**UNIVERSITY OF GHANA**

**COLLEGE OF HEALTH SCIENCES**

**SCHOOL OF PUBLIC HEALTH**

# ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* AND *SALMONELLA* IN PIGS, GREATER ACCRA REGION, GHANA

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## DECLARATION

I hereby declare that this thesis is a record of my own research work, written entirely by me. It has neither in whole nor in part been presented for another degree in this university or elsewhere. Works by other researchers have been duly cited by references to the respective authors and all assistance received acknowledged accordingly.

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## DEDICATION

I dedicate this thesis to God Almighty my creator. He has been the source of my strength throughout this program and on His wings only have I soared. I also dedicate this work to my parents and siblings; who have encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I started.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **AMR** | Antimicrobial Resistance |
| **BPW** | Buffered Peptone Water |
| **CDC** | Centers for Disease Control |
| **CLSI** | Clinical and Laboratory Standards Institute |
| **DDW** | Double Distilled Water |
| **DNA** | Deoxynucleic acid |
| **E. *coli*** | *Escherichia coli* |
| **FAO** | Food and Agricultural Organization |
| **GAR** | Greater Accra Region |
| **GHSA** | Global Health Security Agenda |
| **GLASS** | Global Antimicrobial resistance Surveillance System |
| **GSS** | Ghana Statistical Services |
| **MDR** | Multi-Drug Resistance |
| **MH** | Muller Hinter |
| **MIC** | Minimum Inhibitory Concentration |
| **MLN** | Mesenteric Lymph Node |
| **MOFA** | Ministry of Food and Agriculture |
| **NAP** | National Action Plan |
| **NTS** | Non-typhoidal *Salmonella* |
| **PCR** | Polymerase Chain Reaction |
| **S-S** | Salmonella-Shigella |
| **S. *typhimurium*** - | *Salmonella typhimurium* |
| **Spp** | Species |
| **TSA** | Tryptone Soya Agar |
| **TSI** | Triple Sugar Iron |
| **USA** | United State of America |
| **VSD** | Veterinary Services Department |
| **WHO** | World Health Organisation |
| **XLD** | Xylose Lysine Deoxycholate |

## ABSTRACT

### Background

Food-borne pathogens associated with animal products pose significant threat to individual and population health globally. They cause serious infections in humans which can lead to treatment failures if they develop resistance. *Escherichia coli* (*E. coli*) is a commensal bacterium of the human and animal gut but has the ability to cause illnesses. *Salmonella* is one of the major foodborne pathogens worldwide. The presence of *Salmonella* and *E. coli* in food animals at slaughter and the consequent cross-contamination of edible carcass tissues present a significant food safety hazard. In this study, fecal and mesenteric lymph node (MLN) carriage of *E. coli* and *Salmonella,* their antimicrobial susceptibility profiles and serogroups of *Salmonella* among healthy pigs at slaughterhouses were investigated.

### Methods

Fecal and MLN samples were collected from one-hundred and forty (140) pigs from three (3) selected slaughterhouses in the Greater Accra Region. Approximately, 5g of feces and 5g of MLN were aseptically removed from the rectum and mesentery respectively at slaughter line. Fecal and MLN were pre-enriched in a non-selective medium; buffered peptone water (BPW). For *E. coli* isolates, a loop of the aliquots BPW was cultured on MacConkey agar and incubated at 44$^{o}$ C overnight. For *Salmonella* isolates, a loop full of the pre-enriched aliquots were transferred into a selective enrichment medium; Tetrathionate broth and incubated at 37$^{o}$ C for 24 hours. Enriched aliquots were cultured on xylose lysine deoxycholate (XLD). Based on their colony morphologies, presumptive *E. coli* and *Salmonella* isolates were subjected to biochemical testing for identification. The susceptibility of the pure isolates was tested against ten (10) antibiotics using the Kirby-Bauer disk diffusion method. *Salmonella* serogrouping was done by slide agglutination testing. Slaughterhouse hygiene and slaughter practices were also

observed and recorded. Summary descriptive statistics were performed to characterized antimicrobial susceptibility profiles.

**Results**

Of the 280 samples collected, 67.50% (189/280) were *E. coli* positive and 7.86% (22/280) *Salmonella* positive. Fecal and MLN *E. coli* carriage was 72.14% (101/140); and MLN *E. coli* 62.86% (88/140). Similarly, fecal *Salmonella* carriage was 2.14% (3/140); and MLN *Salmonella* carriage 13.57% (19/140). Antimicrobial susceptibility testing indicated that *E. coli* isolates (fecal and MLN) expressed the most resistance to tetracycline (56.1%), ampicillin (39.2%) and trimethoprim/sulphamethoxazole (35.4%). *Salmonella* isolates showed resistance mainly to ampicillin (31.8%) only. Multi-drug resistance (MDR) was detected in 30.16% of *E. coli* isolates, however, there was no MDR among the *Salmonella* isolates tested. The most frequent MDR profile for both fecal and MLN *E. coli* isolates was to tetracycline, ampicillin and trimethoprim/sulphamethoxazole. Resistance of a single isolate expressed to eight (8) was seen in feces; and seven (7) antibiotics was seen in MLN. All *Salmonella* serogroups identified consisted of non-typhoidal *Salmonella*. Structure design, waste management and hygiene practices were sub-standard per the Ghana Food and Drugs Authority code of practice for slaughterhouses.

**Conclusions**

Fecal and MLN carriage of *E. coli* a and *Salmonella* are high among pigs in Greater Accra Region. High levels of MDR of *E. coli* detected pose a great public health concern as these resistant strains have the potential of transferring their resistant genes to pathogenic organisms leading to increased rates of treatment failures. Misuse of antibiotics, inappropriate structure designs, poor hygiene and slaughter practices may have contributed to MDR. This calls for stricter regulation and monitoring of the use of antibiotics in livestock production and hygiene practices at slaughterhouses.

# CHAPTER 1

# INTRODUCTION

## 1.0 Background

Antimicrobial resistance (AMR) is known as the ability of microorganisms such as bacteria, fungi, viruses and parasites to withstand the effect of standard doses of clinically relevant antimicrobial drugs (Sharma et al., 2018; World Health Organization, 2012). It is an increasing menace to global public health and one of the significant threats to individual and population health as well as food safety and security, livelihoods and animal production (S. Davies & Gibbens, 2013; FAO, 2016a; O'Neill, 2014; World Economic Forum, 2013). AMR does not have geographical boundaries and can pass over among humans, animals across countries, mediated through resistant strains; without any specific information and check resulting in prolonged illness, disability, and death (Littmann & Viens, 2015). Globally, statistics on AMR suggest that the death toll from AMR could be as high as 300 million people by 2050, with an estimated financial loss of $100 trillion (O'Neill, 2014). In the United States of America (USA) as high as $20 billion in direct health care costs and $35 billion in lost productivity are associated with AMR in the economy annually (Centers for Disease Control and Prevention, 2013). In African regions, the understanding and magnitude of AMR related issues are impeded by inadequate data as surveillance on drug resistance is limited to a few countries, and laboratory capacities are limited to monitor AMR. However, despite these impediments, available data suggest that African regions share the worldwide trend of increasing drug resistance (Ndihokubwayo et al., 2013).

Currently, AMR associated with the food chain is a subject of significant interest to global public health (Safefood, 2019). It estimated that every year, almost 1 in 10 people fall ill and 33 million of healthy life years are lost due to food-borne diseases. Most of these food-borne

illnesses have been associated with zoonotic pathogens, and a rising drug resistance prevalence is being recognized in them (Verraes et al., 2013; World Health Organization., 2019). AMR zoonotic pathogens constitute a direct risk for public health. Of these zoonotic pathogens, *Salmonella* and *Campylobacter* are the most common causes of food-borne illnesses in industrialized countries (Hoelzer et al., 2017; Verraes et al., 2013). The consequences of these food-borne pathogens lead to increase number of hospitalizations, the risk of invasive infections and mortality. According to Centers for Disease Control and Prevention, (2015), 192 cases of food-borne illnesses from MDR *Salmonella spp* were recorded, and 523,380 pounds of pork recalled in 2018 due to the presence of *salmonella spp*. AMR in commensal bacteria such as *Escherichia coli,* constitute an indirect risk for public health as these resistant bacteria when given the right conditions, could transfer or disseminate their resistant genes to pathogenic and zoonotic bacteria (Hoelzer et al., 2017; Verraes et al., 2013).

Besides the human health considerations, AMR undermines food security and rural livelihoods (FAO, 2016a). It has been reported that resistance to penicillin, tetracycline and sulphonamides is common among chicken and pig bacterial isolates, as well as significantly higher MDR in these isolates than those from cattle (FAO, 2016b). MDR of *E coli* was detected in pork, ground turkey, chicken and beef. Urahn, Coukell, Eskin, & Hoelzer, (2016) reported significantly increasing resistance of *Salmonella* isolates to ceftiofur among animal over the years from 0% up to 21.5% in cattle, 0.5% to 11.9% in chicken, 3.7% to 15.2% in turkey and 0% to 1.8% in pigs.

The global burden of AMR has no signs of receding; instead, it piles up the pressure on human and veterinary medicine. With this mounting pressure of AMR globally, several countries have initiated surveillance and monitoring programs. Agencies such as the Global Antimicrobial Resistance Surveillance System under WHO was launched in 2015 to aid the global action plan

on antimicrobial resistance. Its aim is to support global surveillance and research in order to strengthen the evidence based on antimicrobial resistance (AMR) and help to inform decision-making and drive national, regional, and global actions. Food and Agriculture Organization, CDC, and Office International des Epizooties are also making substantial efforts to control antibiotic resistance. Other programs such as the Global Health Security Agenda (GHSA), Antimicrobial Resistance Action Package (GHSA Action Package Prevent-1) also deal with the global threat of antibiotic resistance.

In Ghana, on the 11th April 2018, the Antimicrobial Use and Resistance Policy and an accompanying comprehensive National Action Plan (NAP) on AMR were launched. The objectives of the NAP are to improve awareness and knowledge of AMR, provide evidence-based knowledge to reduce the burden of AMR, reduce the occurrence of infections in establishments, optimize antimicrobials' use in human and animal health and create an enabling environment for sustainable investment in AMR reduction. The unit responsible for monitoring the use of antibiotics and surveillance of resistance in animals is the Veterinary Public Health and Food Safety. Its overall goal is to prevent zoonotic diseases in humans and ensure food safety. It also has plans to carry out antibiotic residue testing and antibiotic resistance testing (Ghana Ministry of Health, Ministry of Food and Agriculture, Ministry of Environment, Science, 2018).

The launch of NAP was followed by an Inter-ministerial Committee meeting on AMR in March 2019. In April 2019 NAP piloted the Progressive Management Pathway (PMP) tool developed by FAO; a practical self-assessment tool which was followed by the implementation of NAP in August 2019. As part of the implementation process through the AMR Reference Laboratory at the University of Ghana, Fleming Fund Project is supporting One-Health governance structure surveillance on AMR and antimicrobial use (AMU), a government-led system of collecting, analyzing and reporting AMR, and AMR on national and international platforms.

Still, in the implementation process, FDA is to ensure quality, safe and efficacious antimicrobial agent on the Ghanaian market (G.D. Zaney, 2019; Ki Jung, 2019). Therefore, FDA with support from Fleming Fund has embarked on two (2) projects; Quality of Selected Antimicrobial Agents and Data Capture on Antimicrobial Agents.

### 1.1 Problem statement

The advent of antibiotic use in livestock production largely contributed toward health and productivity, but also played a notable role in the rise of antimicrobial resistance (AMR) (Sharma et al., 2018). Although trends of AMR in animals have not gained much attention compared to the trends in humans, data available show variable levels of the burden of bacteria resistance worldwide (CDDEP, 2016). In developed countries, a European Union summary report on antimicrobial resistance in animals revealed a multi-drug resistance (MDR) in *Salmonella spp.* isolates in meat of up 73% in turkeys, 56% in broilers and 37.9% in pigs. E. *coli* isolates from broilers showed the highest microbial resistance to ampicillin (58.6%) and 52.8% resistance to tetracyclines among pigs (EFSA & ECDC, 2015). Smaller studies in low and middle-income countries revealed similar rates of MDR. For example, Taiwan and Kenya recorded 96% and 7.1% MDR in *Salmonella* isolates in pork respectively (Kikuvi, Ombui, & Mitema, 2010; Kuo et al., 2014), and Cambodia recorded 79% MDR in *E. coli* isolates in pork (Ström et al., 2018). In Ghana, a study conducted in some selected pigs' farms in the Ashanti Region revealed large variety and quantity of antibiotics used (Sekyere, 2014). Still in the Ashanti Regtion, Sekyere & Adu, (2015) found a MDR of 9.7% of S. *Typhimurium* isolates from pigs' feces. MDR of 26.67% was observed among beef *E. coli* isolates in Wa abattoir, in the Upper West Region, Ghana (Frederick Adzitey, 2020).

AMR development occurs primarily as a consequence of selection pressure placed on susceptible microbes by the use of antimicrobial agents (FAO, 2016b) although there are a

variety of other factors. In the case of antibiotic resistance, antibiotics cause selective pressure by killing susceptible bacteria, allowing antibiotic-resistant bacteria to survive and multiply. AMR in animals, on the one hand, is believed to be partly associated with inappropriate use of antibiotics for treatment; such as the use of antibiotics in viral infections and sub-therapeutic dosing. On the other hand, inappropriate use and disposal of antibiotics by farmers also contribute the risk of AMR (Sekyere, 2014); antibiotic-resistant bacteria from the environment can contaminate farm animal feed and can result from careless use of antibiotics in animal production and aquaculture (Odeyemi, 2016).

Antimicrobials are commonly used non-therapeutically in livestock production as a kind of "insurance" in addition to other animal disease risk-management measures. Metaphylaxis which involves administration of an antimicrobial at therapeutic doses to clinically healthy animal within a group in which some individuals have exhibited infection and prophylaxis which involves treatment of healthy animals to prevent diseases are other associated factors for AMR development. Though debatable, the use of antibiotics for growth promotion purposes is also known to contribute to AMR (Tang et al., 2017). There could be cross-contamination across the food chain; environmental and worker contamination.

The occurrence of resistance in bacteria can also be due to mutation. The bacteria build up resistance to antibiotics through mutating existing or exchange of genetic material between organisms; resistant gene transfer to a pathogenic organism.

Resistant bacteria in animals will lead to poor response in treatment which will increase morbidity and mortality. Farmers will suffer economic losses as they lose their animals to sickness and death. Besides, resistant bacteria in animals will provide a direct route for human infection either through direct animal contact or through the food-chain- "Farm to fork principle" (Hong, Yannarell, & Mackie, 2010). An infection with resistant-bacteria through food chain will increase AMR in human, posing the risk of treatment failure which will lead to

high morbidity, mortality, increased cost of healthcare, economic losses, and reduced quality of life.

To mitigate this rising threat of AMR, many countries the world over initiated surveillance and monitoring programs. In Ghana, the National Action Plan was launched. One of its objectives is to provide evidence-based knowledge on AMR.

The purpose of this study is to determine and compare the prevalence, antimicrobial resistance profiles of *E. coli* and *Salmonella* and serogroups of *Salmonella* in fecal and lymph nodes in pigs.

### 1.2 Conceptual framework

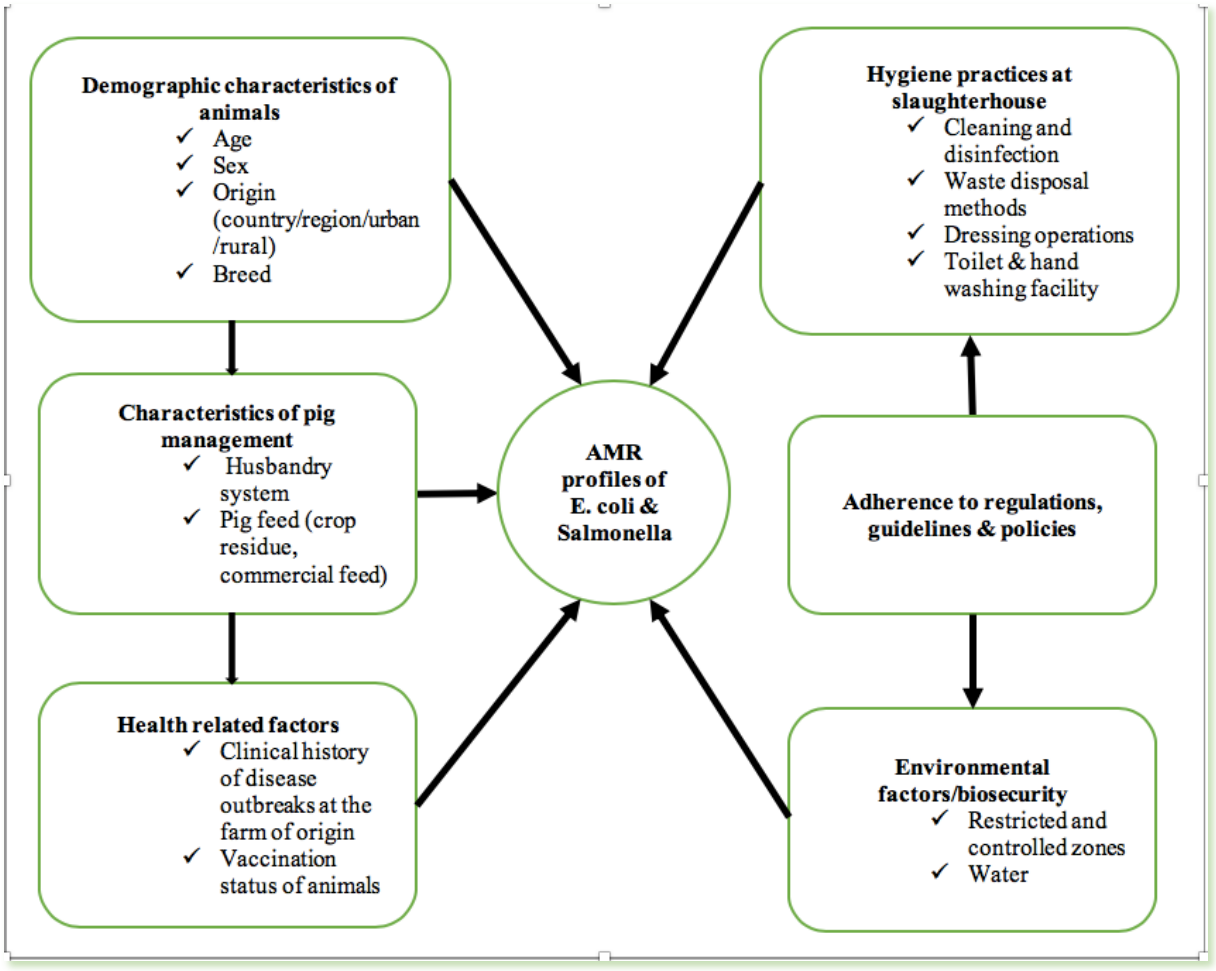


**Figure 1: Conceptual framework**

### 1.3 Narrative Summary of Conceptual Framework

This conceptual framework attempts to explain the various independent variables in assessing AMR profiles of E. *coli* and *Salmonella spp* in pigs. AMR in animals is believed to be directly linked with inappropriate use of antibiotics on one hand and many other factors on the other hand. The influence of demographic characteristics such as age, sex and farm origin of the pig can be argued in the direction of the number and quantity of antibiotics consumed in their lifetime. Older pigs are likely to be exposed in their lifetime to more antibiotics than younger animals, as a result, are more at risk of developing AMR. Younger pigs are also likely to be exposed in their growing stage to more antibiotics as they receive oral antimicrobial for prophylaxis. And it has been reported to increase AMR in commensal *E. coli* from pigs (Burow et al., 2014). The influence of the breed of the pig can be argued in that local breed are more resistant to many diseases; therefore, receive less prophylaxis treatment compared to exotic breed.

Husbandry system is directly linked with medical treatment practices; intensive husbandry systems usually use more antibiotics for prevention and control of diseases compared to extensive system. Husbandry system is also linked with the type of feed given to the pigs. Commercially prepared feeds (some containing growth promoters) and growth promoters are more used in intensive systems compared to extensive systems. Thus, it increases the risk of such pigs to develop AMR.

Information on health factors (clinical history of a disease outbreak at the farm of origin, vaccination status) is directly linked to the understanding of AMR development. Disease outbreak history is linked to treatments given, which will subsequently influence AMR development.

Adherence to regulations, guidelines and policies influences biosecurity factors and hygiene practices at the slaughterhouse, which ultimately influences the risk of AMR contamination in food. Food handling, environmental and worker contamination are risks factors to AMR.

### 1.4 Justification

Strengthening and providing evidence – based knowledge to reduce the burden of AMR through research and national surveillance is foundational to the successful implementation of the Ghana NAP on AMR. Even though research activities on AMR in animals are being conducted in Ghana, not much has been achieved and AMR continues to pose threats to food security and public health in the country and globally. This study will reveal the burden of AMR of *E. coli* and *Salmonella spp* in pork in Greater Accra, which will contribute to existing knowledge on AMR. Consequently, it will help in closing the gap on evidence on AMR in the food chain and livestock production. Findings from this study will assist in the development of strategies for more medically rational use of antimicrobials in animals by the Veterinary Services Directorate of the Ministry of Food and Agriculture, the Ministry of Health and other relevant national and international stakeholders. This will reduce AMR in animals, human as well as in the environment and in turn, reduce the risk of treatment failure, AMR related mortality and cost of healthcare. The findings will also serve as a baseline study for further research in AMR.

### 1.5 Objectives

#### 1.5.1 Main objective

To assess the antimicrobial resistance profiles of E. *coli* and *Salmonella* species isolated from pigs at slaughter.

#### 1.5.2 Specific objectives

1. To determine the prevalence of fecal and mesenteric lymph nodes carriage of E. *coli* and *Salmonella spp* in healthy pigs at slaughter.

2. To determine the antibiotic susceptibility profiles of E. *coli* and *Salmonella* spp isolated from fecal and mesenteric lymph nodes samples of healthy pigs.
3. To determine serogroups of *Salmonella* isolated from fecal and mesenteric lymph nodes samples of healthy pigs
4. To determine the association between AMR of *E. coli* and *Salmonella* and pig management practices

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Food-borne pathogens and public health

The global impact of food borne pathogens on health, trade and development is enormous and is a major public health concern. The possibility of these pathogens to contaminate food can occur at any point of the food chain; from "farm-to-fork" (Carvajal et al., 2013). Worldwide, hundreds of millions of cases of food borne diseases occur every year costing billions of dollars.

According to Scallan, Hoekstra, Mahon, Jones, & Griffin, (2019) an estimation of 9.4 million illnesses, 5,6961 hospitalizations and 1,351 deaths through contaminated food by 31 known pathogens occur every year in the United States of America. Of these known pathogens, *Salmonella spp* (*Non-typhoidal Salmonella*) was the leading cause but overall, 90% of the food borne diseases outbreaks were attributed to seven major pathogens: *Non-typhoidal Salmonella, Escherichia coli 0157, Clostridium perfringes, campylobacter, Listeria monocytogens, norovirus* and *Toxoplasma gondii*.

In Low- and Middle-Income Countries (LMICs) where Ghana belongs, the full burden of food borne diseases is not known. However, experts believe that the situation is worse. This is conceivable given reports of high level of hazards contained in most livestock derived food and food products (Grace et al., 2010) and also reports of high prevalence of potentially food borne pathogens found in hospitals and community surveys of human diarrheal diseases (Fletcher et al., 2011).

Of recent, there has being rising concerns of antibiotic resistance, including multi-drug resistance (MDR) among most of food borne diseases pathogens globally. MDR associated with food borne diseases increases morbidity and mortality resulting in serious economic repercussion for affected countries.

Eight priority bacteria of which *E. coli* and *Salmonella* are among, have been identified by the Global Antimicrobial resistance Surveillance System (GLASS) platform launched in 2015 by the World Health Organization to support the global action plan on AMR.

### 2.2 *E. coli* and *Salmonella* in food- producing animals

Food-producing animals (e.g: chickens, cattle, pigs, sheep and goats) are the major reservoirs for many food-borne pathogens such as *Listeria monocytogenes, non-typhoidal Salmonella species* (NTS), *Campylobacter* species and *Shiga toxin*-producing *E. coli*. Food-borne pathogens cause millions of acute illnesses and chronic complications, as well as large and challenging outbreaks in many countries (Heredia & García, 2018). And most of these pathogens may be spread to human through the food chain during slaughter of the animals, through improper handling of food, or inadequate cooking.

According to the EFSA- European Food & Safety Authority, (2018) 1.5 billion children under 3 years come down with diarrhea caused by enteropathogenic micro-organisms, which results in more than 3 million deaths every year. In the USA, bacterial enteric pathogens cause 55,961 hospitalization and 1,351 deaths every year (Elaine Scallan et al., 2011).

*E. coli* is predominantly non-pathogenic flora of the human and animal gut. It helps in vitamins' production, competes with and suppresses pathogenic bacterial growth however, some strains have the ability to cause gastrointestinal, urinary or central nervous system disease (Feng, 2013). In Nigeria, 88.7%, 81%, 89.5% samples from cattle, chicken, and swine, respectively, were positive for *E. coli* (Adenipekun et al., 2015). Milk from milk shops in Mekelle Tigray, Ethiopia was found to have a prevalence of *E. coli* of 43.35%. In the same study, milk from dairy farms were found to have a prevalence of 27.91% of *E. coli* (Tadesse et al., 2018). Adzitey, (2020) in a study in Wa abattoir, Ghana reported 98.0%, 92.0% and 88.0% of *E. coli* in beef liver, beef kidney and meat muscle respectively. In a study conducted by Pissetti,

Werlang, Kich, & Cardoso, ( 2017) where carcass swabs from pigs collected in three (3) commercial slaughterhouses, *E. coli* was reported to be 100%.

*Salmonella* species especially non-typhoidal *Salmonella* species has long been recognized as an important zoonotic pathogen of economic importance. Pork products are among the main sources of *Salmonella* infection in humans. In Ghana, a study conducted in Ashanti region isolated 66.7% of *S. typhimurium* from pig feces (Sekyere & Adu, 2015). F Adzitey, Teye, Kutah, & Adday, (2011) isolated *Salmonella* species from meat samples from beef in the Tamale Municipality of Ghana. In 2007, a year-long study in the UK found *S. typhimurium* in the ileocecal lymph nodes of 21.2% of pigs at slaughter. A prevalence of as high as 75% of *Salmonella* was reported in beef in the Techiman Municipality of Ghana (Frederick Adzitey et al., 2015). In Abeokuta, Nigeria Ojo et al., (2012) reported 13.1% *Salmonella* isolated from 153 cloacal swabs from free range chickens. A study conducted on serotypes and antimicrobial resistance profiles of *Salmonella* isolates from pigs at slaughter in Kenya reported an overall prevalence of 13.8% of *Salmonella* isolated from feces and carcass swabs (Kikuvi et al., 2010). In cattle, *Salmonella* infections are an important cause of mortality and morbidity and sub-clinical infections are often seen thus, making cattle an important reservoir of *Salmonella* for human infection. Infections in cattle have been associated with serovars *Dublin* and *Typhimurium* (Paul A. Barrow U. Methner, 2013). Sheep salmonellosis is apparently rare in most countries of the world with large sheep population and does not represent a relevant economic issue.

### 2.3 Serotypes of *E. coli* and *Salmonella*

Serotypes or strains of *E. coli* are distinguished based on their somatic (O), flagellar (H) and capsular (K) antigens (Batt, 2014; Tenaillon et al., 2010). About 186 various *E. coli* O-groups and 53 H-types exist. Pathogenic *E. coli* are classified into six (6) pathotypes causing a wide range of gastrointestinal (diarrheagenic *E. coli*) and extra-intestinal (extra-intestinal pathogenic

*E. coli*, ExPEC) diseases (CDC et al., 2020; Fratamico et al., 2016). Of the six pathotypes, the Shiga toxin-producing *E. coli* (STEC) is the most common pathotype associated with food-borne outbreak. The most common STEC *E. coli* is the O157:H7 *E.* coli (Boyer, 2015). Globally, it is estimated that STEC causes 2,801,000 acute illnesses annually. In the USA, STEC *E. coli* leads to 3,890 cases of hemolytic uremic syndrome (HUS), 270 cases of end-stage renal diseases and 230 deaths (Majowicz et al., 2014). Still in the USA, it is estimated that animal contact constitutes 6% of O157:H7 STEC illnesses (Croxen et al., 2013).

Over 2500 different *Salmonella* serovars or serotypes have been identified since its first isolation to date. With the exception of *Salmonella typhi* and *Salmonella paratyphi* (typhoid *Salmonella*), the rest are known as *Salmonella* non-typhoid (NTS) where animals are the major reservoir (Eng et al., 2015). While all serovars can cause disease in humans, a few are host specific in animals; *Salmonella enterica* serovar *Dublin* in cattle and *Salmonella enterica* serovar *Choleraesuis* in pigs. When these serovars cause diseases in humans, it's often invasive and life-threatening (Ranieri et al., 2013; World Health Organization., 2019). The incidence of diseases caused by NTS varies geographically. Annually, it is estimated to cause 690 cases per 100,000 population in Europe while 100 cases per 100,000 population in Israel (Eng et al., 2015). Worldwide, *Salmonella typhimurium* (*S. typhimurium*) and *Salmonella enteritidis* (*S. enteritidis*) are the most dominant serovars and infections with these two pathogens have been mainly linked to ingestion of undercooked meat or ground beef and dairy products and especially eggs (Department for Environment, 2013; EFSA- ECDC, 2016; Mohammed, 2017).

### 2.4 Emergence and spread of antibiotic-resistant *E. coli* and *Salmonella* in food animals

The extensive use of antimicrobials in animals and humans has contributed to the rise in multi-drug resistance among several bacterial strains. Globally, multidrug-resistance (MDR) *E. coli* and *Salmonella* strains in food animals have been among the major public health concern (Adesiji et al., 2014). In the developing countries, the incidence of MDR of bacteria originating

from food animals is not receding. This situation could be attributed to the inappropriate or uncontrolled use of antimicrobials in farming practices. Subsequent antimicrobial resistance transmission to humans can be in the form of either resistant pathogens or commensal organisms such as non-pathogenic *E. coli* with the ability to transfer resistance genes to pathogenic organisms (Morley et al., 2011). High levels of resistance were found to tetracycline (60.00%), gentamicin (53.3%) and trimethoprim-sulfamethoxazole (66.7%) from *Salmonella spp* isolated from human, poultry and seafood sources (Adesiji et al., 2014). In a study conducted in the Ashanti region of Ghana, MDR (40.3%) of *Salmonella enterica* serovar Typhimurium isolated from pig feces was found (Sekyere & Adu, 2015). Resistance to *E. coli* strains has also been reported in food animals and humans. In Brazil, Pissetti et al., (2017) recorded 71.5% MDR of *E.coli* strains from pig carcasses. In Lagos Nigeria, MDR of *E. coli* of 61.3%, 40.8% and 17.9% were recorded from chickens, cattle and swine respectively (Adenipekun et al., 2015).

**2.5 Risk factors of MDR of food-borne pathogens in food animals**

Several antimicrobials used in animal production also serve as important drugs in humans. Studies on antibiotics use in animal husbandry and the impact on antibiotic resistance of pathogenic food-born bacteria and commensal bacteria have been reported in many countries (Lundin et al., 2008; Sumberg et al., 2013). The causes of antibiotic resistant are quite complex and include human behavior at several levels.

Globally, over 70% of antimicrobials produced are used in food-producing animals. Antibiotics are used non-therapeutically as growth promoters and prophylaxis, especially in developing countries (United Nations Environment Programme, 2017). Animal production in many countries transition to more intensive animal farming practices, leading to greater use of antimicrobials (Hedman et al., 2020). Therefore, intensive animal food production can lead to

the selection for the emergence of resistance due to the extended use of antibiotics for growth promotion, disease prevention, and infection treatment.

A review carried out on AMR in poultry farming within low-resource settings classified breeds of poultry in relation to the type of husbandry system. Local breeds are usually raised in small-extensive and extensive systems. This type of classification is not different in other livestock animals such as pigs. In that review, it was revealed that local poultry are generally raised without routine antimicrobial therapy. However, semi-intensive and intensive poultry farmers who raise exotic/commercial poultry breeds, raise the birds with antimicrobials administered in commercial feed and water. Hence, this setting can function as high risk antimicrobial resistant genes (ARGs) development (Hedman et al., 2020).

Studies carried out have highlighted the role of poor regulatory system, policies and veterinary oversight on access and use of antibiotics in food animal production (Maron et al., 2013). Lack of laboratory capacities for testing diseases before treatment, poor knowledge on AMR by farmers especially in developing countries have been implicated on the inappropriate and indiscriminate use of antibiotics (Tang et al., 2017).

The authorized professionals for diagnosis, prescription and administration of antibiotics to farms animals are the veterinarians in many developed countries. However, in most developing countries, studies revealed different situations; antimicrobial use by farmers are not as well controlled or monitored (Mainda et al., 2015). Findings in a study in Ghana indicated that non-veterinary persons administered antibiotics in 98% of poultry farms in three selected regions (Boamah et al., 2016). The consequences of antibiotics administered by such individuals could be an under-dose or over-dose of the animals (Maron et al., 2013) and incompletion of antibiotic treatment courses. Incompletion of antibiotics treatment courses is known to exposing the microorganisms to sub inhibitory concentrations of the antibiotics leading to development of resistance (Davies, 2010; Kohanski et al., 2010).

Hygiene levels of slaughter facilities, personnel and equipment play vital roles in the production of wholesome and safe meat and meat product for human consumption (FDA-Food and Drugs Authority, 2013) as well as the emergence and spread of resistant strains (Carvajal et al., 2013). A comparative study in Khon Kaen province Thailand on *Salmonella ssp* contamination and AMR patterns between 2 slaughterhouses; Hazard Analysis and Critical Control Point (HACCP) standard and poor hygiene practice (PHP) slaughterhouses revealed that in HACCP standard slaughterhouse, the fecal isolates of *Salmonella* (8.61%) were significantly lower than pork isolates (18.33%) in PHP slaughterhouse (Wu et al., 2019). In the same study, the numbers of *Salmonella* serotypes and some AMR from pigs to pork was reduced in HACCP standard slaughterhouse but increased in the PHP slaughterhouse(Wu et al., 2019). Poor personal hygiene, environmental hygiene and used of dirty equipment contributes to meat contamination, a catalyst for development and spread of antimicrobial resistance.

Analysis of studies carried out on pig after they leave the farm; during transport and in lairage highlighted the role that these stages play in the final carcass status. These stages are found to be importance sources of *Salmonella* in pigs (Carvajal et al., 2013). Therefore, improvements in cleansing and disinfection procedures at both transport and lairage in addition to other control measures are necessary to decrease the likelihood of contamination before the pigs enter the slaughterhouse.

# CHAPTER 3

# METHODS

## 3.0 Study design

This was a descriptive and analytical cross-sectional study consisting of a field and sampling frame phase, bacteria isolation and identification phase, phenotype screening and serology (Figure 2). The study period was from February to July, 2020. Fresh fecal and mesenteric lymph nodes samples were collected from pigs, post evisceration at three selected slaughterhouses in GAR. Data on demographic characteristics of the animals and other epidemiological factors were collected using a semi-structured questionnaire. Laboratory investigations involved a pre-enrichment phase for both pathogens and a selective enrichment phase for *Salmonella* pathogen, then bacteria isolation and identification and antibiotic sensitivity testing of pathogens detected. All *Salmonella* isolate were serogrouped. A checklist was used to obtain data on the slaughterhouses' adherence to regulations, guidelines and policies. We performed descriptive statistic on data collected using STATA version 15.

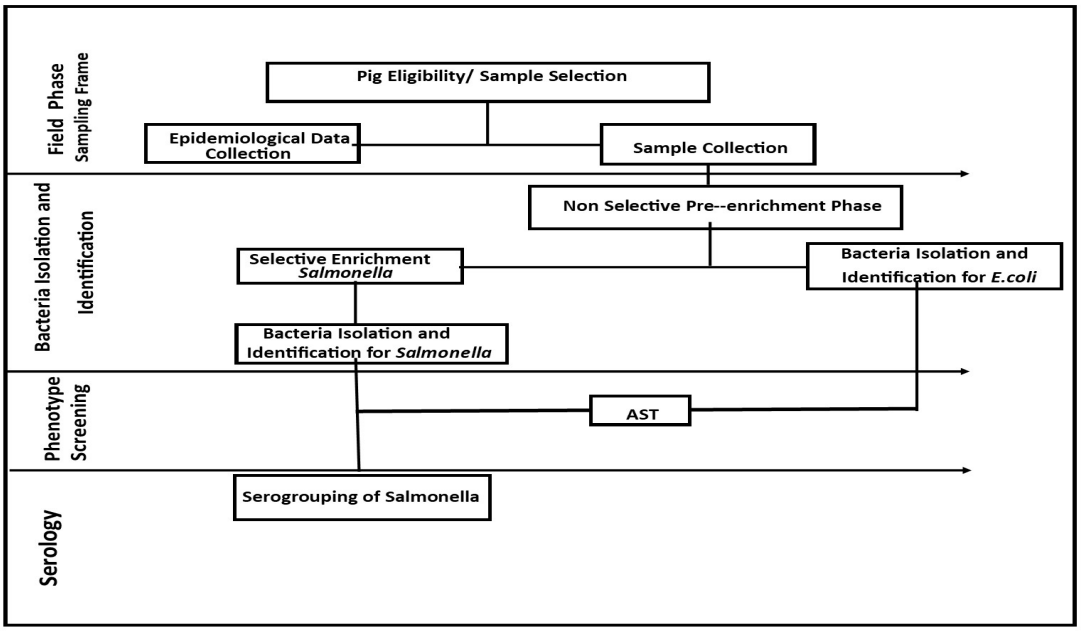


**Figure 2:** Schematic description of workflow of the study
**Source:** Author's own construct

### 3.1 Study area

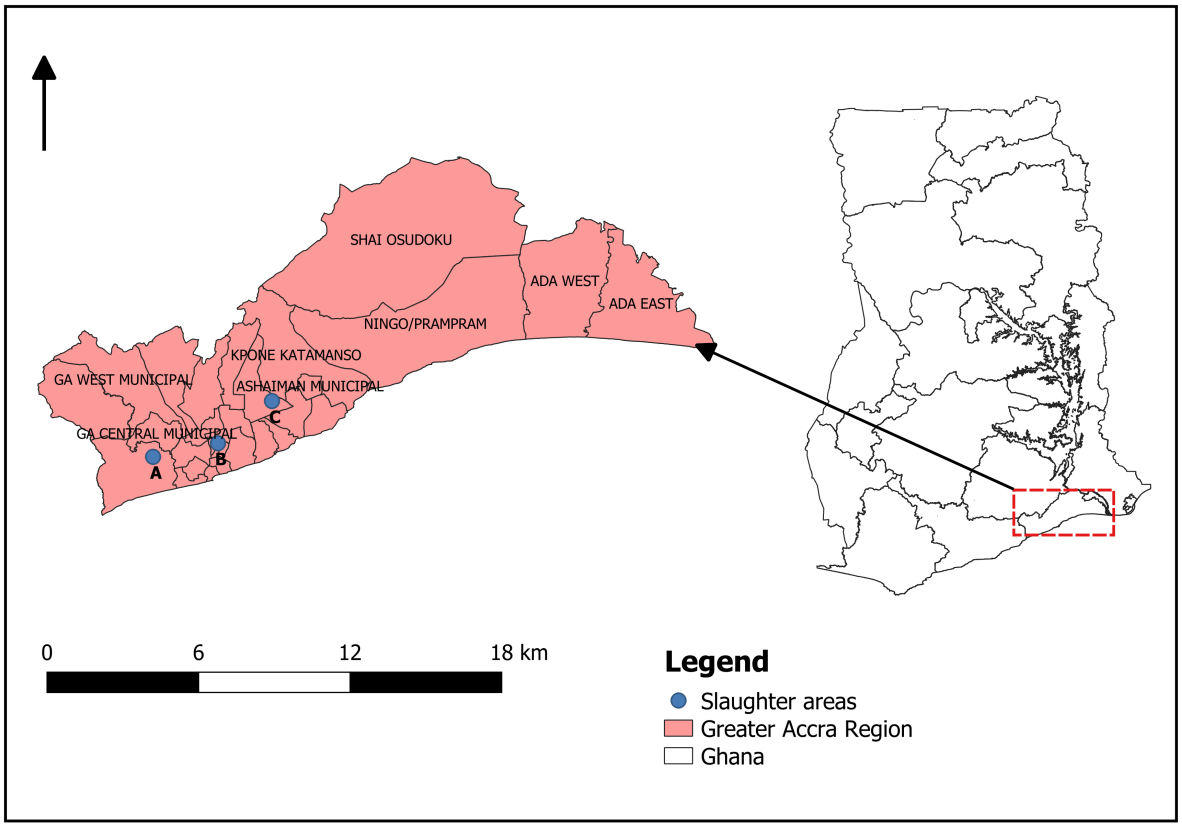


**Figure 3:** Map of Greater Accra Region indicating the three selected slaughter sites

**Source:** Author's own construct

The Greater Accra Region (GAR) is one of Ghana's sixteen (16) administrative regions. Geographically, GAR lies between longitude 5.8143° N and latitude 0.0747° E, occupying a total land surface of 3,245 km$^2$. According to the 2010 population and housing census, the total livestock population in the region was 1,225,229. Swine flu and African swine fever affect several pig farms in Ghana at different periods or seasons (personal communication with Veterinary Services Directorate, 2019). There are few abattoirs in the region in general and due to religious reasons, most pig farmers slaughter on their farms. However, there are three abattoirs/slaughterhouses which are known by the public to be engaged in pig slaughter; one public abattoir and two private slaughterhouses where the samples were collected.

The two private slaughterhouses; **A** and **B** are dedicated swine slaughterhouses where as the public slaughterhouse; **C** is mixed.

At slaughter house **A**, majority of the pigs received are from the northern part of the country and a few from GAR and Central Region (CR). The pigs delivered to the slaughterhouse are reared in a commercial extensive and intensive systems. The slaughterhouse has a capacity of 10 to 30 pigs slaughtered per day. Slaughter house **B** receives majority of its pigs from GAR and its surroundings. Pigs delivered there are often reared in a commercial intensive system. It has a daily slaughter capacity of 20 to 50 pigs.

Slaughterhouse **C** is under an academic/research institution. The institution conducts research into poultry and livestock rearing. It receives its animals from all over the world. Intensive husbandry system is practiced and this slaughterhouse does not run operations on a daily basis. Generally, slaughter is done as and when the rearing animals are ready for slaughter with a slaughter capacity of 1 to 10 pigs.

At all the three slaughterhouses, slaughtering took place via stunning. No processing of pork other than cutting is carried out on site as well as at all the three slaughterhouses.

### 3.2 Study variables

- **Dependent variable(s):** antimicrobial resistance profiles of *E. coli* and *Salmonella* species
- **Independent variables:** age, sex, origin of the animals, type of feeding, clinical history of disease outbreaks on farm of origin, vaccination status of animals, practices of butchers and hygiene conditions/practices, biosecurity measures at the slaughterhouses and environmental factors.

**Table 1:** Characteristics of study variables

| Variables | Operational definition | Measurement scale | Source of data |
|---|---|---|---|
| **Dependent variable(s):** | | | |
| AMR profiles of E. *coli* & *Salmonella spp* | Resistance of E. *coli* & *Salmonella spp* seen via antibiotic susceptibility testing | Categorical | Laboratory |
| **Independent variables:** | | | |
| **Demographic characteristics** | | | |
| Age of animal | Young or adult based on appearance of the animal teeth | Categorical | Observation |
| Sex | Male, Female | Categorical | Movement permit |
| Origin of the animal | Country, region, type of settlement | Categorical | Movement permit |
| Breed | Local black, large white, landrace, cross-breed | Categorical | Interview |
| **Pig management characteristics** | | | |
| Husbandry system | Extensive, intensive, semi-intensive | Categorical | Interview |
| Feed | Crop residue, commercial feed | Categorical | Interview |
| Water source | Well, borehole, rain, tap | Categorical | Interview |
| **Health related factors** | | | |
| Clinical history of disease | History of disease outbreak within the past one year, vaccination status of animals | Categorical | Interview |
| **Adherence to regulations, guidelines and policies** | | | |
| Slaughterhouse structure and practices | Cleaning and disinfection, Waste disposal methods, Dressing | Categorical | Interview/checklist |
| Environmental factors/biosecurity | Restricted and control zones | Categorical | Interview/checklist |

### 3.3 Study Population

The study population included all pigs presented at the three selected slaughterhouses/abattoirs in Greater Accra region.

### 3.4 Inclusion and Exclusion criteria

Samples were collected from all pigs clinically healthy and fit for slaughter. Carcasses that were not found wholesome for human consumption after meat inspection were excluded from the sampling.

### 3.5 Sample size determination

The minimum sample size was calculated using the Cochran's formula (Singh & Masuku, 2011). A prevalence of 8.6% of salmonella in pig feces (Kikuvi et al., 2010) obtained from a study conducted in Kenya was used.

$$\mathbf{n = [(Z_{\alpha/2})^2 * p(1-p)] / d^2}$$

where, n = sample size , p (prevalence) = 8.6%, Z= z-score of 1.96 at 95% confidence level and d (precision) = 0.05

$$n= [1.96^2 * 0.086(1-0.086)] / (0.05)^2$$

$$\mathbf{n = 121}$$

### 3.6 Sampling procedure

The three slaughterhouses were purposively selected based on the level of slaughter, structural designs of the slaughterhouses and the ownership type of slaughterhouses (public and private). The sample size was proportionately allocated to the three slaughterhouses based on the average number of pigs slaughtered per week. At the slaughterhouse, following the selection of the number of pigs to be slaughtered for the day, random numbers were generated and used to select the carcasses for sampling. An even and odd numbers were written on a paper and balloting was done. If an even number is picked, all carcasses with even numbers were sampled and vice-versa for the day.

### 3.7 Sample collection

Fresh fecal and mesenteric lymph nodes collected from each pig randomly selected. Approximately 5g of feces and 5g of mesenteric lymph nodes were aseptically removed from the rectum and mesentery respectively at the slaughter line. Once the abdomen of the carcass was opened, the rectum is located and the area wiped with a sterile gauze. A new surgical blade is used to incise the wiped area, then feces was manually scooped out from the rectum into a sterile stool container. The mesentery was also located and at least 5g of mesenteric lymph node tissue was excised using a separate sterile scalpel blade and placed into sterile zip lock bags. The samples were maintained on ice at temperature between 0 – 4$^{o}$C and transported to the microbiology laboratory at Noguchi within 2- 4 hours. They are either processed the same day or stored at -20$^{o}$C for processing.

### 3.8 Data collection technique and tools

Using a semi-structure questionnaire and interviewing butchers/middle-men/livestock owners present at the slaughterhouses, data was collected on demographic characteristics (age, sex, breed, etc), characteristics of pig management (type of husbandry, feeding) and health related factors (clinical history of disease outbreaks on farm of origin, vaccination status).

Data on the pig origin was extracted from movement permits available at the slaughterhouses.

Using a checklist following the Ghana FDA guidelines, data were collected on slaughter house structure (location and design), hygiene practices (sanitary facilities, personnel hygiene and meat handling) and environment (biosecurity measures).

Data on *Salmonella* and *E. coli* isolation and identification was obtained by colonial morphology and biochemical testing methods on agar media.

Antibiotic susceptibility testing was assessed by determining the diameter of the zone of complete inhibition for ten (10) antibiotics tested. All *Salmonella* isolates were screened for serogroups.

### 3.9 Laboratory analysis

#### 3.9.1 *Salmonella* isolation and identification

**a) Pre-enrichment**

Samples were defrosted under optimal room temperature for 2 – 3 hours. Feces samples were fractioned in 1g and transferred into separate sterile falcon tubes. The exterior of each mesenteric lymph node was briefly placed into a flame for decontamination and 1g of mesenteric lymph node from each pig was placed into separate stomacher bags. For each bag and falcon tube was added 9ml of 2% Buffered Peptone Water (BPW), homogenized, and incubated for 18 hours at 37$^{o}$C.

**b) Selective enrichment**

Following pre-enrichment, a selective enrichment was done by transferring 1ml of each pre-enriched sample into separate sterile falcon tubes containing 9ml of Tetrathionate broth and incubated at 37$^{o}$C for 24 hours.

**c) Isolation and identification**

Following the selective enrichment, ten (10µl) microliter loop full of each broth was streaked on petri dishes containing a *Salmonella* selective medium; Xylose Lysine Deoxycholate (XLD) agar and incubated overnight at 36.0°C ± 1°C for 18-24 hours. The streak plates were assessed; a typical *Salmonella* colony is red or pink with a black center of about 1-2 mm in size. *Salmonella-like* or presumptive *Salmonella* colonies were stabbed in Triple Sugar Iron (TSI) agar and urea and incubated at 36.0°C ± 1°C overnight for 18-24 hours (preliminary biochemical identification). The isolates with biochemical characteristics typical of *Salmonella* were confirmed by Analytical Profile Index (API) test using API 20 E (Figure 4).

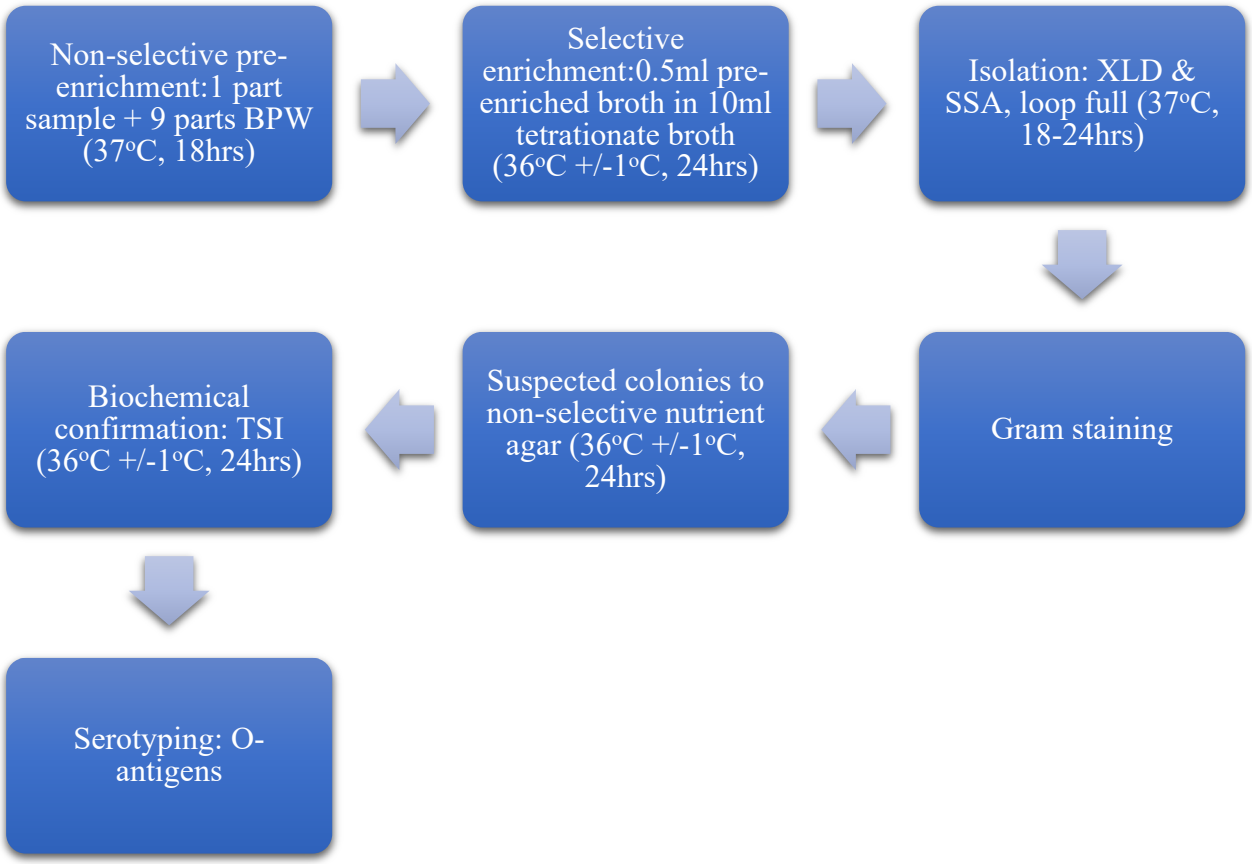


**Figure 4:** Flow diagram of *Salmonella* isolation and identification

### 3.9.2 *Escherichia coli* isolation and identification

For E. *coli spp* isolation, samples were similarly pre-enriched in 9ml of 2% BPW. Then 10µl loop full of each pre-enriched broth was streak on petri dishes containing MacConkey agar and incubated at 44° C overnight. A typical *E. coli* colony is red or pink and about 1-4 mm in size. Presumptive E. *coli* isolates were sub-cultured on MacConkey agar, incubated at 44° C overnight and were confirmed by TSI and Sulphur Indole Motility (SIM) biochemical testing. Pure colonies of the confirmed *Salmonella* and *E. coli* isolates were inoculated into nutrient agar plates at 36.0°C ± 1°C overnight for 18-24 hours prior to antibiotic susceptibility testing and serotyping (Figure 5).

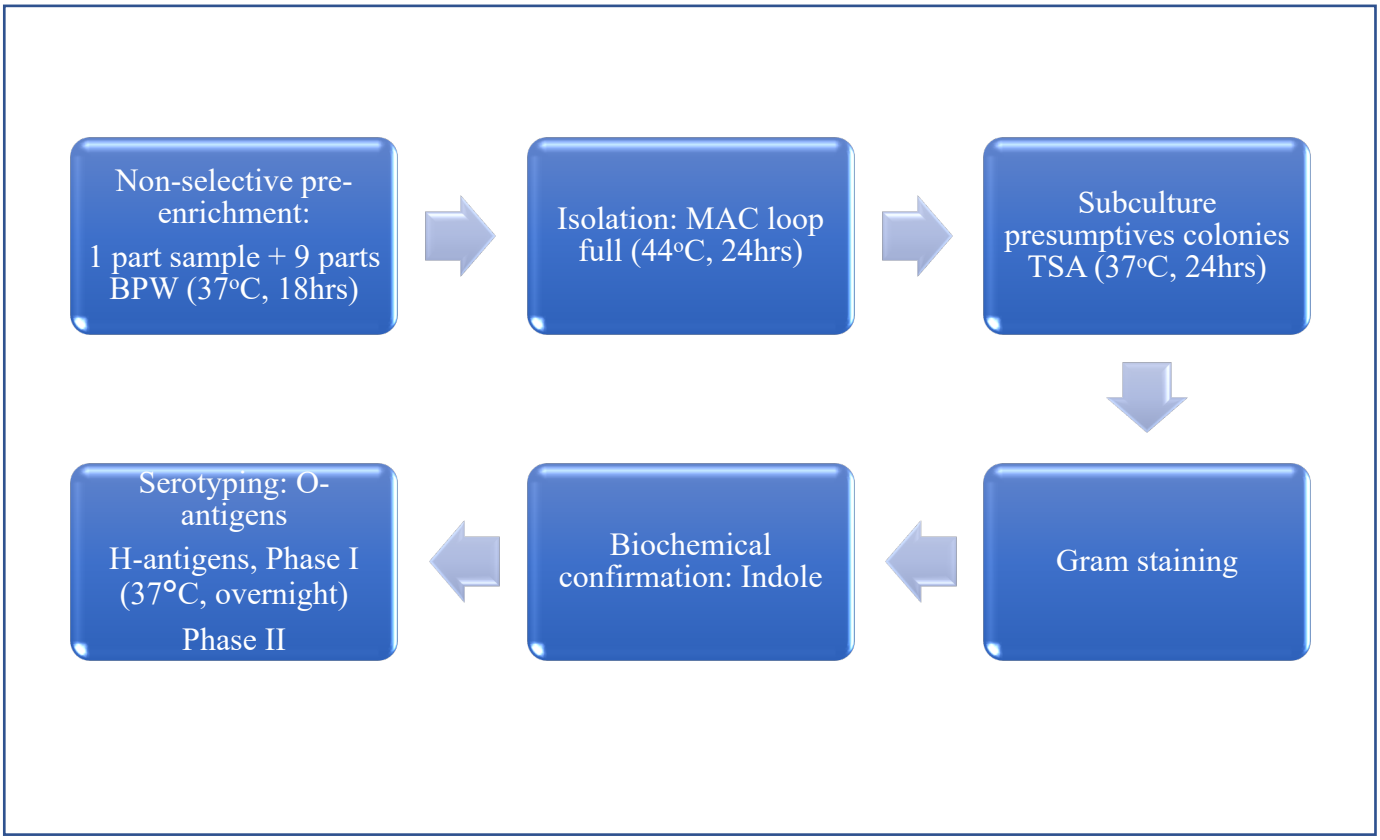


**Figure 5:** Flow diagram of *E. coli* isolation and identification

All the media used are products from **Oxoid Ltd, Wade Road, Basingstore, Hants RG24 8FW, UK**.

### 3.9.3 Antimicrobial susceptibility testing/diameter of zone of complete inhibition determination

The pure isolates were subjected to Kirby- Bauer disk diffusion method, according to the guidelines recommended by Clinical and Laboratory Standards Institute (CSLI, 2018). Muller Hinton (MH) agar medium was prepared according to manufacturer's instructions. It was sterilized by autoclaving at 121°C for 15 minutes, cooled to 45 – 50°C and poured on petri plates to a depth of 4mm (Hudzicki, 2012). Suspension containing approximately 1 to 2 x $10^8$ colony forming units/ml or 0.5 McFarland E. *coli* and *Salmonella* was inoculated on the petri plates containing the MH agar. Commercially prepared disks, impregnated with a standard concentration of antibiotics (Oxoid) were placed and lightly pressed onto the inoculated agar

surfaces. The plates were incubated at 35 $^{o}$C± 2$^{0}$C; ambient 16-18 hours and evaluated according to the specifications of the Clinical and Laboratory Standards Institute (CLSI) documents. Multi-drug resistance (MDR) was defined as described by Magiorakos et al., (2011) for an isolate to be resistant to three or more antimicrobial classes.

**Table 2:** Antibiotic agents and interpretative criteria used for AST in *E. coli* and *Salmonella* isolated from fecal and mesenteric lymph nodes samples

| **Antibiotic class** | **Antibiotic agent** | **Concentration (µg/disk)** | **CLSI zone of inhibition diameter** | | |
|---|---|---|---|---|---|
| | | | **S≥** | **I** | **R≤** |
| Penicillins | Ampicillin (AMP) | 10 | 17 | 14-16 | 13 |
| Fluoroquinolones | Ciprofloxacin (CIP) | 5 | 21* | 16-20* | 15* |
| | Ciprofloxacin (CIP) | 5 | 31 | 21-30 | 20 |
| Tetracyclines | Tetracycline (TET) | 30 | 15 | 12-14 | 11 |
| Aminoglycosides | Gentamycin (CN) | 10 | 15 | 13-14 | 12 |
| Sulfonamides | Trimethoprim/sulphamethoxazole (SXT) | 1.25/23.75 | 16 | 11-15 | 10 |
| Amphenicols | Chloramphenicol (C) | 30 | 18 | 13-17 | 12 |
| Cephalosporins | Cefuroxime (CXM) | 30 | 18 | 15-17 | 18 |
| | Cefotaxime (CTX) | 30 | 26 | 23-25 | 22 |
| | Ceftazidime (CAZ) | 30 | 21 | 18-20 | 17 |
| Polymyxins | Colistin (CL) | 10 | 14 | - | 11 |

(*) all Enterobacteriaceae except *Salmonella* isolates

### 3.9.4 Serogrouping of *Salmonella*

Using commercially polyvalent and monovalent Salmonella O antisera (**DENKA SEKEN CO., LTD**), serogrouping was carried out by slide agglutination tests according to the manufacturer's instruction. Agglutination with polyvalent sera was performed initially, then with specific monovalent sera. Polyvalent antisera O, O1 and Vi were used in this study. Approximately 10 – 15 $\mu$L drop of each polyvalent anti-sera was deposited onto a glass slide. A volume of 1 $\mu$L of fresh, pure *Salmonella* isolate was added onto the slide and mixed with a loop. The mixture on the glass side was rocked back and forth for 1 – 10 seconds while

observing for agglutination. An isolate was considered to be positive if agglutination occurred. Similarly slide agglutination with specific monovalent antisera was performed for isolates identified to be positive for any of the polyvalent antisera.

**3.10 Quality control**

At the slaughterhouse and sample collection level, trained laboratory technicians from the National Veterinary Laboratory assisted with the sample collection exercise. For fecal sample collection, the surface of the intestines was wiped with sterile gauze to dry any blood on the surface. This was to ensure that there is no contamination of the fecal samples with organisms could be in the blood.

A sterile scalpel blade was used to incise the intestine and 5g of feces was scooped directly from the intestine using the spoon of the stool specimen container. For the lymph nodes, the mesentery was located and lymph nodes exposed. A rat-tooth forceps was used to grab the lymph nodes and a sterile scalpel blade was used to excise the tissue.

At the laboratory level, plates were labeled with date of preparation and batch number and sterility tests conducted on them by testing samples for performance, using stable, reference strains. The culture media plates were stored upside down at 2-8$^0$C sealed in plastic bags to reduce chances of contamination. E. *coli* ATCC 25922 was used as quality control for *E. coli* and the wild type *Salmonella* for *Salmonella* for the AST.

Questionnaires and checklist were pretested among five (5) veterinarians at the School of Veterinary Medicine, University of Ghana. During the pretest, some questions were removed and some added. This was to ensure that the data to be gathered achieve the purpose of the study.

The sample collectors were trained veterinary technicians from the Accra Veterinary Laboratory. The administration of the questionnaire and checklist was done by the principal investigator.

### 3.11 Data processing and analysis

#### 3.11.1 Descriptive analysis

Data was entered into Microsoft Excel 2016, cleaned, coded and merged taking into consideration of the pig as the unit of analysis before importing it to STATA /IC version 15 for analysis. Descriptive statistics was performed and data was summarized by pathogen type isolated, the source of sample and AMR profiles of *E. coli* and *Salmonella* isolates from the pig fecal and mesenteric lymph nodes samples; using frequencies and proportions and presented in tables and charts.

#### 3.11.2 Inferential analysis

Differences in antimicrobial resistance proportions of the isolates from the different slaughter sites were compared using Pearson's Chi-square tests. Simple binary logistic regression models were used to assess the crude odds ratios of isolates being resistant to at least one of the ten (10) antibiotics. A nested model building approach was used in the multiple regression analysis. Statistical significance of all measures was considered at p-values less than 0.050. For the purpose of this study, all isolates with intermediate susceptibility were classified as susceptible/sensitive for statistical analysis.

### 3.12 Ethical Considerations

**Ethical approval**

Ethical approval was sought from the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC). Permission was sought from the office of the Director of the Veterinary Services Directorate (VSD) and the Greater Accra regional agricultural office of the Ministry of Food and Agriculture (MOFA) to access the slaughterhouses.

**Possible risks and benefits**

It was anticipated that there were no direct risks involved. The participant however, was required to spare some few minutes to answer few questions from the researcher. Similarly, it

was anticipated that there were no direct benefits involved to the study participants. The indirect benefit is that results from this study will be used to strategize the use of antibiotics in animals that will ultimately reduce antimicrobial resistance in animals as well as in human and in turn, reduce the risk of treatment failure, antimicrobial related mortality and cost of healthcare in humans.

**Informed consent**

A written consent was given to the middlemen/butchers and explained to them to sign for participation without any form of coercion.

**Confidentiality**

Confidentiality and anonymity of the slaughterhouse where samples were collected was ensured throughout the investigation. The slaughterhouses were identified by alphabets and unique identification numbers were assigned to the samples collected. Names of origin of the animals were kept only to the investigation team.

# CHAPTER 4

# RESULTS

## 4.0 Demographic characteristics of pigs slaughtered

One hundred and forty (140) pigs were sampled, of which 50.7% (71/140) were females and 68.6% (96/140) were adults (determination by dentition). The most common breed sampled was a crossbreed 59.3% (83/140); likely a mixture of large white/land race and a local breed. The pigs were received from Sudan savanna (Upper West and Upper East regions), costal savanna (Greater Accra and Central regions) and rain forest zones (Western region). A small number of pigs received, were from France (Table 3).

**Table 3:** Distribution of number of pigs sampled by origin and slaughterhouses, GAR, February – July, 2020

| Origin of pigs | Slaughter sites | | | Total |
|---|---|---|---|---|
| | A | B | C | |
| | n =54, (38.6%) | n=77, (55.0%) | n=9, (6.4%) | n=140 |
| **Sudan savanna** | 47 (100.0) | 0 (0.0) | 0 (0.0) | 47 |
| **Costal savanna** | 7 (11.1) | 56 (88.9) | 0 (0.0) | 63 |
| **Rain forest** | 0 (0.0) | 21 (100.0) | 0 (0.0) | 21 |
| **Europe (France)** | 0 (0.0) | 0 (0.0) | 9 (100.0) | 9 |

## 4.1 Farm management practices and health related characteristics

Table 4 shows that majority 47.9% (67/140) of the pigs received for slaughter were from farms which practiced intensive husbandry system as compared to extensive husbandry system 35.7% (50/140). Crop residues feeding type was the most 50.0% (70/140) seen among pig farms followed by commercially prepared feed 47.9% (67/140). The water source was mainly borehole 57.9% (81/140) followed by well water 35.7% (50/140).

**Table 4:** Distribution of pigs by slaughter sites and in relation to farm management practices, GAR, February – July, 2020

| Variables | A n (%) | B n (%) | C n (%) | Total n (%) |
|---|---|---|---|---|
| **Husbandry system:** | | | | |
| Intensive | 4 (7.4) | 54 (70.1) | 9 (100.0) | 67 (47.9) |
| Extensive | 50 (92.6) | 0 (0.0) | 0 (0.0) | 50 (35.7) |
| Semi-intensive | 0 (0.0) | 23 (29.9) | 0 (0.0) | 23 (16.4) |
| **Feeding type:** | | | | |
| Crop residue | 47 (67.1) | 23 (32.9) | 0 (0.0) | 70 (50.0) |
| Garbage | 3 (5.6) | 0 (0.0) | 0 (0.0) | 3 (2.1) |
| Commercial feed | 4 (7.4) | 54 (70.1) | 9 (100.0) | 67 (47.7) |
| **Water source:** | | | | |
| Well | 50 (92.6) | 0 (0.0) | 0 (0.0) | 50 (35.7) |
| Borehole | 4 (7.4) | 77 (100.0) | 0 (0.0) | 81 (57.9) |
| Tap water | 0 (0.0) | 0 (0.0) | 9 (100.0) | 9 (6.4) |
| **Outbreaks at pig farm:** | | | | |
| Yes | 52 (96.3) | 0 (0.0) | 0 (0.0) | 52 (37.1) |
| No | 2 (3.7) | 77 (100.0) | 9 (100.0) | 88 (62.9) |

Most (62.9 %) of the pigs sampled in this study were from farms that had not experienced any disease outbreak for the past one year. It was not possible to reliably determine whether antibiotics had been used in water and feed as well as whether *Salmonella* or any other type of vaccine was given to the pigs, as the animals were brought to the slaughter houses/slabs by traders and not the owners. In nine (9) cases where this information was given, there was no disease outbreak on the farm for the past year and no antibiotics were used in the water and feed.

### 4.2 Assessment of slaughterhouse structure, practices and environment

Slaughter site **C** had a better structure compared to **A** and **B** but all had zinc roofing with no ceiling**.** **C** had a fencing to prevent the entrance of unauthorized persons and animals. It also had a separate holding area for the animals before slaughter and access to the premises was controlled or guarded. Site **A** and **B** were found not to have appropriate structure for slaughter activities. At site **A**, clients were seen moving in and out of the premise for the selection of their animals. And slaughter was done in the open under zinc shed.

There was poor ventilation at **B** and its size was not suitable**.** The structure is located in the backyard of the owner and was also used for cooking and frying of some of the meat, which is sold directly to the public. Fire-wood is used for the cooking which causes smoke and soot build-up as well as oil stains.

Slaughter site **B** and **C** had adequate supply of running tap water however, **A** used well water for washing carcasses and cleaning the surfaces and equipment.

There was no demarcation between clean and dirty areas in all slaughter sites. Containers and equipment were passed from dirty to clean areas and carcasses were cleaned and dressed on the floor. All three-slaughter sites had no carcass carriers for moving carcasses between the different sections. Staffs were seen carrying carcasses using uncleaned pans and none of the staff wore PPEs during the entire study period. The butchers at site **A** and **B** were not professional butchers. They were usually family relatives or acquaintances.

On-site toilet and hand washing facility for the personnel were both absent in **A.** Slaughter site **B** and **C** had toilet facilities but lacked on-site hand washing facility after using the toilet.

All three-slaughter sites did not have effective waste management system for solid and liquid waste. The most common type of waste management used in all 3 facilities was flush-open gutter and flush-open slats systems.

### 4.3 Distribution of *E. coli* and *Salmonella* in fecal and lymph nodes

A total of 280 samples (fecal, n = 140 and MLN, n = 140) were collected and analyzed from the 140 pigs sampled. The overall prevalence of *E. coli* regardless of source of sample was 89.3% (125/140) and that of *Salmonella* was 15.7% (22/140). Overall, 14.3% (20/140) pigs had both *E. coli* and *Salmonella* isolated in them (Table 5).

**Table 5:**Distribution of *E. coli* and *Salmonella* in pigs from all sampling sites GAR, February – July, 2020

| Presence of pathogen | Number of pigs | Isolates | | |
|---|---|---|---|---|
| | N | *E coli* | *Salmonella* | Both *E. coli* & *Salmonella* |
| **Positive n (%)** | 140 | 125 (89.3) | 22 (15.7) | 20 (14.3) |
| **Negative n (%)** | 140 | 15 (10.7) | 118(84.3) | 120 (85.7) |

Of the 280 samples 67.5% (189/280) were positive for *E. coli* and 7.9 % (22/280) were positive for *Salmonella*. *E. coli* was isolated from 72.1% (101/140) and 62.9% (88/140) of the pigs in the feces and MLN respectively. Similarly, *Salmonella* was isolated from 2.1% (3/140) and 13.6% (19/140) of the pigs in the feces and lymph nodes respectively (Table 6).

**Table 6:** Distribution of *E. coli* and *Salmonella* by source and slaughter sites, GAR, February – July, 2020

| Isolates/source | | Slaughter Sites | | | |
|---|---|---|---|---|---|
| | | A (n=54) | B (n=77) | C (n=9) | Total (n=140) |
| ***E. coli*** | **Fecal** | 45 (83.3%) | 49 (63.6%) | 7 (77.8%) | 101(72.1%) |
| | **MLN** | 42 (77.8%) | 41(53.3%) | 5(55.6%) | 88(62.9%) |
| ***Salmonella*** | **Fecal** | 0 (0.0%) | 3 (3.9%) | 0(0.0%) | 3(2.1%) |
| | **MLN** | 11(20.4%) | 7 (9.1%) | 1(11.1%) | 19(13.6%) |

**MLN**- Mesenteric Lymph Node

*E. coli* was isolated from feces and MLN of the same animal in sixty-four (64) cases. We found *E. coli* only in feces and not in MLN in thirty-seven (37) cases as compared to only twenty-four (24) cases in which *E. coli* was found in MLN but not feces of the same animal (Table 7).

**Table 7:** Distribution of fecal and MLN test outcomes for *E. coli* in pigs, GAR, February – July, 2020

| | | ***E. coli* outcome** | | | |
|---|---|---|---|---|---|
| | | | **Positive** | | |
| **Level Variables** | **Total N** | **All negative n (%)** | **Fecal only n (%)** | **MLN only n (%)** | **Both fecal and MLN n (%)** |
| **Total** | 140 | 15 (10.7) | 37 (26.4) | 24 (17.1) | 64 (45.7) |
| **Site:** | | | | | |
| A | 54 | 2 (3.7) | 10 (18.5) | 7 (13.0) | 35 (64.8) |
| B | 77 | 12 (15.6) | 24 (31.2) | 16 (20.8) | 25 (32.5) |
| C | 9 | 1 (11.1) | 3 (33.3) | 1 (11.1) | 4 (44.4) |
| **Sex:** | | | | | |
| Male | 69 | 8 (11.6) | 21 (30.4) | 12 (17.4) | 28 (40.6) |
| Female | 71 | 7 (9.9) | 16 (22.5) | 12 (16.9) | 36 (50.7) |
| **Age:** | | | | | |
| Young | 44 | 3 (6.8) | 11 (25.0) | 6 (13.6) | 24 (54.5) |
| Adult | 96 | 12 (12.5) | 26 (27.1) | 18 (18.8) | 40 (41.7) |
| **Breed:** | | | | | |
| Cross breed | 83 | 5 (6.0) | 22 (26.5) | 11 (13.3) | 45 (54.2) |
| Large white | 45 | 7 (15.6) | 12 (26.7) | 11 (24.4) | 15 (33.3) |
| Ashanti black(local) | 10 | 2 (20.0) | 3 (30.0) | 2 (20.0) | 3 (30.0) |
| Unknown | 2 | 1 (50.0) | 0 (0.0) | 0 (0.0) | 1 (50.0) |
| **Region/zone:** | | | | | |
| Sudan Savannah | 47 | 2 (4.3) | 10 (21.3) | 6 (12.8) | 29 (61.7) |
| Coastal savannah | 63 | 9 (14.3) | 15 (23.8) | 11 (17.5) | 28 (44.4) |
| Rain forest | 21 | 3 (14.3) | 9 (42.9) | 6 (28.6) | 3 (14.3) |
| N/A | 9 | 1 (11.1) | 3 (33.3) | 1 (11.1) | 4 (44.4) |
| **Type of settlement:** | | | | | |
| Rural | 92 | 6 (6.5) | 26 (28.3) | 16 (17.4) | 44 (47.8) |
| Urban | 48 | 9 (18.8) | 11 (22.9) | 8 (16.7) | 20 (41.7) |
| **Husbandry system:** | | | | | |
| Intensive | 67 | 12 (17.9) | 21 (31.3) | 13 (19.4) | 21 (31.3) |
| Extensive | 50 | 2 (4.0) | 10 (20.0) | 7 (14.0) | 31 (62.0) |
| Semi-intensive | 23 | 1 (4.3) | 6 (26.1) | 4 (17.4) | 12 (52.2) |
| **Feeding type:** | | | | | |
| Crop residue | 70 | 3 (4.3) | 16 (22.9) | 10 (14.3) | 41 (58.6) |
| Gabbage | 3 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (100.0) |
| Commercial | 67 | 12 (17.9) | 21 (31.3) | 14 (20.9) | 20 (29.9) |
| **Source of water:** | | | | | |
| Well | 50 | 2 (4.0) | 10 (20.0) | 7 (14.0) | 31 (62.0) |
| Borehole | 81 | 12 (14.8) | 24 (29.6) | 16 (19.8) | 29 (35.8) |
| Tap water | 9 | 1 (11.1) | 3 (33.3) | 1 (11.1) | 4 (44.4) |
| **Transp. to slaughterhouse:** | | | | | |
| Ingroups | 131 | 14 (10.7) | 34 (26.0) | 23 (17.6) | 60 (45.8) |
| Driven on foot | 9 | 1 (11.1) | 3 (33.3) | 1 (11.1) | 4 (44.4) |
| **Outbreak at purchasing farms:** | | | | | |
| Yes | 52 | 2 (3.8) | 9 (17.3) | 7 (13.5) | 34 (65.4) |
| No | 88 | 13 (14.8) | 28 (31.8) | 17 (19.3) | 30 (34.1) |

There was no case in which *Salmonella* was isolated both in the feces and MLN of the same animal (Table 8).

**Table 8:** Distribution of fecal and MLN test outcomes for *Salmonella* in pigs, GAR, February – July, 2020

| | | ***Salmonella* outcome** | | | |
|---|---|---|---|---|---|
| | | | **Positive** | | |
| **Variables** | **Total N** | **All negative n (%)** | **fecal only n (%)** | **MLN only n (%)** | **Both Fecal and MLN n (%)** |
| Total | 140 | 118 (84.3) | 3 (2.1) | 19 (13.6) | 0 (0.0) |
| **Site:** | | | | | 0 (0.0) |
| A | 54 | 43 (79.6) | 0 (0.0) | 11 (20.4) | 0 (0.0) |
| B | 77 | 67 (87.0) | 3 (3.9) | 7 (9.1) | 0 (0.0) |
| C | 9 | 8 (88.9) | 0 (0.0) | 1 (11.1) | 0 (0.0) |
| **Sex:** | | | | | 0 (0.0) |
| Male | 69 | 56 (81.2) | 1 (1.4) | 12 (17.4) | 0 (0.0) |
| Female | 71 | 62 (87.3) | 2 (2.8) | 7 (9.9) | 0 (0.0) |
| **Age:** | | | | | 0 (0.0) |
| Young | 44 | 32 (72.7) | 1 (2.3) | 11 (25.0) | 0 (0.0) |
| Adult | 96 | 86 (89.6) | 2 (2.1) | 8 (8.3) | 0 (0.0) |
| **Breed:** | | | | | 0 (0.0) |
| Cross breed | 83 | 67 (80.7) | 3 (3.6) | 13 (15.7) | 0 (0.0) |
| Large white | 45 | 40 (88.9) | 0 (0.0) | 5 (11.1) | 0 (0.0) |
| Ashanti black (local) | 10 | 9 (90.0) | 0 (0.0) | 1 (10.0) | 0 (0.0) |
| Unknown | 2 | 2 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **Region/zone:** | | | | | 0 (0.0) |
| Sudan Savannah | 47 | 36 (76.6) | 0 (0.0) | 11 (23.4) | 0 (0.0) |
| Coastal savannah | 63 | 55 (87.3) | 3 (4.8) | 5 (7.9) | 0 (0.0) |
| Rain forest | 21 | 19 (90.5) | 0 (0.0) | 2 (9.5) | 0 (0.0) |
| N/A | 9 | 8 (88.9) | 0 (0.0) | 1 (11.1) | 0 (0.0) |
| **Type of settlement:** | | | | | 0 (0.0) |
| Rural | 92 | 77 (83.7) | 1 (1.1) | 14 (15.2) | 0 (0.0) |
| Urban | 48 | 41 (85.4) | 2 (4.2) | 5 (10.4) | 0 (0.0) |
| **Husbandry system:** | | | | | 0 (0.0) |
| Intensive | 67 | 58 (86.6) | 2 (3.0) | 7 (10.4) | 0 (0.0) |
| Extensive | 50 | 39 (78.0) | 0 (0.0) | 11 (22.0) | 0 (0.0) |
| Semi-intensive | 23 | 21 (91.3) | 1 (4.3) | 1 (4.3) | 0 (0.0) |
| **Feeding type:** | | | | | 0 (0.0) |
| Crop residue | 70 | 57 (81.4) | 1 (1.4) | 12 (17.1) | 0 (0.0) |
| Garbage | 3 | 3 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Commercial | 67 | 58 (86.6) | 2 (3.0) | 7 (10.4) | 0 (0.0) |
| **Source of water:** | | | | | 0 (0.0) |
| Well | 50 | 39 (78.0) | 0 (0.0) | 11 (22.0) | 0 (0.0) |
| Borehole | 81 | 71 (87.7) | 3 (3.7) | 7 (8.6) | 0 (0.0) |
| Tap water | 9 | 8 (88.9) | 0 (0.0) | 1 (11.1) | 0 (0.0) |
| **Transp. to slaughterhouse** | | | | | 0 (0.0) |
| Ingroups | 131 | 110 (84.0) | 3 (2.3) | 18 (13.7) | 0 (0.0) |
| Driven on foot | 9 | 8 (88.9) | 0 (0.0) | 1 (11.1) | 0 (0.0) |
| **Outbreak at purchasing farms:** | | | | | 0 (0.0) |
| Yes | 52 | 41 (78.8) | 0 (0.0) | 11 (21.2) | 0 (0.0) |
| No | 88 | 77 (87.5) | 3 (3.4) | 8 (9.1) | 0 (0.0) |

### 4.4 Antibiotics susceptibility testing

#### 4.4.1 Antimicrobial resistance profiles of *E. coli* and *Salmonella* in fecal and MLN

Fig. 6 shows the distribution of all 189 *E. coli* and 22 *Salmonella* isolates identified, subjected to antibiotic susceptibility testing (AST). Respectively, 64.6% (122/189) and 36.4% (8/22) of *E. coli and Salmonella* isolates showed resistance to the antibiotics tested. Of these resistance proportions resistant the distribution of *E. coli* isolates by source of samples in pigs was: (feces: 45.7%; 64/140) and (MLN: 41.4%; 58/140). Similarly, resistant *Salmonella* isolates was: (feces 2.1%; 3/140) and (MLN: 3.6%; 5/140).

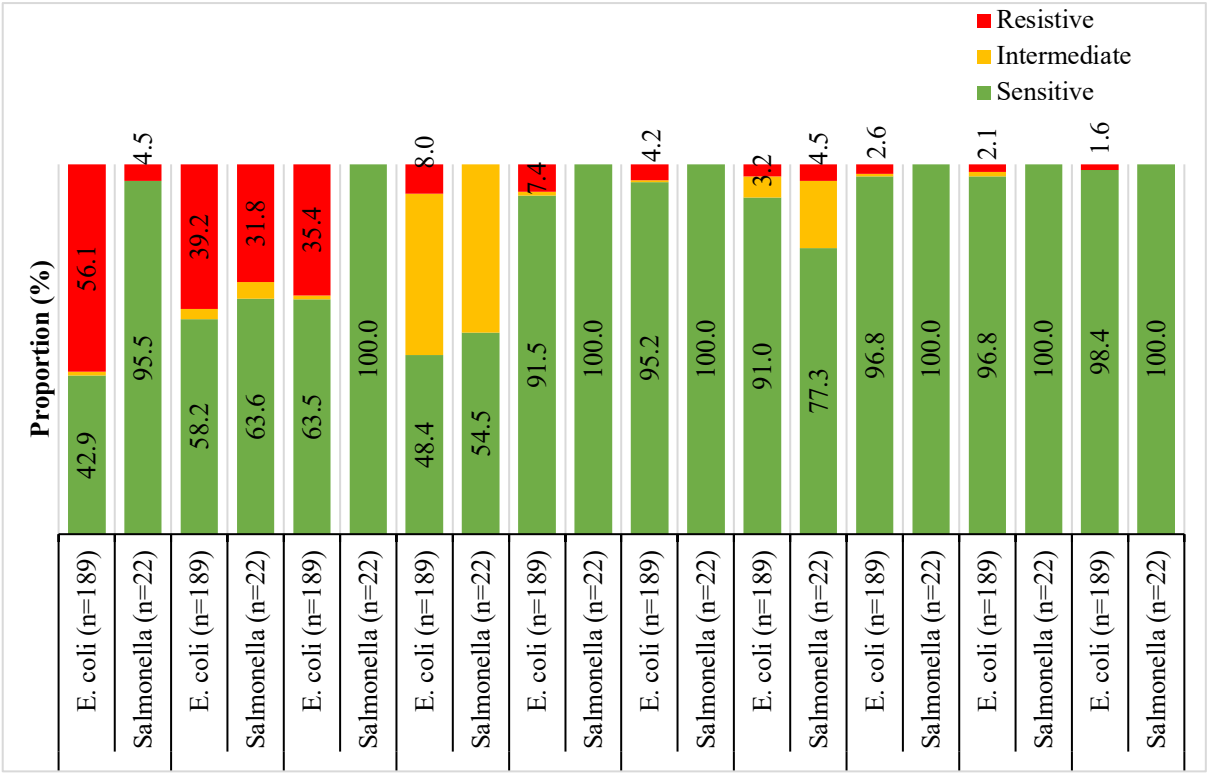


**Figure 6:** Distribution of antibiotic sensitivity in fecal and MLN samples in pigs at slaughter, GAR, February – July, 2020

*E coli* isolates showed resistant mainly to tetracycline (56.1%), ampicillin (39.2%), trimethoprim/sulphamethoxazole (35.4%) and colistin (7.9%). High susceptibility (> 90%) was observed in cefuroxime (98.4%), ceftazidime and gentamicin (96.8%) each, cefotaxime (95.2%), chloramphenicol (91.5%) and ciprofloxacin (91.0%). Intermediate resistances were

observed for all antibiotics tested except cefuroxime and with the highest rate (43.9%) expressed in Colistin.

*Salmonella* isolates showed mainly resistance to ampicillin (31.8%) only. *Salmonella* isolates were fully susceptible to six (6) antibiotics; trimethoprim/sulphamethoxazole, chloramphenicol, cefotaxime, gentamicin, ceftazidime and cefuroxime. The remaining four (4) antibiotics; CIP, TET, AMP and CL susceptibility rates were 96.8%, 95.5%, 63.6% and 54.5% respectively. Intermediate resistances were observed with the highest (45.5%) expressed in colistin.

In line with the profile by the *E. coli* resistant isolates among the three slaughterhouses (Table 9), tetracycline (54.5%), ampicillin (38.6%) and trimethoprim/sulphamethoxazole (36.6%) were most frequent resistances observed in the feces. Similarly, tetracycline (58.0%), ampicillin (39.8%) and trimethoprim/sulphamethoxazole (35.0%) were most frequent resistances observed in the MLN. The resistance frequency to antimicrobials was not significantly different among the isolates obtained from the three slaughter houses, except for chloramphenicol (p-value $< 0.001$) and gentamicin (p-value $= 0.012$) in the feces and; only ampicillin in the MLN (p-value $= 0.040$).

**Table 9:** Distribution of antibiotic resistance of fecal and MLN *E. coli isolates* from pigs *by* slaughterhouses, GAR, February – July, 2020

| Antibiotic | %resistance(fecal) | | | | | % resistance (MLN) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **A (n=45)** | **B (n=49)** | **C (n=7)** | **Total (n=101)** | | **A (n= 42)** | **B (n=41)** | **C (n=5)** | **Total (n=88)** | |
| | n(%) | n(%) | n(%) | n(%) | P-value | n(%) | n (%) | n(%) | n(%) | P-value |
| AMP | 15 (33.3) | 19 (38.8) | 5 (71.4) | 39(38.6) | 0.170[a] | 12 (28.6) | 22 (53.7) | 1 (20.0) | 35(39.8) | **0.040[a]** |
| TET | 22 (48.9) | 28 (57.1) | 5 (71.4) | 55(54.5) | 0.492[a] | 22 (52.4) | 26 (63.4) | 3 (60.0) | 51(58.0) | 0.574[a] |
| CTX | 4 (8.9) | 0 (0.0) | 0 (0.0) | 4(4.0) | 0.116[a] | 3 (7.1) | 1 (2.4) | 0 (0.0) | 4(4.6) | 0.697[a] |
| CAZ | 1 (2.3) | 0 (0.0) | 0 (0.0) | 1(1.0) | 0.515[a] | 1 (2.4) | 2 (4.9) | 0 (0.0) | 3(3.4) | 0.678[a] |
| SXT | 12 (26.7) | 21 (42.9) | 4 (57.1) | 37(36.6) | 0.139[a] | 12 (28.6) | 15 (36.6) | 3 (60.0) | 30(34.1) | 0.357[a] |
| C | 0 (0.0) | 8 (16.3) | 3 (42.9) | 11(10.9) | **<0.001[a]** | 1 (2.4) | 2 (4.9) | 0 (0.0) | 3(3.4) | 0.678[a] |
| CIP | 1 (2.2) | 3 (6.1) | 2 (28.6) | 6(5.9) | 0.070[a] | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0(0.0) | - |
| CN | 0 (0.0) | 1 (2.0) | 2 (28.6) | 4(4.0) | **0.012[a]** | 0 (0.0) | 2 (4.9) | 0 (0.0) | 2(2.3) | 0.325[a] |
| CL | 10 (23.8) | 3 (6.1) | 0 (0.0) | 13(12.9) | 0.051[a] | 2 (4.9) | 0 (0.0) | 0 (0.0) | 2(2.3) | 0.550[a] |
| CXM | 2 (4.4) | 0 (0.0) | 0 (0.0) | 2(2.0) | 0.330[a] | 1 (2.4) | 0 (0.0) | 0 (0.0) | 1(1.1) | 1.000[a] |

a: Fischer's exact

The rates observed for MLN *Salmonella* isolates to all antimicrobial agents among the slaughter sites were not significant. All resistant *Salmonella* strains from fecal samples were from slaughterhouse **C**.

### 4.4.2 Multi-drug resistance profile of *E. coli* and *Salmonella* isolated from fecal and MLN

Overall, 64.6% (122/189) of *E. coli* isolates tested against the ten (10) antimicrobials showed resistance to at least one (1) antimicrobial. Single and double antimicrobial resistance were expressed by 19.6% and 14.8% of the isolates respectively (Table 10). A total of 57 (30.2%) *E. coli*

strains were multi-drug resistant- they were resistant to three (3) or more antimicrobials (Table 8). The highest MDR rate was found in isolates from feces (20.8%) as compared to 19.3% in isolates from lymph nodes.

There was no multi-drug resistance among the *Salmonella* isolates tested. However, 31.8% (7/22) and 4.6% (1/22) showed single and double antimicrobial resistance respectively.

**Table 10:** Distribution of multi-drug resistance profile of E. coli and Salmonella by sample source in pigs, GAR, February – July, 2020

| Number of anti-biotic resistant | *E. Coli* | | | *Salmonella* | | |
|---|---|---|---|---|---|---|
| | **Fecal (n=101)** | **MLN (n=88)** | **Total (N=189)** | **Fecal (n=3)** | **MLN (n=19)** | **Total (N=22)** |
| | **n (%)** | **n (%)** | **n (%)** | **n (%)** | **n (%)** | **n (%)** |
| **0** | 37 (36.6) | 30 (34.1) | 67 (35.5) | 0 (0.0) | 14 (73.7) | 14 (63.6) |
| **1** | 17 (16.8) | 20 (22.7) | 37 (19.6) | 3 (100.0) | 4 (21.1) | 7 (31.8) |
| **2** | 13 (12.9) | 15 (17.1) | 28 (14.8) | 0 (0.0) | 1 (5.3) | 1 (4.6) |
| **3** | 21 (20.8) | 17 (19.3) | 38 (20.1) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **4** | 6 (5.9) | 2 (2.3) | 8 (4.2) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **>4** | 7 (6.9) | 4 (4.6) | 11 (5.8) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **MDR** | 34(27.1) | 23 (26.1) | 57 (30.2) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

The simple binary logistics regression analysis identified “feeding type” and “husbandry system” as the factors significantly associated with fecal *E. coli* antibiotic resistance (Table 11). The odds of developing resistance was 3.86 times higher in feces sampled from pigs fed with commercially prepared feed as compared to those fed with crop residue and it is statistically significant (CI= 1.57 – 10.00; p= 0.004). The odds of developing resistance among pigs bred in extensive husbandry system is 0.09 times and in semi-intensive 0.37 times; indicating lower probability of *E. coli* resistance compared to pigs sampled from intensive husbandry system.

All variables with more than two levels tested to assess the joint contribution of the variables in the model, indicated that “husbandry system” ($p< 0.001$) and “feeding type” ($p< 0.009$) still contribute significantly in predicting the outcome.

**Table 11:** Association of fecal *E. coli* antibiotic resistance and independent variables in pigs, GAR, February – July,2020

| | ***E. coli* fecal sample** | | | | |
|---|---|---|---|---|---|
| | | **Non-Resistance** | **Resistant** | | |
| **Variables** | **N** | **n (%)** | **n (%)** | **COR [95% CI]** | **P-value** |
| **Total** | 101 | 37 (36.6) | 64 (63.4) | | |
| **Site:** | | | | | **0.892[a]** |
| A | 45 | 17 (37.8) | 28 (62.2) | 1 | |
| B | 49 | 18 (36.7) | 31 (63.3) | 1.05 [0.45-2.41] | 0.917 |
| C | 7 | 2 (28.6) | 5 (71.4) | 1.52 [0.26-8.71] | 0.640 |
| **Sex** | | | | | |
| Male | 49 | 16 (32.7) | 33 (67.3) | 1 | |
| Female | 52 | 21 (40.4) | 31 (59.6) | 0.72 [0.32-1.62] | 0.421 |
| **Age** | | | | | |
| Young | 35 | 15 (42.9) | 20 (57.1) | 1 | |
| Adult | 66 | 22 (33.3) | 44 (66.7) | 1.50 [0.65-3.48] | 0.346 |
| **Breed** | | | | | |
| Cross breed | 67 | 29 (43.3) | 38 (56.7) | 1 | |
| Large white/local | 33 | 8 (24.2) | 25 (75.8) | 2.38 [0.94-6.05] | 0.067 |
| Unknown | 1 | 0 (0.0) | 1 (100.0) | [empty] | |
| **Sex** | | | | | |
| **Region/zone:** | | | | | |
| Sudan Savannah | 39 | 15 (38.5) | 24 (61.5) | 1 | |
| Coastal savannah | 43 | 18 (41.9) | 25 (58.1) | 0.87 [0.36-2.10] | 0.754 |
| Rain forest | 12 | 2 (16.7) | 10 (83.3) | 3.12 [0.60-16.26] | 0.176 |
| N/A | 7 | 2 (28.6) | 5 (71.4) | 1.56 [0.27-9.10] | 0.620 |
| **Settlement:** | | | | | |
| Rural | 70 | 30 (42.9) | 40 (57.1) | 1 | |
| Urban | 31 | 7 (22.6) | 24 (77.4) | 2.57 [0.98-6.76] | 0.055 |
| **Husbandry system** | | | | | **0.001[a]** |
| Intensive | 42 | 8 (19.0) | 34 (81.0) | 1 | |
| Extensive | 41 | 16 (39.0) | 25 (61.0) | 0.37 [0.14-0.99] | 0.048 |
| Semi-intensive | 18 | 13 (72.2) | 5 (27.8) | 0.09 [0.02-0.33] | <0.001 |
| **Feeding type** | | | | | |
| Crop residue/ garbage | 60 | 29 (48.3) | 31 (51.7) | 1 | |
| Commercial | 41 | 8 (19.5) | 33 (80.5) | 3.86 [1.53-9.72] | 0.004 |
| **Source of water** | | | | | |
| Well | 41 | 16 (39.0) | 25 (61.0) | 1 | |
| Borehole/Tap water | 60 | 21 (35.0) | 39 (65.0) | 1.19 [0.52-2.70] | 0.680 |
| **Form of transport:** | | | | | |
| In groups | 94 | 35 (37.2) | 59 (62.8) | 1 | |
| Driven on foot | 7 | 2 (28.6) | 5 (71.4) | 1.48 [0.27-8.06] | 0.648 |
| **Outbreak at purchasing farm:** | | | | | |
| Yes | 43 | 17 (39.5) | 26 (60.5) | 1 | |
| No | 58 | 20 (34.5) | 38 (65.5) | 1.24 [0.55-2.81] | 0.603 |

a: p-value testing the overall significance of the differences observed in the respective variables. Empty: all observations expressed resistance. COR: crude odds ratio. CI: confidence interval.

In the multiple binary logistic regression model, "husbandry system" was the only factor found to be significantly associated with the development of fecal *E. coli* antibiotic resistance (Table 12).

**Table 12**: Predictors of E. *coli* antibiotic resistance in fecal samples of pigs, GAR, February–July, 2020

| | ***E. coli* fecal sample** | | | | | |
|---|---|---|---|---|---|---|
| | | **Non-Resistant** | **Resistant** | | | |
| **Variables** | **N** | **n (%)** | **n(%)** | **COR [95% CI]** | **AOR [95% CI]** | **P-value** |
| **Total** | 101 | 64 (63.4) | 37(36.6) | | | |
| **Sex** | | | | | | |
| Male | 49 | 16 (32.7) | 33 (67.3) | 1 | 1 | |
| Female | 52 | 21 (40.4) | 31 (59.6) | 0.72 [0.32-1.62] | 0.69 [0.27-1.80] | 0.452 |
| **Age** | | | | | | |
| Young | 35 | 15 (42.9) | 20 (57.1) | 1 | 1 | |
| Adult | 66 | 22 (33.3) | 44 (66.7) | 1.50 [0.65-3.48] | 1.92 [0.70-5.30] | 0.207 |
| **Breed** | | | | | | |
| Cross breed | 67 | 29 (43.3) | 38 (56.7) | 1 | 1 | |
| Large white/local | 33 | 8 (24.2) | 25 (75.8) | 2.38 [0.94-6.05] | 1.07 [0.31-3.69] | 0.914 |
| Unknown | 1 | 0 (0.0) | 1 (100.0) | [empty] | [empty] | |
| **Type of settlement** | | | | | | |
| Rural | 70 | 30 (42.9) | 40 (57.1) | 1 | 1 | |
| Urban | 31 | 7 (22.6) | 24 (77.4) | 2.57 [0.98-6.76] | 0.72 [0.13-3.96] | 0.709 |
| **Husbandry system** | | | | | | **0.086[a]** |
| Intensive | 42 | 8 (19.0) | 34 (81.0) | 1 | 1 | |
| Extensive | 41 | 16 (39.0) | 25 (61.0) | 0.37 [0.14-0.99] | 0.59 [0.05-6.66] | 0.672 |
| Semi-intensive | 18 | 13 (72.2) | 5 (27.8) | 0.09 [0.02-0.33] | 0.05 [0.00-0.75] | 0.030 |
| **Feeding type** | | | | | | |
| residue/ garbage | 60 | 29 (48.3) | 31 (51.7) | 1 | 1 | |
| Commercial | 41 | 8 (19.5) | 33 (80.5) | 3.86 [1.53-9.72] | 0.81 [0.06-11.16] | 0.878 |
| **Source of water** | | | | | | |
| Well | 41 | 16 (39.0) | 25 (61.0) | 1 | | |
| Borehole/Tap water | 60 | 21 (35.0) | 39 (65.0) | 1.19 [0.52-2.70] | [omitted] | |
| **Outbreak at purchasing farm** | | | | | | |
| Yes | 43 | 17 (39.5) | 26 (60.5) | 1 | 1 | |
| No | 58 | 20 (34.5) | 38 (65.5) | 1.24 [0.55-2.81] | 2.36 [0.20-28.24] | 0.497 |

a: p-value testing the overall significance of the differences observed in the respective variables. Empty: all observations expressed resistance. COR: crude odds ratio. AOR: adjusted odds ratio. CI: confidence interval. Omitted: due to collinearity.

For MLN *E. coli* antibiotic resistance, only "age" was found as the factor significantly associated with an odds ratio (OR): 2.87 (CI = 1.14 – 7.24, p= 0.026) in the simple regression, indicating higher probability of predicting the outcome in older pigs compared to younger pigs (Table 13).

**Table 13:** Association of MLN *E. coli* antibiotic resistance and independent variables in pigs, GAR, February – July, 2020

| | *E. coli* MLN sample | | | | |
|---|---|---|---|---|---|
| | | Resistance | Non-resistant | | |
| **Variables** | **N** | **n (%)** | **n (%)** | **COR [95% CI]** | **P-value** |
| **Total** | 88 | 58 (65.9) | 30 (34.1) | | |
| **Site:** | | | | | **0.249[a]** |
| A | 42 | 24 (57.1) | 18 (42.9) | 1 | |
| B | 41 | 30 (73.2) | 11 (26.8) | 2.05 [0.81-5.14] | 0.128 |
| C | 5 | 4 (80.0) | 1 (20.0) | 3.00 [0.31-29.18] | 0.344 |
| **Sex:** | | | | | |
| Male | 40 | 26 (65.0) | 14 (35.0) | 1 | |
| Female | 48 | 32 (66.7) | 16 (16.0) | 1.08 [0.44-2.61] | 0.870 |
| **Age:** | | | | | |
| Young | 30 | 15 (50.0) | 15 (50.0) | 1 | |
| Adult | 58 | 43 (74.1) | 15 (25.9) | 2.87 [1.14-7.24] | 0.026 |
| **Breed:** | | | | | |
| Cross breed | 56 | 37 (66.1) | 19 (33.9) | 1 | |
| Large white | 26 | 15 (57.7) | 11 (42.3) | 0.70 [0.27-1.82] | 0.464 |
| Ashanti black(local) | 5 | 5 (100.0) | 0 (0.0) | (empty) | |
| Unknown | 1 | 1 (100.0) | 0 (0.0) | | |
| **Region/zone:** | | | | | **0.357[a]** |
| Sudan Savannah | 35 | 20 (57.1) | 15 (42.9) | 1 | |
| Coastal savannah | 39 | 29 (74.4) | 10 (25.6) | 2.18 [0.81-5.81] | 0.121 |
| Rain forest | 9 | 5 (55.6) | 4 (44.4) | 0.94 [0.21-4.10] | 0.932 |
| N/A | 5 | 4 (80.0) | 1 (20.0) | 3.00 [0.30-29.66] | 0.347 |
| **Settlement:** | | | | | |
| Rural | 60 | 36 (60.0) | 24 (40.0) | 1 | |
| Urban | 28 | 22 (78.6) | 6 (21.4) | 2.44 [0.86-6.92] | 0.092 |
| **Husbandry system:** | | | | | **0.167[a]** |
| Intensive | 34 | 26 (76.5) | 8 (23.5) | 1 | |
| Extensive | 38 | 21 (55.3) | 17 (44.7) | 0.38 [0.14-1.05] | 0.063 |
| Semi-intensive | 16 | 11 (68.8) | 5 (31.2) | 0.68 [0.18-2.54] | 0.563 |
| **Feeding type:** | | | | | |
| Crop residue | 51 | 31 (60.8) | 20 (39.2) | 1 | |
| Garbage | 3 | 3 (100.0) | 0 (0.0) | (empty) | |
| Commercial | 34 | 24 (70.6) | 10 (29.4) | 1.55 [0.61-3.91] | 0.356 |
| **Source water:** | | | | | **0.184[a]** |
| Well | 38 | 21 (55.3) | 17 (44.7) | 1 | |
| Borehole | 45 | 33 (73.3) | 12 (26.7) | 2.23 [0.89-5.58] | 0.088 |
| Tap water | 5 | 4 (80.0) | 1 (20.0) | 3.24 [0.33-31.74] | 0.313 |
| **Form of transport:** | | | | | |
| In groups | 83 | 54 (65.1) | 29 (34.9) | 1 | |
| Driven on foot | 5 | 4 (80.0) | 1 (20.0) | 2.15 [0.23-20.12] | 0.503 |
| **Outbreak at purchasing farm:** | | | | | |
| Yes | 41 | 23 (56.1) | 18 (43.9) | 1 | |
| No | 47 | 35 (74.5) | 12 (25.5) | 2.28 [0.93-5.62] | 0.072 |

a: p-value testing the overall significance of the differences observed in the respective variables. Empty: all observations expressed resistance. COR: crude odds ratio. CI: confidence interval.

In the multiple regression model (Table 14), age still the only factor found to be significantly associated with MLN *E. coli* antibiotic resistance (OR): 3.24 (CI= 1.08 – 9.66, p= 0.035).

**Table 14***:* Predictors of E. *coli* antibiotic resistance in MLN samples of pigs, GAR, February – July, 2020

| | | ***E. coli* MLN sample** | | | | |
|---|---|---|---|---|---|---|
| | | **Resistance** | **Non-resistant** | | | |
| **Variables** | **N** | **n (%)** | **n (%)** | **COR [95% CI]** | **AOR [95% CI]** | **P-value** |
| **Total** | 88 | 58 (65.9) | 30 (34.1) | | | |
| **Sex:** | | | | | | |
| Male | 40 | 26 (65.0) | 14 (35.0) | 1 | 1 | |
| Female | 48 | 32 (66.7) | 16 (16.0) | 1.08 [0.44-2.61] | 0.99 [0.36-2.76] | 0.984 |
| **Age:** | | | | | | |
| Young | 30 | 15 (50.0) | 15 (50.0) | 1 | 1 | |
| Adult | 58 | 43 (74.1) | 15 (25.9) | 2.87 [1.14-7.24] | 3.24[1.08-9.66] | 0.035 |
| **Region/zone:** | | | | | | **0.312[a]** |
| Sudan Savannah | 35 | 20 (57.1) | 15 (42.9) | 1 | 1 | |
| Coastal savannah | 39 | 29 (74.4) | 10 (25.6) | 2.18 [0.81-5.81] | 0.12[0.01-2.61] | 0.176 |
| Rain forest | 9 | 5 (55.6) | 4 (44.4) | 0.94 [0.21-4.10] | 0.07[0.00-1.52] | 0.089 |
| N/A | 5 | 4 (80.0) | 1 (20.0) | 3.00 [0.30-29.66] | 0.24[0.00-21.41] | 0.539 |
| **Settlement:** | | | | | | |
| Rural | 60 | 36 (60.0) | 24 (40.0) | 1 | 1 | |
| Urban | 28 | 22 (78.6) | 6 (21.4) | 2.44 [0.86-6.92] | 1.8[0.33-10.39] | 0.490 |
| **Husbandry system:** | | | | | | |
| Intensive | 34 | 26 (76.5) | 8 (23.5) | 1 | 1 | |
| Extensive | 38 | 21 (55.3) | 17 (44.7) | 0.38 [0.14-1.05] | 0.08[0.01-1.29] | 0.075 |
| Semi-intensive | 16 | 11 (68.8) | 5 (31.2) | 0.68 [0.18-2.54] | (omitted) | |

a: p-value testing the overall significance of the differences observed in the respective variables. Empty: all observations expressed resistance. COR: crude odds ratio. AOR: adjusted odds ratio. CI: confidence interval. Omitted: due to collinearity.

### 4.4.3 Multi-drug resistance profile of *E. coli* and *Salmonella* in fecal and MLN

Distribution of MDR profiles by source of sample (Table 15) revealed that the multi-resistant isolates presented profiles that conferred resistance to between three (3) and eight (8) antimicrobials in the case of fecal *E. coli* isolates and; between three (3) to seven (7) antimicrobials in the case of lymph nodes *E. coli* isolates.

In the fecal E. coli isolates, resistance to only one (1) antibiotic; colistin was most frequent in slaughter house **A** and to only tetracycline in slaughterhouse **B.** Whereas in lymph *nodes E. coli* isolates it occurred mostly to tetracycline in slaughterhouse **A.** Resistance of one isolate to three (3) and four (4) among fecal and lymph nodes, was seen in slaughter house **A** and **B** respectively. Resistance of a single isolate to eight (8) antibiotics (AMP + TET + CTX + CAZ

+ SXT + CIP + CL + CXM) in feces and seven (7) antibiotics (AMP + TET + CTX + CAZ + SXT + CL + CXM) in the mesenteric lymph nodes occurred in slaughterhouse.

**Table 15:** Antimicrobial resistance profile of *E. coli* and *Salmonella* isolates by slaughterhouses in pigs, GAR, February – July, 2020

| | **Slaughter sites** | | |
|---|---|---|---|
| | **A** (n) | **B** (n) | **C** (n) |
| **FECAL SAMPLE** | | | |
| CL | 2 | 1 | 0 |
| TET | 5 | 7 | 0 |
| TET+CL | 3 | 1 | 0 |
| TET+SXT | 1 | 1 | 0 |
| TET+SXT+C | 0 | 2 | 0 |
| TET+CTX | 1 | 0 | 0 |
| TET+CTX+CL | 1 | 0 | 0 |
| AMP | 1 | 1 | 0 |
| AMP+CL | 2 | 0 | 0 |
| AMP+SXT | 1 | 1 | 0 |
| AMP+TET | 1 | 0 | 1 |
| AMP+TET+SXT | 7 | 10 | 1 |
| AMP+TET+SXT+CL | 1 | 0 | 0 |
| AMP+TET+SXT+CIP | 0 | 1 | 0 |
| AMP+TET+SXT+C | 0 | 4 | 0 |
| AMP+TET+SXT+C+CN | 0 | 0 | 1 |
| AMP+TET+SXT+C+CIP | 0 | 1 | 1 |
| AMP+TET+SXT+C+CIP+CN | 0 | 0 | 1 |
| AMP+TET+SXT+C+CIP+CN+CL | 0 | 1 | 0 |
| AMP+TET+CTX+SXT+CXM | 1 | 0 | 0 |
| AMP+TET+CTX+CAZ+SXT+CIP+CL+CXM | 1 | 0 | 0 |
| **MESENTERIC LYMPH NODE SAMPLE** | | | |
| SXT | 0 | 1 | 0 |
| TET | 7 | 6 | 0 |
| TET+SXT | 4 | 1 | 3 |
| TET+CTX+SXT+C+CL | 1 | 0 | 0 |
| AMP | 2 | 3 | 1 |
| AMP+TET | 2 | 5 | 0 |
| AMP+TET+C | 0 | 1 | 0 |
| AMP+TET+SXT | 6 | 9 | 0 |
| AMP+TET+SXT+CN | 0 | 1 | 0 |
| AMP+TET+SXT+C | 0 | 1 | 0 |
| AMP+TET+CAZ+SXT+CN | 0 | 1 | 0 |
| AMP+TET+CTX | 1 | 0 | 0 |
| AMP+TET+CTX+CAZ+SXT | 0 | 1 | 0 |
| AMP+TET+CTX+CAZ+SXT+CL+CXM | 1 | 0 | 0 |

Figure 7 and 8 showed that the most frequent resistance profiles for both fecal and lymph nodes *E. coli* isolates was AMP+TET+SXT (fecal: 52.9% and lymph nodes: 65.2%).

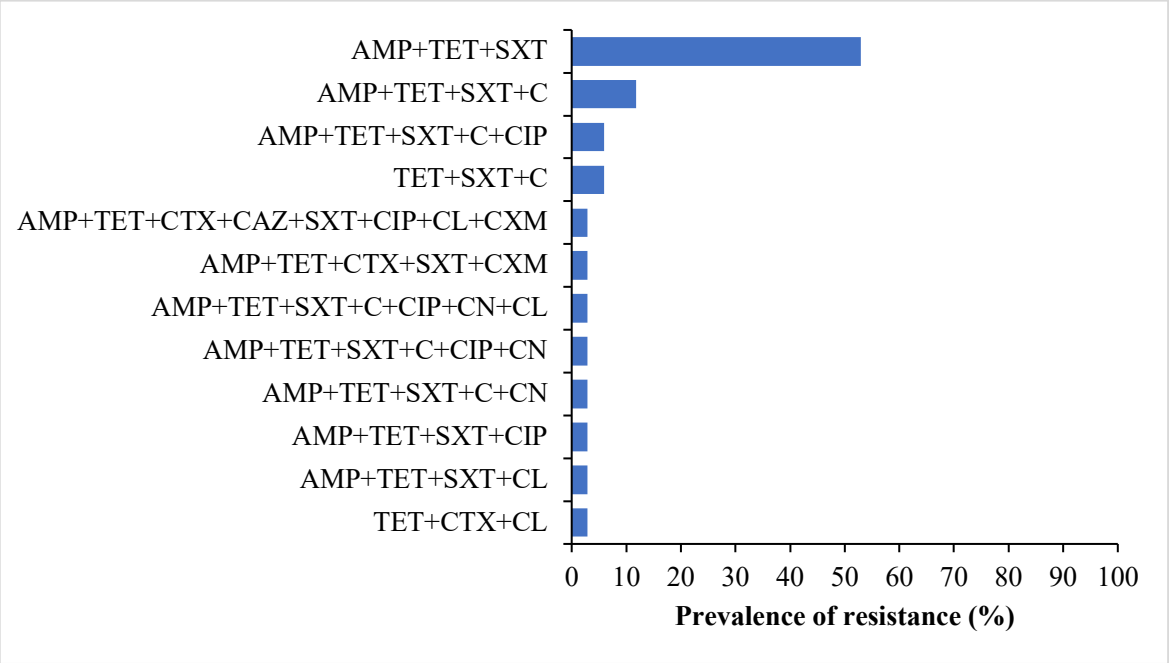


**Figure 7:** MDR profile of *E. coli* isolates from pigs' feces, GAR, February – July, 2020

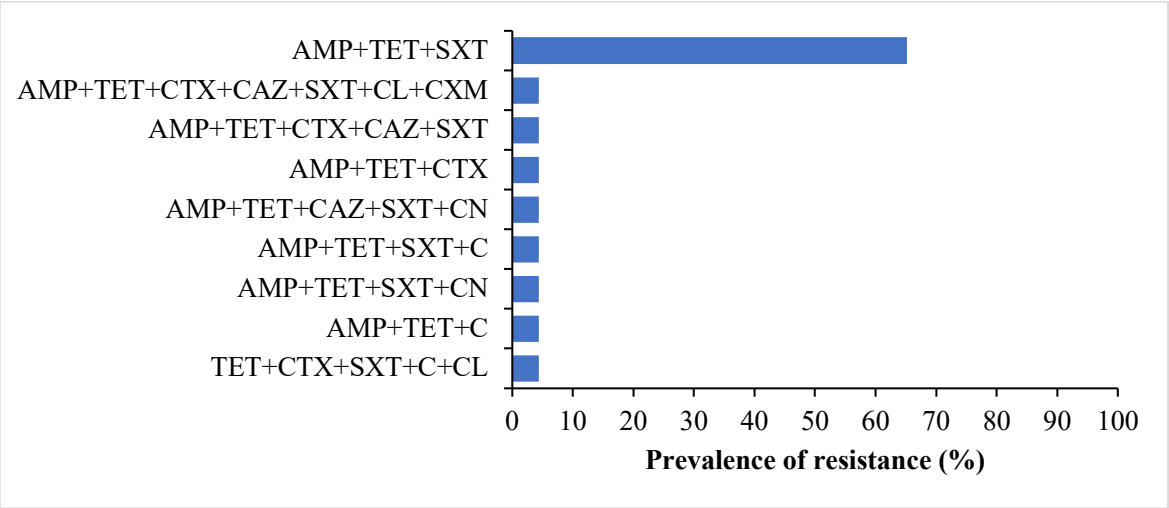


**Figure 8:** MDR profile of *E. coli* isolates from pig MLN, GAR, February – July, 2020

### 4.5 Serogroup distribution pattern of *Salmonella* isolates obtained from fecal and MLN

Table 16 illustrates that of the 22 *Salmonella spp* isolated, fourteen (14) and three (3) isolates were typeable using polyvalent O and O1 antisera respectively; groups A-I. The non-typeable O antigen was expressed by five (5) isolates which were recorded as NT. The monovalent specific serotyping indicated that majority of the isolates 58.8% (10/17) belonged to serogroup B, serogroup C2 16.7% (3/17) and serogroup I 11.8% (2/17). The remaining serogroups C1 and G each consisted of only one (1) isolate representing 11.8% (2/17).

**Table 16:** Distribution of *Salmonella* isolates by source according to serogroups in pigs, GAR, February – July, 2020

| Serogroups | Fecal (n=3) | MLN (n=19) | Total (n=22) |
|---|---|---|---|
| **C1 & G** | 0 | 2 | 2 |
| **B** | 0 | 10 | 10 |
| **C2** | 1 | 2 | 3 |
| **I** | 0 | 2 | 2 |
| **NT** | 2* | 3* | 5 |

(*): Non-typeable

# CHAPTER 5

## DISCUSSION

### 5.0 Fecal and mesenteric lymph nodes carriage *E. coli* and *Salmonella*

This study sought to characterize fecal and mesenteric lymph node samples carriage of *E. coli* and *Salmonella* isolates in pigs as well as uncovering the resistance profiles of the isolates and the phenotypic characterization of *Salmonella.*

In this study the overall proportion of fecal *E. coli* carriage was high (72.1%) but lower than what was reported in a similar study in Nigeria where a carriage of 89.5% was observed for *E.coli* in swine feces (Adenipekun et al., 2015). In the mesenteric lymph nodes, the proportion of *E. coli carriage* (62.9%) was also lower compared to reports in other organs where proportions of 98.0% (beef liver) and 92.0% (beef kidney) were observed (Adzitey, 2020). These lower proportions observed could probably be associated with long distance travel to the slaughter site or the effect of diet. Grispoldi et al., (2020) demonstrated that there was a negative correlation between the farm-slaughterhouse distance and the prevalence of *E. coli* with lower prevalence in cattle transferred over longer distance. Animals that have travelled for longer distance have had longer feed withdrawal resulting in fasting and reduction in the *E. coli* population. In this study, majority (110/140; 78.6%) of these animals were brought from outside the GAR for slaughter with most of them being slaughtered upon arrival. Moreover, though controversial, there is literature regarding the effect of diet on *E. coli* population in cattle (Callaway et al., 2009). There are suggestions that changing from high grain diet to hay can reduce the *E. coli* population. In this current study, majority (70.0%) of the pigs sampled were fed on crop residues. It should be however noted that the shedding of any bacteria group in the gut of animals are complex and can be affected by numerous other factors. Therefore, fasting should not be used as a measure to reduce shedding and subsequent carcass contamination with enteric *E. coli*.

The proportion of *E. coli* at the three slaughter sites varied from 63.6% to 83.3% in fecal samples and 53.3% to 77.8% MLN and was found to be significant. These differences could also be attributed to diet effect.

The swine GIT and its adnexa (lymph nodes) are the greatest bacterial contamination sources in pig slaughtering. In sixty-four (64) cases in this study, *E. coli* was found in the feces and lymph nodes of the same animal. This finding is in line with previous studies describing translocation of bacteria to lymph nodes with *E. coli* isolates being highly abundant (Mann et al., 2015). In 24 cases, *E. coli* was found only in the lymph nodes but not in the feces. This may have resulted in cross-contamination from the abattoir equipment on the dressing line or floors (animals were dressed on the floor at all three slaughter sites), or from the waste materials from the dirty area as there was no clear demarcation between the dirty and clean areas.

Apart from laying hens and poultry, asymptomatically *Salmonella*-infected pigs are a major source of salmonellosis in human (Mandilara et al., 2013; Pires et al., 2011). In this study, the proportion of *Salmonella* was 2.1% in feces and 13.6% in the lymph nodes. The proportion in the feces was lower than what was reported in a similar study in Kenya, where a proportion of 8.6% was observed (Kikuvi et al., 2010) but was similar to what was observed (14.5%) in the mesenteric lymph nodes of breeding sows (Garrido et al., 2020). However, a higher proportion of *Salmonella* in the lymph nodes than in feces was observed in this study and it was in agreement with data in literature which suggests that the pathogen is intermittently shed in pigs' feces. Most likely, the pathogen might have been absent in the feces at the time of the sample collection (Garrido et al., 2014). In Ghana, Frederick Adzitey, (2015) reported proportions of *Salmonella* from beef to be 31.0%.

The presence *E. coli* and *Salmonella* in the lymph nodes poses a great food safety hazard as most often these lymph nodes are usually not removed from the carcasses. And when opened up during dressing or cutting can contaminate the carcass (Mann et al., 2015).

### 5.1 Distribution of drug resistance among *E. coli* and *Salmonella* isolates

Both fecal and MLN *E. coli* isolates showed varying levels of multi-drug resistance, as well as resistance to most of the individual drugs. This trend of high antibiotic resistance level of *E. coli* in pigs is in agreement with reports by other authors in Ghana who found high antibiotic resistance levels of *E. coli* in cattle, sheep, goat and poultry feces (Donkor et al., 2012) and beef (Frederick Adzitey, 2020). Drug resistance has been associated with indiscriminate use of antimicrobial drugs which provide selective pressure favoring the emergence of resistant strains. The high resistance rates are probably indicative of high level of exposure to antimicrobial drugs usage among livestock farmers in Ghana. Though we could not reliably determine the use of antimicrobials by the pigs farmers in this study, other studies reported high rates of misuse of antimicrobial drugs among livestock farmers (Donkor et al., 2012; Sekyere, 2014). In this study, most (56.1%) of the *E. coli* isolates were resistant to Tetracyclines. This finding is in accordance with reports in a study in Nigeria, where the highest resistance level (59.0%) of *E. coli* isolates tested against 22 antibiotics was tetracycline (Adenipekun et al., 2015). In addition, high levels of resistance of *E. coli* to tetracycline was also found in beef in Ghana (Frederick Adzitey, 2020). Following the high resistance shown to tetracyclines, fecal *E. coli* isolates also showed high resistance to ampicillin (39.2%) and trimethoprim/sulphamethoxazole (35.4%). These results observed were comparable to other studies. *E. coli* of poultry fecal origin in Lagos, Nigeria were 34.1% and 40.0% resistant to ampicillin and trimethoprim/sulphamethoxazole respectively (Adenipekun et al., 2015).

With regards to *Salmonella* resistance, highest resistance was observed to ampicillin alone. Owing to the relatively cheap cost and ready availability of ampicillin, the drug is widely used by farmers for both prophylactic and therapeutic purposes.

This study found increased odds of developing *E. coli* resistance in older pigs compared to younger pigs. This finding is in contrast with Ström et al., 2018 findings where bacterial

isolated from growers pigs (younger pigs) showed significantly high resistance prevalence than bacteria from fatteners. Our findings can be explained by the fact that older pigs might be probably more exposed to antibiotics in their lifetime compared to younger pigs. The study also found that pigs bred in intensive husbandry system have increased odds of developing antibiotics resistance than those bred in extensive and semi-intensive husbandry systems. This increase in odds can be associated to the fact that animals in intensive husbandry system are usually subjected to prophylaxis treatment thereby exposing the animals to more antibiotics leading to the risk of developing resistance. Pigs fed with commercially prepared feed were also found to have increase odds of developing antibiotics resistance. The use of antibiotics for growth promotion purposes is also known to contribute to AMR, although it is debatable (Tang et al., 2017). Some commercially prepared feed contains growth promotors.

### 5.2 General distribution of *Salmonella* serogroups

This study sought to characterize *Salmonella* isolated from feces and MLN of pigs by serogroups. The monovalent specific showed all isolates to be Non-Typhoidal Salmonella (NTS). Not surprising, farm animals are known to be the major reservoir of NTS (Eng et al., 2015; Kikuvi et al., 2010). NTS have zoonotic or potentially zoonotic ability. The most common presentation of NTS is gastro-enteritis however up to 5.0% of NTS are reported in developed countries to be implicated in extra-intestinal diseases resulting to bacteremia and focal systemic infections (Feasey et al., 2012) .

About 59.0% of all isolates belonged to serogroup B followed by serogroup C2 (16.7%). *Salmonella Typhimurium* (*S. Typhimurium*) and *Salmonella Newport* belong to these serogroups respectively; some of the most NTS. *S. Typhimurium* has a wide range of hosts.

Serogroup C1 & G comprises *Salmonella choleraesuis* (*S. choleraesuis.* This finding is comparable to other studies. *S. choleraesuis* is the main culprit of salmonellosis in pigs. It has a narrow host range and occasionally infect humans (Chiu et al., 2005). It causes invasive

infections, one of the most common causative organisms of infective aneurysms; a devastating endovascular infection in humans. However, although in epidemiological studies this grouping system is useful and be used clinically to confirm genus identification, it cannot identify whether the pathogen can cause enteric fever, as significant coss-reactivity among serogroups arises.

### 5.3 Risk factors of occurrence of multi-drug resistant strains of bacteria in feces and mesenteric lymph nodes

In Ghana, regulations and policies guiding the administration of antimicrobials by professional veterinarians are poorly enforced. Non-veterinary persons administer antibiotics to livestock animals (Boamah et al., 2016). Easy access to antibiotics by farmers from veterinary offices, veterinary chemical shops and even mobile salesmen furthermore, exacerbates the problem of antibiotics use and handling practices in disease management (Boamah et al., 2016; Sekyere, 2014). These practices and factors influence greatly the development of resistance of commensal bacteria and possible transfer of resistant genes to pathogenic organisms.

Ghana FDA code of practices for slaughterhouses for the maintenance of food safety were not adhered to in this study. In all three slaughter facilities, carcasses were cleaned and dressed on the floor where the staff were also walking with the foot-wares. There was also no demarcation between dirty and clean areas in all three facilities and no effective waste management. Only one slaughter site had a better structure design but was not up to the standard. These findings were similar to other findings by other authors in Ghana (Dsani et al., 2020) and in South-Sudan (Andrea & Aburi, 2012). Frederick Adzitey, (2015) found that good hygiene practices in slaughterhouses is associated with lower levels of *E. coli* in a study in Ghana. All three slaughterhouses recorded high proportions of *E. coli* contamination both in fecal and MLN and these are potential sources of meat contamination. In terms of MDR, the two (**A** & **B**) out of the three slaughter sites that did not have adequate structure design at all had isolates that were

resistant to up to seven and eight antibiotics. This study reveals that hygiene conditions and slaughterhouse practices need to be monitored by the authorities in charge to ensure the production of uncontaminated meat by bacteria and more importantly, prevent the exposure of consumers to MDR bacteria.

**Limitations of the study**

Though, this study provided evidence of the presence of *E. coli* and *Salmonella* resistance in feces and MLN of pigs, it exhibited a number of limitations. Ideally, some molecular analysis would have been done to characterize the strains and resistant genes of the *E. coli* and *Salmonella* bacteria isolated. A comparison to know whether there are similarities between these pathogens isolated in the feces and MLN should have also been done. However, due to the ongoing pandemic, all laboratories were booked. Samples are stored for the molecular aspect to be done.

Aside from the lack of molecular work, we should also consider limitations associated with the study inability to assess factors of resistance in the production chain as environmental determinants such as the water and the soil were not assessed. Water used during the dressing especially the well water could serve as a possible source of contamination for the mesenteric lymph nodes. The improper waste management at all the slaughterhouse could cause heavy contamination of the soil which could contribute to contamination of water. Further studies would consider these so as to provide a comprehensive understanding of the source of contamination.

Another limitation is that, this study could not reliably ascertain whether antibiotics were used in the pigs feed or drinking water as information was collected from butchers/middlemen and not the owners. For future similar studies, would exclude animals from which the information cannot be obtained. This will enable the study give a fair judgement as whether use of antibiotic in feed or water contributed to the resistance or not.

# CHAPTER 6

## 6.0 Conclusions

This study detected high proportion of *E. coli* in both fecal and MLN from selected slaughterhouse in healthy pigs at slaughter in the GAR. Fecal *E. coli* proportion was slightly higher than that in the MLN. Proportion of *Salmonella* in the feces was lower compared to MLN. *E. coli* isolates showed high variability levels of MDR as well as individual resistance to drugs tested. Tetracycline, ampicillin and trimethoprim/sulphamethoxazole were the antibiotics that showed most resistance for *E. coli* isolates and ampicillin for *Salmonella* isolates. The most common MDR pattern for *E. coli* was to tetracycline, ampicillin and trimethoprim/sulphamethoxazole. There was no MDR detected for *Salmonella* isolates. Husbandry system and feeding type were identified as factors closely related to fecal *E. coli* antibiotic resistance development and age as factor closely related to MLN *E. coli* antibiotic resistance development. Moreover, inappropriate structure designs, poor hygiene and slaughter practices found in this study may have contributed to MDR. NTS belonging to serogroup B, C2, C1 & G were detected in this study.

The present study revealed the occurrence of antimicrobial resistant *E. coli* and *Salmonella* in pigs at slaughter in Greater Accra Region, Ghana. This observation indicates the potential importance of pigs as a source of single and multiple antimicrobial-resistant *E. coli* and *Salmonella* isolates to commonly used antimicrobials including ampicillin, tetracycline and trimethoprim/sulphamethoxazole. This calls for measures to control the occurrence of *E. coli* and *Salmonella* in pigs and, stricter regulation and monitoring the use of antibiotics in livestock production.

## 6.1 Recommendation

**Animal health sector**

- Sensitize and train farmers and middle-men on good practices during transportation of livestock
- Train slaughterhouse workers on good hygiene practices and importance of zoonosis

**Food and Drug Authority (FDA)**

- Ensure that the Ghana FDA code of practice for slaughterhouses is adhered to by slaughter facilities

**Animal health sector and FDA**

- Scientists should conduct in depth research to understand the scope of meat contamination and ARM

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# APPENDIX

## STUDY SPECIFIC PROCEDURE

### I. Purpose

To be used to determine the proportion of fecal and mesenteric lymph nodes carriage of E. *coli* and *Salmonella species*, using colonial morphology identification and biochemical testing; to conduct the antibiotic susceptibility profiles of E. *coli* and *Salmonella species* isolated using the Kirby Bauer's Disc Diffusion method; and determine the serogroup of *Salmonella* using slide agglutination test.

### II. Scope

This document covers steps used to investigate the antimicrobial resistance profiles of E. *coli* and *Salmonella species* and the serogroup of *Salmonella* isolated and identified from pigs at slaughter in three slaughterhouses in Greater Accra Region.

### III. Materials

1. Weighing boat
2. Xylose lysine deoxycholate agar
3. MacConkey agar
4. Triple sugar iron agar
5. Mueller Hinton agar
6. 0.5 MacFarland Standard
7. Quality control standard bacteria strains
8. Four-sided rack
9. Normal saline solution
10. Pasteur pipette
11. Cryotubes
15. Gram negative AST discs
16. *Salmonella* O antisera
17. Buffered peptone water
18. Sterile alginate swab sticks
19. Disc dispenser / Forceps
20. Vortex
21. Ruler/measuring calliper
22. Falcon tubes
23. Glycerol
24. Petri dish
25. Bunsen burner

12. 1 & 10 ul Inoculating Loops
13. Sterile wooden applicator sticks
14. Sterile polyester-tipped swab
26. Microscope slides
27. Stool containers
28. Sterile 5-10ml tubes

## IV. Procedure

**Media preparation**

**MacConkey agar**

1) Prepare MacConkey media following manufacturer's instructions
2) Autoclave at 121$^{o}$C for 15 minutes and allow medium to cool to about 45-50 $^{0}$C
3) Pour media onto sterile petri dishes and leave standing for 30 minutes to solidify

**Xylose lysine Deoxycholate agar**

1) Prepare following manufacturer's instructions
2) Heat under constant stirring until the medium starts to boil but avoid overheating
3) Cool it to 45-50 $^{0}$C
4) Pour on petri dish and allow it to solidify for 30 min
5) Store in plastic bags in the dark at about 4 $^{0}$C.

**Precaution**

a) Label plates with date of preparation, batch number and sterility conducted by testing samples for performance using stable reference strains
b) Store culture media plates upside down at 2-8$^{0}$C sealed in plastic bags to reduce chances of contamination

**Bacteria isolation and identification**

***Salmonella* isolation and identification**

1) Place the exterior of each mesenteric lymph node samples briefly into a flame to remove any residual exterior contamination.
2) Place 1g of mesenteric lymph node (composed of parts of five mesenteric lymph nodes from multiple locations in the gastrointestinal tract) and 1 g of feces from each pig into separate stomacher bags containing 9ml 2% BPW, homogenize and incubate it at 37°C for 18hours.
3) 0.5ml of the pre-enrichment broth transferred into 10 ml Tetrathionate broth and incubate at 36°C +/- 1°C for 24 hours.
4) Spread a 10µl loop full from the inoculated and incubated Tetrathionate broth on XLD agar plates and incubate at 36.0°C ± 1°C overnight (18-24 hours).
5) Primary cultures were sub-cultured onto XLD, stabbed into TSI and urea
6) Plate suspected colonies onto non-selective media; nutrient agar at 37°C for 24hours for biochemical confirmation and serogrouping.
7) Biochemical identification was done using API 20 E (gram negative oxidase negative, Enterobacteriaceae spp), following manufacturer's instruction
8) Serogrouping; used Reagent (DENKA SEKEN CO., LTD) antisera. Test was done according to manufacturer's instructions

***Escherichia coli* isolation and identification**

1) Place the exterior of each mesenteric lymph node samples briefly into a flame to remove any residual exterior contamination.

2) Place 1g of mesenteric lymph node (composed of parts of five mesenteric lymph nodes from multiple locations in the gastrointestinal tract) and 1 g of feces from each pig into separate stomacher bags containing 9ml 2% BPW, homogenize and incubate it at 37°C for 18hours.
3) Spread a 10µl loop full from the pre- enriched broth on MacConkey agar plate and incubate at 44°C overnight (18-24 hours).
4) Subculture of presumptive *Escherichia coli* colonies on MacConkey agar and incubate it at 37°C overnight (18-24hrs) prior to biochemical testing.
5) Secondary cultures were subjected to TSI and sulfur indole motility testing for biochemical analysis.

**Antimicrobial susceptibility testing**

**Procedure for Agar Disk Diffusion**

**Preparation of Mueller-Hinton Agar**

1) Prepare Mueller-Hinton media following manufacturer's instruction with supplementation for fastidious organisms.
2) Autoclave at 121$^{o}$C for 15 minutes and allow medium to cool to 50$^{o}$C.
3) Measure 60 to 70ml of medium per plate into 15x150mm plate or measure 25 to 30ml per plate into 15x100mm plates.
4) Pour agar into petri dishes on a level pouring surface, to a uniform depth of 4mm.
5) Just before use, place the plate in an incubator (35$^{o}$C to 37$^{o}$C) if excess moisture is on surface until the moisture evaporates. This is usually done for 10 to 30 minutes. If possible, lids could be removed.

**Precaution**

a) Use freshly prepared plates same day or store in a refrigerator (2° to 8°C) for up to 2 weeks.

**McFarland Turbidity Standard**

A commercially prepared MacFarland Standard 0.5 from **Pro-Lab Diagnostics, 3 Bassendale Road, Bromborough, CH62 3QL, UK** was used.

**Preparation of Inoculum**

1. The primary isolated colony was unto a non-inhibitory agar medium; nutrient agar, to obtain isolated colonies for the sensitivity test.
2. Incubate at 35 to 37$^{o}$C overnight (18 to 24hours).
3. Select 4 or 5 well-isolated colonies with an inoculating loop, and transfer the growth to a tube of **sterile saline**
4. Vortex the suspension.
5. Compare the bacterial suspension to the 0.5 McFarland standard

**Inoculation procedure**

1. Dip a sterile cotton swab into the suspension.
2. Remove excess liquid by press firmly against the inside wall of the tube
3. Streak the swab over the entire surface of the medium 3 directions, rotating the plate approximately 60 degrees
4. Swab finally all around the edges of the agar surface.
5. Apply disks within 15 minutes of inoculation.

**Antimicrobial disks**

**Procedure:**

1. Using sterile forceps, place the disks individually onto the agar, gently pressing it down onto it, within 15 minutes of inoculation.

2. Invert agar plates and make sure disks do not fall off the agar surface.
3. Incubate pates at 35-37$^{o}$C within 15 minutes of disk application overnight.

**Recording and Interpreting results**

1. Measure the diameter of the zones of complete inhibition (including the diameter of the disk) with a ruler or calliper and record to the nearest millimetres.
2. Compare the zones of growth inhibition with the **zone-size interpretative table** Interpret zone diameters into susceptibility categories according to the "*Clinical and Laboratory Standards Institute"* (CLSI, 2018) document current breakpoint tables at www.clsi.org .

## V. Quality Control

ATCC 25922 was used as control for the E. *coli* control strain used when testing *Enterobacteriaceae* and *V. cholerae*) with each test. Wild type *Salmonella* was used for the *Salmonella* control strain

## VI. Quality Control and Quality Assurance

Quality Control Strains were run alongside every batch of sensitivity being worked on to ensure accuracy of tests done.

## VII. Definition

**SOP-** Standard Operating Procedure

**SSP-** Study Specific Procedure

**CLIS-** Clinical and Laboratory Standards Institute

**ATCC-** American Type Culture Collection

**QC-** Quality Control.

**Record sheets**

**Animal ID**: ……. **Lab ID**…… **Sex:** F / M **Age:** ……

**Abattoir**…… **Sample source**……..... **Date received**……. **Date processed**……

## MICROBIOLOGICAL RESULTS

**Colonial morphology**

| Media | Color | Results | Comments |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

**Biochemical identification**

| Screening medium | Test reaction | Results | comments |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

**Antibiotic sensitivity testing**

| No | Antimicrobial agent tested | Resistant | Susceptible |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |

**ETHICAL APPROVAL LETTER**


**UNIVERSITY OF GHANA**

**University of Ghana Institutional Animal Care and Use Committee (UG-IACUC)**

Phone:
Email: UG-IACUC@ug.edu.gh

P.O. Box LG 581
Legon, Accra
Ghana

**Office Location: Department of Animal Experimentation Building, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana**


16/01/2020

**ETHICAL CLEARANCE**
(UG-IACUC 008/19-20)

Your protocol for an ethical clearance has been reviewed by the University of Ghana Institutional Animal Care and Use Committee and has been approved as follows:

**TITLE OF PROTOCOL:** Antimicrobial Resistance of *Escherichia Coli* and *Salmonella* in Pigs, Greater Accra, Ghana

**PRINCIPAL INVESTIGATOR:** Dr Bonodong Z. Guri (DVM)

Please note that the final review report must be submitted to the Committee at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to UG-IACUC for review and approval prior to implementation.

Please report all serious adverse events related to this study to UG-IACUC within seven (7) days verbally and in writing within fourteen (14) days.

This certificate is valid till 15th January, 2021. You are to submit annual reports for continuing review.

Professor Major (Rtd.) George A. Asare
**CHAIRMAN**
**Consent form**

**General information about research**

You have been invited to take part in a research study on the antimicrobial resistance of *Escherichia coli* and *Salmonella* in pigs in Greater Accra region of Ghana. The researcher will first explain the study and will ask you to participate by signing this agreement which states that the study has been explained to you, that your questions have been answered and that you agree to participate. The researcher will explain the purpose of the study. He will explain how the study will be carried out and what you will be expected to do.

The researcher will also explain the possible risks and benefits of participating in the study. You should ask the researcher any questions you have about any of these things before you decide whether you wish to take part in the study. Please read the form and talk to the researcher about any questions you may have. Then, if you decide to participate, please sign and date this form in front of the person who explained the study to you.

**General objective:** The main aim of this study is to assess the antimicrobial resistance of *Escherichia coli* and *Salmonella* species from pigs at slaughter in Greater Accra region, Ghana.

**Purpose(s) of research:** The purpose of this research is to determine the presence of the microbes *Escherichia coli* and *Salmonella*, their resistance to antimicrobial and use this information to develop strategies/plans of the better use of antibiotics in animals that will ultimately reduce antimicrobial resistance in animals as well as in human and in turn, reduce the risk of treatment failure, antimicrobial related mortality and cost of healthcare.

**Procedure of the research:** You will be asked some few questions on your pig, the farm origin, disease outbreak history and management practices of the farm. Your responses to these questions will be ticked appropriately on the questionnaire. Your movement and slaughter permit will also be reviewed to fill in some of the questions. When your is slaughter and passed for human consumption, fecal samples and carcass swabs will be taken for testing to find out

if there are *Escherichia coli* and *Salmonella* microbes. In total we expect to take samples from 121 pigs in addition to key informants.

**Possible Risks and Discomforts**: There are no direct risks involved in participating in this study. You may however be required to spare few minutes of your time to answer few questions from research assistants.

**Possible Benefits**: There are no direct benefits to the study participant at the point of data collection in this study. However, results from this study will be used to strategize the use of antibiotics in animals that will ultimately reduce antimicrobial resistance in animals as well as in human and in turn, reduce the risk of treatment failure, antimicrobial related mortality and cost of healthcare in humans.

**Confidentiality:** Be assured that all information obtained from you will be treated with utmost confidentiality and used strictly for the purposes of this research. You will not be associated with the information provided during the study. Anonymity will be ensured and you will be identified by a code which will be known only to the research team Information collected on study questionnaires will be given codes numbers. All information you provide will be for academic purposes alone and other than other purpose. The findings from this study may be reported in publications or reports but no name will be mentioned. Also, as part of my responsibility to conduct this research properly, I may allow officials from the Ethics Committee, academic supervisors and sponsors to have access these records.

**Compensation:** There will not be any monetary payment that will be required of you, and you will not also be compensated or paid any money for participating in this study.

**Voluntary Participation and Right to Leave the Research:** Participation is solely voluntary and you can decide at any point of the study to withdraw from participating. You do not have to offer reasons for your withdrawal from the study and no explanation is required for redrawing from the study.

**VOLUNTEER AGREEMENT**

The above document describing the benefits, risks and procedures for the research title ***Antimicrobial Resistance of Escherichia coli and Salmonella in Pigs, Greater Accra, Ghana, 2019*** has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____ _____

Date Name and signature or mark of volunteer

**If volunteers cannot read the form themselves, a witness must sign here:**

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Name signature of person who obtained consent

## Questionnaire

**Demographic Characteristics**

1. Pig ID number ☐ Owner's contact ☐
2. Date:…….
3. Name/location of slaughterhouse:……
4. GPS coordinates: Latitude…….. Longitude…….
5. Age of animal: ☐ Young ☐ Adult
6. Sex: ☐ Male ☐ Female
7. Breed of animal: ☐ Local/Ashanti black ☐ Large white ☐ Land race ☐ Cross-breed ☐ Unknown
8. Origin of animal: ☐ Ghana ☐ Burkina ☐ Togo ☐ Cote d'Ivoire ☐ Mali ☐ Other (specify)
9. If Ghana (for question 7), which region: ……..
10. Type of settlement: ☐ Rural ☐ Urban

**Pig management practices**

11. Husbandry type/system: ☐ Intensive ☐ Semi-intensive/mixed ☐ Extensive/free-range
12. Feeding type: ☐ Crop residue ☐ Garbage/scavenging ☐Commercial feed
13. Source of water: ☐ Bore hole ☐ Well ☐ Rain
14. Transportation of animal to the slaughterhouse: ☐ Singly on motorbike ☐ In group by vehicle
15. Do you transport pigs mixed with other animals to the slaughterhouse? ☐ Yes ☐ No

**Vaccination records**

16. Is a *Salmonella* vaccine used on the farm?

□ Yes □ No □ Don't know

17. Any other vaccine given apart from *Salmonella?*

□ Yes □ No □ Don't know

**Clinical history of disease outbreak and record of antibiotics use**

18. Do you have a temporary detention area for the pigs before slaughter?

□ Yes □ No

19. Have you ever had any problems with the pigs you bought for slaughter? (if no, skip Q19) □ Yes □ No

20. What did you do? …….

21. For the past one year, do you know if the farm you bought the animal has any disease outbreak? □ Yes □ No □ Don't know

22. Do you know if a Veterinary Officer take care of the health of animals you buy ?

□ Yes □ No □ Don't know

23. Does the farm owner treat the animals by himself?

□ Yes □ No □ Don't know

24. What type of medication practices are done on the farm?

□ Herbal/local treatment □ Modern/orthodox treatment

25. Use antibiotics in the pigs' water? □ Yes □ No □ Don't know

26. Use of antibiotics in the pigs feed? □ Yes □ No □ Don't know

**Checklist**

**Slaughterhouse assessment for adherence to regulation, guidelines and policies**

| FACILITY INFORMATION | |
|---|---|
| Name of Facility: _____<br>District / Location: _____ | Facility Type: _____<br>No. of staff employed: ____ Doctors<br>_____Technician _____Cleaners |

| A. RESTRICTED AND CONTROL ACCESS ZONES | | |
|---|---|---|
| | **Answers** | **Comments** |
| 1. The roofing is with ceiling of adequate head room, smooth and flat that allow for easy cleaning | ☐ YES ☐ NO ☐ N/a | |
| 2. The area is secured to prevent the entrance of unauthorized persons or animals | ☐ YES ☐ NO ☐ N/a | |
| 3. Access to the premises is controlled or guarded | ☐ YES ☐ NO ☐ N/a | |
| **B. WATER SOURCE FOR WASHING CARCASS** | | |
| 1. Running tap water | ☐ YES ☐ NO ☐ N/a | |
| 2. Well water | ☐ YES ☐ NO ☐ N/a | |
| 3. Rain water | ☐ YES ☐ NO ☐ N/a | |
| **C. TOILETS AND HAND WASHING FACILITIES** | | |
| | **Answer** | **Comments** |
| 1. Toilet facility on-site? | ☐ YES ☐ NO ☐ N/a | |
| 2. Running water available for washing hands | ☐ YES ☐ NO ☐ N/a | |
| 3. Sinks or basins and soap available for washing hands | ☐ YES ☐ NO ☐ N/a | |
| 4. Workers on slaughter line able to wash their hands and disinfect equipment without leaving their work stations? | ☐ YES ☐ NO ☐ N/a | |
| **D. WASTE MANAGEMENT PRACTICES** | | |
| 1. Type of waste management most used in facility:<br>a) None? | ☐ YES ☐ NO ☐ N/a | |

| | | |
|---|---|---|
| b) Pit-holding? | ☐ YES ☐ NO ☐ N/a | |
| c) Mechanical scraper or tractor? | ☐ YES ☐ NO ☐ N/a | |
| d) Flush-open gutter? | ☐ YES ☐ NO ☐ N/a | |
| e) Flush-open slats? | ☐ YES ☐ NO ☐ N/a | |
| **E. CLEANING AND DESINFECTION** | | |
| 1. Are all floors in the slaughterhouse thoroughly washed each day as soon as slaughtering is completed? | ☐ YES ☐ NO ☐ N/a | |
| 2. Are utensils and equipment used in slaughtering and dressing cleaned immediately after completion of work? | ☐ YES ☐ NO ☐ N/a | |
| 3. Are utensils and equipment used in slaughtering and dressing washed and disinfected with hot water before each of work and/or between period of work? | ☐ YES ☐ NO ☐ N/a | |
| 4. Are utensils and equipment used in slaughtering and dressing cleaned and sanitized immediately when coming into contact with abnormal or diseased tissue? | ☐ YES ☐ NO ☐ N/a | |
| 5. Are containers and equipment passed from dirty to clean edible area? | ☐ YES ☐ NO ☐ N/a | |

# GALLERY

**Figure 9:** Sample collection of feces in one of the three slaughterhouses

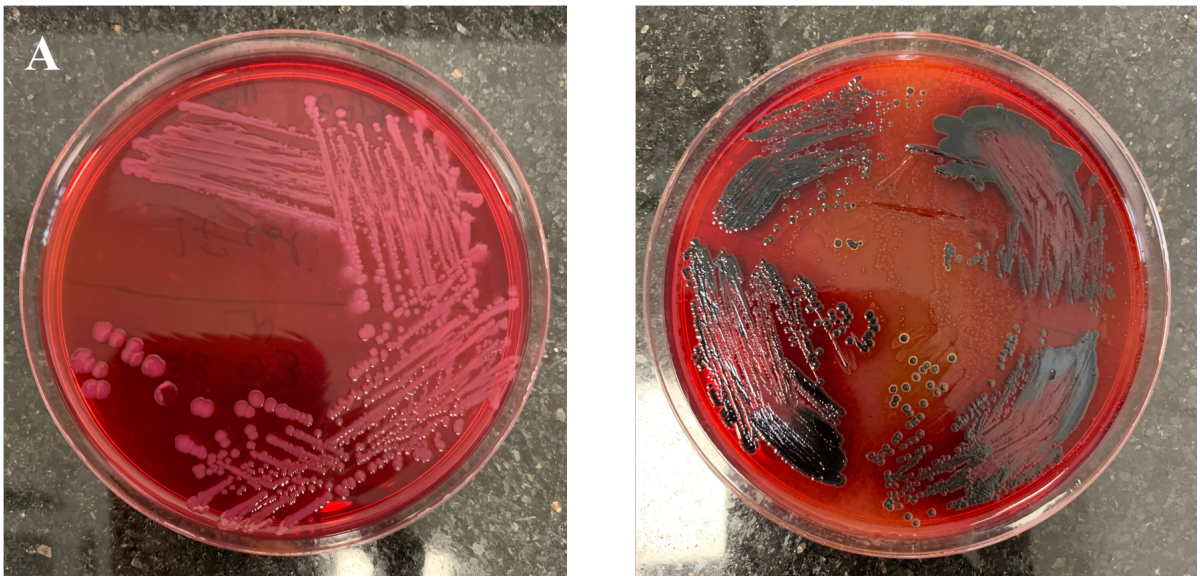


**Figure 10:** "A" presumptive E. coli isolate and “B” presumptive Salmonella isolate

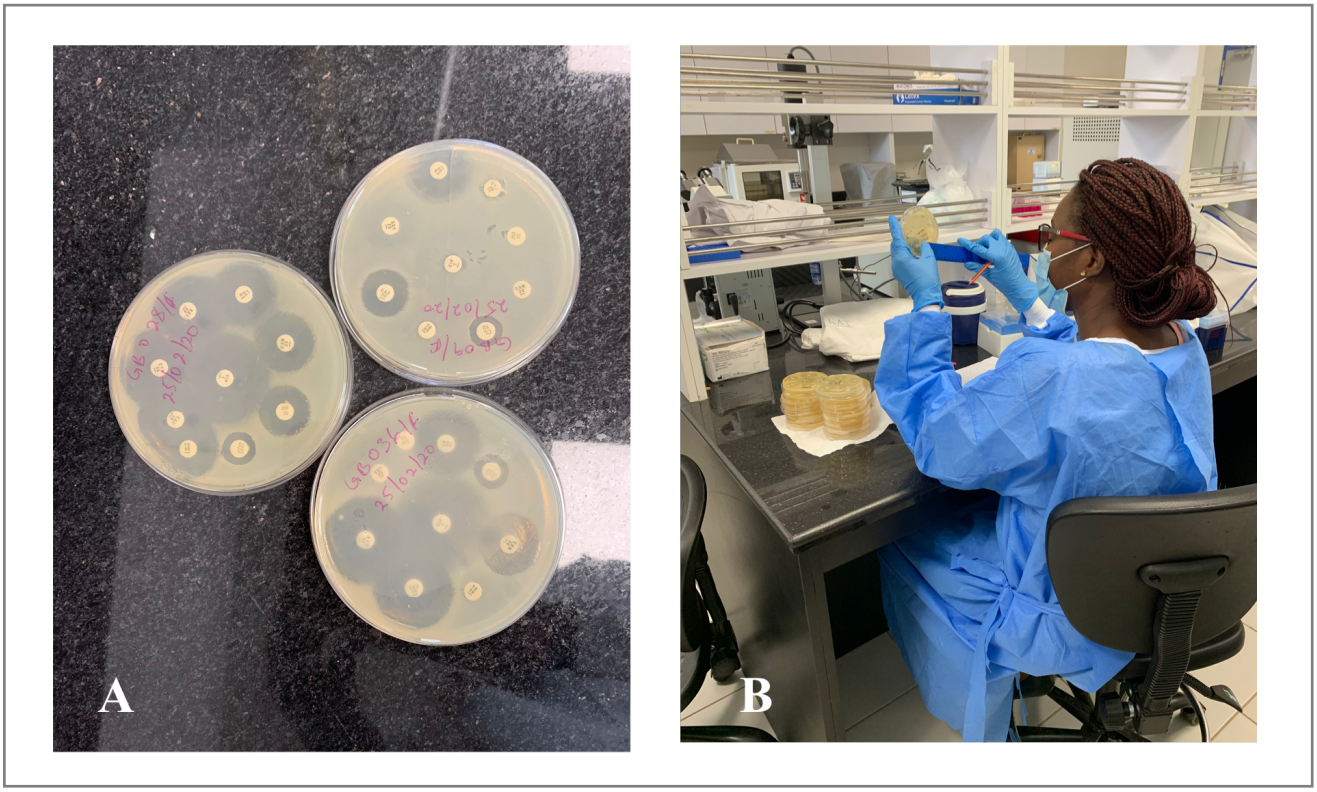


**Figure 11:** "A" some AST plates for reading and "B" measuring zone of inhibitions of AST plates

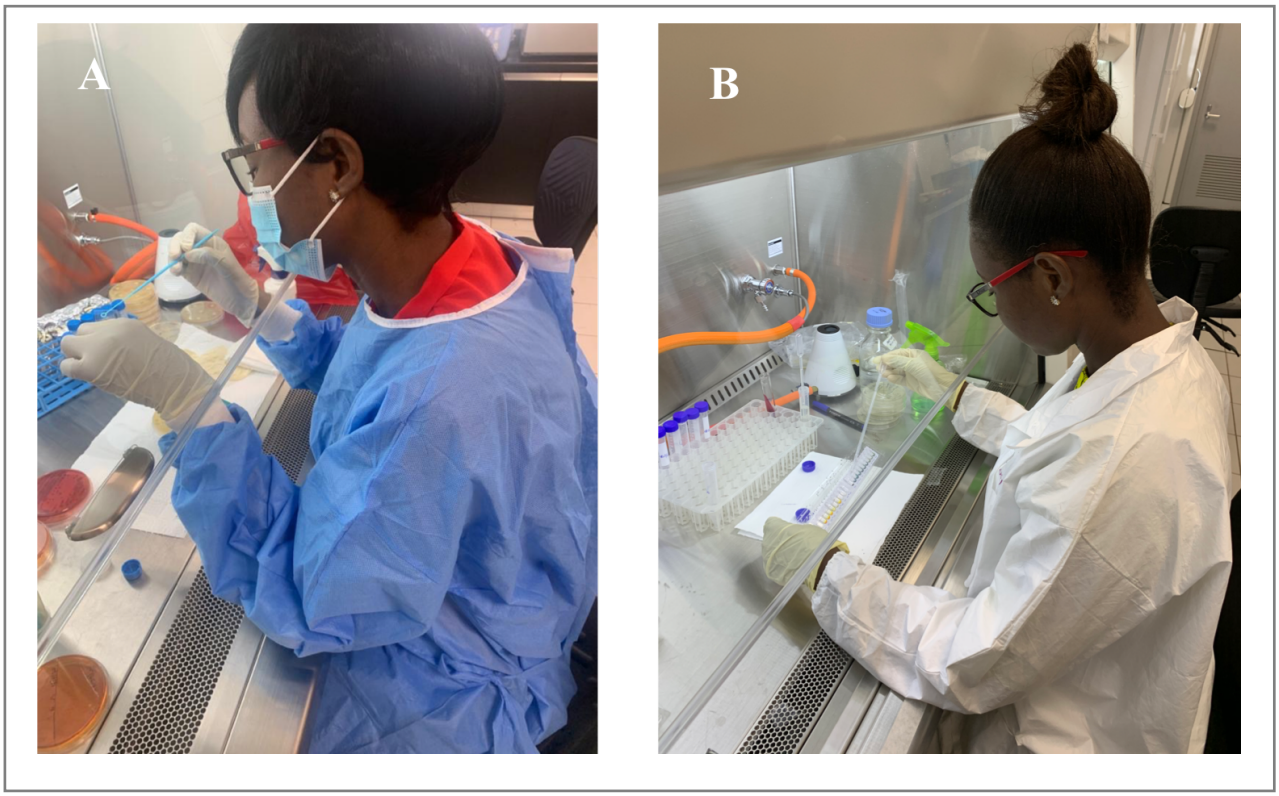


**Figure 12:** "A" Culture of samples on agar media and "B" performing API test for presumptive Salmonella isolates