raw beef and raw pork, produced relatively higher resistance values compared with those obtained from cloacal swabs, faecal swabs and drinking water in poultry farms. Each isolate was at least resistant to ten (10) antibiotics. Meanwhile, the values obtained especially in poultry farms could be linked to the level of supply of clean drinking racks, neat feeding racks and the ability to separate unhealthy chickens from healthy ones (Lee et al., 2020). These practices might influence the different levels of multidrug resistance in the three farms.

Both *E. coli* and *K. pneumoniae* obtained from the raw meat and rectal swabs of cows and pigs were very susceptible to colistin and amikacin. Colistin and amikacin were the only tested antibiotics that were very active against *E. coli* isolated from raw meat and rectal samples. However, between the two bacterial isolates, *K. pneumoniae* of raw meats produced the lowest susceptibility to colistin and amikacin compared to *E. coli*. This development resulted from the presence of SHV-12, an ESBLs resistant gene located in the plasmid (Kola et al., 2012). When tested against isolates from rectal swab samples. Colistin is a class of antibiotic that targets the outer membrane of Gram-negative bacteria making it an excellent bactericidal agent against most enteric bacteria (Falagas & Kasiakou, 2005). From the study, the increased activity of colistin against the bacteria isolates could be attributed to its ability to displace magnesium and calcium divalent ions, which normally stabilize the lipopolysaccharides molecules which in turn increases in the penetrability of the cell wall, outflow of cell organelles and molecules, and, then, cell death (Falagas & Kasiakou, 2005). While the use of either colistin or amikacin treated multidrug-resistant *K. pneumoniae* infections, their combination might have a significant effect (Ontong, Ozioma, Voravuthikunchai & ChusriI, 2021).

ESBLs

The ESBL-degrading proteins have been isolated from beef, pork, and poultry (Bergšpica, 2020). In research conducted by Rega, Carmosino, Bonilauri, Frascolla, Vismarra & Bacci (2021), it was discovered that pork had higher ESBL value than wild boar. Similarly, Meissner et al. (2022) in Germany corroborated that pig farms had significant levels of ESBLs. In contrast, the study performed by Galler et al. (2021) and Aworh et al. (2022) on rectal samples of pig and raw beef respectively produced relatively low values. However, in this study, ESBLs were found to be more in poultry than pigs and cows. This high value could be attributed to the CTX-M-15 ESBLs gene, which is one of the resistant genes in poultry (Falgenhauer et al., 2019). Between the two bacterial species understudy, K. *pneumoniae* was seen to be the highest producer of ESBLs, this could be connected to the presence of movable plasmid-borne genes which are easily transmissible to the environment and human host (Ahmed, Elshafiee, Khalefa, Kadry & Hamza, 2019). Modification of the cellular structure of and synthesis of antibiotic-degrading proteins also promulgates ESBLs in *K. pneumoniae* (Zeng & Lin, 2013).

Genotypic characterization

The 32 bacterial coded samples identified phenotypically and ESBLs producers were used for molecular studies were confirmed genotypically. Six (6) isolates were genotypically confirmed each to be *E. coli* and *K. pneumoniae*; three (3) were *E. cloaca* and two (2) were *S. enterica*. Only one (1) isolate was confirmed *P. mirabilis*. The non-determined strains were 14 in number. The 6 isolates of *E. coli* genotypically confirmed had obtained r*RNA* genes (100%); 4 (66.7%) possessed KPC. Only two (2) (33.3%) had CTX-M and TEM while 1 (16.7%) had NDM. Out of the 6 genotypically confirmed 6 *K. pneumoniae*, only 4 (66.7%), 2 (33.3%) and 1 (16.6%) had *rRNA* genes, CTX-M and QnrA respectively. Aside *E. coli* and *K. pneumoniae*, all the bacterial isolates had *rRNA* genes.

The result obtained from this study differs from that obtained by Dahms et al. (2015). Dahms et al. (2015) obtained more CTX-M, and TEM from pig and cow farms, than any other ESBLs however, it shared a similar trend with our study because SHV-producing bacteria were not isolated in all the livestock farms studied. From our study, both ESBLs and carbapenemase producers were obtained. While KPC-producing *E. coli* obtained was prevalent, CTX-M-producing *K. pneumoniae* were prevalent. As expected, *K. pneumoniae* can produce CTX-M groups we ended up detecting and isolating carbapenemase producers. The prevalence of KPC and NDM was uncommon as only CTXM, SHV and TEM producers were commonly expected (Paterson & Bonomo, 2005). The presence of KPC and NDM creates carbapenem resistance among strains of *E. coli* (Goodman, Simner, Tamma & Milstone, 2016). With this, AMR resistance will spread in the farms and upon consumption of meats, it could spread to humans. The presence of KPC, NDM, CTX-M, and TEM contributes to the significant increase in the resistance of *E. coli* and K. pneumoniae seen in the study.

Nanoparticles

Comparing AgNp and ZnONP, the AgNPs were observed to be very active against some *Escherichia coli, Salmonella enterica, Enterobacter aerogenes, Shigella* sp *Vibrio cholerae, Vibrio parahaemolyticus* and *Klebsiella pneumoniae* isolate studied both at low and high concentrations. Zones of inhibitions resulting from the activities of AgNPs ranged between 7.0 mm to 26.0 mm while the zones of inhibitions of ZnONPs ranged between 7.0 mm and 8.9 mm. The dash (-) in all the tables represents the inability of the nanoparticles to inhibit the isolated bacteria. It was observed that the inhibitions appeared to increase with the increased concentrations of AgNPs and ZnONPs of *Gongronema latifolium, Newbouldia laevis Vermonia amygadalina* and *Ocimum gratissum*Comparably, even at high concentrations, AgNPs were more active when than ZnOPs. The increased antibacterial activity of AgNP is attributed to the ability of Ag^{+} to reduce to Ag_2O.

In the electrochemical series, Ag^+ is lower than H+ and thus, less electropositive but readily available to react with reducing agents to form complex reactions. The maximum performance of $AgNO_3$ depends on the bioactive components of the plants used for the synthesis of AgNPs. They are responsible for reducing the silver ions (Teimuri-Mofrad et al., 2017).

AgNP and ZnONP synthesized from *Gongronema latifolium* (utazi) had the most antibacterial activities followed by *Newbouldia laevis* (ogirishi). The *Ocimum gratissium* (scent leaf) of both nanoparticles had the least activity. *G. latifolium* is a tropical plant used for herbal remedies especially for the treatment of infections. It can be used in preparing delicacy or could be eaten raw or boiled (Teimuri-Mofrad et al., 2017). In other words, the antibacterial activity of *G. latifolium* nanoparticles was partly as a result of the presence of bioactive components and antioxidants of the plant (Gutiérrez Rodelo et al., 2022) which prevent oxidation of lipids in body tissues. The bioactive components from the study affected the antibacterial activity of AgNPs. Gutiérrez Rodelo et al. (2022) posited that *G. latifolium* was effective in the treatment of inflammation in rats. The low antibacterial activity of *O. gratissium* could be attributed to some environmental factors (Teimuri-Mofrad et al., 2017). Fact such as drought, oxidation, humidity, and low temperature reduced eugenol and methyl eugenol quantities. Thes molecules are necessary for antibacterial activities of *O. gratissium*. Infact, methyl eugenol and eugenol were reported for their toxicity to cells and certain organs. Excess use can result in the deterioration of some organs (Teimuri-Mofrad et al., 2017).

The silver and zinc nanoparticles of the four plants extract studied were used to induce antibacterial activity against some isolates. *S. aureus* produced the highest resistance by the size of the zone of inhibition (26.0 mm), followed by *S. enterica* (16.0 mm). *K. pneumoniae* had the lowest resistance followed by *Salmonella enterica*. It was only at the high concentrations of the

nanoparticles were *E. coli* and *V. parahaemolyticus* were inhibited. The result obtained from the study was in contrast with that obtained in Mani et al. (2021). The authors used *Allium cepa* for the synthesis of nanoparticles and was effective against *S. aureus.* However, in our study the pathogen was inhibited. It is known that the main targets for antibiotics in *Staphylococcus aureus* are the cell wall, the ribosome, DNA and RNA (Foster, 2017). The resistance to nanoparticles exhibited by *S. aureus* is likely to be as a result of the mobile genes acquired via horizontal gene transfer (Foster, 2017). Some antibiotic producers protect themselves from potentially inhibitory molecules, or in their competitors. However, the pathogen has several ways to eliminate or degrade the nanoparticles or any conventional antibiotics (Hemlata Singh & Tejavath, 2020). Through such processes, the pathogen persists and cause infections.

Ordinarily, *K. pneumoniae* being a capsule producer should have been resistant to the antibiotics based on previous studies (Struve & Krogfelt, 2003). It is an opportunistic pathogen that is resistant to carbapenem drugs (Fontoura, Veriato, Raniero & Castilho, 2023). The susceptibility of this pathogen to nanoparticles synthesized from the plant extracts in this study could be influenced by the type of plants. It might be that the plants' bioactive molecules were more powerful than those studied previously. Moreover, the genetic component in this pathogen responsible for resistance might have been downregulated which is dependent on the time of exposure of the pathogen to nanoparticles (Pareek et al., 2021). The antibacterial activity of the nanoparticles against *K. pneumoniae* is novel as it will bring solution to the resistance and aid in treating infections.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Livestock farming has remained one of the veritable means of improving the livelihood of the Southeasterners. The challenge often experienced is the spread of pathogenic bacteria either directly from the farm animals or the agents that surround them such as air, water and soil. Of all the cities studied, Aba had the highest loads of bacteria. This calls for concerted effort by all stakeholders residing in the city and in extension the entire South-east as the disease resulting from the spread of these microbes from Aba could reach to other South-east states. Holistic hygienic practices should be promoted by livestock keepers as health workers should routinely conduct inspection on those farms and markets where these animals are reared and consumed, respectively. Most ESBLs genes responsible for multidrug resistance in humans can be transferred to human populations through contact with the farm animals, consumption of their flesh and use of their droppings as manures. In order to eliminate or reduce the spread of ESBLs producing bacteria and the genes responsible for the spread, silver and zinc nanoparticles should constantly be used as alternative regimen for treatment and management of infectious diseases in both humans and farm animals since theses microorganisms are resistant to most conventional antibiotics, even the most active ones. New approach involving proper surveillance of disease spread in the environment, animal farms and abattoirs should also be adopted by government. Importantly, basic sites of interaction between the farms, farmers and the environment should be blocked to forestall further spread.

5.2 Recommendations

1. Proper sanitation mechanisms in livestock farms and abattoirs should be introduced and enforced by relevant government agencies.
2. Personal hygiene should be practiced and maintained
3. Livestock feeds should not be produced or processed with antibiotics as these my entrenched multidrug resistant in animals and livestock workers.
4. In the identification of microorganisms, PCR has been identified as an effective diagnostic tool when compared with biochemical assay and cultural characteristics. Therefore, for a more efficient identification method, quantitative polymerase chain reaction (q-PCR) is needed.
5. If conventional antibiotics must be used for the management of recalcitrant bacterial infections, colistin is recommended due to its efficacy.
6. Silver and zinc nanoparticles are also recommended in lieu of conventional antibiotics as they are better antibiotic agents at high concentrations than colistin.

5.3 Contributions to Knowledge

1 Aba, from the results has the highest isolated THBC, TCC and TPPBC when compared with other studied towns. The increase in THBC, TCC and TPPBC in Aba could be due to increased economic activities and improper livestock wastes management and disposal.

2 Poultry and pig farms from the study areas had the highest microbial loads.

3 The use of colistin from the study was very effective when compared with other conventional antibiotics.

4 From the genotypic characterization of livestock bacterial isolates, *E. coli and K. pneumoniae* were the most abundant, and they possessed extended spectrum beta-lactamase enzymes.

5 In all the farms, non-ESBLs producers are more than ESBLs producers.

6 Polymerase chain reaction (PCR) identified microorganisms other than those isolated phenotypically

7 The silver and zinc nanoparticles were effective even more than colistin, being the most active antibiotic, the bacterial isolates were susceptible to.

8 The antibacterial activities of the nanoparticles increased with increasing concentrations at each molarity.

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APPENDIX

Table 4.1: THBC, TPPBC and TCC of air, water soil, hand swabs and feed samples of pig farm in Aba, Umuahia, Okigwe and Mbaise.

	Aba			Umuahia			Okigwe			Mbaise		
	THBC ×10^5	TPPBC ×10^5	TCC ×10^5	THBC ×10^5	TPPBC ×10^5	TCC ×10^5	THBC ×10^5	TPPBC ×10^5	TCC ×10^5	THBC ×10^5	TPPBC ×10^5	TCC ×10^5
Air (CFU/plate/hour)	18.96±0.4 a^1	21.33±0.4 b^1	ND	11.36±0.4 a^2	20.23±0.4 b^2	ND	12.20±0.3 a^3	17.50±0.5 b^3	ND	13.20±0.3 a^4	19.00±0.3 b^4	ND
Water (CFU/ml)	20.33±0.6 a^1	12.73±0.7 b^1	22.16±0.7 c^1	12.30±0.4 a^2	11.66±0.2 b^2	16.86±0.3 c^2	13.23±0.3 a^3	20.06±0.3 b^3	17.80±0.6 c^3	13.96±0.2 a^3	12.30±0.5 b^1	21.00±0.4 c^4
Soil (CFU/ml)	28.43±0.3 a^1	23.10±0.3 b^1	23.26±0.4 c^1	27.50±0.5 a^2	22.06±0.2 b^2	21.16±0.4 c^2	23.33±0.3 a^3	20.33±0.5 b^3	19.20±0.4 c^3	28.16±0.5 a^1	15.56±0.6 b^3	20.46±0.4 c^4
Hand swabs CFU/ml)	19.43±0.5 a^1	15.46±0.6 b^1	20.50±0.7 c^1	11.43±0.4 a^2	12.83±0.7 b^2	14.76±0.6 c^2	6.1 3±0 .6a^3	9.8 3±1 .0b^3	12.73±0.5 c^3	11.46±0.5 a^4	26.23±0.4 b^4	20.36±0.6 c^3
Feeds (CFU/ml)	21.16±0.4 a^1	20.36±1.3 b^1	24.06±0.4 c^1	14.93±0.3 a^2	19.60±0.7 b^1	21.53±0.5 c^2	12.90±0.3 a^3	17.56±0.5 b^2	22.33±0.5 c^3	14.60±0.5 a^4	19.23±0.3 b^1	19.23±0.5 c^4

Values with different numbers as superscripts within a row for the same parameter are significantly different ($p<0.05$; $p<0.01$; $p<0.001$; $p<0.0001$). Values with similar numbers as superscripts within a row for the same parameter are not significantly different ($p>0.05$). Each alphabet represents similar parameter within a column.

Key: THBC-Total heterotrophic Bacteria Count; TPPBC-Total Potential Pathogenic Bacteria Count; TCC-Total Coliform Count; ND- Not determined.

Table 4.2: THBC, TPPBC and TCC of air, water soil, hand swabs and feed samples of cow abattoir in Aba, Umuahia, Okigwe and Mbaise

	Aba			**Umuahia**			**Okigwe**			**Mbaise**		
	THBC ×10^5	**TPPBC× 10^5**	**TCC× 10^5**	**THBC ×10^5**	**TPPBC× 10^5**	**TCC× 10^5**	**THBC ×10^5**	**TPPBC× 10^5**	**TCC× 10^5**	**THBC ×10^5**	**TPPBC×10^5**	**TCC× 10^5**
Air(CFU/plate/hour)	18.56±0.3a[1]	11.70±0.4b[1]	ND	10.50±0.6a[2]	10.3±0.5b[2]	ND	10.66±0.5a[3]	10.8±0.3b[3]	ND	11.46±0.5a[4]	10.66±0.3b[3]	ND
Water (CFU/ml)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Soil (CFU/ml)	26.70±0.7a[1]	11.70±0.4b[1]	17.93±0.6c[1]	26.16±0.5a[1]	16.4±0.4b[2]	16.16±0.3c[2]	26.33±0.2a[1]	15.60±0.3b[3]	16.33±0.5c[2]	25.40±0.4a[2]	16.20±0.3b[2]	15.36±0.4c[3]
Hand swabs (CFU/ml)	20.16±0.5a[1]	10.80±0.3b[1]	10.96±0.2c[1]	18.20±0.4a[2]	9.26±0.5b[2]	9.03±0.6c[2]	17.80±0.9a[3]	9.33±0.5b[2]	9.3±0.3c[2]	17.16±0.3a[4]	9.33±0.4b[2]	9.50±0.4c[2]
Feeds (CFU/ml)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Values with different numbers as superscripts within a row for the same parameter are significantly different ($p<0.05$; $p<0.01$; $p<0.001$; $p<0.0001$). Values with similar numbers as superscripts within a row for the same parameter are not significantly different ($p>0.05$). Each alphabet represents similar parameter within a column.

Key: THBC-Total heterotrophic Bacteria Count; TPPBC-Total Potential Pathogenic Bacteria Count; TCC-Total Coliform Count; ND-Not determined.

Table 4.3: THBC, TPPBC and TCC of air, water soil, hand swabs and feed samples of poultry farm in Aba, Umuahia, Okigwe and Mbaise

	Aba			**Umuahia**			**Okigwe**			**Mbaise**		
	THBC ×10⁵	**TPPBC ×10⁵**	**TCC ×10⁵**	**THBC ×10⁵**	**TPPBC ×10⁵**	**TCC ×10⁵**	**THBC ×10⁵**	**TPPBC ×10⁵**	**TCC ×10⁵**	**THBC ×10⁵**	**TPPBC ×10⁵**	**TCC ×10⁵**
Air (CFU/ plate/ hour)	15.20±0.4a[1]	20.13±0.5b[1]	ND	9.538±0.2b[2]	18.23±0.5b[2]	ND	10.40±0.6a[3]	19.50±0.5b[3]	ND	10.26±0.5a[3]	16.30±0.5b[4]	ND
Water (CFU/ ml)	20.70±0.6a[1]	10.33±0.5b[1]	21.26±0.4c[1]	11.53±0.3a[2]	11.90±0.2b[2]	17.00±0.5c[2]	12.36±0.4a[3]	9.864±0b[1]	16.80±0.4c[3]	13.56±0.6a[4]	12.03±0.4b[2]	19.03±0.4c[4]
Soil (CFU/ ml)	26.26±0.4a[1]	13.23±0.5b[1]	22.36±0.4c[1]	22.36±0.5a[2]	12.33±0.4b[2]	20.96±0.5c[2]	26.10±0.4a[1]	12.36±0b[2]	18.73±0.4c[3]	26.26±0.5a[1]	12.46±0.5b[2]	19.26±0.4c[3]
Hand swabs (CFU/ ml)	19.06±0.4a[1]	20.20±0.5b[1]	15.00±0.8c[1]	11.76±0.5a[2]	13.16±0.7b[2]	13.26±0.4c[2]	12.86±0.2a[3]	14.26±0.3b[3]	9.978±0c[3]	12.66±0.4a[3]	14.26±0.3b[3]	15.36±0.5c[1]
Feeds (CFU/ ml)	20.16±0.6a[1]	16.53±0.3b[1]	12.43±0.4c[1]	14.36±0.5a[2]	13.23±0.4b[2]	11.56±0.5c[1]	12.76±0.6a[3]	12.46±0b[3]	10.23±0.6c[2]	13.36±0.5a[4]	13.86±0.3b[2]	11.26±0c[1]

Values with different numbers as superscripts within a row for the same parameter are significantly different ($p<0.05$; $p<0.01$; $p<0.001$; $p<0.0001$). Values with similar numbers as superscripts within a row for the same parameter are not significantly different ($p>0.05$). Each alphabet represents similar parameter within a column.

Key: THBC-Total heterotrophic Bacteria Count; TPPBC-Total Potential Pathogenic Bacteria Count; TCC-Total Coliform Count; ND-Not determined.

Table 4.4: Prevalence of bacteria isolated from livestock farms and workers in Aba, Umuahia Mbaise and Okigwe

Name of organism	Total	Cow	Pig	Poultry	Poultry workers only
Escherichia coli	933	327(35.0%)	318(34.1%)	186(19.9%)	102(10.9%)
K. pneumoniae	505	118(23.4%)	126(25.0%)	168(33.3%)	93(18.4%)
S. aureus	123	21(17.1%)	18(14.6%)	18(14.6%)	66(53.7%)
S. enterica	15	0(0.0%)	3(20.0%)	12(80.0%)	0(0%)
E. aerogenes	9	3(33.3%)	6(66.7%)	0(0%)	0(0.0%)
***Vibrio* sp.**	9	3(33.3%)	6(66.7%)	0(0%)	0(0%)
***Shigella* sp.**	3	0(0.0%)	3(00%)	0(0%)	0(.0%)
Total	1,597	472(29.6%)	480(30.1%)	384(24.0%)	348(21.8%)

Table 4.5: Prevalence of *Escherichia coli, Klebsiella pneumoniae.,* and *Salmonella enterica* isolated from livestock farms and workers in Aba

Name of organism	Total	Cow (n=210)	Pig (n=110)	Poultry (n=165)
Escherichia coli	218	77(35.3%)	75(34.4%)	43(19.7%)
K. pneumoniae	183	65(35.5%)	67(36.6%)	35(19.1%)
Salmonella enterica	5	0(0%)	0(0%)	5(100%)
Total	406	142(35.0%)	142(35.0%)	83(20.4%)

Table 4.6: Prevalence of *Escherichia coli, Klebsiella pneumoniae.,* and *Salmonella enterica* isolated from livestock farms in Umuahia.

Name of organism	Total	Cow (n=200)	Pig (n=100)	Poultry (n=100)
Escherichia coli	202	67(33.2%)	62(32.2%)	52(25.7%)
K. pneumoniae	45	14(31.1%)	16(35.6%)	9(20.0%)
S. enterica	1	0(0%)	0(0%)	1(100%)
Total	248	81(32.7%)	78(31.5%)	62(25.0%)

Table 4.7: Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella* *enterica* isolated from livestock farms and farmers in Mbaise

Name of organism	Total	Cow (n=107)	Pig (n=110)	Poultry (n=105)
Escherichia coli	185	62(33.5%)	65(35.1%)	40(21.6%)
K. pneumoniae	84	24(28.5%)	28(33.3%)	22(26.2%)
S. enterica	2	0(0%)	0(0%)	2(100%)
Total	271	86(31.7%)	93(34.3%)	64(23.6%)

Table 4.8: Prevalence of *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* isolated from livestock farms and workers in Okigwe

Name of organism	Total	Cow (n=107)	Pig (n=110)	Poultry (n=105)
Escherichia coli	175	32(34.4%)	31(33.3%)	19(20.4%)
K. pneumoniae	42	15(35.7%)	15(35.7%)	8(19.1%)
S. enterica	0	0(0%)	0(0%)	0(0.0%)
Total	217	47(34.8%)	46(34.1%)	(20.0%)

Table 4.9: Prevalence of *Escherichia coli*, *Klebsiella pneumoniae.*, and *Salmonella enterica* isolated from all the poultry workers in Aba, Umuahia, Mbaise and Okigwe

Name of organism	Total	Aba (n=80)	Umuahia (n=40)	Mbaise (n=40)	Okigwe (n=30)
Escherichia coli	73	23(31.5%)	21(28.8%)	18(24.7%)	11(15.1%)
K. pneumoniae	40	16(40.0%)	10(25.0%)	10(25.0%)	4(10.0%)
S. enterica	0	0(0%)	0(0%)	0(0%)	0(0%)
Total	113	39(34.5%)	31(27.4%)	28(24.8%)	15(13.3%)

Table 5: Gram staining, Motility and Biochemical characteristics of bacteria isolated on some selective culture media.

GR	MOT	CAT	OXI	COAG	IN	MR	VP	CIT	URE	H_2S	IDENTITY OF ISOLATES
-	+	-	-	-	+	+	-	-	-	+	***Escherichia coli***
-	-	-	-	-	-	-	+	+	+	+	***Klebsiella sp.***
+	-	+	-	+	-	+	-	-	-	+	***Staphylococcus aureus***
-	+	-	-	-	-	-	-	-	-	+	***Salmonella sp.***
-	-	-	-	-	-	-	+	-	-	+	***Enterobacter sp.***
-	+	-	+	-	-	-	+	-	-	-	***Vibro sp.***
-	-	-	-	-	-	+	-	+	-	+	***Proteus sp.***
-	-	-	-	-	-	+	-	-	-	-	***Shigella sp***

Key:GR, gram staining; MOT, motility test; Cat, catalase; Oxi, oxidase; Coag, coagulase; In, indole; MR, methyl red; VP, Voges Proskaeur; Cit, citrate; Ure, urease; -, negative; +, positive.

Table 7: Antibiotic used and their CISL, 2016 recommended breakpoints

Antibiotics	Symbol	Concentration (µg)	Zone breakpoint(mm) for disc diffusion tests	
			Resistant, ≤	Susceptible, ≥
Ceftazidime	CAZ	30	17	21
Amoxicillin/clavulanic acid	AMC	30	13	18
Amikacin	AK	30	14	17
Gentamicin	CN	10	12	15
Imepenem	IMP	10	19	23
Cloxacillin	OB	5	NA	NA
Cefepime	CFP	30	18	25
Ciprofloxacin	CIP	5	15	21
Cefotaxime	CTX	30	22	26
Cefpodoxine	CPD	10	17	21
Ceftriazone	CRO	30	19	23
Aztreonam	AZT	30	14	18
Meropenem	MER	10	19	23
Colistin	COL	10	10	11

Table 8: Primers and nucleotide sequences

Primers	Nucleotide sequences 5'-3'	Expected Apmplicon size
16SrRNA		1500bp
27F:	5'-AGAGTTTGATCMTGGCTCAG-3'	
1492R:	5'-CGGTTACCTTGTTACGACTT-3'	
SHVF:	5' -CGCCTGTGTATTATCTCCCT-3'	281bp
SHV R:	5'-CGAGTAGTCCACCAGATCCT-3'	
TEMF:	5'-ATGAGTATTCAACATTTCCGTG-3'	400bp
TEMR:	5'-TTACCAATGCTTAATCAGTGAG-3'	
CTX-MF:	5'-CGCTTTGCGATGTGCAG-3'	550bp
CTX-MR:	5'-ACCGCGATATCGTTGGT-3'	
KPCF:	5'-GCTCAGGCGCAACTGTAAG-3'	150 bp
KPCR:	5'-AGCACAGCGGCAGCAAGAAAG-3'	
NDMF:	5′-GGTTTGGCGATCTGGTTTTC-3′	621 bp
NDMR:	5′-CGGAATGGCTCATCACGATC-3	
QnrBF:	5′-GATCGTGAAAGCCAGAAAGG-3′	450 bp
QnrBR:	5′-CGATGCCTGGTAGTTGTCC-3′	