

**IDENTIFICATION OF MULTI-DRUG RESISTANT BACTERIAL
ISOLATES IN SOIL CONTAMINATED WITH ABATTOIR
EFFLUENTS.**

BY

JOSEPH JOHN OSHIOMHA

LSC2009936

UNIVERSITY OF BENIN

BENIN CITY

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF
MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF
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REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN
MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.**

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CERTIFICATION

This is to certify that this project work was successfully carried out by **JOSEPH JOHN OSHIOMHA** with matriculation number **LSC2009936**, of the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria, under my supervision.

DR. (MRS.) O.A. OLOGBOSERE

(Project Supervisor)

DATE

APPROVAL

This project work was carried out by **JOSEPH JOHN OSHIOMHA** with matriculation number **LSC2009936** in partial fulfillment of the award of a Bachelor of Science, B.Sc. (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

PROF. (MRS.) F.I AKINNIBOSUN

Date

(Head of Department)

DEDICATION

This report is primarily dedicated to the Divine Providence for His abundant blessings, mercy, and guidance throughout my university journey.

I am also dedicating this project to my parents for their constant love and support.

ACKNOWLEDGEMENTS

First and foremost, I wish to give my profound gratitude to God Almighty for His faithfulness, goodness and grace throughout my life and academic journey.

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ABSTRACT

The discharge of untreated abattoir effluents into the environment poses significant public health risks, particularly due to the spread of multidrug-resistant (MDR) bacteria. This study aimed to isolate and characterize MDR bacteria from soil samples contaminated with abattoir effluents in Benin City, Edo State, Nigeria. Soil samples were collected from five abattoirs Ekehuan, Santana Market, University of Benin (UNIBEN), Oluku, and Ikpoba—at a depth of 5–10 cm and analyzed microbiologically. The highest bacterial load was recorded at UNIBEN abattoir (5.56 log₁₀ CFU/mL), while Ekehuan had the lowest (3.96 log₁₀ CFU/mL). Morphological and biochemical identification revealed *Escherichia coli*, *Bacillus* sp., *Staphylococcus* sp., *Proteus* sp., *Pseudomonas* sp., *Salmonella* sp. and *Klebsiella* sp. as the predominant isolates. *Escherichia coli* was the most prevalent (23.33%), followed by *Bacillus* sp. (21.05%) and *Staphylococcus* sp. (16.34%). Antibiotic susceptibility testing showed high resistance levels, with Gram positive bacteria, *Staphylococcus Aureus* having a multiple resistant index of 0.4 while *Pseudomonas* sp and *E coli* exhibit the highest resistant index of 0.6 respectively. The presence of MDR bacteria in abattoir effluents highlights the need for improved wastewater management and antimicrobial resistance monitoring to mitigate environmental and public health risks.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The proliferation of multidrug-resistant (MDR) bacteria represents a significant public health challenge globally. Multidrug resistance (MDR) refers to the ability of bacteria to resist the effects of multiple antibiotics, complicating treatment and increasing morbidity and mortality rates (Laxminarayan *et al.*, 2016). The World Health Organization (WHO) has highlighted antimicrobial resistance (AMR) as one of the most pressing health issues of the 21st century, necessitating urgent interventions (WHO, 2020). While much focus has been placed on clinical settings, the role of environmental reservoirs, particularly soil contaminated with abattoir effluent, has received less attention.

Abattoirs discharge a variety of waste materials, including blood, feces, and urine, directly into the environment. These effluents often contain antibiotics, pathogens, and organic matter that create conducive conditions for bacterial proliferation and horizontal gene transfer (HGT), fostering the spread of resistance genes (Adekanmbi and Falodun, 2015). Repeated exposure to antibiotic residues exerts selective pressure, promoting the survival and multiplication of MDR bacteria in soil (Christou *et al.*, 2017). This situation poses risks to public health by contaminating food crops, water sources, and indirectly contributing to zoonotic infections. The contamination of soil with abattoir effluent also provides a fertile ground for the persistence of antimicrobial residues, which can further accelerate the development of resistant bacterial strains (Wellington *et al.*, 2013).

Research has demonstrated that abattoir effluents can serve as hotspots for antibiotic-resistant bacteria (Igbinosa *et al.*, 2017). In Nigeria, the unregulated disposal of abattoir waste exacerbates environmental contamination and facilitates the spread of MDR bacteria

(Nwankwo *et al.*, 2021). Studies by Uddin *et al.* (2020) have shown that bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolated from abattoir effluent-contaminated soil exhibited resistance to multiple antibiotics, including third-generation cephalosporins and carbapenems. Similarly, research conducted by Dulo *et al.* (2015) in Ethiopia isolated *Staphylococcus aureus* and *Acinetobacter baumannii* from abattoir-contaminated environments, highlighting their resistance to methicillin and aminoglycosides.

The indiscriminate discharge of untreated abattoir effluent into the environment is a major contributor to the proliferation of MDR bacteria in developing countries (Olowe *et al.*, 2019). This practice not only leads to environmental pollution but also increases the risk of food and water contamination, threatening public health. MDR bacteria in soil can colonize crops, enter the food chain, and ultimately contribute to the emergence of difficult-to-treat infections (Pruden *et al.*, 2013).

Despite the evident environmental and health risks, limited research has been conducted to isolate and identify MDR bacteria from abattoir-contaminated soil in Nigeria. This knowledge gap undermines efforts to develop effective mitigation strategies to curb the spread of antimicrobial resistance (Ogbonna *et al.*, 2021). In regions with inadequate waste management infrastructure, the accumulation of antibiotic residues and resistant bacteria in the environment poses a significant challenge to public health (Iwu *et al.*, 2022). Understanding the extent of MDR bacterial contamination in soil can provide insights into environmental reservoirs of resistance genes and their potential to transfer to human pathogens.

Furthermore, this research aligns with the One Health approach, which emphasizes the interconnectedness of human, animal, and environmental health in combating AMR (Van

Boeckel *et al.*, 2019). By addressing environmental reservoirs of resistance, this study aims to contribute to the growing body of knowledge on environmental AMR by isolating and characterizing MDR bacterial strains from soil contaminated with abattoir effluent.

1.2. AIM AND OBJECTIVES

The aim of this study was to identify multidrug-resistant bacterial isolate from soil contaminated with abattoir effluent.

The specific objectives of this research were to:

1. Enumerate and isolate the bacterial isolates present in the contaminated soil samples.
2. Identify the bacterial isolates in the contaminated soil samples
3. Determine the Antibigram of the bacterial isolates
4. Assess the prevalence of multidrug resistance bacteria in abattoir effluent contaminated soil.

CHAPTER TWO

LITERATURE REVIEW

2.1. ABATTOIR AND ABATTOIR EFFLUENT

Abattoirs, commonly referred to as slaughterhouses, are facilities where animals are processed for meat production. The discharges from slaughterhouses fall under the group of industrial wastes and it can be considered as the hazardous waste as it is harmful to the human and animal health and deteriorates environment if it is not properly treated (Chukwu, 2008). These operations generate significant amounts of effluent, which poses a considerable environmental and public health risk due to the potential for contamination with a variety of microorganisms, including pathogenic bacteria. One of the most concerning groups of bacteria found in abattoir effluent is Gram-negative bacteria (Ogbomida *et al.*, 2016). Gram-negative bacteria are of particular interest because they are known to harbor numerous pathogenic species and have a natural propensity for developing antibiotic resistance, which makes their presence in the environment a significant public health issue (Ajayi *et al.*, 2023).

In developing countries like Nigeria, abattoirs often lack proper waste treatment systems, allowing untreated effluent to be released into the environment, including rivers, farmlands, and surrounding communities (Manjunath *et al.*, 2000). This can lead to the contamination of soil and water sources, ultimately exposing the population to pathogenic microorganisms, particularly in areas with poor sanitation infrastructure.

In Nigeria, there are numerous abattoirs that serve the meat consumption needs of the region. However, these abattoirs also produce large quantities of effluent, which is often improperly

managed which subsequently result in environmental pollution and the dissemination of pathogenic microorganisms, especially Gram-negative bacteria, which are known to be prevalent in such settings (Ogbomida *et al.*, 2016).

Abattoir effluent typically contains blood, feces, urine, undigested food, fat, and other animal by-products (Akinro *et al.*, 2009). It also contains high concentrations of organic matter and nutrients such as nitrogen and phosphorus, which promote microbial growth. The improper disposal of such effluent leads to significant pollution in the receiving water bodies and surrounding environments (Adelegan, 2002). Abattoir effluent serves as a reservoir for various microorganisms, including Gram-negative bacteria, which can spread through water, soil, and even air, affecting both animal and human health (Akpan *et al.*, 2020).

One key characteristic of Gram-negative bacteria is the structure of their cell walls, which contains an outer membrane rich in lipopolysaccharides (LPS). This LPS layer not only contributes to their pathogenicity but also makes these bacteria more resistant to antibiotics and disinfectants. Notable pathogenic Gram-negative bacteria include *Escherichia coli*, *Salmonella spp.*, *Klebsiella spp.*, and *Pseudomonas aeruginosa*, all of which are commonly found in abattoir effluent (Ajayi *et al.*, 2020). These bacteria have been linked to various infections in humans, including gastrointestinal diseases, urinary tract infections, and even septicemia (Antunes *et al.*, 2014).

The health risks associated with the release of untreated abattoir effluent are multifaceted. Pathogenic Gram-negative bacteria, which thrive in such environments, pose a significant threat to public health. When such effluent is released into nearby water bodies without proper treatment, it contaminates drinking water supplies, which can lead to waterborne diseases. In areas surrounding abattoirs, residents are often exposed to these microorganisms through direct contact with contaminated water or soil, consumption of contaminated crops irrigated with such water, or through the air in the form of bioaerosols (Molla *et al.*, 2017).

Furthermore, Gram-negative bacteria are notorious for their ability to acquire and transfer antibiotic resistance genes. The increasing prevalence of multidrug-resistant (MDR) strains of Gram-negative bacteria in abattoir effluent has been documented in multiple studies (Odonkor and Ampofo, 2013). The excessive use of antibiotics in livestock production—both for therapeutic purposes and as growth promoters—contributes to the development of resistant bacteria that can then enter the human population through contaminated water and food sources (Hawkey, 2015).

The environmental implications of releasing untreated abattoir effluent into ecosystems are severe. These effluents, laden with high organic loads, significantly reduce the dissolved oxygen levels in water bodies, leading to the death of aquatic organisms. Additionally, the presence of nutrient-rich effluent can cause eutrophication, further disrupting aquatic ecosystems (Akatah, *et al.*, 2018). The persistence of Gram-negative bacteria in these environments exacerbates the problem by making the water unsuitable for human use and agricultural purposes.

In Benin City, the proliferation of small-scale and unregulated abattoirs exacerbates the problem. Most abattoirs lack appropriate waste treatment facilities, and the effluent generated is typically discharged directly into nearby rivers or the municipal drainage system, which eventually contaminates larger water bodies (Falodun and Rabi, 2017). According to Adesemoye *et al.* (2006), the levels of microbial contamination, especially by Gram-negative bacteria, in abattoir effluent in Nigeria are often far beyond acceptable limits, posing a serious threat to both the environment and public health. Given the critical public health and environmental challenges posed by the discharge of untreated abattoir effluent, particularly in regions like Benin City, it is important to isolate and characterize Gram-negative bacteria from these effluent sources. This will not only help in identifying potential pathogenic strains but also in assessing their antibiotic resistance profiles. Understanding the prevalence of

antibiotic-resistant bacteria in such environments is key to implementing effective public health interventions, as well as for guiding policymakers in the development of regulations to manage abattoir effluent properly. Moreover studies on the microbiological quality of abattoir effluent are essential in highlighting the need for improved waste management practices in these facilities. Despite the known risks, many abattoirs in Nigeria still operate without adequate waste treatment mechanisms, increasing the likelihood of environmental contamination and public health outbreaks (Ezeugwunne *et al.*, 2009).

2.2 CHARACTERISTICS ABATTIOR EFFLUENT

Globally, the treatment of meat processing wastewater is a growing concern due to its complex and highly variable composition. Meat processing facilities, including abattoirs, generate large amounts of wastewater, which contain a mixture of fats, proteins, carbohydrates, fibers, and pathogens, as well as residues of veterinary drugs. These components make the wastewater difficult to treat using conventional methods. The fats and proteins tend to form scum layers or cause blockages in wastewater treatment systems, while the high organic content contributes significantly to biochemical oxygen demand (BOD) and chemical oxygen demand (COD). This high organic load can lead to severe environmental degradation if the wastewater is not properly treated before being discharged into water bodies (Brennan *et al.*, 2021).

One of the major issues associated with abattoir wastewater is its variability. Depending on the type of animals slaughtered, the practices employed in the facility, and the specific slaughtering techniques, the composition of the effluent can vary widely. This variability complicates the development of standardized treatment methods for abattoir wastewater. For instance, waste from poultry processing may contain more feathers and lighter organic material, whereas beef or pork slaughterhouses generate higher levels of fats, proteins, and heavier solids. These differences in waste composition mean that a one-size-fits-all approach

to wastewater treatment is rarely effective, necessitating tailored solutions based on the specific characteristics of the effluent (Fasanmi *et al.*, 2017).

Abattoir wastewater typically contains high concentrations of total suspended solids (TSS), biochemical oxygen demand (BOD), total nitrogen (TN), total phosphorus (TP), total organic carbon (TOC), and fats, oils, and grease (FOG). For example, total suspended solids (TSS) in the wastewater arise from the accumulation of blood, tissue, fat particles, and other solid materials from the slaughtering process. These solids can clog treatment systems and waterways, creating a dense sludge that is difficult to break down. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) reflect the amount of oxygen required to biologically or chemically degrade the organic matter in the wastewater. Abattoir effluent typically has very high BOD and COD values due to the large amounts of proteins, fats, and organic material present (Zweifel *et al.*, 2016). If released into water bodies without adequate treatment, this high organic load can result in oxygen depletion, which in turn leads to the death of aquatic organisms and the disruption of aquatic ecosystems.

In addition to BOD and TSS, total nitrogen (TN) and total phosphorus (TP) are also critical pollutants in abattoir wastewater. These nutrients, if not removed or properly managed, can contribute to eutrophication when they enter lakes, rivers, and other water bodies. Eutrophication is the excessive growth of algae and aquatic plants caused by an oversupply of nutrients, primarily nitrogen and phosphorus. This process depletes the oxygen levels in the water, leading to the collapse of aquatic ecosystems and the death of fish and other marine life. Eutrophication also impairs the usability of water bodies for recreational, industrial, and domestic purposes (Nwanta *et al.*, 2011).

Veterinary drug residues also pose significant concerns in abattoir wastewater. Antibiotics, antiparasitics, and other drugs used to maintain animal health can remain in the tissues and fluids of the animals, eventually making their way into the wastewater. The presence of these

compounds in wastewater can lead to the development of antibiotic-resistant bacteria in the environment, posing a major public health threat. This is particularly concerning as antibiotic resistance continues to rise globally, making infections harder to treat and increasing the risk of disease outbreaks (Marshall and Levy, 2011).

The large volumes of water used during various stages of meat processing further exacerbate the issue of abattoir wastewater. Water is essential for the washing of carcasses, cleaning of facilities, and general sanitation practices. However, this widespread use of water also leads to the generation of significant volumes of wastewater. Depending on the scale of operations, abattoirs can consume thousands of liters of water per day, resulting in a substantial amount of wastewater that needs to be managed. The combination of high water use and the complex composition of the effluent make meat processing wastewater one of the most significant pollution sources within the agribusiness sector (Fasanmi *et al.*, 2017).

It is very important to improve slaughtering and processing techniques in order to minimize water use and reduce the generation of pollutants at the source. For instance, introducing dry cleaning methods to remove solid waste before rinsing carcasses with water, or recycling water within the facility to reduce overall consumption, are practical measures that can help mitigate the environmental impact of abattoir operations. Additionally, implementing advanced wastewater treatment technologies, such as anaerobic digestion, constructed wetlands, or membrane bioreactors, can help to remove suspended solids, organic matter, and nutrients more effectively, thereby improving the quality of the discharged water (Jiang *et al.*, 2019).

2.3 MICROBIAL LOAD OF WASTEWATER

Meat plant wastewater may acquire pathogens naturally from the hides and digestive tracts of the slaughtered animals and unhygienic practices of personnel and equipment involved during slaughter (Mittal, 2004). Abattoir wastewater contains several million CFU/100mL

of total coliform, faecal coliform and *Streptococcus* groups of bacteria which indicates the possible presence of pathogens of enteric origin such as *Salmonella* spp. and *Campylobacter jejuni* and some gastrointestinal parasites (USEPA,2002). Marriott (1999) reported that *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7 etc. and other like some typical pathogens in meat plant wastewater.

A number of pathogenic species of *Salmonella*, including *Salmonella enteritidis* are common inhabitants of the enteric tracts of livestock and poultry and may be present in meat and poultry processing wastewaters. *Salmonella* was considered as a pollutant for possible regulation in these wastewaters because of its potential risk to public health through public and private water supplies, contact forms of recreation and wild life exposure to effluents discharged to natural waters (USEPA, 2002). Stolle (1981) investigated the contamination of *Salmonella* during the slaughter of cattle in Berlin and was isolated from swab samples (4.3% +ve incidence) and from faecal samples (0.75% only positive). He stated that among all the processing steps the cutting off the hooves and loosening the skin of legs gave highest recovery of *Salmonella* and noticed that the mud on the hooves and skin are the most important sources of *Salmonella*.

Listeria monocytogenes are found in the intestinal tracts of sheep, cattle, chickens and swine as well as in soil and decaying vegetation. *L. monocytogenes* has been found both in pork and beef slaughterhouses (van den Elzen and Snijders, 1993; Gill and Jones, 1995; Sammarco *et al.*, 1997; Korsak *et al.*, 1998). The sites positive for *L. monocytogenes* were non-food contact surfaces such as floors, walls, trucks, drains, shoes, doors, door handles including sanitized floor mats and foot baths (Klausner and Donnelly, 1991; Samelis and Metaxopoulos, 1999; Miettinen *et al.*, 2001; Vogel *et al.*, 2001).

Bacillus cereus spores are ubiquitously present in the soil, feed, faeces and farm environment, and may easily contaminate the skin, hides, udders, raw milk and processing equipment's

(Christiansson *et al.*, 1999). During slaughtering process, the meat is exposed to many sources of *B. cereus* contamination (Lawrie, 1998) and it enters the meat and meat products through cross contamination also *B. cereus* is one of the potential spoilage bacteria associated with red meat (Nel *et al.*, 2004). Adeyemo *et al.* (2002) studied the water quality and sanitary condition in Bodija abattoir in Nigeria and they reported that the faecal *Streptococcus* count in the water from wells that within the abattoir and 50-80m away from the abattoir as 8.8×10^4 CFU/mL and 4.3×10^4 CFU/mL, respectively.

Pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* can be found in animal carcasses and waste. In a study conducted by Ullah *et al.*, (2022) in Nigeria's Minna abattoir, numerous bacteria were isolated from 12 waste, including *Bacillus*, *Streptococcus*, *Escherichia*, *Klebsiella*, *Staphylococcus*, *Pseudomonas*, *Salmonella*, and *Penicillium* spp. These findings are consistent with of studies by Coker *et al.*, (2001) and Svanstrom (2014) on antibiotic-resistant bacteria in abattoir wastes.

2.3.1. *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive bacterium that is commonly found on the skin and mucous membranes of humans and animals. It is one of the most frequent causes of skin infections, respiratory diseases, and food poisoning. In abattoir effluent, *S. aureus* contamination can occur during the slaughtering process, especially when hygiene practices are poor. The bacteria can survive in water and on surfaces for extended periods, making them a persistent contaminant in environments affected by abattoir waste (Olanrewaju *et al.*, 2021).

Of particular concern is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in abattoir effluent. MRSA is resistant to several commonly used antibiotics,

making infections caused by these strains difficult to treat. The presence of MRSA in abattoir effluent can contribute to the spread of antibiotic resistance in the environment, posing a serious public health threat (Marshall and Levy, 2011).

2.3.2. *Clostridium* spp.:

The genus *Clostridium* includes several pathogenic species, such as *Clostridium perfringens* and *Clostridium botulinum*, which are known to cause food poisoning and other serious illnesses in humans. *C. perfringens* is commonly found in the intestines of animals and can contaminate meat during the slaughtering process. The bacteria produce toxins that can cause necrotic enteritis and gas gangrene in humans (Adesemoye *et al.*, 2006).

Clostridium bacteria are spore-forming organisms, which allows them to survive in harsh environmental conditions for long periods. The spores can persist in abattoir effluent and soil, making them difficult to eradicate. When abattoir waste is discharged into water bodies, the spores can contaminate water supplies and pose a risk of infection to humans who come into contact with the contaminated water (Wizor and Nwankwoala, 2019).

2.3.3. *Escherichia coli* (*E. coli*)

E. coli is a Gram-negative bacterium that is commonly found in the intestines of animals and humans. While most strains of *E. coli* are harmless, certain strains, such as ***E. coli* O157**, are pathogenic and can cause severe gastrointestinal illnesses in humans. *E. coli* is an indicator of fecal contamination in water bodies because it is a normal part of the intestinal flora of warm-blooded animals. In abattoir effluent, the high organic content promote the growth of *E. coli*, and its presence in contaminated water bodies can lead to outbreaks of foodborne illnesses, especially when the water is used for drinking or irrigation (Darah and Opigo, 2020).

Pathogenic *E. coli* strains produce toxins, such as Shiga toxins, that can cause hemolytic uremic syndrome (HUS) in humans, a condition that leads to kidney failure and, in some cases, death. The transmission of *E. coli* to humans can occur through direct contact with contaminated water or through the consumption of undercooked meat or vegetables irrigated with contaminated water (Nwanta et al., 2008).

E. coli has been identified as a key ARB in abattoir effluent. Resistant strains of *E. coli*, particularly those resistant to beta-lactams, tetracyclines, and fluoroquinolones, have been detected in wastewater from abattoirs. This raises serious concerns about the contamination of food and water sources, as *E. coli* is a known cause of gastrointestinal infections in humans. The presence of antibiotic-resistant strains in effluent underscores the risk of resistance genes spreading to other bacteria in the environment, further exacerbating the public health threat (Igbinosa and Obuekwe, 2014).

2.3.3. *Salmonella* spp.

Salmonella is a genus of Gram-negative bacteria that includes several pathogenic species responsible for illnesses such as salmonellosis and typhoid fever. *Salmonella* is commonly found in the intestines of animals, particularly cattle and poultry, and can be excreted in large quantities through feces. In abattoirs, the handling of animal intestines and the slaughtering process can lead to the contamination of effluent with *Salmonella*, which can subsequently enter nearby water bodies (Bhunja, 2018). Exposure to *Salmonella*-contaminated water can cause gastroenteritis in humans, characterized by diarrhea, abdominal cramps, and fever. In severe cases, especially when the bacteria spread to the bloodstream, it can lead to life-threatening conditions. The risks of *Salmonella* contamination is particularly high in areas where abattoirs discharge untreated wastewater into rivers and streams that are used for domestic purposes (Ogbonna et al., 2014).

Salmonella are significant contributors to foodborne illnesses, and multidrug-resistant (MDR) *Salmonella* strains have been isolated from abattoir effluent. MDR *Salmonella* is particularly concerning due to its ability to resist multiple antibiotics, which makes infections difficult to treat. The detection of these resistant strains in abattoir effluent suggests that they could spread through food chains and water systems, potentially affecting both human populations and animal husbandry practices (Van Boeckel *et al.*, 2015).

2.3.5. *Pseudomonas* spp.

Pseudomonas is a genus of Gram-negative bacteria that are widely distributed in the environment, including in soil, water, and plants. One of the most important species in this genus is *Pseudomonas aeruginosa*, which is known for its resistance to antibiotics and its ability to cause infections in humans, particularly in individuals with compromised immune systems (Marshall and Levy, 2011).

Pseudomonas aeruginosa is an opportunistic pathogen that can cause a variety of infections, including respiratory tract infections, urinary tract infections, and wound infections. In abattoir effluent, *P. aeruginosa* can thrive in the nutrient-rich environment and be carried into nearby water bodies. The bacteria's ability to form biofilms makes it particularly resilient in the environment, allowing it to persist in contaminated water and increase the risk of infection (Adeyemi *et al.*, 2020).

2.4 IMPACTS OF ABATTOIR WASTEWATER ON ENVIRONMENT

The increased demand for meat as a result of urbanization has prompted the establishment of numerous abattoirs. Abattoir operations in low-income nations are technologically less developed, with poor waste handling and disposal systems, when compared to industrialized countries where waste creation, analysis, and treatment are prerequisites for building abattoirs (Coker *et al.*, 2001; Chukwu, 2008).

Poor abattoir waste disposal can have negative effects on the environment including, human health. Failure to manage this waste can lead to environmental hazards, public health problems, and ecological contamination. Improper management practices in slaughterhouses will affect the quality of water, soil and air which endanger of the aquatic life (Raymond, 1977).

2.4.1 Impact on the soil

Due to contamination of the high polluted water in the soil, the oxygen is utilized as an electron acceptor by aerobic microbes for the degradation of organic matter leads to prompting denitrifying microflora to reduce available nitrate into gaseous N_2 leading to cause some negative effects i.e. anaerobic methanogen bacteria produce more methane than aerobic methanogenic bacteria, thus causing greenhouse effect and global warming as methane is five times more effective than CO_2 (Tortora *et al.*, 1997; Rusanov *et al.*, 2002; Tourova *et al.*, 2002; Madigan *et al.*, 2003).

The leaching of the wastewater through soil into ground water will cause some adverse effects due to increased concentration of elements like nitrates (George, 1987) and microbial contamination (Bitton and Harvey, 1992; Federov *et al.*, 1993; Tortora *et al.*, 1997; Lapygina *et al.*, 2002; Shah and Thakur, 2002). During passage of wastewater through soil, clay, fine sandy materials and organic matter by adsorption and trapping process can remove pathogenic microbes and some dissolved organic matter, but the passage of wastewater beyond the carrying capacity of the natural processes, the diversity of autochthonous species could diminish while, the individual species may able to survive more to cause grave consequences in ground water (Baker and Herson, 1994; Atlas and Bartha, 1998; Lapygina *et al.*, 2002).

Osemwota (2009) studied the effects of abattoir effluent on the physic-chemical properties of

soil, which was treated to different rates of abattoir effluent under natural environment and in pot experiment then they found that the exchangeable bases (Ca, Mg, K and Na) were reduced whereas the available phosphorus and some micro nutrients were significantly increased.

2.4.2 Impact on water

Poorly disposed effluent wastewater from abattoirs on surface water bodies has been found to raise the pH, temperature, BOD, COD, total solids (TS), and turbidity (Weobong *et al.*, 2011; Adewumi *et al.*, 2016). Because of their proximity to abattoir 14 waste disposal sites, leachates pollute aquifers and transfer enteric pathogens, parasites, and nutrients into waterways (Adegbola *et al.*, 2012; Hassan *et al.*, 2014). There is also the possibility of pathogenic infectious organisms with antibiotic resistance patterns being transferred to humans (Coker *et al.*, 2001; Svanstrom (2014)).

The effluent discharges into receiving waters and the cumulative hazardous effects on the environment have received much attention due to rapid industrialization in modern society. Industrial and abattoir wastes containing high concentration of microbial nutrients in turn promote growth of high coliform type and other microbial forms significantly, both in effluent and receiving waters (Ezeronye and Amogu, 1998).

When the organic matter present in the wastewater exceeds the capacity of its microbial break down and recycle of the organic matter in water, which promotes the rapid growth or blooms of algae leading the eutrophication of the water bodies (Omole and Longe, 2008). Cooper *et al.* (1979) reported that abattoir effluents significantly increased the levels of nitrogen, phosphorus, BOD and other nutrients causing river pollution and the quality of ground water in vicinity also adversely effected due to seepage of abattoir effluents and pose health risks for butchers and users of well water (Sangodoyin and Agbawhe, 1992).

Sangodoyin and Agbawhe (1992) also studied the effect of slaughter house wastewater on the ground and surface water and reported the contents of COD and total solids 2410 and 13100 mg/L respectively and further increased the contents of COD, calcium, chlorides and nitrates of the underlying aquifer.

Ezeronye and Ubalua (2005) studied the effect of abattoirs and industrial effluents on the contents of heavy metals and microbial quality of Aba river in dry and wet seasons and reported little higher and non-significant levels of lead (0.064 mg/L), iron (0.81 mg/L), arsenic (0.1 mg/L), chromium (0.006 mg/L) and mercury (0.009 mg/L), and within the USEPA permissible limits. The mean total viable count for the bacterial colonies ranged from 0.059×10^6 to 1.01×10^8 CFU/mL. The highest bacterial count was observed at the abattoir site followed by the downstream during dry and rainy seasons. The predominant bacteria include *Staphylococcus* species, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella*, *Bacillus* and *Clostridium* species were isolated from the river water.

Chukwu (2008) has analysed the pollution effect from abattoir waste in Minna, Nigeria on the nearby ground water by sampling from the two wells A and B, which were 10-12 m and 300-320m away from the slaughtering area and abattoir, respectively and they reported levels of BOD (3.25, 2.41 mg/L); COD(2.11,19.2 mg/L); Nitrates (1.0, 0.6 mg/L) and Phosphates (2.04, 0.6 mg/L)for both wells respectively and the values are more than that of the recommended limits by WHO for drinking water. Chukwu *et al.* (2008) evaluated the Tayi stream water quality, in which the waste effluents of Minna abattoir, Nigeria is mixed by analyzing water quality at upstream, point stream and downstream during the wet season and they found that iron content (0.91, 0.52 and 0.53 mg/L), pH (6.8, 8.8 and 6.8), total alkalinity (164.0,104.0 and 90 mg/L) at 3 sites respectively and they revealed that effluent has lowered the quality of Tayi stream.

2.4.3 Impact on environment

Guo and Sims (2000) investigated the effects of slaughterhouse effluents irrigation on soil, tree biomass production and nutrient uptake in *Eucalyptus globus* seedling on growth cabinets at various temperatures (5, 15 and 25°C) and at various irrigation rates of 0 (control), 10, 20, 30 or 40 mm/wk. They found reduction in pH of solids, increase in soil nutrient concentration such as soil nitrogen and phosphorus and enhanced tree leaf area. Further, they reported that 20 mm/ wk irrigation rate has about twice the leaf area of other seedlings, enhanced biomass production, enhanced nutrient uptake for nitrogen (84 mg/tree) and phosphorus (9 mg/tree). Based on the results, they concluded that temperature and irrigation rates significantly influenced the production of certain crops.

Bello and Oyedemi (2000) reported that noise pollution abattoir activities and its location has influence on prevalence of some diseases i.e. pneumonia, diarrhoea, typhoid, asthma, wool sorter's diseases, respiratory and chest diseases etc. Lot of metals present in slaughterhouse wastewater are serious concern due to their potential toxicity to aquatic plant and animal species through bioaccumulation and bio magnification in the food chains. The high concentrations of heavy metals in water toxic to organisms and wild life leading to problem of ultimate disequilibria in the natural ecological balance (Babich and Stoczky, 1985) and the toxicity of a moderately toxic metal could be enhanced by synergisms and population of organisms present may decline (Laws, 1981) and the accumulation of these toxic metals in the aquatic food is a potent threat to public health (Ezeronye and Ubalua, 2005).

Suspended solids in wastewater can clog fish gills, and cause a decrease in oxygen transport and increase in turbidity. Particulate materials in wastewater block the liquid distribution systems, dilute the sludge with inactive material and favour the growth on particle surface rather than in a granular biomass which cause washout (Mittal, 2004). Few epidemiological

studies have established definitive adverse health impacts attributable to pathogenic organisms in agricultural reuse of wastewaters from abattoirs. Helminthic diseases caused by *Ascaris* and *Trichuris* spp. are endemic in areas of the world where raw untreated sewage is used to irrigate salad crops and vegetables eaten uncooked (Shuval *et al.*, 1985).

2.5. PRESENCE OF MULTI-DRUG RESISTANT BACTERIA IN ABATTOIR EFFLUENT

Antibiotic-resistant bacteria (ARB) in abattoir effluent present a significant concern for both public health and the environment. Numerous studies have highlighted the presence of ARB in these wastewaters, with particular focus on zoonotic pathogens bacteria that can be transmitted from animals to humans. These pathogens not only pose a direct threat to human health through potential exposure but also contribute to the broader dissemination of antibiotic resistance within ecosystems. The following are some of the most commonly reported Antibiotic-resistant bacteria in abattoir effluent.

2.5.1. *Staphylococcus aureus*

Among the most concerning Antibiotic-resistant bacteria in abattoir effluent is Methicillin-resistant *Staphylococcus aureus* (MRSA), a well-known cause of severe infections in humans and animals. The presence of MRSA in abattoir wastewater is alarming due to the high risk of occupational exposure for slaughterhouse workers, who may come into direct contact with contaminated water and surfaces. Moreover, MRSA can easily spread within the community through direct contact or via environmental pathways. This highlights the potential for abattoir effluent to serve as a reservoir for resistant pathogens, posing a broader risk to public health (Wright, 2011).

The detection of Antibiotic-resistant bacteria such as *E. coli*, *Salmonella* spp., and MRSA in abattoir effluent underscores the potential risks posed by untreated or inadequately treated waste from slaughterhouses. The presence of these resistant pathogens in wastewater not only increases the likelihood of environmental contamination but also facilitates the spread of resistance genes, ultimately impacting both human health and the safety of ecosystems.

2.5.2. *Escherichia coli*

E. coli, a bacterium commonly found in the intestines of animals and humans, has been identified as a key ARB in abattoir effluent. Resistant strains of *E. coli*, particularly those resistant to beta-lactams, tetracyclines, and fluoroquinolones, have been detected in wastewater from abattoirs. This raises serious concerns about the contamination of food and water sources, as *E. coli* is a known cause of gastrointestinal infections in humans. The presence of antibiotic-resistant strains in effluent underscores the risk of resistance genes spreading to other bacteria in the environment, further exacerbating the public health threat (Adeleke *et al.*, 2020).

2.5.3. *Salmonella* spp.

Salmonella is another major pathogen found in abattoir wastewater. These bacteria are significant contributors to foodborne illnesses, and multidrug-resistant (MDR) *Salmonella* strains have been isolated from abattoir effluent. MDR *Salmonella* is particularly concerning due to its ability to resist multiple antibiotics, which makes infections difficult to treat. The detection of these resistant strains in abattoir effluent suggests that they could spread through food chains and water systems, potentially affecting both human populations and animal husbandry practices (Van Boeckel *et al.*, 2015).

2.6. ROUTES OF THE SPREAD OF ANTIBIOTIC RESISTANCE

The spread of antibiotic resistance from abattoir effluent to the broader environment and human populations occurs through multiple interconnected pathways. Each of these pathways allows antibiotic-resistant bacteria (ARB) and resistance genes to permeate ecosystems, contributing to the rise of resistance in microbial communities. The primary routes of dissemination include surface water contamination, soil contamination, occupational exposure, and food chain contamination. Understanding these pathways is crucial to developing effective mitigation strategies and safeguarding public health.

2.6.1. Surface Water Contamination

One of the most significant pathways through which antibiotic resistance spreads from abattoir effluent is the contamination of surface water bodies. When untreated or insufficiently treated effluent is discharged into rivers, lakes, streams, or other water systems, antibiotic-resistant bacteria are introduced into these aquatic ecosystems. These bacteria can persist in the water column and sediments for extended periods, interacting with other aquatic microorganisms and potentially transferring resistance genes through horizontal gene transfer (HGT). This not only increases the prevalence of resistant bacteria in aquatic environments but also poses direct risks to human health. People who come into contact with contaminated water through recreational activities, agriculture, or even drinking water may be exposed to these resistant bacteria. Furthermore, aquatic organisms, such as fish and shellfish, can become carriers of antibiotic-resistant bacteria, contributing to the spread of resistance through the food chain. Consumption of contaminated water or aquatic organisms that harbor

resistant pathogens can lead to infections that are more difficult to treat, particularly in regions where water treatment facilities are inadequate (Baquero *et al.*, 2008).

2.6.2. Soil Contamination:

Abattoir effluent is often used for land application, particularly in agricultural settings where it serves as a source of irrigation or fertilizer. However, the disposal of effluent on land can result in the contamination of soil with Antibiotic-resistant bacteria and antibiotic resistance genes. Resistant bacteria in effluent can survive in soil environments for long periods, especially under favorable conditions such as high moisture and nutrient levels. Once in the soil, ARB can interact with soil-dwelling microbes, including those that naturally inhabit agricultural fields. This interaction facilitates the transfer of resistance genes to non-pathogenic soil bacteria, which can act as reservoirs of resistance, potentially transmitting these genes to pathogenic organisms. In turn, these resistant bacteria can reach crops grown in contaminated soils, posing a risk to consumers who ingest fresh produce. Moreover, the leaching of antibiotic-resistant bacteria from soil into groundwater supplies can further extend the spread of resistance, contaminating drinking water sources and affecting both rural and urban populations (Wellington *et al.*, 2013).

CHAPTER THREE

MATERIALS AND METHOD

3.1. SAMPLING AREA

The study was conducted in Benin City, Edo State, Nigeria, at five different abattoirs: Ekehuan, Santana Market, University of Benin (UNIBEN), Oluku, and Ikpoba abattoirs. These sites were selected based on their high levels of activity and proximity to major urban settlements.

3.2. SAMPLE COLLECTION

Soil samples were collected from five different abattoirs known to have direct effluent discharge into the surrounding environment. At each abattoir, five samples were randomly collected from areas with visible effluent discharge and soil contamination. The samples were collected at a depth of 5-10 cm using sterile soil augers and transferred into sterile polythene

bags. The samples were immediately transported to the Microbiology Laboratory at the University of Benin for analysis.

3.3. STERILIZATION OF MATERIALS

Materials such as Petri-dishes, pipette, glass containers (conical flask, round bottom flask) and bottles were washed, drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160°C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol.

3.4 PREPARATION AND STERILIZATION OF MEDIA

Materials used include; Glass wares such as test tubes, beakers, conical flasks, Petri-dishes, McCartney bottles, stirring glass rod and measuring cylinder. Media and Biochemical test reagents and Gram's staining kit. All glassware which include MacCartney bottles, Petri dishes, test tubes, conical flasks, measuring cylinders and pipettes, were sterilized at 160 °C for 1 hr in a hot-air-oven before use. The media used in this study were sterilized at 121 °C for 15 min in an autoclave. Agar media, agar slant and biochemical reagents were prepared freshly and refrigerated at 3-4 °C. Aseptic conditions were ensured during inoculation and subculturing.

3.4.1 Preparation of Nutrient agar

Twenty eight grams (28 g) of nutrient agar was dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium will be the placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask will be allowed to cool.

3.5. ENUMERATION AND ISOLATION OF TOTAL HETEROTROPHIC BACTERIAL COUNT

Soil samples were air-dried at room temperature for 24 hours to remove excess moisture. Each sample was sieved through a 2 mm mesh to remove large debris and stones. A 1g portion of each soil sample was suspended in 10 mL of sterile distilled water. Serial dilutions (10-fold) of the samples were prepared and dilutions (10^3 - 10^5) of samples were plated out on freshly prepared Nutrient Agar (NA) using the pour plate method, and plates incubated at 37°C for 24 hrs. Colonies were observed, and the number of colonies on each agar plate were counted and recorded. Distinct colonies were purified by subculture on nutrient agar plates and later transferred into agar slants for preservation at 4°C, for further analysis. The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula below:

$$\frac{cfu}{ml} = \frac{\text{number of colonies} \times \text{dilution fold/series}}{\text{volume of inoculum}}$$

(Willey *et al.*, 2008)

3.6. PHENOTYPIC IDENTIFICATION OF BACTERIA FROM SAMPLES

Following successful pour plate technique, isolation and culture was made from a single colony and characterized using cultural, morphological and biochemical methods using the Bergey's manual. Several tests such as Gram reaction, catalase, urease, indole, oxidase, sugar fermentation, citrate utilization, respective reaction on triple sugar iron agar tests were carried out to presumptively identify bacterial isolates (Holt *et al.*, 1994).

3.6.1. Morphology identification

The morphological identity of each bacteria isolate was obtained by Gram staining so as to know the gram reaction, cell morphology and arrangement by viewing under the microscope.

The Gram stain procedure is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1min then washed with distilled water. Subsequently the slides were flooded with Lugol's iodine solution for 30sec and then washed off with distilled water. 95% alcohol was used for decolorization for 10sec and immediately washed off with distilled water. Finally, the smear was counter stained with safranin for 1min and washed off. The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of $\times 100$ magnifications to view the slides.

3.7. BIOCHEMICAL IDENTIFICATION

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

3.7.1 Oxidase test

This is mainly used to differentiate between *Pseudomonas* from other Gram-negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochrome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are Gram positive and Gram negative respectively were employed as control. A piece of filter paper using sterilized wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous

tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 seconds.

3.7.2 Urease test.

This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 degree centigrade for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

3.7.3 Indole production test

An indole test was carried out to demonstrate the ability of certain bacteria that can decompose amino acid tryptophane to indole. The indole production test is essential in identifying the *Enterobacteriaceae* family that breaks down the amino acid tryptophan by releasing indole in the presence of intracellular enzymes called "tryptophanase." Several drops of Kovac's indole reagent were placed on a filter paper. A portion of a pure isolated colony picked from the TSA pure culture with an inoculating loop was smeared onto the reagent-saturated area of the filter paper. It was allowed and examined to observe for colour development within 2 - 3 minutes. In this spot test, indole combined with the reagents in the filter paper matrix to produce a blue-to-blue-green colour change on the bacterial smear and adverse reactions remained colourless or light pink.

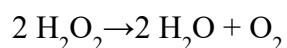
3.7.4 Citrate utilization test

The citrate utilization test is a part of the test used to differentiate organisms on their ability to utilize citrate as the primary energy source. A citrate test was performed to differentiate

members of Enterobacteriaceae capable of fermenting citrate in the presence of the enzyme citrate. Simon's citrate agar contained citrate as significant energy and was prepared for inoculation on Petri dishes. Well-prepared and sterilized citrate agar plates were inoculated from the pure isolated culture by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours. There were changes in colour due to bacterial growth of the organisms on the medium due to citrate metabolism, which gave a positive citrate test. The shift in pH turns the bromothymol blue indicator in the medium from green to blue (positive result). A negative test was demonstrated with no growth, no colour change, or the colour of the medium remains green

3.7.5 Catalase test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.



3.7.6. Triple sugar iron (TSI) agar test

The Triple Sugar Iron (TSI) test is an ability to test an organism's capability to ferment sugars and to produce hydrogen sulphide (H₂S) or gas (O₂), or both. The test was used primarily to differentiate members of the *Enterobacteriaceae* family based on their sugar fermentation patterns and from other Gram-negative rods. An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations,

the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H₂S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant indicated H₂S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO₂ and H₂) (Fawole and Oso, 2007).

3.8 ANTIBIOTIC SUSCEPTIBILITY TEST

The identified colonies of bacteria were used to determine the susceptibility and resistance of bacterial isolates, which were subjected to standard antibacterial susceptibility testing (AST) to decipher their resistance or susceptibility to common antibiotics used for treatment within the locality. The standard discs were produced by Oxoid, UK, which was used to execute the disc diffusion method employed in this study. For this assay, a fully grown bacterial culture (from 18-24 hours) was cultured on MHA. The inoculum corresponding to 1.5 x 10⁸ cells/ml McFarland standard was streaked using a sterile loop onto the MHA plates before the introduction of antibiotic discs and were added with extreme care to the plates with the aid of sterile forceps. The susceptibility results were recorded after incubation for 24 hours at 37 °C. Following the standard or rules of AST established in 2017 by CLSI (Clinical Laboratory Standards Institute). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on the 2020 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S).

3.9. MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDEX

This index is obviously a good tool which identifies the region where the isolates were obtained. Whether they are from places of high or low risks or from areas where antibiotics are abused. This tool becomes necessary for health risk assessment. According to Davis and Brown (2016), an index of ≥ 0.2 and above is indicative of a 'high-risk' contamination source. In this study the MAR index was determined by employing the methods delineated by Chitanand *et al.* (2010). The formula below was used to decipher MAR index of bacterial isolates.

$$MAR\ index = \frac{y}{nx}$$

where y = number of resistance scored,

n = number of isolates and

x = total number of antibiotics

It is a general established rule that MAR index greater than 0.2 is indicative of the fact that the bacterium originates from areas where antibiotics have been abused (or regularly used) or worse still from areas of high-risk source of contamination.

3.10. STATISTICAL ANALYSIS

Data obtained in this study were collected and analysed using Microsoft excel and by statistical package for social scientist (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Normal distributed data was expressed as mean \pm standard deviation and means were compared by analysis of variance (Ogbeibu, 2014).

CHAPTER FOUR

RESULTS

Figure 4.1, Present the total bacterial count in soil samples contaminated with effluent from selected abattoirs in Benin City varied across the study locations. The count ranged from 5.56 \log_{10} cfu/ml to 3.96 \log_{10} cfu/ml. The highest bacterial count was recorded for soil samples collected from the University of Benin (UNIBEN) while the least bacterial count was recorded for soil samples collected from Ekehuan.

Table 4.1. Shows the results for the cultural, morphological and biochemical characteristics of the bacterial isolates in soil samples contaminated with effluent from selected abattoirs in Benin City. Morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation were taken into account. Biochemical tests were also carried out to further identify the bacterial isolates. Among the biochemical tests used were catalase, oxidase, indole and citrate tests. The bacteria isolated from the soil samples include, *Bacillus* sp, *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Samonella* sp.

Table 4.2. Present the distribution of bacterial isolates in soil samples contaminated with abattoir effluent from selected locations in Benin City. *Escherichia coli* was the most distributed bacterial isolates *across* the various study sites.

Figure 4.2. Shows the percentage occurrence of bacterial isolates in soil samples contaminated with abattoir effluent revealed variations in the prevalence of different bacterial species across the study sites. Among the isolates identified, *Escherichia coli* had the highest occurrence, accounting for 23.33% of the total isolates. *Bacillus* sp. was the second most prevalent isolate, with a percentage occurrence of 21.05%, *Staphylococcus* sp. accounted for 16.34%, *Proteus* sp., *Pseudomonas* sp. and *Salmonella* sp. each had a percentage occurrence

of 15.79%, *Klebsiella* sp. was the least frequently occurring isolate, with a percentage occurrence of 10.53%,

Table 4.3. shows the result of the antibiotic sensitivity test on Gram positive and Gram negative bacterial isolates in palm-wine samples from various markets in Benin City. The *in vitro* antimicrobial assay was carried out using the Kirby Bauer disk diffusion technique. The antibiotics utilized for the assay were; Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Amoxicillin, Rocephin, Ciprofloxacin, Erythromycin, Saprifloxacin, Tarivid and Augmentin. For Gram positive bacteria isolates, *Staphylococcus* sp. was observed to exhibit the highest bacterial resistance with a MAR index of 0.4 while *Bacillus* sp. exhibited the lowest bacterial resistance with a MAR index of 0.3. For Gram negative bacteria isolates, *Pseudomonas* sp. and *Escherichia coli* was observed to exhibit the highest bacterial resistance with a MAR index of 0.6 while *Salmonella* sp. and *Klebsiella* sp. exhibited the lowest bacterial resistance with a MAR index of 0.4 respectively.

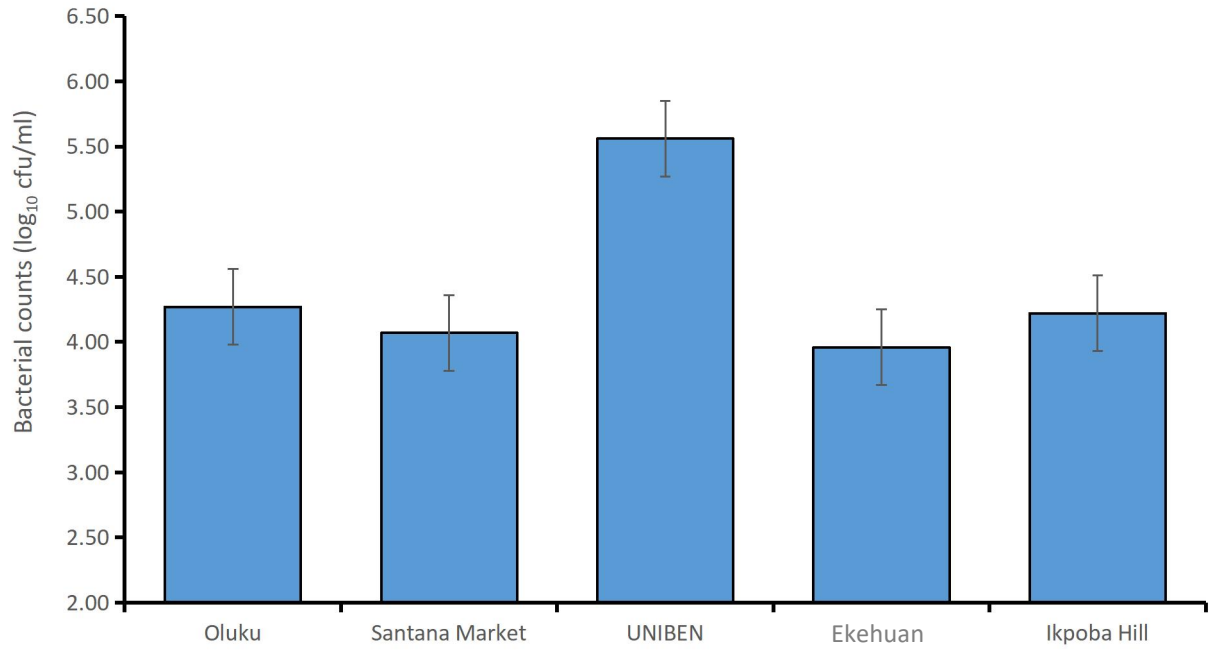


Figure 4.1:

Total bacterial count (log₁₀ cfu/ml) in soil samples contaminated with effluent from selected abattoir in Benin City.

Table 4.1: Identification off bacterial isolate in soil samples contaminated with effluent from selected abattoir in Benin City.

Elevation	Raised	Flat	Flat	Flat	Flat	Raised	Flat
Margin	Entire	Entire	Undulate	Undulate	Entire	Entire	Undulate
Color	Cream	Cream	Cream	Cream	Cream	Cream	Greenish
Shape	Circular	Circular	Irregular	Irregular	Circular	Circular	Irregular
Size	Medium	Small	Large	Large	Medium	Small	Medium
Gr. diff. agar	SSA	EMB	BCA	EMB	SSA	MSA	Cetrimide agar
Colour	Black	Pink	Straw	Green	Black	Yellow (fermentation)	Green
Staining							
Gram stain	-	-	+	-	-	+	-
Cell type	Rod	Rod	Rod	Rod	Rod	Cocci	Rod
Arrangement	Pair/Chains	Disperse	Disperse	Disperse	Disperse	Clusters	Disperse
Color	Pink	Pink	Purple	Pink	Pink	Purple	Pink
Biochemical							
KOH String Test	+	+	-	+	+	-	+
Catalase	+	+	+	+	+	+	+
Indole	-	-	-	+	-	-	-
Citrate	-	+	+	-	+	-	+
Oxidase	-	-	-	-	-	-	+
Glucose	+	+	+	+	+	+	+
Sucrose	-	+	+	-	-	+	-
Lactose	-	+	+	+	-	+	-
Gas formation	+	(+/-)	-	+	+	-	-
H2S formation	+	-	-	-	+	-	-
TSI (Slant/Butt)	K/AG H2S	A/A (G*)	A/A	A/AG	K/AG H2S	A/A	K/AG

Identity	<i>Salmonella</i> sp.	<i>Klebsiella</i> sp.	<i>Bacillus</i> sp.	<i>Escherichia coli</i>	<i>Proteus</i> sp.	<i>Staphylococcus</i> sp.	<i>Pseudomonas</i> sp.
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Key: (-) negative test; (+) positive test; (Gr. diff. agar) Gram differential agar; (BCA) Bacillus Cereus Agar; (EMB) Eosin Methylene Blue agar; (PCA) Plate count agar; (A) Acid; (K) Alkaline; (G) Gas production (bubbles); (H₂S) Hydrogen sulphide (black precipitate); (KOH) Potassium hydroxide test; (TSI) Triple sugar iron test.

Table 4.2: Distribution of bacterial isolates in soil samples contaminated with effluent from selected abattoir in Benin City.

Isolates	Oluku	Santana Market	Ekehuan	UNIBEN	Ikpoba Hill
<i>Salmonella</i> sp.	+	+	-	+	+
<i>Klebsiella</i> sp.	-	+	-	+	+
<i>Bacillus</i> sp.	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+
<i>Proteus</i> sp.	+	+	-	+	+
<i>Staphylococcus</i> sp.	+	+	-	+	+
<i>Pseudomonas</i> sp.	+	-	+	+	-

Key: Present (+), Absent (-)

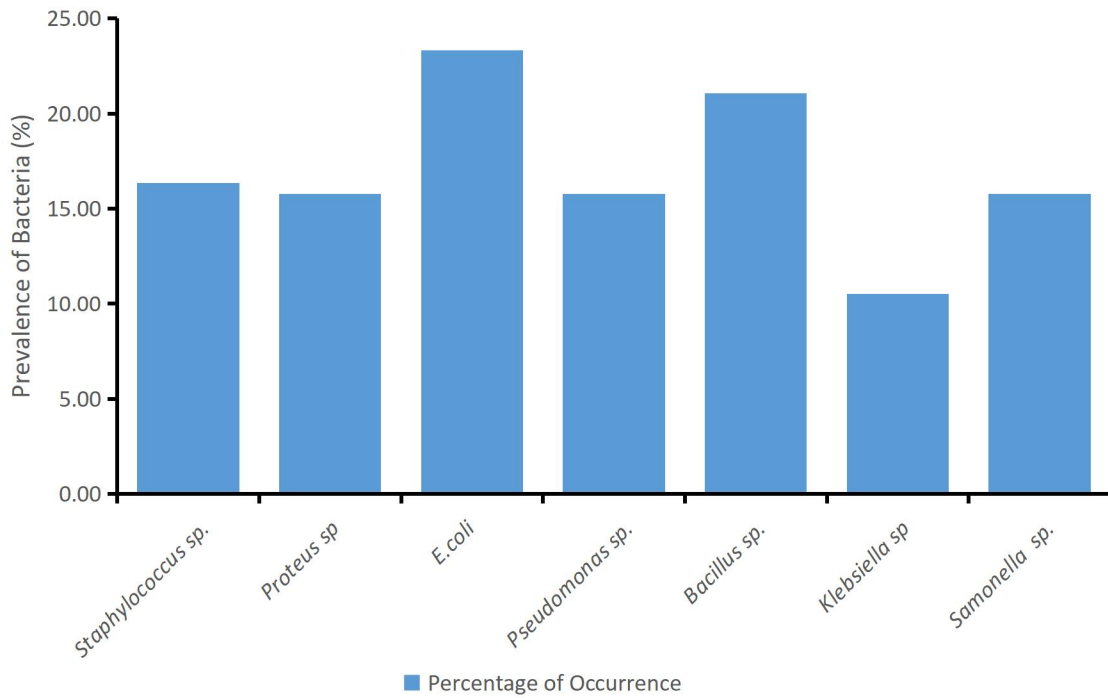


Figure 4.2: Frequency of occurrence of bacterial isolates in soil samples contaminated with effluent from selected abattoir in Benin City.

Table 4.3 Antibiotics sensitivity test of the isolated bacterial

Bacterial isolates										
Gram positive	PEF	CN	APX	Z	AM	R	CPX	AZ	LEV	E
Staphylococcus sp.	18(S)	9(R)	3(R)	10(R)	6(R)	16(I)	18(S)	12(I)	18(S)	16(I)
Bacillus sp	22(S)	14(I)	9(R)	0(R)	10(R)	16(I)	18(S)	18(S)	20(S)	14(I)
Gram negative	LEV	CF	SP	CPX	AM	AU	CN	PEF	OFX	AZ
Pseudomonas sp.	12(I)	10(R)	9(R)	3(R)	18(S)	10(R)	9(R)	10(R)	20(S)	14(I)
Salmonella sp.	14(I)	6(R)	14(I)	18(S)	3(R)	0(R)	11(I)	14(I)	14(I)	10(R)
Proteus sp	10(R)	12(I)	12(I)	18(S)	10(R)	0(R)	12(I)	18(S)	16(I)	10(R)
Klebsiella sp.	20(S)	10(R)	14(I)	16(I)	0(R)	10(R)	3(R)	18(I)	14(I)	18(S)
Escherichia coli	14(I)	3(R)	8(R)	10(R)	10(R)	0(R)	0(R)	16(I)	10(I)	12(I)

Resistant (R) = 0-10mm

Intermediate (I) = 11-16mm

Sensitive (S) =17mm and above

KEY:

Resistant (R) = 0-10mm

Intermediate (I) = 11-16mm

Sensitive (S) =17mm and above

Tetracycline (TE); Gentamicin (GEN); Amoxicillin + Clavulinic Acid (AG); Clindamycin (CD); Ciprofloxacin (CIP); Colistin (CS); Erythromycin (E); and Carbenicillin (CB).

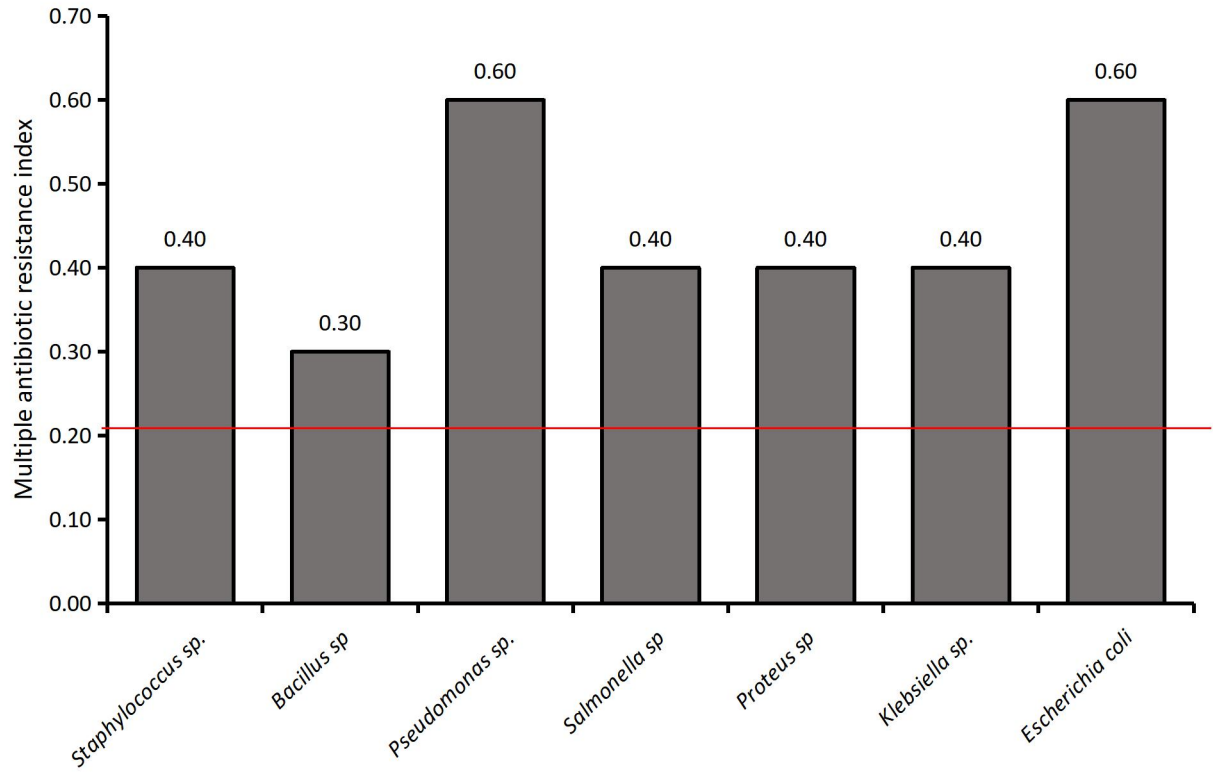


Figure 4.4: Multiple Antibiotics resistance (MAR) index

CHAPTER FIVE

DISCUSSION

Soil contamination with abattoir effluent is a significant environmental and public health concern, particularly in developing countries where waste management systems are often inadequate. Abattoir effluents are liquid wastes generated during the slaughtering and processing of animals. These effluents contain high levels of organic matter, blood, fats, proteins, and nutrients, as well as potentially harmful microorganisms, including pathogenic bacteria (Bandaw and Herago, 2017). When discharged untreated into the environment, abattoir effluents can infiltrate surrounding soils, altering their microbial composition and physicochemical properties. This study focus on the isolation and identification multi drug resistance bacterial isolates from soil samples contaminated with abattoir effluents in selected abattoir locations in Benin City, Edo State, Nigeria.

The total bacterial count in soil samples contaminated with abattoir effluents varied significantly across the study locations, as shown in Table 4.1. Soil from the University of Benin (UNIBEN) recorded the highest bacterial count, with a value of 5.56 log₁₀ CFU/ml. This could be because the effluents may contain many growth factors that could be easily utilized by the organisms which are not available in the uncontaminated soil. It could also be due to the destabilization of the soil's ecological balance as a result of the contamination from the discharge of abattoir wastewater into the soil environment. These results agree with the reports of Rabah *et al.* (2010) and Eze *et al.* (2013).

Moderate bacterial counts were observed in Oluku and Ikpoba Hill, indicating moderate contamination levels, while the least bacterial count was recorded in Ekehuan, with a value of 3.96 log₁₀ CFU/ml. The relatively low bacterial load in Ekehuan may be attributed to the reduced frequency of effluent discharge or better natural attenuation processes, such as

microbial degradation and adsorption by soil particles. The differences in bacterial loads among the study locations underline the role of effluent management practices, soil properties, and environmental conditions in shaping the microbial composition of contaminated soils. Improper disposal of abattoir effluents introduces pathogenic microorganisms into the soil, posing risks to public health and the environment (Akinnibosun and Ayejuyoni, 2015).

The cultural, morphological, and biochemical identification of bacterial isolates revealed the presence of several species, including *Escherichia coli*, *Bacillus* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Salmonella* sp. This agrees with the results of Adesemoye *et al.* (2006) and Ezeronye and Ubalua (2005), who also isolated similar organisms. The diversity of bacterial species identified underscores the extent of contamination and the public health risks associated with abattoir effluents.

Among the isolates, *Escherichia coli* had the highest frequency of occurrence, constituting 23.33% of the total isolates. This is consistent with the findings of Gufe *et al.* (2021) where *E. coli* (23.33%) and *Bacillus species* (21.85%) were the most prevalent and *Staphylococcus intermedius* (1.30%) the least prevalent. This highlights significant fecal contamination, as *E. coli* is a reliable indicator of fecal pollution. The prevalence of *E. coli* in soil samples contaminated with abattoir effluents has been similarly reported by Ediene and Iren, (2017) who emphasized the role of untreated effluents in the dissemination of fecal bacteria in the environment.

Bacillus sp., an ubiquitous soil bacterium, constituted 21.05% of the isolates. The presence of *Bacillus* sp in the contaminated soil is expected as this organism is indigenous to the soil environment, and are known to persist in such environment (Atlas and Bartha, 2007). Similar observations were made by Ezeronye and Ubalua (2005), Bala (2006) and Rabah *et al.* (2010). *Pseudomonas* spp. is liable to survive and multiply in almost any moist situation and cause

extensive rashes. Food-borne diseases caused by *E. coli* occurs in most slaughter houses due to their unhygienic practices especially when the cow meat comes in contact with faecal material. The detection of other bacteria such as *Staphylococcus* sp., *Klebsiella* sp., *Proteus* sp. and *Salmonella* sp. further reflects the complexity of microbial communities in contaminated soils. The presence of these organisms is of particular concern, as they are known pathogens capable of causing diseases in humans and animals (Akinnibosun and Ayejuyoni, 2015).

The antibiotic resistance profiles of the bacterial isolates revealed varying degrees of resistance to commonly used antibiotics, highlighting the potential of abattoir effluents as reservoirs of antibiotic-resistant bacteria (Akpan *et al.*, 2020). Among the Gram-positive isolates, *Staphylococcus* sp. exhibited the highest resistance, with a Multiple Antibiotic Resistance (MAR) index of 0.4. This suggests that the isolates may have been exposed to sub-lethal concentrations of antibiotics in the effluents, which could select for resistant strains. *Bacillus* sp. had a comparatively lower MAR index of 0.3, indicating less exposure or susceptibility to antibiotics.

For Gram-negative isolates, *Pseudomonas* sp. and *Escherichia coli* exhibited the highest resistance, with MAR indices of 0.6. This is alarming, as these organisms are known for their intrinsic resistance mechanisms and their ability to acquire resistance genes. The lowest resistance was observed in *Salmonella* sp. and *Klebsiella* sp. which had MAR indices of 0.4. These findings are consistent with those of Gufe *et al.* (2021), who reported high levels of antibiotic resistance among bacterial isolated from abattoir effluents in Bulawayo, Zimbabwe.

The MAR indices observed in this study indicate the potential for the dissemination of resistance genes in the environment. The findings underscore the need for stringent regulations on antibiotic use in livestock and proper treatment of abattoir effluents to prevent

the proliferation of resistant bacteria. The presence of multidrug-resistant bacteria in soil samples contaminated with abattoir effluents poses significant public health risks. Such bacteria can spread to humans and animals through direct contact, agricultural activities, or contamination of water sources. The findings of this study highlight the urgent need for improved effluent management practices to mitigate these risks (Gufe *et al.*, 2021).

Abattoir effluents are rich in organic matter, nutrients, and residual antibiotics, creating a conducive environment for the proliferation of antibiotic-resistant bacteria. If not properly managed, these effluents can contaminate nearby ecosystems, disrupt soil microbial communities, and facilitate the spread of resistance genes (Masse *et al.*, 2021)..

5.1. CONCLUSION

The findings of this study provide critical insights into the microbial and antibiotic resistance profiles of bacteria isolated from soils contaminated with abattoir effluent in Benin City, Nigeria. The high bacterial counts observed across the study locations, particularly in soil samples from the University of Benin, underscore the significant impact of untreated abattoir effluent on the microbial load in the environment. The presence of pathogenic bacteria, including *Escherichia coli*, *Bacillus* sp., *Pseudomonas* sp., *Salmonella* sp., *Klebsiella* sp., *Proteus* sp. and *Staphylococcus* sp., highlights the potential health risks associated with exposure to these contaminated soils.

The antibiotic susceptibility profiles of the isolates revealed concerning levels of multidrug resistance, particularly among Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas* sp., with multiple antibiotic resistance (MAR) indices exceeding the safety threshold of 0.2. These findings emphasize the potential role of contaminated soils as reservoirs for antibiotic-resistant bacteria, which could exacerbate the global public health threat of antimicrobial resistance (AMR).

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