ISOLATION AND ANTIBIOTICS SUSCEPTIBILITY OF BACTERIA ISOLATES FROM PIGS PENS IN IKORODU AREA OF LAGOS

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DEDICATION

This effort is dedicated to my cherished parents and teachers, who taught me how to think, comprehend, and express myself. I sincerely believe that without their inspiration, capable direction, and devotion, I would not be able to complete the project's exhausting procedure.

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ABSTRACT

Antibiotics are the most widely used in Sub-Saharan Africa for the treatment of diseases prevention, growth promotion, and prophylaxis, all of which are critical for the animal husbandry industry's long-term viability. This study was aimed to examine the susceptibility of the isolated bacteria to antimicrobials from a pig farm in Lagos State's Ikorodu area. The aggregate number of 42 rectal and 10 fecal samples was collected from three (3) different pig pen and was examined using Nutrient agar, EMB agar, MacConkey agar. The pour plate technique was used were MacConkey agar, Nutrient agar, and EMB agar were placed onto a Petri-dish holding 1ml of the appropriate dilution to isolate bacteria. After incubating the plates at 37°C for 24 hours, colony counts were taken, and the bacterial isolates were preserved by sub culturing them to get pure cultures, which were used for biochemical tests. For primary isolation of bacteria from the rectal swab samples, MacConkey agar, Blood agar, and EMB agar were used. The blood agar was prepared as Tryptone Soya Agar mixed with 5% of blood that served for detection of haemolytic activity of bacteria. Blood agar, MacConkey agar and EMB agar plates were incubated aerobically at 37 ° C for 24hours. The bacterial isolates were preserved by sub culturing them to get pure cultures which were used for biochemical tests. The isolates were subjected into morphological examination and biological test and their identity were confirmed using the characteristics. The test carried out include Gram' s staining, Catalase Test, Potassiumhydroxide test (KOH), Haemolytic Test, Sugar Fermentation Test, Indole Test, Starch Hydrolysis Test, Mannitol broth Test, Urease Test, Hydrogen sulphide Test, Motility Test, and Citrate Test. The number of pure bacteria isolates were found from37 rectal and 5fecal samples. The cultural and biochemical characteristic of the bacterial isolates showed the fact that 12 of the isolates (28.6) were *Staphylococcus spp*, 7 (16.7%) were *Bacillus spp*, 6 (14.2%) were *Enterobacter spp*, 7 (16.7%) were *Klebsiella spp*, 7 (16.7%) *Citrobacter spp,* 2 (4.76%) were *E.coli,* 1 (2.38%) was *Samonella spp*. Antibiotic susceptibility testing revealed that; For the gram positive isolates the highest susceptibility of the isolate was Gentomycin

(100%), while the lowest susceptibility was Cloxacillin (0%). The highest resistance of the isolates (100%) was recorded with Cloxacillin, while the lowest (0%) was recorded with Gentomycin. The antibiotics susceptibility of gram negative isolates showed that the highest susceptibility of the isolate was Ofloxacin (95.2%), Nitrofurantoin (95.2%), Gentamicin (95.2%), while the lowest susceptibility was Cefuroxime (23.8%). The highest resistance of the isolates (76.2%) was recorded with Cloxacillin while the lowest (4.76%) was recorded with Gentamicin, Ofloxacin and Nitrofurantoin. This investigation displayed multi drug resistance to antibiotics in varying degrees and this may be caused by the misused of antimicrobials by pig producers without veterinarian consultation or prescription.

TABLE OF CONTENTS

TITLE

PAGE..i

CERTIFICATION..ii

DEDICATION..iii

ACKNOWLEDGEMENT……………………………….………………….……iv

ABSTRACT………………………………………………………………….…….v

CHAPTER 1

1.0. INTRODUCTION...1

1.1 AIM OF THE STUDY………………...3

1.2 OBJECTIVE OF THE STUDY…………………………………...….3

1.3 LIMITATION OF THE STUDY……………………………….…...3

CHAPTER 2

2.0 LITERATURE

REVIEW..2

2.1 PREVALENCE OF BACTERIA IN PIGS…………………………….....…5

2.2 COMMON BACTERIA ASSOCIATED WITH PIG PRODUCTION……………..5

2.3 MECHANISM OF PRODUCTION OF ANTIMICROBIAL……………………...…7

2.4 ANTIMICROBIAL USE……………………………………………….………9

2.5 COMMON ANTIBIOTICS USED FOR SWINE

PRODUCTION……………………………………………………………...…10

2.6 QUANTITY OF ANTIBIOTICS AND OTHER DRUGS SOLD IN NIGERIA……………………………………………………………………………....11

2.7 METHOD USED TO DETERMINE ANTIBIOTICS SUSCEPTIBILITY……………………………………………………………………....11

2.8 EMPERICAL REVIEW…………………………………………….…………………...15

CHAPTER 3

3.0 MATERIALS AND METHODS...16

3.1 DESCRIPTION OF STUDY AREA……………………………………………………………………………...16

3.2 METHODS……………………………………………………………………………….16

3.2.1 SAMPLE COLLECTION…………………………….……………......…....16

3.2.2 STERILIZATION TECHNIQUES……………………….……………………17

3.2.3 ISOLATION OF THE ORGANISM………………………….……………..18

3.3 BIOCHEMICAL TESTS AND IDENTIFICATION OF THE ISOLATES……………………………………………………………………..…19

3.3.1 GRAM'S STAINING…………………………………………………...……19

3.3.2 CATALASE TEST……………………………………………………………………………...19

3.3.3 HAEMOLYSIS TEST………………………………………………….……….19

3.3.4 POTASSIUM HYDROXIDE (KOH) TEST………………………….……….19

3.3.5 SUGAR FERMENTATION TEST…………………………………..………....19

3.3.6 STARCH HYDROLYSIS TEST………………………………………...…..20

3.3.7 MOTILITY TEST…………………………………………………..…...21

3.3.8 UREA HYDROLYSIS………………………………..……...……21

3.3.9 INDOLE TEST……………………………………………..……………21

3.4.0 CITRATE UTILIZATION TEST………………………………….……..21

3.4.1 HYDROGEN SULPHIDE PRODUCTION TEST…………………….…...21

3.4.2 ANTIMICROBIAL SUSCEPTIBILITY TEST………………..……..…..…...22

CHAPTER 4

4.0

RESULTS……………………………….………......................................24

4.1 DISTRIBUTION OF THE ISOLATES FROM THE PIG FARMS……………24

4.2 CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF THE

BACTERIA ISOLATE …………………………………………………………25

4.3 FREQUENCY OF OCCURENCE OF THE ISOLATED

BACTERIA…………………………………………………………………….29

4.4 ANTIBIOTICS SUSCEPTIBILITY OF THE GRAM-POSITIVE

ISOLATES……………………………………………………………….…29

4.5 ANTIBIOTICS SUSCEPTIBILITY OF THE GRAM-NEGATIVE

ISOLATES…………………………………………………………………..29

CHAPTER 5

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION……………….…..30

5.1 DISCUSSION………………………………………………………………...31

5.2 CONCLUSION…………………………………………………………………..32

5.3 RECOMMENDATION……………………………………………...……..33

CHAPTER 6

6.0 REFERENCES………………………………….…………………………34-37

CHAPTER ONE

INTRODUCTION

Worldwide, pig production varies from highly intensive farming to subsistence (Aerestrup, 2008). Pig production and consumption are primarily concentrated in Nigeria's southern regions. In Nigeria, this sector is still underdeveloped when compared to poultry and cattle (Adebowale et al., 2020). Pig production gives a way to contribute to the key goals of sustainable growth, removing poverty and hunger, and enhancing good health and wellbeing. (Oloso *et al.,* 2018). This is due to the distinctive characteristics of pigs, such with rapid growth rates and fast feed conversion rates. However, considering its high potential, there are various limitations of pig production, including insufficient policies and services for disease prevention and management in animals, poor sanitation and management methods, high mortality rates and limited or non-existent health treatment (Ezeibe, 2010). As a result, this affects the production of this animal hence the need to use antibiotics to prevent and improve the feeding of pathogens from attacking the livestock. This method is very popular in the development of pigs on a wide scale.

Antimicrobials (AMs) are also misused by pig producers without veterinarian consultation or prescription. Because the majority of the antimicrobials implicated are also used to treat human infections, the widespread use of AMs in cattle creates a worldwide health risk (Adesokan *et al.,* 2015). Different animal species can be carriers who are asymptomatic of bacteria for various periods of time in carrier pig foals who are healthy, bacteria can be shed intermittently. Among veterinary doctors and farmers, this has called for public interest. These asymptomatic carriers are a means of transmitting diseases (Wales, 2011). Bacteria can be borne by sheep and cattle for a maximum of ten weeks and for up to 14 months by horses. Pigs, on the other hand, will shed up to 28 weeks of bacteria in their faeces without clinical symptoms (Wilson, 2018).

Antibiotics have been used widely in both developed and emerging countries around the world. It was used for a number of purposes, such as growth enhancement, disease prevention and feed efficiency. This was used to minimize morbidity and mortality in farm animals. (Moyane *et al.,*2013). The main supplier of antimicrobial drugs in Nigeria is private pharmaceuticals who have little or no knowledge about the usage of these drugs. Many antibiotics are being sold un-prescribed by necessary personnel hence prompting their abuse and over usage. This, on the other hand, adds to the misuse of these drugs, hence the animals developing resistance to the drugs. Antibiotics are the most widely used antibiotics in Sub-Saharan Africa for the treatment of diseases prevention, growth promotion, and prophylaxis, all of which are critical for the animal husbandry industry's long-term viability (Eagar, 2008).

However, the use of these antibiotics in animals, particularly food animals, may cause to the creation of antibiotic-resistant bacteria, which might subsequently infect both animals and people (Mellon *et al.,* 2001). Due to the structure of antibiotic consumption in Nigeria, information on the use of antibiotics medications is scarce. Also most of the antibiotics used are not being prescribed by the necessary personnel. Antibiotics, particularly tetracycline and streptomycin, are overused in piggery, according to a study by Sawant et al., (2005). They went on to say that antibiotic use differs from farm to farm and country to country, based on the targeted policies and outcomes. There has been a lot of discussion in veterinary medicine over the antibiotic usage in animals in animals reared for human consumption (food animals). Antibiotic resistance develops natural section as a result as a consequence exposure to antibiotic chemicals (Aminor and Mackie, 2007). In lieu of all these, there is a need to isolate and examine the susceptibility of the isolated bacterial to antimicrobials in selected pig pen in Ikorodu Lagos state.

1.1 AIM OF THE STUDY

The major goal of this project is to examine the susceptibility of the isolated bacterial to antimicrobials from a pig farm in Lagos State's Ikorodu area.

1.2 OBJECTIVES OF THE STUDY

In a bid to fulfill the aim of this research, the following specific objectives were set;

i. To isolate the bacteria from the pigs from selected pig farms in Ikorodu, Lagos.
ii. To characterize and identify the bacteria isolated.
iii. To test the bacterium isolate's antibiotic susceptibility.

1.3 LIMITATION OF STUDY

As there is no success without challenge, the success of this research work is not without some limitations and some of the problem encountered during the course of carrying out this research work include;

a. Unavailability of some resource materials as at when due.
b. Contamination of plates and some other apparatus due improper maintenance thereby leading to incorrect values and spending of more money to buy another plate.
c. Unwillingness of the farm owners and handlers to allow the researcher to take samples from their pen as they presume the researcher is an undercover from ministry of health and environment that is trying to snitch into their activities.

Despite widespread antibiotic abuse in many underdeveloped nations, there is a scarcity of data on antibiotic use and resistance in farm animals. Many farms in Nigeria have swine production that is prone to filth, which draws bacteria. Antimicrobials are used frequently to keep bacteria from hurting this animal so that production can be maximized. Despite this, there are few published studies on the transmission of germs between pigs and people. The limited reports that are available mainly focus on a specific species of bacteria, such as *Klebsiella* (Thongpan *et al*), *Salmonella* (Wilson, 2018), or *E.coli* (Thongpan *et al*) (Rizaldi *et al*, 2019). In this

context, the goal of this research is to isolate, identify, and investigate the susceptibility of bacteria to antibiotics at pig pens in Gberigbe piggery estate, Ikorodu, Lagos.

CHAPTER TWO

LITERATURE REVIEW

2.1 Prevalence of bacteria in pigs

In terms of dental, ophthalmic, cutaneous, cardiovascular, renal, and digestive systems, pigs and humans are physically and biologically similar. The pathogen's ability to penetrate the pig-human species barrier is increased by these common biological traits (WHO, 2009; Morens and Taubenberger, 2010). With influenza viruses, for example, host switching has been reported frequently (Morens and Taubenberger, 2010).

2.2 Common bacteria associated with pig productions

A significant part of animal husbandry in Nigeria is the pig production industry. The development of the relatively underdeveloped swine industry in Nigeria has been severely hampered by the proliferation of bacterial infections. The following are some of the bacteria infection and diseases in the pig industry;

BRUCELLOSIS: Brucellosis in pigs is a zoonotic disease**,** affecting pigs, affected by the bacterium *Brucellasuis*. This species has five biovars, however *Brucellasuis* Biovars 1, 2, and 3 are the most common in pigs. With the exception for South America and Southeast Asia, where substantial incidence has been documented, Porcine brucellosis is widespread but low in prevalence (OIE, 2009). Porcine brucellosis has been linked to loss of development in pregnant cows in India (Singh *et al*.,2015)

COLIBACILLOSIS: Colibacillosis, caused by pathogenic *Escherichia coli*, one of the most common causes of illness and death in piglets is a disease called porcine encephalitis. Enteritis (*enteric colibacillosis*), which is associated with diarrhea, occurs at three main periods of pig's life; neonatal diarrhea occurs at 0–4 days of age, neonatal weaning diarrhea at 4 days to 3 weeks and post-weaning diarrhea of pigs at 5weeks of age (Roy, 2014). About 50 percent cases of neonatal diarrhea were described to be due to *E. coli.*

LISTERIOSIS: Listeriosis is an infectious and deadly disease caused by *Listeria monocytogenes* in animals, birds, and humans. Infection in animals is often asymptomatic, although severe cases sometimes occur (OIE, 2014). The symptoms of the disease include septicemia, encephalitis, meningitis, meningoencephalitis, rhombencephalitis, abortion, stillbirth, neonatal infections, and gastroenteritis (Barbuddhe and Chakraborty, 2009; Barbuddhe *et al.,*2012). The organism has an intracellular life cycle that allows it to go from cell to cell without being released. This ability explains the etiology and clinical symptoms of the disease by allowing it to penetrate the placental and blood–brain barriers. Temperatures ranging from 4°C to 37°C are tolerated by the bacterium (Janakiraman, 2008). *Listeria monocytogenes* is extremely important since it has the ability to cause enormous economic losses in the cattle sector, as well as abortion in pregnant women, and is one of the most common causes of food poisoning that leads to death.

LEPTOSPIROSIS: Infection with any of a vast number of *Leptospira spp*. serovars causes leptospirosis, an infectious disease that affects pigs and many other animals (including humans). Pigs can be infected with a variety of serovars. Leptospirosis is found in pigs all over the world when the conditions are conducive for the disease's survival (Rajkowa, 2018).

***STREPTOCOCCUS SUIS* INFECTION:** *Streptococcus suis (S. suis*) is a very common swine pathogen that causes a variety of illnesses in pigs, including meningitis, arthritis, septicemia, endocarditis, encephalitis, abortions, polyserositis, and bronchopneumonia, all of which have a detrimental impact on pig productivity (Okwumabua *et al.,* 2003). *S. suis* strains are classified into serotypes based on polysaccharide capsular antigens. There are 35 capsular serotypes known (types 1/2 and 1 through 34), with type 2 being the most common cause of sickness. Other serotypes, such as 1/2, 1, 7, 9, and 14, can also cause illness (Staats, 1997). Carriers in the subclinical stag are the most common means of transmission of the bacterium to susceptible young pigs. Many pig-producing countries throughout the world have reported outbreaks.

TUBERCULOSIS: Tuberculosis (TB) is a granulomatous infection caused by acid-fast bacteria from the genus Mycobacterium. Among the mammals affected are humans and birds. The causal organism, Mycobacterium, is split into three types: human, bovine, and avian. The organism that causes tuberculosis in primates is *Mycobacterium tuberculosis*. Other mammals have *Mycobacterium bovis*, and birds have *Mycobacterium avium*. Pigs are susceptible to all three forms of tubercle bacilli, however the first two are rarely affected. It has been proposed that the prevalence of tuberculosis in pigs is linked to contact (direct or indirect) with tuberculous humans, livestock, or birds. The occurrence of tuberculosis in pigs has long been documented in practically all nations where pigs are raised. Bacteria had become well-established in the pig population as it developed (Khan *et al.,* 2013; Kramear and Oppliger, 2017). This had health ramifications because of pig mortality and productivity losses, as well as the financial implications because of trade penalties of pigs and pig products that have been contaminated, and disease management measures (Culling) (Khan, 2013). Pigs are known to carry a variety of germs. Some amongst them on the World Organization for Animal Health's (OIE) diseases' list to report in 2019 (OIE, 2019). Some of them were also on the list of Federal Select Agents in the United States.

2.3 MECHANISM OF OPERATION OF ANTIMICROBIAL

Antimicrobials have five main mechanisms of action; Interference with the synthesis of the cell wall, disruption of cell membrane structure, protein synthesis suppression, interference with nucleic acid synthesis, and metabolic pathway inhibition (Tenover, 2006; Giguere *et al.,* 2013) Interference with bacterial cell walls: -lactams (penicillin and cephalosporins) and glycopeptides (vancomycin) are two types of antibiotics that hinder bacterial cell wall production (Tenover, 2006). The β-lactam drugs interfere with transpeptidase and transglycosylase enzymes, by binding to penicillin proteins (PBPs), required for normal peptidoglycan cross-linking. The D-alaninetermini of the peptidoglycan strands are

sequestered by glycopeptides, limiting normal cross-linking. When peptidoglycan cross-links are not formed, the cell wall becomes mechanically weak and vulnerable to osmotic lysis (Walsh, 2000).

Disruption of cell membrane structure: This is the mechanism of action of the polymyxins (colistin) and daptomycin. Polymyxins bind to the lipopolysaccharides (LPS) of the bacteria cell's outer and inner membranes, compromising the phospholipid layers' stability (Yu *et al.*, 2015). This increases membrane permeability, allowing cell contents to flow out and bacterial cells to die. Daptomycin binds to the membrane of the bacterial cell, producing depolarization n and cell death (Taylor and Pal).

Inhibition of protein synthesis: Ribosomes are the architecturally different functional units involved in protein synthesis in bacterial and eukaryotic cells (Walsh, 2000). Because they bind to the structural components of ribosomes, antibiotics limit protein syntheses. Macrolides and chloramphenicol connect to the 50S subunit (Tenover, 2006).

Interference with nucleic acid synthesis: Quinolones (nalidixic acid) and fluoroquinolones (ciprofloxacin) target DNA gyrase and DNA topoisomerase IV, respectively, to decrease nucleic acid synthesis. Positive DNA supercoils are relaxed by these enzymes, allowing polymerase enzymes to replicate DNA without interruption. The DNA synthesis inhibited is caused by the binding of these enzymes**.** Rifampicin also affects nucleic acid synthesis by reducing RNA synthesis by targeting bacterial DNA-dependent RNA polymerase (Walsh, 2000; Tenover, 2006).

Inhibition of metabolic pathways: By inhibiting the folate synthesis route, trimethoprim and sulfonamides inhibit DNA synthesis. Trimethoprim inhibits the dihydrofolate reductase enzyme directly, inhibiting the production of tetra hydrofolic acid, whereas sulfonamides compete with para-aminobenzoic acid (PABA) for binding to the dihydropteroate synthase enzyme, preventing the generation of dihydrofolic acid (Tenover, 2006). When taken together,

trimethoprim and sulfonamides have a synergistic impact because they disrupt the folate production route in a sequential manner (McManus, 1997).

2.4 ANTIMICROBIAL USE

Antibiotics have made a significant contribution to the treatment of fatal catastrophes in animals and people (Muurinen, 2015). For the first time in the twentieth century, Flemmings and Erlich discovered this. Antibiotics are required for the treatment of diseases in both humans and animals. Antibiotics are given to farm animals in order to prevent and manage sickness as well as to promote their growth (Benedict, 2011). The majority of medications used in swine development are antibiotics (Tetracycline, Amoxicillin, streptomycin etc.). Many farmers prefer to change the antibiotics prescribed if the medicinal regimen used for the initial treatment of the animal fails. Intensive farmers are more likely to use antibiotics as against subsistence farmers. Antibiotics can be injected, fed, or dissolved in water as a health management strategy. According to Carson *et al.,* oxytetracycline, macrolides, penicillin, florfenicol, and spectinomycin are injected, and monensin, tylosin, lasolocid, and tetracyclines are included in animal diets, lincomycin, spectinomycin, chlortetracycline, and oxytetracycline are added to their water, and oxytetracycl (2008).

Usage of antibiotics is very common in sub-Saharan Africa. Veterinary doctors consider a host of factors before concluding on the choice of antibiotics to be administered (Benedict, 2011). According to a poll of veterinary specialists, moral views have different implications for behavioral attitudes depending on the situation, such as the level of risk involved in treating or not treating an animal and the efficacy of antibiotics in acute sickness. (McIntosh *et al.* ,2009). Another consideration included in the choosing of the antibiotics prescribed is the potency and safety of the antibiotics. In consideration of the health risk involved with the administration of antibiotics and the possible connection with the application of antibiotics, there is certainly a need for evidence of the efficacy of these medications. The FDA's approval process for new

animal medication applications ensures that antibiotics meet efficacy and safety requirements. In the agriculture industry, however, FDA approval to test antibiotics further is frequently accompanied by additional independent field trials. Antibiotics are usually more effective when the infection is detected early on, especially in the case of respiratory infections (Cusack *et al.,* 2003). Many antibiotics were compared to see which one had the best efficacy and effectiveness. Another important element to consider when selecting antibiotics for a certain purpose is their cost effectiveness (Perett *et al*.,) 2008

Table 2.5: Common antibiotics used for swine production

Antibiotics	Treatment objectives
Lincomycin	For controlling of diseases, to feed efficiency and for growth promoter
Penicillin	For controlling of diseases, to feed efficiency and for growth promoter
Virginiamycin	For controlling of diseases, to feed efficiency and for growth promoter
Tetracycline	For controlling of diseases, to feed efficiency and for growth promoter
Erythromycin	For controlling of diseases.

Source: Aiyegprp *et al.,* (2013)

Table 2.6 Quantity of antibiotics and other drugs sold in Nigeria in 2014-2015

Antibiotics	2014 (Qty in Kg)	2015 (Qty in Kg)
Aggregated antibiotics	188,339	190,219

Tetracyclines	8,147	168,880
Fluoroquinolones	5,115	3,146
Macrolides	3,349	9,798
Sulf1onamides	1,060	687
Polypeptides	459	142,333
Amphenicol	268	658
Penicillins	193	-
Aminoglycosides	46	131
Glucopeptides	24	40
Total	207,000	515,892

Source: Federal ministry of Agriculture, Environment and Health, DR ChikweIhekweazu (2017).

2.7 METHODS USED TO DETERMINE ANTIBIOTIC SUSCEPTIBILITY

In this article, antibiotic susceptibility testing methods are discussed, which are important in determining treatment outcomes, drug discovery, and even epidemiological studies. The testing protocols are determined by the concept of each system, the culture process, and the test to discover antimicrobial compounds that kill bacteria (Balouri *et al,* 2011). When antibiotic susceptibility patterns are checked as soon as feasible, the time and cost of hospitalization, as well as the rate of morbidity and mortality, are all drastically reduced (Ataee *et al.,* 2012). Among the methods are:

Agar/Disc diffusion antibiotic testing: This test looks at bacterial growth on commercially available agar plates with paper discs that have been treated with a standard concentration of antimicrobial agent. A disc diffusion method is based on the determination of an inhibitory zone that is proportional to the bacterial susceptibility to the antibiotic present in the disc during the diffusion process itself. When the antimicrobial concentration is so low, the inhibition zone

is specified that it does not prevent the increase in test mication. In relation to the minimum inhibitory concentration (MIC) of that specific bacterium / antimicrobial mixture is the area of inhibition surrounding the antimicrobial disc. The area of inhibition is in reverse correlation with the MIC in the test bacterium. The larger the inhibition area, the reduced the antibiotic dose required to prevent the multiplication of bacteria. This depends, however, on the antibiotic and disco infusability (OIE, 2012).

Broth and agar dilution method: The purpose of this procedure is to identify the lowest concentration of the antibiotic tested which prevents the visible growth of bacteria (MIC, usually in g/ml or mg/l)). The MIC does not always imply an absolute value, on the other hand. "The true: MIC" is defined as the threshold when the lowest test concentration inhibits bacterial growth and bacterial growth is inhibited by the next lowest test level. This might be seen as one-dilution intrinsic variation in the MIC data gathered from a dilution series. Both interpretive criteria (sensitive, intermediate and resistant) and appropriate quality control reference agencies for the particular bacterium/antibiotic combination should include antimicrobial ranges. Diffusion of agar disc is less reproducible and quantitative than dilution of the antimicrobial sensitivity. On the other hand, antibiotics are often tested at doubling dilutions which result in inaccurate MICs. All parameters that could have an impact on the choice of an AST technique include cost, replicability, reliability, accuracy, species and antimicrobials of that OIE Member and enough validation data for the spectrum of organisms to be susceptibility tested (OIE, 2012).

2.8 EMPIRICAL REVIEW

Hussein (2016) studied pathogenic microorganisms recovered from clinical samples and their identification, isolation, and susceptibility. From January to June 2016, he collected 150 samples. He recovered gram-negative bacteria from this, including 50 *Pseudomonas*

aeruginosa, 50 *E.coli,* and 50 *Enterobacteriocae*, as well as *Proteaus mirabilis*. Ten biochemical assays were used to identify the isolates. In comparison to other antibiotics, the antibiotics described the varied modes of action in their activity, resistance, and sensitivity. He pointed out that bacteria have varying degrees of antibiotic resistance, with Aztreonam being more active than other antibiotics.

They utilized polymerase chain reaction to confirm two genotypes isolates containing eaeA, stx1 and stx2 as a result of their investigation. They showed that pigs in the United States may carry E. coli bacteria. In Zaria and its surrounds in Kaduna North, Maaji, Kia, and Bello (2020) studied the prevalence of *Clostrium difficile* and *Clostridumpefrigens* in pigs, as well as the related risk factors. Rectal swabs from 132 pigs were taken and cultivated anaerobically in fluid thioglycate and clostridia agar. Despite the absence of *C. difficile, C. perfrigens* was found at a frequency of 16.7%. Antimicrobial susceptibility was evaluated on the isolates using 13 different antimicrobials. Vancomycin, gentamicin, chloramphenicol, and erythromycin susceptibility was found in just one isolate (4.55 percent). They advised that pig farmers be educated about the disease and how to prevent it through appropriate management and hygiene measures. *Yersinia enterocolitica* isolated from chitterlings, raw milk, and swine feces was tested for antimicrobial susceptibility by Drake, Davis, Khatiwada, and Williams (2018). The isolates were described using MacConkey and CIN agar, and they were confirmed using polymerase chain reaction. Direct plating on selective agars revealed that 4.4 percent of swine fecal samples were presumptively positive for Y. enterocolitica. The fecal samples were found to be non-pathogenic after virulence testing were performed. The disc diffusion method was used to determine the antibiotic susceptibility of the isolates. More than half of the antimicrobials were found to be effective, with ciprofloxacin, kanamycin, trimethoprim, and gentamycin being completely ineffective against the isolates. The least effective antibiotic was found to be ampicillin. The major reason of this pathogen's prevalence in farm animals and

food supplies was determined to be cross contamination at the farm level, posing a danger to human health when food is contaminated.

Burow *et al.,* (2018) investigated antimicrobial susceptibility in pig feces following enrofloxacin treatment in an experimental setting. In contrast to control (CON) and resistances to other antimicrobials, fluoroquinolones (E. coli) in OT, PC, CPT and OT were considered in the blood serum, and resistance to (fluorocarbon) quinolons in commensal Escherichia coli (E. coli) was compared to the starting situation in OT, PT, COT and CPT. Five sets of 14 weaners were held in three different rooms (OT with COT, PT with CPT, CON alone). Five days of donation of enrofloxacin to OT and PT. Rectal swab and blood samples were obtained before, during and 51 days following therapy. All treatment pigs, COT, and CPT contained enrofloxacin and ciprofloxacin. The therapy and contact groups both identified ciprofloxacin and palidixic acid resistance. They found that antimicrobials are and can consume animals that are in contact with treated animals.

Dinesh *et al.,(*2018) investigated the isolation, identification, and characterization of enteric bacteria from post-weaning diarrheic pigs, as well as antibiotic resistance in these bacteria. A culture method was used to examine 25 fecal samples from post-weaning pigs for the presence of *Enterobacteriacae* members, resulting in 18 lactose fermenter and two non-lactose fermenter bacterial colonies were isolated. *E.coli,* two *Enterobacterasburiae*, and one *Klebsiella pneumonia* were found in 15 of the 18 colonies. The sensitivity patterns of chloramphenicol, gentamicin, and ampicillin in *E.coli* isolates show 100 percent sensitivity in vitro. All *E.coli* isolates are resistant to at least four antimicrobials, while four isolates are resistant to at least six Strom *et al*., (2017) They investigated antibiotic use and susceptibility in E. coli in small and medium-sized pig farms in the northeastern part of Thailand, which is located in the country's northern portion. They obtained information through the use of questionnaires. In each farm, three fecal samples were obtained from healthy sows, and *E. coli* was grown and antibiotic sensitivity was determined using the broth micro dilution technique.

Antimicrobial susceptibility was compared between isolates from small and medium scale farms using multilevel regression models. They discovered that most small-scale farmers choose when to use antimicrobials and purchased them from local stores, but medium-scale farmers always consult with veterinarians about antimicrobial therapy. The small- and medium-scale farms have different processes for accessing and administering antimicrobials. Although isolates from medium-scale farms have higher levels of antimicrobial resistance and multidrug resistance, it is unclear if this is due to increased antimicrobial use or changes in administration techniques.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Description of study area

Ikorodu is a city and Local Government Area in Lagos State, Nigeria. It shares boundary with Ogun State. Its geographical latitude is 6°36'3"N and geographical longitude 3°29'17" E.

Apparatuses and Equipment used

Conical flask, Test tubes, Needle and syringe, Pipette, Volumetric flask, Microscope slides, Petri dish, McCartney bottles, measuring cylinders, Durham tubes. Incubator, Refrigerator, Autoclave, Microscope, Electric hot plate, Spatula, Inoculating loop, weighing balance. Test tube rack, Hand gloves, Aluminum foil, Wire loop, Face mask, Distilled water, Cotton wool, Paper tape, Sanitizer, Matches, Alcohol, Plastic bowls, Morning fresh liquid soap, Detergent, Marker and Cotton wool.

Media used

Nutrient agar, EMB agar, MacConkey agar.

Reagents used

Ethanol, Safranin 70%, Crystal violet, Iodine, Decolorizer, Hydrogen peroxide, KOH solution oil immersion.

3.2 METHODS

3.2.1 Sample Collection

The aggregate number of 42 rectal and 10 fecal samples was collected from three (3) different pig pen at Gberigbe Piggery Estate, Ikorodu, Lagos state. The samples were (20) rectal samples (10 rectal samples collected from the adult male, 7 from adult female, 3 from the female piglets)

and (4) fecal samples collected from the first pig pen, (10) rectal samples (4 rectal samples collected from the adult male, 4 from the adult female, 2 from the female piglets) and (3) fecal samples collected from the second pig pen, (12) rectal samples (5 rectal samples collected from the adult male, 5 from adult female, 1 from a male piglet, 1 from a female piglet) and (3) fecal samples collected from the third pig pen. Fecal samples were collected aseptically into sterile universal bottles while rectal sample were collected with swab sticks from pig and piglets. To ensure that the samples were examined as soon as possible, they were transferred to the Microbiology Laboratory of the Department of Biological Sciences and Biotechnology at Caleb University in Imota.

3.2.2 STERILIZATION TECHNIQUES.

Sterile conditions were ensured by carrying out all procedures under aseptic conditions to prevent external and internal contamination which may lead to wrong analysis and wrong results. The media were prepared in clean conical flasks; poured into test tubes in the case of left in the flask for agar, corked with cotton wool and placed in an autoclave for sterilization for 121°C for 15 minutes. The agar was then poured into sterile Petri dishes and allowed to set before being inoculated or put in tightly closed plastic bag in refrigerator for storage. Before pouring into Petri dishes, the bench was swabbed with a disinfectant, the windows closed and the Bunsen burner lighted to the flame mouth of the conical flask while pouring the agar.

Used cultures and contaminated items to be discarded were also being sterilized before being discarded. Inoculating loop and stabbing needle were flamed until they are red before and after use. The loop was however allowed to cool before use. The forceps were sterilized by being inserted into a bottle of alcohol and flamed before use. Working benches sand surfaces of equipment were disinfected before use.

3.2.3 ISOLATION OF THE ORGANISM

According to the normal methodology, the fecal (soil) samples were serially diluted five times before being used. The pour plate technique is used to determine the total viable heterotrophic aerobic numbers in a given population of organisms. MacConkey agar, Nutrient agar, and Eosin methylene blue agar were placed onto a Petri-dish holding 1ml of the appropriate dilution to isolate bacteria. After incubating the plates at 37°C for 24 hours, colony counts were taken, and the bacterial isolates were preserved by sub culturing them to get pure cultures, which were used for biochemical tests.

For primary isolation of bacteria from the rectal swab samples, MacConkey agar, Blood agar, and EMB agar were used. The blood agar was prepared as Tryptone Soya Agar mixed with 5% of blood that served for detection of haemolytic activity of bacteria. Blood agar, MacConkey agar and EMB agar plates were incubated aerobically at 37°C for 24hours. The bacterial isolates were preserved by sub culturing them to get pure cultures which were used for biochemical tests. (Jana *et al.,*2018).

After the culturing and sub-culturing of the isolates to get pure isolates, the pure isolates that was gotten and was used for the biochemical test was; The aggregate number of 37 rectal and 5 fecal samples; (18) rectal and (2) fecal samples from the first pig pen, (9) rectal and (1) fecal samples from the second pig pen, (10) rectal samples and (2) fecal samples from the third pig pen.

3.3 BIOCHEMICAL TESTS AND IDENTIFICATION OF THE ISOLATES

3.3.1 Gram's Staining

The Gram stain divides bacteria into groups i.e. Gram positive (purple color) and Gram negative (pink or reddish color), it was conducted as follows; A smear of the culture was prepared on a clean, grease free slide by emulsifying a little quantity of the growth on a drop of distilled water. The smear was heat fixed and replaced on the slide which was then stained with crystal violet for 30-60 seconds and rinsed off. The slide was further stained with iodine solution and allowed for 60 seconds then rinsed with water but not blotted, then smear was briefly decolorized for about 5 seconds with acetone-alcohol, the smear was rinsed quickly to avoid excess discoloration but not blot dried. The smear was counter stained with safranin for 30-60 seconds; this was rinsed off and allowed to dry. A drop of oil immersion was placed on the stained smear and was viewed under a microscope using objective lens x100. Then the cell shape and Gram's reaction was determined and recorded.

3.3.2 Catalase Test

Colonies with isolation were gathered using a sterile loop and placed on a microscopic slide without grates, each colony receives 3 percent hydrogen peroxide. Bubble effervescence has shown a favorable response for catalase production, but the absence of bubbles have shown a negative response for formation of catalase. "The (Olútiola*et al.* 2000.")

3.3.3 Haemolytic Test

Blood agar was prepared, put onto a sterile petri-dish and allow to set. The plate was dried at a temperature of 25°C in an oven. After that, the culture was streaked over the medium and anaerobically incubated for 24 hours at 37°C.Clear zone around the colony indicates beta haemolysis, partial indicates alpha haemolysis and no clear zone indicates gamma haemolysis (Olutiola *et al.,* 2000)

3.3.4 Potassium hydroxide (KOH) Test

Weighing 0.3 grams of KOH and dissolving it in 10 mL of water A drop of the prepared KOH solution was placed on a glass slide, and colonies of the isolates were spread over the solution using a well-known inoculating loop. A positive result suggests non-mucoid formation, while a negative result shows mucoid production.

3.3.5 Sugar Fermentation Test

1.75 g peptone water was weighed and mixed with 50 mL distilled water, and 0.5 g of each sugar (maltose, lactose, fructose, and sucrose) was weighed and placed into 10% peptone water. Then, until blood red, phenol red indicator was added and distributed into cryovial bottles, which were then sterilised at 121°C for 15 minutes and allowed to cool before being inoculated with isolates and stored at 37°C for 48 hours. A favourable result is indicated by a colour change from red to yellow, whilst a negative result is indicated by no colour change from red.

3.3.6 Starch Hydrolysis Test

This test determines whether or not an organism can hydrolyze starch. 2g of soluble starch was measured into 200ml Nutrient Agar and sterilized at 121°C for 15 minutes to make 1% starch agar. After that, the mixture was placed into petri plates, speckled with inoculums, and incubated at 37°C for 24 hours. By flooding the plates with iodine, the hydrolysis of starch was examined. A clear zone surrounding the line of growth indicates a positive result, whereas no clear zone indicates a negative result.

3.3.7 Motility Test

Testing for bacteria motility with a semi-solid medium Agar is dissolved in 250ml of water, and then homogenized in a water bath with 3.5g of nutritional Agar. An autoclave was used to

sterilize a small amount of the homogenized material after it had been homogenized and placed into multiple cryovial bottles. They were then incubated for four days after being sterilized and allowed to cool and solidify.

3.3.8 Urea Hydrolysis

It was incubated aerobically at 37°C for 48 hours after the isolates were inoculated on the agar slants, which were prepared using urea base agar and pure urea. In the case of good results, the agar was pink (Olutiola *et al.,* 2000).

3.3.9 Indole Test

An organism's capacity to breakdown tryptophan and create indole can be determined using the indole test. The organism was cultured for 24 hours in peptone broth. If indole has been created, KOVAC's reagent was added to the media to detect it. This is an indication of a positive result, which can be seen by looking for a ring of cherry red hue on top of the media. This color is absent in negatives.

.3.4.0 Citrate Utilization Test

This test is used to determine a bacterium's ability to use citrate as the sole source of carbon in its environment. There are just two sources of carbon in this solution: sodium citrate as the single carbon source and ammonium dihydrogen phosphate as the sole nitrogen source. Other nutrients and the Ph indicator bromomothymol blue are also included. It was inoculated on Citrate Agar slant with stabs and streaks, and the results were positive. It took a 24-48-hour

incubation time before there was any noticeable change in color. Positive results are indicated by a color change from green to blue on the screen (Olutiola*et al.,* 2000).

3.4.1 Hydrogen Sulfide Production Test

This test determines whether or not hydrogen sulphide is being produced. Microbes' ability to ferment up to three sugars is tested using a triple sugar iron agar (TSIA). This was accomplished by aseptically inoculating a sterile triple sugar iron (TSIA) slant with pure culture. The inoculated tube was incubated at 35°C to 37°C for 24 hours, and the results revealed that H2S reacts with iron to form Fes, a black compound. In TSIA tubes containing bacteria that produce hydrogen sulphide, the agar turns black, suggesting a positive outcome, whereas no change implies a poor outcome.

3.4.2 Antimicrobial Susceptibility Test

As per normal procedures, the Mueller-Hinton Agar (MHA) (Oxoid, UK) and Kirby-Bauser Disc diffusion techniques for each bacterial isolate were examined for antibiotic susceptibility, using Abetek antibiotics disk. Three to five pure colonies of bacteria were chosen and carefully combined to make a homogeneous solution with 5 ml of sterile normal saline. The suspension turbidity was calibrated according to the McFarland turbidity standard of 0.5. The bacteria were spread equally across the whole surface of MHA using a sterile brush. For 3 to 5 minutes, the inoculation plates could dry in room temperature until sterile forceps were placed on top of a set of antibiotics disks and may stand for 30 minutes. The plates were incubated at 35 ° C for 16 to 18 hours, and the diameter of the inhibitory zone, measured with a ruler according to the 2014 criteria of the Clinical and Laboratory Guidelines Institute, determined by antimicrobial disk breaks (CLSI, 2014).

CHAPTER FOUR

RESULTS

Table 4.1 shows the distribution of the isolates from the pig pens in Gberigbe Piggery Estate, Ikorodu, Lagos state.

Table 4.2 and their likely identity show a cultural and biochemical feature of the bacterial isolates. This indicated 12 of the isolates (28.6), 7 (16.7%) were *bacillus spp*, 6 (14.2%) was *Enterobacter spp*, 7 (16.7%) was *bacteria spp*, 7 (16.7%) were *klebsiella spp*, and 2 (4.76%) were *e.coli*, and 1 (2.38%) was *salmonella spp*. The results have also shown that 12 (28.6%) of the isolates are gram-positive cocci, that 11 (26.2%) are gram-negative coccibacilli, 12 (28.6%) are gram-negative rod, and 7 (16.7%) are gram-positive rod.

The frequency of occurrence of the isolated bacteria is shown in Table 4.3, with *Staphylococcus spp* having the highest frequency percentage of (28.6%) while and Salmonella *spp* has the lowest frequency percentage of (2.38%).

The antibiotics susceptibility of the gram positive isolates is shown in Table 4.4. The results showed the fact that the highest susceptibility of the isolate was Gentomycin (100%), while the lowest susceptibility was Cloxacillin (0%). The highest resistance of the isolates (100%) was recorded with Cloxacillin, while the lowest (0%) was recorded with Gentomycin. The antibiotics susceptibility of gram negative isolates is shown in Table 4.5. The results showed the fact that the highest susceptibility of the isolate was Ofloxacin (95.2%), Nitrofurantoin (95.2%), Gentamicin (95.2%), while the lowest susceptibility was Cefuroxime (23.8%). The highest resistance of the isolates (76.2%) was recorded with Cloxacillin while the lowest

(4.76%) was recorded with Gentamicin, Ofloxacin and Nitrofurantoin.

Table 4.1 Distribution of isolates from the pig pen.

N/S	FARM/SAMPLE	NO OF ISOLATE	PERCENTAGE
A.	Rectal	18	42.9
	Fecal	2	4.76
B.	Rectal	9	21.4
	Fecal	1	2.38
C.	Rectal	10	23.8
	Fecal	2	4.76

Table 4.2; Cultural and Biochemical characteristics of the bacterial isolates

ISOLAT E	CATAL ASE	KO H	GRAMSTAI NING	SUCR OSE	MALT OSE	FRUCT OSE	LACT OSE	CITRA TE	INDO LE	STAR CH	MOTILI TY	UREA SE	HAEMOL YSIS	H2 S	GLUCO SEA	GLUCO SEG	*POSSIBLE MICROORGA NISM*	
OO29M (R3)	+	+	COCCI	+	+	+	+	-	+	+	+	-	+	+	+	+	*Staphylococcu s spp*	
S31 B (R2)	+	+	ROD	+	+	+	+	+	-	-	+	+	+	-	+	+	*Bacillus spp*	
S32B (R2)	+	-	ROD	+	-	+	+	+	-	-	+	+	+	+	+	+	*Enterobacter spp*	
OOO8M (R1)	+	+	COCCI	+	+	+	+	-	-	-	-	+	-	+	-	-	*Staphylococcu s spp*	
OO2O1M (R3)	+	-	ROD	+	+	+	+	-	-	-	+	+	-	-	+	+	*Enterobacter spp*	
OO15B (R1)	+	+	ROD	-	+	+	-	-	-	+	-	+	+	-	+	+	*Bacillus spp*	
OOO7M (R1)	+	-	COCCIBACI LLI	+	+	+	+	-	+	-	-	+	-	-	+	+	*Klebsiella spp*	
OO191M (R3)	-	+	COCCI	+	+	+	+	+	+	+	-	+	+	-	+	+	*Staphylococcu s spp*	
OO15B (R1)	-	+	ROD	+	+	+	-	-	+	-	-	+	+	-	+	+	*Bacillus spp*	
OO1OM (R1)	+	+	COCCI	-	+	+	-	-	-	+	+	+	-	+	+	+	*Staphylococcu s spp*	
OO15M2 (R1)	+	+	COCCI	+	+	+	+	+	-	+	-	-	+	+	+	+	*Staphylococcu s spp*	
OO26M (F1)	+	-	COCCIBACI LLI	+	+	+	+	+	-	+	+	+	-	+	+	+	*Enterobacter spp*	
OO13M (F3)	+	-	COCCIBACI LLI	+	-	+	+	-	+	-	+	-	+	+	+	+	*E.Coli*	
OO16M (R1)	+	-	COCCIBACI LLI	+	-	+	-	-	-	-	-	+	-	+	+	+	*Salmonella spp*	
OO21M (R3)	+	+	ROD	+	+	+	+	+	-	-	-	+	-	-	+	+	*Bacillus spp*	
OO192M (R2)	-	+	COCCI	+	+	+	+	+	-	+	+	+	-	-	+	+	*Staphylococcu s spp*	
OO7B (R1)	+	-	ROD	+	+	+	+	-	+	-	-	+	+	+	+	+	*Klebsiella spp*	
OO262M (R3)	-	-	COCCIBACI LLI	+	+	+	+	+	-	+	+	+	-	-	-	-	*Enterobacter spp*	
S33B (R2)	+	+	COCCI	-	+	+	+	-	+	-	+	+	+	+	-	-	*Staphylococcu s spp*	
OO263M (F2)	+	-	COCCIBACI LLI	+	+	+	+	+	+	-	+	+	-	-	+	+	*Citrobacter spp*	

OO3M (R1)	+	-	ROD	+	+	+	+	-	+	-	+	+	+	-	+	+	*E.Coli*	
OOO3B (R2)	+	-	COCCIBACI LLI	+	+	+	+	-	+	-	-	+	-	-	+	+	*Klebsiella spp*	
S35B (R2)	+	-	ROD	+	-	+	-	+	-	+	-	+	-	+	+	+	*Klebsiella spp*	
OO3O1M (R2)	+	-	COCCIBACI LLI	+	+	+	+	+	+	-	-	+	+	+	+	+	*Klebsiella spp*	
S31M (R2)	+	-	ROD	+	+	+	-	-	+	+	-	+	-	+	+	+	*Enterobacter spp*	
OO262M (F1)	+	-	COCCIBACI LLI	+	+	+	+	+	+	+	+	+	+	-	+	+	*Citrobacter spp*	
OO30B (R3)	+	+	COCCI	+	+	+	+	-	+	-	+	+	-	+	+	+	*Staphylococcu s spp*	
OOO5B (R1)	+	-	ROD	+	+	+	+	+	-	-	-	+	-	+	+	+	*Citrobacter spp*	
OO15B (R1)	+	+	ROD	+	+	+	+	-	-	+	+	+	+	-	-	-	*Bacillus spp*	
OOO7B (R1)	+	-	ROD	+	+	+	+	+	-	-	+	+	-	+	+	+	*Citrobacter spp*	
OOO6M (R1)	+	+	COCCI	+	+	+	+	+	+	-	+	+	-	-	+	+	*Staphylococcu s spp*	
S36B (R2)	+	-	ROD	+	+	+	+	+	+	+	-	+	+	+	+	+	*Citrobacter spp*	
OO6M (R1)	+	+	COCCI	+	-	+	-	-	-	+	+	-	-	-	+	+	*Staphylococcu s spp*	
OO22B (R3)	+	+	ROD	+	+	+	+	+	+	+	-	+	-	-	+	+	*Bacillus spp*	
OO15M1 (R1)	+	+	COCCI	+	+	+	-	+	-	-	+	+	-	-	-	-	*Staphylococcu s spp*	
OO25M (R3)	+	-	COCCIBACI LLI	+	+	-	+	+	+	+	+	+	-	+	+	+	*Enterobacter spp*	
OOO52B (R2)	+	-	ROD	+	+	+	+	-	-	-	-	+	+	+	+	+	*Klebsiella spp*	
OO11M (F3)	+	-	COCCIBACI LLI	-	+	+	+	+	-	+	+	+	-	+	+	+	*Klebsiella spp*	
OOO2B (R1)	+	-	ROD	+	+	+	+	-	+	+	+	+	+	-	+	+	*Citrobacter spp*	
OO28M (R3)	+	+	ROD	+	+	+	+	+	+	+	-	+	-	+	+	+	*Bacillus spp*	
S5M (R2)	+	-	ROD	+	-	+	+	+	+	-	+	+	-	+	+	+	*Citrobacter spp*	
OO3OB (R3)	+	+	COCCI	-	+	+	-	-	+	+	-	+	-	+	+	+	*Staphylococcu s spp*	

KEY

- **-- NEGATIVE**

\+ **-- POSITIVE**

(R1) RECTAL SAMPLES FROM THE FIRST PIG PEN (F1) FAECAL SAMPLES FROM THE FIRST PIG PEN

(R2) RECTAL SAMPLES FROM THE SECOND PIG PEN (F2) FAECAL SAMPLES FROM THE SECOND PIG PEN

(R3) RECTAL SAMPLES FROM THE THIRD PIG PEN (F3) FAECAL SAMPLES FROM THE THIRD PIG PEN

Table 4.3; The number of isolated microorganisms that occur on a regular basis

S/N	Microorganism	Number of Isolates	Frequency (%)
1	*Klebsiella spp*	7	16.7
2	*Salmonella spp*	1	2.38
3	*Citrobacter spp*	7	16.7
4	*Bacillus spp*	7	16.7
5	*Enterobacter spp*	6	14.3
6	*Staphylococcus spp*	12	28.6
7	*E.coli*	2	4.76
	Total	42	100

Table 4.6The Antibiotic Susceptibility of the Gram Positive Isolates

Antibiotics	Number of susceptibility %	Number of resistance %
CAZ	3 (16.7)	15 (83.3)
CRX	5 (27.7)	13 (72.2)
GEN	18 (100)	0 (0)
ERY	12 (66.7)	6 (33.3)
CXC	0 (0)	18 (100)
AUG	12 (66.7)	5 (27.7)
OFL	11 (61.1)	7 (38.9)

KEY: CAZ- Ceftazidime , CRX- Cefotaxime, GEN- Gentamicin, ERY- Erythromycin, CXC- Cloxacillin, AUG- Augmentin, OFL- Ofloxacin.

Table 4.7The Antibiotic Susceptibility of the Gram Negative Isolates

Antibiotics	Number of susceptibility %	Number of resistance %
CXM	5 (23.8)	16 (76.2)
OFL	20 (95.2)	1 (4.76)
AUG	13 (61.9)	8 (38.1)
NIT	20 (95.2)	1 (4.76)
CPR	15 (71.4)	6 (28.5)
CAZ	6 (28.5)	15 (71.4)
CRX	15 (71.4)	6 (28.5)
GEN	20 (95.2)	1 (4.76)

KEY: CXM- Cefuroxime, OFL- Ofloxacin, AUG- Augmentin, NIT- Nitrofurantoin, CPR- Ciproflaxacin, CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin.

NB: The aggregate number of 35 samples was used for the antibiotics susceptibility of the isolates, 3 of the samples got contaminated.

CHAPTER FIVE

DISCUSSION, RECOMMENDATION AND CONCLUSION

5.1 DISCUSSION

Bacterial infections in animals continue to be a serious issue across the world. The motility and poor development of infected animals, as well as the potential of transmission to humans via the food chain or direct animal contact, resulted in significant economic losses. As a result, detecting bacteria in animals is critical, as is having the necessary tools to manage germs on-farm and prevent their spread to the general population (Rotimi, 2008).

Table 4.1 Showed the fact the distribution of samples (Isolates) taken from three pig pens in Gberigbe Piggery Estate, Ikorodu, Lagos state. From the table, it is seen that most of the isolates (samples) were taken from pig pen A. 18 rectal samples and 2 fecal samples were taken from pig pen A while 9 rectal samples and 1 fecal samples was picked from pig pen B and 10 rectal samples and 2 fecal samples were taken from pig pen C.

A Cultural and Biochemical characteristic of the bacterial isolates is seen in Table 4.2and their probable identity. The summary of table 4.2 as shown in table 4.3 revealed that12of the isolates (28.6%) were *Staphylococcus spp*, 7 (16.7%) were *Bacillus spp,*6 (16.7%) were *Enterobacterspp,*7(16.7%) were *Klebsiella spp,* 7 (16.7%) *Citrobacter spp,* 2 (4.8%) were *E.coli,* 1(2.4%) was *Samonella spp.* The result also showed the fact that 12 (28.6%) of the isolates were gram-positive cocci, 11 (26.2%) were gram-negative coccobacilli, 12 (28.6%) were gram-negative rod, and 7 (16.7%) were gram-positive rod.The frequency of occurrence of the isolated bacteria is shown in Table 4.3, showed the fact that *Staphylococcus spp* is having the highest frequency percentage of (28.6%) in which a similar result was also reported in a study (Khanna*et al.,*2008) where *Staphylococcus aureus* was detected on 79 (64.2%) farms from 14 provinces, among this farms live-stock animal *Staphylococcus aureus* positive farms dominated (71/79, 89.9% , 95%. 81.0% and 95.5%). Also a similar study also reported

Staphylococcus aureus found in 44% of the rectal swabs from 377 pigs. (Dimitracopoulos *et al.,*2021) while *Salmonella spp* has the lowest frequency percentage of (2.4%).

The prevalence of *Salmonella spp* in the samples was estimated to be 2.4%. This is against previous studies from *Salmonella* infection in piglets 2021 (Bernad-Roche *et al.,*2002) their studies reported that the overall percentage of *Salmonella*-infected piglets was 36.5% but was variable across pig farms from 17.5% to 59.2%.

The antibiotics susceptibility of the gram positive isolates is shown in Table 4.4. The results show that the highest susceptibility of the isolate was Gentomycin (100%), while the lowest susceptibility was Cloxacillin (0%). The highest resistance of the isolates (100%) was recorded with Cloxacillin, while the lowest (0%) was recorded with Gentomycin. The antibiotics susceptibility of gram negative isolates is shown in Table 4.5. the results show that the highest susceptibility of the isolate was Ofloxacin (95.2%), Nitrofurantoin (95.2%), Gentamicin (95.2%), while the lowest susceptibility was Cefuroxime (23.8%). The highest resistance of the isolates (76.2%) was recorded with Cloxacillin while the lowest (4.76%) was recorded with Gentamicin, Ofloxacin and Nitrofurantoin which is similar to where *E.coli* from conventional pig farms were resistance to gentamicin 0.7% (Nulsen*et al.,*2008).

5.2 CONCLUSION

In summary, our result shows the presence of *Staphylococcus spp*, *E. coli, Bacillus spp, Enterobacter spp, Klebsiella spp, Citrobater spp* and *Salmonella spp*. The isolation and characterization of bacteria from pigs is alarming. Antimicrobial resistance can rise when antimicrobial exposure rises because bacteria can quickly adapt to new environmental conditions, such as the presence of antimicrobial compounds. The zoonotic pathogens Staphylococcus and other microorganisms (bacteria) found here are zoonotic pathogens, and their prevalence in animals provides a constant threat to humans. Most of these diseases are related to outbreaks with food, live contact with animals, poor hygiene and environmental exposure. Bacterial infections can lead to antibiotics being used. This comprises *Bacillus spp, Enterobacter spp., Paenibacillus spp*., *Listeria spp, Koluria spp., Cocobacillus spp*. These illnesses require broad spectrum of antibiotics to be used in feed, drinking water or through injections, including erythromycin, gentomycin, ceproflavin or other medications.

It is critical to determine the etiological agent of illnesses in order to sustain a viable swine business. In the case of bacterial agents, it is critical to analyze antibiotic susceptibility patterns in order to provide prompt treatment and avoid needless costs and the development of antibiotic resistance. As organisms acquire resistance to frequently used antibiotics, determining antibiotic sensitivity becomes important in order to avoid overuse of antibiotics and the development of resistance. This would assist to prevent antibiotic-resistant germs from spreading to other livestock and people.

5.3 RECOMMENDATION

In the Ikorodu Area of Lagos, food animal carcasses (pigs) prepared as meat for human consumption are possible reservoirs of certain bacterial infections that might result in or constitute a severe public health danger. As a result of the findings of this study, the following suggestions are made:

- In both animal husbandry and human medicine, there should be more demands for surveillance and monitoring of antibiotic use across the world.
- In Nigerian slaughterhouses, public health intervention strategies at the pre- and post-slaughter stages should be explored.
- Susceptibility tests should be performed on animals, particularly pigs (because to their proclivity for collecting dirt's), at specific periods of the year to determine the presence of bacteria and when antibiotics should be administered.
- Before providing medicines to animals, appropriate testing should be performed, and drugs should be recommended by a competent veterinary practitioner.
- Further research into the environmental impact of bacteria-contaminated manure in endemic areas is required.

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Antibiotics Susceptibility of the Gram-positive isolates

ISOLATE	CAZ	CRX	GEN	CTR	ERY	CXC	AUG	OFL
0015MAC	0	0	16.5	19.5	10	0	17	0
0013MAC	0	0	13.5	18.5	0	0	0	16
0016MAC	0	0	12	10	15	0	15.5	20.5
0028MAC	0	0	11	19.5	0	0	0	0
0019MAC	0	0	16.5	15	15	0	0	0
0015BA2	0	0	20.5	12	22	0	20	14
0026MAC2	0	12.5	15	15.5	0	0	0	19.5
0030BA1	14	0	13.5	0	0	0	16.5	21.5
0029MAC	0	0	13	0	16	0	10	20
0002BA	19.5	19.5	19	21	17	s0	20.5	24.5
0019MAC2	23.5	19.5	15	29.5	0	0	12.5	30
0022BA	0	10	18.5	10.5	25.5	0	31.5	20
0026MAC3	0	0	14.5	10.5	20	0	12.5	12
0026MAC4	0	0	18	12	21	0	15.5	13
0006MAC	0	0	18	19.5	14.5	0	19	0
006MAC	0	0	14.5	19	12.5	0	13.5	0
0015MAC2	0	0	15	14.5	11.5	0	15	0
0020MAC	0	15	15.5	0	0	0	0	0

Antibiotics Susceptibility of the Gram-negative isolates

ISOLATE	CXM	OFL	AUG	NIT	CPR	CAZ	CRX	GEN
0011MAC	20.5	19.5	20.5	17	14	0	0	15
0007BA2	0	19.5	0	12.5	0	0	13.5	16
0007MAC	0	20	0	11	0	0	11.5	15.5
0030BA1	0	23.5	0	11.5	19.5	20	19.5	14.5
0003MAC	21	22.5	19.5	22.5	20.5	22.5	0	13.5
0003BA	0	21.5	0	10.5	16.5	0	0	13.5
0013MAC	0	15.5	10	11.5	0	0	14.5	11.5
S35 BA	0	29.5	15	20.5	20.5	0	0	11.5
0005BA	24.5	18.5	24.5	26.5	20.5	20	25.5	15.5
0026MAC								
1	15.5	23.5	22.5	18.5	24.5	14.5	18.5	14.5
0030MAC	23.5	21.5	21.5	19.5	21	21.5	23.5	13
0008MAC	0	18.5	0	16.5	18.5	0	0	19.5
0015BA	0	20.5	0	10.5	23.5	0	0	0
S51MAC	0	24.5	0	15.5	15.5	0	10.5	18.5
S31MAC	0	24.5	20	28.5	0	0	10	14.5
S31BA	0	22.5	19	0	15	0	12	15
S33BA	0	22	17.5	28	21	10.5	10	13
S2BA	0	22.5	0	29.5	0	0	12	16
S3BA4	0	0	22	29	28	0	11.5	14.5
0005BA	0	22	21	20	21	0	10.5	15
0010MAC	0	20	25.5	21	0	0	10	18.5