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School of Public Health**



**ANTIMICROBIAL RESISTANCE PATTERNS OF *ESCHERICHIA COLI*
ISOLATED FROM BEEF, MUTTON AND CHEVON IN THE GREATER
ACCRA REGION OF GHANA**

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**A THESIS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE IN
MASTER OF PHILOSOPHY [APPLIED EPIDEMIOLOGY AND DISEASE CONTROL]**

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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 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

This work is dedicated to the Almighty God for his guidance and grace for completion of this work. It is also dedicated to my parents and siblings for all their support and care towards my education and the successful completion of this work.

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LIST OF ABBREVIATIONS

AMR- Antimicrobial resistance

AMU- Antimicrobial use

CDC - Centers for Disease Control and Prevention

CFU - Colony forming unit

CLSI - Clinical and Laboratory Standards Institute

EUCAST - European Committee on Antimicrobial Susceptibility Testing

FBP's - Food borne pathogens

JFC - Private slaughter facility

MALDI-TOF - Matrix- assisted laser desorption ionization–time of flight mass spectrometry

MDR - Multi Drug Resistance

PPE - Personal Protective Equipment

SOP - Standard Operating Procedure

SXT - Sulphamethoxazole/Trimethoprim

TCC - Total coliform count

TSH - Tema slaughterhouse

TSS - Tulaku slaughter slab

TVC - Total viable count

UTI - Urinary tract Infection

WHO - World Health Organization

DEFINITION OF KEY TERMS

Meat: The flesh of an animal, typically a mammal or a bird, used as food.

Chevon (goat meat): The flesh of a goat used as food

Mutton: The flesh of a fully grown sheep used as food.

Beef: The flesh of a cow, bull or ox used as food

Slaughterhouse: An establishment where animals are killed for their meat. It is also known as a building or place where animals are butchered for food.

ABSTRACT

Background

Antimicrobial resistance (AMR) poses a challenge to the health of the populace. Food producing animals (FPA) are major reservoirs for food-borne pathogens, which may be resistant to critically needed antimicrobials in human and veterinary medicine. Contamination of raw meat with *Escherichia coli* (*E. coli*) strains may occur during slaughter and sale. The presence of *E. coli* that have the ability to produce extended spectrum beta lactamase (ESBL), affect treatment outcomes for life-threatening infections in humans. We determined the presence of *E. coli* in raw meat, characterized their antimicrobial susceptibility patterns and detected the resistant genes associated with the extended spectrum beta-lactamase enzyme.

Methods

We collected surface swabs from cattle (81), sheep (16) and goats (108) after slaughter from three slaughterhouses in the Greater Accra Region. With the aid of a template and sterile cotton swab, we sampled an area of 300 cm² from the flank, brisket and upper thigh in cattle, and an area of 75 cm² from the flank, brisket and mid-loin for sheep and goats. We performed total viable counts, coliform counts, and cultured samples on MacConkey agar following enrichment in Brain Heart Infusion (BHI). Based on their colonial morphology, *E. coli* isolates were identified. Confirmation was carried out using the Matrix- assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) system. We tested the susceptibility of isolates to 11 antibiotics using the Kirby Bauer disk diffusion method. We performed the combination disk test for screening of ESBL's in all *E. coli* isolates that had resistance to cephalosporins. We subsequently screened these isolates for ESBL resistant genes (*CTX*, *SHV* and *TEM*) by Polymerase Chain Reaction (PCR). We observed slaughter practices. Descriptive statistics was used to characterize antimicrobial susceptibility patterns.

Results

The mean total viable count was 5.03 (\pm 1.06) log CFU/cm² for the 205 samples collected. This exceeds the limit of 5.0 log CFU/cm², for total viable microbial counts for cattle, sheep and goat carcasses. For Enterobacteriaceae, high counts were reported at Tema slaughterhouse and Tulaku slaughter slab (3.12 \pm 0.2 and 3.87 \pm 0.7). Overall, prevalence of *E. coli* was 48% (98/205) in all meat types sampled. Antimicrobial susceptibility testing showed that isolates exhibited resistance to ampicillin (57%; 56/98), tetracycline (45%; 44/98), sulfamethoxazole-trimethoprim (21%; 21/98), cefuroxime (17%; 17/98), ciprofloxacin (8% ;8/98) and cefotaxime (2%; 2/98). Meropenem showed the highest rate susceptibility(100%). Multi-drug resistance was identified in 22% (22/98) of isolates. Four (4) isolates were found to have the *TEM* gene. Water supplies,

sanitary facilities, work area and equipment at all sites were found to be sub-standard per the guidelines set by the Ghana Food and Drugs Authority (FDA).

Conclusion

Contamination with *E. coli* was found to be high in raw meat. The presence of ESBL-producers among *E. coli* found in meat, has implications for the management of local and systemic infections in humans. Sub-optimal slaughter practices might have facilitated contamination. There is an urgent need to monitor the hygiene process at slaughter sites and to educate slaughterhouse workers on hygienic practices.

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Annual deaths attributable to antimicrobial resistant infections have sparked global concern for the need to the development of strategies for its control (Robinson et al., 2016). AMR threatens food security and health, particularly in developing countries still burdened with poverty and infectious diseases(WHO, 2017). The slow discovery of novel antimicrobial drugs in recent years compounds the problem of AMR and highlights the need to adopt practices targeted at preserving critically needed antimicrobial drugs(Jim O’Neill, 2014). The One Health nature of AMR requires a multi-faceted approach for combatting its development and spread.

Antimicrobial resistant organisms are implicated in a variety of diseases in humans. Infections caused by antimicrobial resistant organisms may lead to prolonged morbidity, result in complications and are three times more likely to result in death (Cecchini et al, 2015). Ten million deaths will be attributable to AMR annually by 2050 according to a WHO report (2016). According to the Global Risks report (2013), over 100,000 AMR related deaths were recorded in US hospitals in 2013. The economic loss incurred due to these deaths was estimated at 1.6% of GDP in that same year. These losses are attributed to the length of stay in hospitals, intensive antimicrobial therapy, and medical procedures for AMR related infections.

Antimicrobial exposure in healthcare settings, agriculture and the environment have been cited as major determinants of AMR (Moore et al., 2015). While the burden of AMR in humans stems mainly from exposure in health care due to the misuse of antimicrobial drugs, foods found to contain resistant bacteria may provide a pathway for humans to become colonized (Lowe, 2016)(Doyle, 2015). Food producing animals are major reservoirs for foodborne pathogens. Cattle,

sheep and goats are reported to be asymptomatic carriers of *Escherichia coli* O157:H7. *Listeria monocytogenes* was confirmed as the cause of an outbreak, killing 176 people in South Africa in 2018 (Ogunbanjo, 2018). The outbreak was linked to the consumption of processed meat products. The rising trend of multi drug resistance (MDR) among these pathogens poses a major public health threat to consumers of foods of animal origin as well as farm attendants.

Antibiotics are routinely used in intensive farming systems to prevent livestock losses and improve production outcomes (Sarkar, 2018). The use of antibiotics in livestock is frequently linked to the presence of AMR in livestock (J. O'Neill, 2015). With an increasing world population and an increasing demand for food, agricultural systems have come under pressure to meet the growing demand. Intensive farming systems often rely on growth promotion methods that may involve the routine use of antibiotics (Sarkar et al., 2018). Determining the causal relationship between antimicrobial use and the occurrence of AMR in livestock is however complicated by the disparate data on antimicrobial use and poor cumulative data (Hernández Rodríguez, 2014).

Livestock may also become exposed to antimicrobial resistant bacteria through the feeding systems, environment, and other animal species (FAO, 2016). Products of animal origin can get contaminated with such bacteria during processing and handling attributable to unhygienic slaughter and sale conditions. *E. coli* showing multidrug resistance has been detected in pork, ground turkey, beef, and chicken (Urahn, 2016). Residues of antibiotics present in animal products further increases concern of its contribution to AMR in humans. Chicken meat samples have been reported to contain high residues of antimicrobial drugs such as tetracycline. (Doyle, 2015). The problem of AMR from animal sources to the health of humans comprises then, not only of the transmission of resistant bacteria in the food chain, but also from the consumption of foods that contain antibiotic residues.

Global action plan for AMR promotes the optimal use of antimicrobial drugs ,improvement in knowledge and surveillance required for its control.

1.2. Problem Statement

Food safety is a crucial component of public health and food security. Ensuring the safety of food products will reduce food losses and lead to major gains in improving food security (Codex Alimentarius Commission, 2003). Strengthening food safety has the potential to reduce poverty, minimize the burden of foodborne diseases and lead to the fulfilment of sustainable development goals that target poverty eradication.

Foodborne pathogens are frequently known to cause disease and contribute to the growing phenomenon of antimicrobial resistance. Most recent foodborne outbreaks have been attributed to zoonotic pathogens, some of which are resistant to antimicrobials used in infectious disease treatment in humans. Over 200 reported cases of *E. coli* O103 infection due to beef consumption were reported in the USA in 2019 (CDC, 2019). According to CDC, 120 cases of foodborne infections from MDR *Salmonella* were recorded in 2018 and 6.5 billion pounds of beef products were recalled in the same year due to the presence of *Salmonella*.

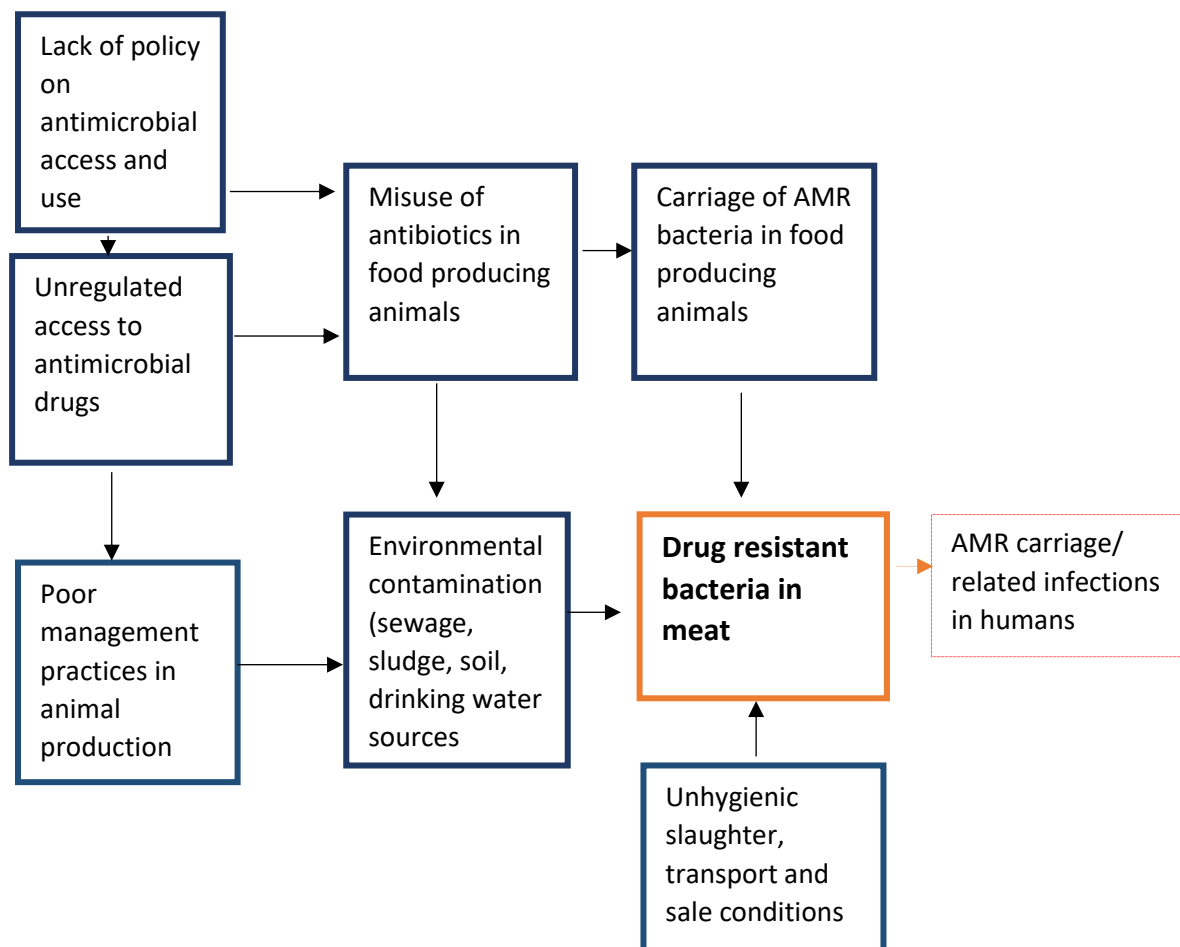
Increasing resistance of foodborne pathogens to antimicrobials of critical importance to human health risks becoming a pandemic threat.

While the magnitude of the presence of drug resistant bacteria in food products in Ghana has not been adequately described, recent studies on clinical bacterial isolates in Ghana have provided a scope on the intensity and increasing trend of antimicrobial resistance in both agriculture and health care (Mohammed et al, 2018). In Ghana, it has been found that, resistance to antimicrobial

agents that are common and cheap is widespread. These include resistance to drugs such as ampicillin, co-trimoxazole and tetracycline. (Newman et al., 2015). Commercial poultry production systems in two regions in Ghana were found to have high rates of multi-resistant *Salmonella* (Andoh et al., 2016).

The absence of policy that provides guidelines for antibiotic use and prevention of resistance in Ghana has been identified as a factor in the upsurge in abuse of antibiotics in the health and agricultural sectors (Boamah et al., 2016). With limited data on the risks of AMR transmission through the food chain in Ghana, the development of strategies to control AMR may be stalled, leading to consequences for human health and economy.

1.3. Conceptual Framework



1.3.1. Narrative of conceptual framework

Several factors may account for the presence of AMR bacteria in food products. Lack of policies that regulate access and use of antimicrobials in farming may promote its misuse and abuse in food producing animals. Most of the antimicrobial agents used for livestock rearing are also used in humans. Misuse of such antimicrobial agents increase selective pressure thereby leading to high carriage rates of resistant bacteria in livestock. Unhygienic and unsafe slaughter conditions may lead to the introduction of AMR bacteria to meat during the slaughter process. Poor farm

management practices such as improper waste disposal may lead to environmental contamination with AMR bacteria. Humans can become exposed to AMR bacteria through the consumption of foods contaminated with AMR bacteria. This may lead to an increase in carriage rates and the possibility of AMR related infections in humans.

1.4. Justification

Currently, no structured system exists for monitoring and surveillance of AMR bacteria in food producing animals in Ghana. This makes it difficult to ascertain the risk that AMR poses through the food chain. Data on AMR carriage in isolates of food borne pathogens will guide future antimicrobial therapy, thus improving treatment outcomes, reducing mortality and improving livelihoods. Knowledge on carriage of resistant strains of food borne pathogens in livestock will help characterize the burden of AMR globally and inform policy on surveillance of AMR through the food chain. This study sought to determine the occurrence of *E. coli* and characterize their antimicrobial susceptibility to critically needed antimicrobial drugs to improve practice and inform policy on AMR control.

1.5. Objectives

1.5.1. General objective

To assess the antimicrobial resistance patterns of *E. coli* contamination in raw beef, mutton and chevon in slaughterhouses in the Greater Accra Region.

1.5.2. Specific objectives

1. To determine the proportion of beef, mutton and chevon contaminated with *E. coli*
2. To determine the antimicrobial resistance of *E. coli* from beef, mutton and chevon.
3. To determine the proportion of meat with ESBL-producing *E. coli* and presence of resistance genes

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Foodborne pathogens and food safety

Foodborne pathogens are parasites, bacteria, virus, fungi and other organisms that can cause disease to humans and animals through the food chain. These organisms can enter the food chain at any point from farm where livestock are raised up to the point of consumption and cause foodborne illness (WHO, 2010).

WHO estimates that globally, 1 in 10 people become ill each year after exposure to food contaminated with any of the 31 foodborne agents that cause disease in humans. According to CDC estimates, approximately 48 million new cases of foodborne disease occur annually in the USA. With these cases resulting in approximately 3,000 deaths and 128,000 hospitalizations in USA, foodborne illnesses have consequences for economies and livelihoods (CDC, 2011). Foodborne diseases place an additional burden on the economies of both developed and developing nations as massive costs are incurred in its control. The cost of foodborne illnesses in the UK was estimated to be £1.5 billion in 2008 (Tam et al., 2011). In a study reported that, 18 million Disability Adjusted Life Years (DALYs) were related to foodborne diarrheal disease causing agents (Zein et al., 2018). Foodborne pathogens also have implications for international trade in food products as they pose a risk for the spread of new pathogens into countries.

Of the foodborne pathogens that may cause disease in humans, *Campylobacter*, *Listeria monocytogenes*, pathogenic *E. coli* strains, *Staphylococcus aureus*, non-typhoidal *Salmonella enterica*, *Salmonella Typhi*, *Clostridium botulinum*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are commonly associated with most foodborne illnesses (CDPH, 2015).

Recurrent foodborne outbreaks in many countries underline the need to conduct foodborne disease surveillance. Foodborne disease surveillance is useful for estimating the magnitude of disease, monitoring trends and detecting outbreaks. Foodborne disease surveillance typically involves systems for alerting, reporting of notifiable diseases and reporting of laboratory isolation of enteric pathogens. Similarly, incidents of suspected foodborne outbreaks are investigated.

Under International Health Regulations (2002), all member countries are mandated to report events that include outbreaks attributed to the presence of a foodborne pathogen (WHO, 2012).

Based on the frequency and type of cases reported in health facilities in Ghana, four main foodborne diseases are targeted for surveillance. They include Viral Hepatitis (Hepatitis A and E), Cholera (*Vibrio cholerae*), Dysentery (*Shigella sp.*) and Typhoid fever (*Salmonella sp.*) (FDA, 2016). Foodborne disease outbreak investigations are also conducted in accordance with the Ghana Food and Drugs Authority (FDA) guidelines.

2.2. Microbial Contamination of food products

In order for foods to be categorized as safe for consumption, they must be free from physical, biological and chemical contaminants. As a result of this, the presence of microbial agents and their toxins in food products should be regularly monitored as they constitute a public health threat (Alum, 2016). The Codex Alimentarius Commission adopts and periodically revises guidelines and quality standards necessary to ensure food safety with the goal of protecting the health of consumers. Using a set of criteria the Commission addresses trade and consumer concerns on safety globally, while ensuring that food safety methods in its member countries are harmonized (Codex Alimentarius Commission, 2016). The guidelines adopted by the commission for the control of selected bacteria for a particular food type highlight the control measures necessary from primary production, through processing and distribution of the food products. The Code of

Hygiene Practice for meat adopted by the commission details the conditions for transport, lairage conditions, dressing of carcasses, design, facilities and equipment present at slaughter sites. Within countries food safety organizations may exist separately as an authority or may be embedded in departments responsible for agriculture and health. The Ghana Food and Drugs Authority (FDA) has established guidelines for operations at slaughterhouses and slabs, practice codes for meat sale points and practice codes for processing facilities (Food and Drugs Authority, 2013).

The microbial limits of food are usually centered on aerobic colony counts, hygiene indicator organisms and some specific foodborne pathogens (Center for Food Safety, 2014). Process hygiene criteria for carcasses of cattle, sheep and goats should not exceed a value of 5.0 log CFU/cm² daily mean log for aerobic colony counts. This criterion holds for carcasses that have been dressed and are yet to be chilled. Similarly, process hygiene criteria for carcasses of cattle, sheep and goats should not exceed 2.5 log CFU/cm² daily mean log for Enterobacteriaceae (Ghana Standards Authority, 2018). Meat and surface samples from equipment used in the processing of meat in Pakistan were found to have total plate counts to be between 10⁸–10¹⁰ CFU/g or cm² (Ali et al, 2010). A study conducted in municipal slaughter houses in India found that 7.33% of meat samples had high levels of *E. coli* (Kumar et al., 2014). Meat samples from sale points in Nigeria were found to have a mean viable count of 4.52x10⁶ CFU/ cm² with an average coliform count value of 2.34x10⁶ CFU/ cm² (Salihu, 2013). Beef from selected retail shops and markets in the Central region of Ghana were found to have bacterial counts as high as 1.15x10⁸ on nutrient agar. In the same study, chevon was found to have bacterial counts as high as 1.67x10⁸ on nutrient agar (Yafetto et al., 2019). High levels of microbial contamination in meat has been attributed to poor hygiene and sanitation at points of sale and slaughter (Camargo et al., 2019).

2.3. Foodborne transmission of *E. coli*

Escherichia coli is a common inhabitant of the intestinal tract of humans and animals. Its role in the functioning of the digestive system is of primary importance, particularly for food absorption and waste processing. *E. coli* is found in the environment in large numbers. Its presence in water is frequently used to detect the presence of contamination with faeces in food and water (Cooke et al., 1985).

Some types of *E. coli* are known to cause diarrheal disease and are categorized as entero-pathogenic (Kornacki et al., 2016). While most *E. coli* are beneficial to humans, a few strains are pathogenic (Boyer, 2015). *E. coli* O157:H7, often produce shiga-toxins capable of causing life-threatening infections in humans. Clinical manifestations include diarrhea, cramping, vomiting, blood-clotting problems, and in some cases, death may occur. These strains have been implicated in the development of hemolytic uremic syndrome (HUS), resulting in kidney failure (CDPH, 2015). A foodborne disease outbreak due to *E. coli* O157:H7 contamination of spinach occurred in 2006 in the USA. Over 200 people were affected across 26 states in the USA (Gelting, 2007). A foodborne outbreak from consumption of ground beef in 8 states of the USA in 2007 was also attributed to *E. coli* O157:H7 (Tauxe, 2008). Although the persistence of pathogenic *E. coli* threatens food safety, commensal *E. coli* are playing an increasing role due to the ability to move mobile genetic elements among similar species and other bacteria.

2.4. Multidrug resistance development of *E. coli*

The rising trend of resistance to critically needed antimicrobial drugs observed in *E. coli* has raised interest globally. More recently, *E. coli* is often used as a measure of antimicrobial resistance of bacteria at the community level (Pessoa-Silva, 2018). Horizontal gene transfer between organisms

that are not closely related have been reported in previous studies (Lawrence, 2002). *E. coli* have the ability to exchange mobile genetic elements with other bacteria present in the environment. As such, the role of *E. coli* in disease occurrence and treatment failures in humans is not limited to pathogenic strains but also to commensal organisms as well, as they serve as reservoirs of resistant genes.

Resistant strains of *E. coli* have been reported in healthy livestock and humans globally. Multidrug resistance of *E. coli* from chicken, beef, mutton and chevon samples in Ethiopia was found to be 46%. High rates of resistance were reported for ampicillin (71.4%) and tetracycline (47.6%) in the same study (Messele et al., 2017). In a study conducted in Ethiopia, *E. coli* isolates from urine and wounds, and exhibited high resistance to erythromycin, tetracycline and amoxicillin (Kibret et al., 2011). In another study conducted in India, 85% of *E. coli* from water showed an intermediate response to rifampicin while 98% showed resistance to erythromycin (Rather et al., 2013). A study among children of school going age in rural areas in India showed that *E. coli* exhibited resistance to antimicrobial drugs such as ampicillin (92%), ceftazidime (90%) and cefoxitin (88%) (Singh et al., 2018). Findings from a study conducted in Zambia show that resistance to tetracycline was highest in *E. coli* from dairy cattle (Mainda et al., 2015). Knezevic et al (2008) reported that *E. coli* from pigs, chicken and cattle had high resistance rates for tetracycline, and moderate resistance rates for streptomycin and ampicillin (Petar, 2008). A study on trends of resistance in commensal *E. coli* over a 20-year period found that antimicrobial resistance trends among animal species in that geographic region were similar (Hesp et al, 2019). This study reinforced the importance of monitoring trends of resistance in commensal pathogens over a period.

2.5. Extended Spectrum Beta Lactamase production in *E. coli*

Extended spectrum beta lactamases are enzymes present in a variety of gram-negative bacteria, causing them to become resistant to commonly used antibiotics. They cleave to the β lactam ring thereby conferring resistance to antimicrobial classes such as penicillins, cephalosporins and monobactams (Rupp et al., 2003). As such, the presence of ESBL producing bacteria are a threat to healthcare delivery since they often lead to increase in morbidity and mortality. ESBL producing bacteria have been reported to have a high prevalence in patients with UTI worldwide (Stadler et al., 2018). Long hospital stays and carriage of other multidrug resistant bacteria are often cited as risks associated with carriage of ESBL producing bacteria (Valenza et al., 2014).

ESBL genes are found on plasmids, meaning that they are readily transmissible from one bacteria to the other. Bla_{TEM} and bla_{SHV} genes are classified as derivatives of plasmid mediated β lactamases, while the bla_{CTX-M} gene is known to be mobilized from environmental bacteria (Overdeest et al., 2011).

A high prevalence of ESBL producing bacteria (55.4%) was found to be associated with catheterization of hospital patients in Iran (Yousefipour, 2018). High prevalence was observed for ESBL producing bacteria in livestock in Madagascar. In the same study, the most prevalent ESBL gene was found to be *bla_{CTX-M-1}* (Gay et al., 2018). *E. coli* was reported as the major producer of ESBL's in a study conducted on pigs in India. *Bla_{CTX-M-1}* was the most abundant ESBLs type in this region (Warjri et al, 2013).

2.6. Risk factors for MDR in food pathogens

Antimicrobials are used routinely in the food production chain worldwide. The correlation between antibiotic use in the livestock sector and resistance in commensal bacteria of livestock from seven

countries is well documented by Chantziaras *et al.*, in the year 2014. The frequency of antibiotic use at the national level was found to influence the emergence of resistance in commensal *E. coli* in pigs, poultry and cattle in the countries surveyed (Chantziaras, 2017).

Preliminary data gathered and few studies carried out have highlighted the role of poor regulatory systems on access and use of antimicrobial drugs in livestock (Boamah et al., 2016). Inadequacy of assay testing laboratories, porous and unregulated borders and poor knowledge on AMR, particularly in developing countries have been identified as factors for indiscriminate antibiotic use in livestock farms.

Studies conducted in Ghana have reported that farmers frequently associate good health in livestock to antimicrobial use although some farmers reported having knowledge of the harmful effects of too much antibiotics in food (Tang et al., 2017). The observed increase in antibiotic use in livestock rearing systems is compounded by the intensification of farming systems in areas where extensive farming systems prevailed. The persistence of diseases coupled with low patronage for animal disease prophylaxis is reported to facilitate the use of antimicrobial drugs particularly in poultry production systems. Inappropriate use of antimicrobials in food animals can result in increased drug resistance. Livestock may serve as carriers of drug resistant foodborne pathogens or develop infections leading to losses in production.

Poor sanitary conditions during slaughter may result in contamination of meat with heavy bacterial loads through cross contamination. The hygiene level of the slaughter environment significantly influences the emergence of resistant bacteria in that environment. Interventions that dwell on the principles of Infection, Prevention and Control (IPC) were reported to contribute to a decrease in the emergence of resistance strains in health care settings (Jim O'Neill, 2016). Carcasses can become contaminated with fecal contents in unhygienic conditions of slaughter. Practices noted to

contribute to this include slaughtering on dirty surfaces and dressing carcasses in the same slaughter space (Aidarose, 2015). Minimizing contamination of meat during slaughter is essential for reducing the risk of transfer of bacteria from the environment.

Transport conditions and hygiene situation of holding pens also influence the probability of cross contamination among livestock species. Where transport conditions are sub-optimal, livestock may become stressed and this may increase microbial shedding. Poor handling of livestock and major disruptions in access of livestock to feed and water during this period also contribute to microbial shedding. Food handlers are touted as source for possible contamination of meat with various species of bacteria. Poor personal and environmental hygiene may lead to direct contamination of raw meat at retail points (Adesokan, 2014). Studies conducted among food handlers in Qatar reported high rate of MDR (27%) in *E.coli* isolates from the food handlers (Eltai et al., 2018). The slaughterhouse design and infrastructure plays an immense role in ensuring that food safety standards are met. International and local guidelines exist for the humane movement and slaughter of different livestock species (Food and Drugs Authority, 2013). Local guidelines set by the Food and Drugs Authority in Ghana give a detailed description of key components of slaughterhouse design and practices required for its operation.

2.7. Integrated Surveillance of AMR in foodborne pathogens

The use of comparable methods for sampling and testing for antimicrobial susceptibility in bacteria are important for comparing antimicrobial testing results in different countries, sub regions and across continents. An integrated surveillance approach is required to ensure that all possible sources of AMR are identified and monitored over time. While integrated AMR surveillance is existent in some developed countries, it is non-existent in most developing countries making it difficult to characterize the burden of AMR globally. Data on AMR is required from all possible

data sources in healthcare, agriculture and the environment, particularly in countries with less resources to improve the implementation of actions designed to stop the spread of antimicrobial resistance (FAO, 2017).

CHAPTER THREE

3. METHODS

3.1. Study design

This is a cross sectional study. Surface swabs were collected from cattle, sheep and goats post evisceration at three selected slaughterhouses in the Greater Accra Region. Data were collected on the type of livestock, sex, breed and other epidemiological data obtained from slaughter records at each study site using a pretested sample record sheet. Data were also collected on slaughterhouse design, operations and slaughter hygiene. Surface swabs from carcasses were processed at the Bacteriology Department of Noguchi Memorial Institute for Medical Research (NMIMR) at the University of Ghana. Laboratory testing primarily included the enumeration of bacteria, a pre-enrichment step, microbial culture, antibiotic sensitivity testing, ESBL detection and screening for resistance genes. Data were analyzed descriptively using STATA version 15.

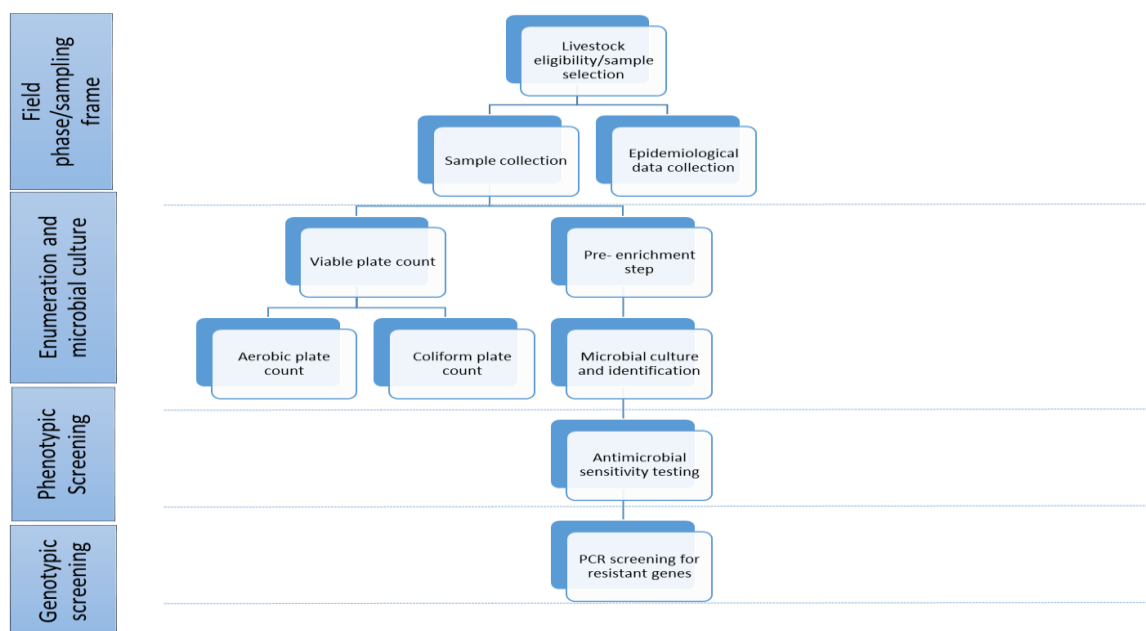


Figure 1. Schematic description of workflow of study.

3.2. Study area

The study area is the Greater Accra region, situated in southern Ghana. The region has a human population of 4,010,054 according to the 2010 population and housing census, representing 16.3% of the populace in Ghana. Eighty (80.2%) percent agricultural households in the region actively engage in crop farming. Close to one third of agricultural households are involved in livestock rearing (35%). The total livestock population in the region was estimated to be 1.5 million in 2017 (Veterinary Services Department, 2018)

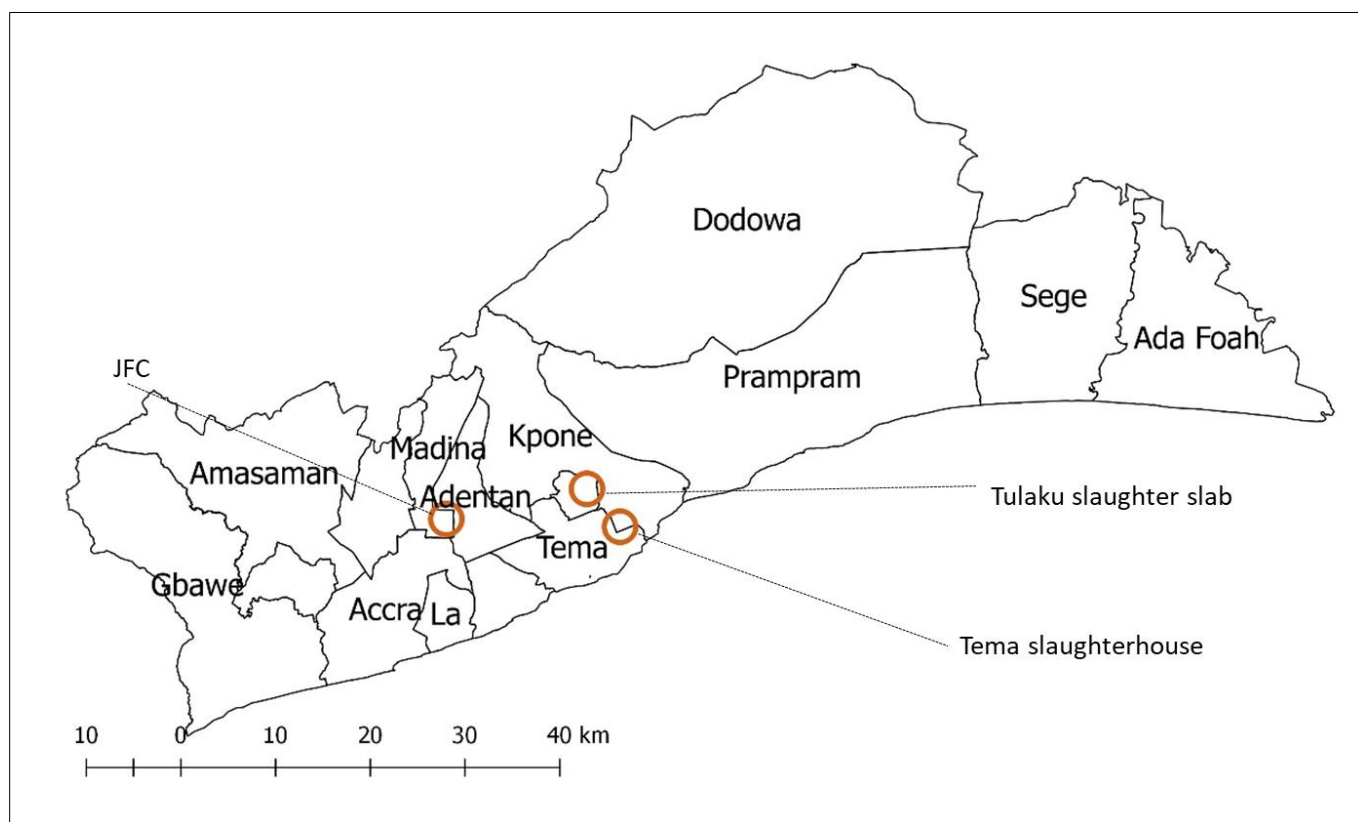


Figure 2. Map of Greater Accra Region showing selected slaughter sites

Livestock slaughter in the region takes place in public and private abattoirs, slaughterhouses and slabs in some districts of the region. Slaughter may also be done in individual households. This

study was conducted at two public slaughter sites (Tema and Tulaku) and one private slaughterhouse (JFC).

3.3. Sample size

Based on a review of literature, the minimum sample size was calculated using a prevalence of *E. coli* in meat of 11.7%. This was obtained from studies conducted in Egypt by Moawad *et al.*, (2017) for the proportion of meat contaminated with *E. coli*.

The sample size was derived by the Cochran's formula:

$$n = \frac{Z^2 \times p \times (1-p)}{E^2}$$

n is the minimum sample size;

Z is the Z-score 95% confidence level;

p is the population proportion, and

E is the allowable error.

Using a prevalence of 11.7 %, Z-score of 1.96 at 95% confidence interval and allowable error (E) of 5%, the minimum sample size for the study will be:

$$n = \frac{1.96^2 (0.117) (1-0.117)}{(0.05)^2}$$

$$n = \frac{3.8416 * 0.117 * 0.883}{0.0025}$$

$$n = 159$$

Thus, the minimum sample size required for this study was 159.

3.4. Sampling procedure

The three slaughterhouses were selected purposively, to cater for the varying design of slaughter structures, level of slaughter and livestock species slaughtered. Though cattle, sheep and goats are slaughtered at all three sites, they vary in their distribution. Public and privately owned slaughter sites also differ in the availability of appropriate structures and equipment for livestock slaughter. The sample size was stratified among the slaughterhouses according to the number of livestock slaughtered. Within each slaughterhouse, the sample size was proportionately distributed among the different livestock species. Based on the estimated number of livestock slaughtered in a day at each facility, random numbers were generated and used in the selection of carcasses for sampling.

3.5. Sample collection

All samples were collected after slaughter and dressing of carcasses. Sterile cotton swabs were used to collect surface swabs from the thigh, brisket and flank/mid-loin of carcasses (Herenda et al., 2000) , (Gill et al., 2001). A sterile template was used to define the specific area to be swabbed. Three (3) sites (flank, brisket and upper thigh) each of 100cm² were sampled for all bovine carcasses. Similarly, for goat and sheep carcasses, samples were obtained from three (3) sites (flank, brisket and mid-loin), 25 cm² in area. Swabs were placed in 10ml of Buffered Peptone Water (BPW) in sterile falcon tubes. All samples were transported to the laboratory within 2-4 hours following sample collection on ice, making sure to maintain a temperature between 0-5°C. Samples were processed in the laboratory immediately.

3.6. Inclusion and Exclusion criteria

Samples were obtained from livestock (cattle, sheep and goats) at selected sites that have been passed for slaughter and sale, as determined by a Meat Inspection Officer or Food Veterinarian.

Carcasses that were found not to be wholesome for consumption post evisceration were excluded from the study.

3.7. Data collection and tools

Data was abstracted from slaughter permits available at each slaughterhouse. Using a sample record sheet, data were collected on the type of animal species, breed and sex of slaughtered animal, origin of the animal and date of slaughter. Where data was not available from records, butchers, middle-men and livestock farmers present at slaughter were interviewed.

Using the Ghana FDA guidelines, we collected data on the location of slaughter site, structure of slaughter facility (fencing, roofing, floors), water supply, lighting, ventilation, ante-mortem examination of livestock, postmortem examination of carcasses, availability of carcass carriers and PPE's, the presence of designated areas for slaughter activities, sanitary status of slaughter sites and training for butchers on slaughter hygiene.

3.8. Enumeration of bacteria

Microorganisms contained in samples were enumerated. Briefly, serial dilutions of tenfold units of each sample were plated on Plate Count Agar (OXOID) using the pour plate method. Samples were left to incubate at a temperature of 37°C for a period 24 hours. Following incubation, plates with colonies ranging from 30-300 were counted. This was achieved with the aid of a colony counter. The total coliform count was performed in the same way using Brilliance *E. coli*/ Coliform selective agar (OXOID). To obtain the total count in 1ml of the original sample (CFU/ml) , we multiplied the number of colony forming units on a dilution plate by the corresponding dilution

factor and divided it by the volume plated (Bassiri, 2016). CFU/cm² was calculated by multiplying the CFU/ml by the volume of original sample, and further dividing it by the area sampled.

3.9. Microbial culture and identification

For *E. coli* isolation, samples were pre-enriched in Brain Heart infusion at 37°C for a period of 24 hours. Ten (10µl) microliter of each sample was then plated on MacConkey agar, which is a selective and differential media often used for the isolation of gram-negative organisms from clinical materials, food and water (Zimbro et al, 2009). Identification of *E. coli* was done by colonial morphology and confirmed using MALDI-TOF MS at the Noguchi Memorial Institute for Medical Research. Briefly, part of a colony from fresh overnight cultures was spotted on the target plate for a MALDI-TOF MS. One (1) µl of formic acid was added to it and allowed to dry for 15 min. 1µl of matrix preparation was placed on each sample and left to dry for a further 15 minutes. MALDI-TOF MS was then conducted.

3.10. Antimicrobial susceptibility testing of *E. coli* isolates

The Kirby Bauer method for disk diffusion was used to test the susceptibility of *E. coli*. The procedure consisted of the preparation of an inoculum standardized to 0.5 McFarland and inoculation on Mueller Hinton (MH) agar plates for 18-24 hours (Zimbro et al., 2009). Briefly, a few well isolated colonies from overnight cultures were selected, transferred to a tube with 5ml sterile saline solution and emulsified inside the tube. The inoculum was then adjusted to 0.5 McFarland standard with the aid of a nephelometer (BD). A cotton swab was placed in the inoculum suspension and swirled several times to absorb it. To remove unwanted fluids from the swab, we placed the swab against the side of the tube and applied moderate pressure. The Mueller Hinton agar plate was then streaked. The plate was turned at an angle of 90 degrees following each

round of streaking. Antibiotic disks (Oxoid) were dispensed with the aid of a disk dispenser. The plates were then left to incubate at 37°C overnight. We interpreted the results using guidelines provided by EUCAST (Hudzicki, 2016).

E. coli ATCC 25922 was used to monitor the performance of the test throughout the study. In this study, we defined multidrug resistance to be resistance to three or more antimicrobial agents.

Table 1. Antimicrobial agents and interpretive criteria used for AST in *E.coli* isolated from raw meat

Antimicrobial category	Antimicrobial agent	Concentration (µg/disk)	EUCAST zone diameter breakpoint values	
			S ≥	R <
Penicillins	Ampicillin	10	14	14
Tetracyclines	Tetracycline	30	15	11
	Cefuroxime	30	19	19
Cephalosporins	Cefotaxime	5	20	17
	Ceftriaxone	30	25	22
Aminoglycosides	Amikacin	30	18	15
	Gentamycin	10	17	14
Amphenicols	Chloramphenicol	30	17	17
Fluoroquinolones	Ciprofloxacin	5	25	22
Carbapenems	Meropenem	10	22	16
Folate Inhibitors	Pathway SXT	1.25-23.75	14	11

3.11. Phenotypic detection of ESBL producing *E. coli*

The combination disk method was used to identify isolates suspected of producing ESBL's based on their antimicrobial susceptibility profiles. The inoculum was standardized to a turbidity of 0.5 McFarland prior to inoculation on Mueller Hinton agar plates. Ceftazidime disks and ceftazidime combined with clavulanic acid disks were positioned 30 mm apart on the inoculated plates, and left to incubate overnight at a temperature of 37°C. The zone of inhibition for the disk with ceftazidime was compared to the zone of inhibition for disk with ceftazidime and clavulanic acid combined. Isolates were classified as ESBL positive if the difference in the inhibition zone diameter of ceftazidime combined with clavulanic acid was ≥ 5 mm greater than the diameter of the inhibition zone for the ceftazidime-only disk. Cefotaxime disks and cefotaxime combined with clavulanic acid disks were used concurrently with ceftazidime for confirmation of ESBL production.

3.12. Detection of ESBL- encoding genes by Polymerase Chain Reaction

Table 2. Oligonucleotide primers

Genes	Primer sequences	Expected amplicon size(bp)
TEM-1	Forward: 5'-GAGACAATAACCCTGGTAAAT-3' Reverse: 5'-AGAAGTAAGTTGGCAGCAGTG-3'	459
CTX-M	Forward: 5'-GAAGGTCATCAAGAAGGTGCG-3' Reverse: 5'-GCATTGCCACGCTTTTCATAG-3'	560
SHV	Forward: 5'-GTCAGCGAAAAACACCTTGCC' Reverse: 5'-GTCTTATCGGCGATAAACCCAG-3'	383

Source: (Strommenger et al., 2003),(Ma et al., 2018)

Bacterial DNA extraction consisted of placing two colonies of overnight growth in a test tube containing 1ml of distilled water. The mixture was boiled for 10 minutes and centrifuged for 5 minutes, at 1000 rotations per minute. The supernatant was collected and used for molecular analysis (Dashti, Jadaon, & Dashti, 2009). PCR was used to detect the presence of ESBL resistant genes, based on phenotypic resistance of *E. coli*. Those isolates found to have ESBL production were screened for bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes. PCR was conducted using 2µl of the bacterial lysate as template DNA in a final volume of 25 µl, containing 10mM of each primer, PCR grade water and multiplex PCR master mix (QIAGEN). Multiplex PCR was carried out in a thermal cycler with a first denaturation step at a set temperature of 95°C for a duration of 5 minutes. After this, 35 cycles of amplification was performed with a denaturation step at a set temperature of 95°C for 30 seconds, followed by an annealing step at a temperature of 60°C for 30 seconds, and an extension lap at 72°C for 2 minute. The cycles end with a final extension lap at a temperature of 72°C for 10 minutes.

3.13. Quality control

All culture media were prepared making sure to adhere to guidelines provided by the manufacturer. Guidelines for the appearance of prepared media and the cultural response to known control strains were observed following media preparation. Control strains were run alongside every batch of antimicrobial sensitivity being worked on to ensure accuracy of tests done.

3.14. Data management and analysis

The field and laboratory data collected were entered manually into MS-EXCEL. All data were cleaned and checked for errors and duplication. Descriptive statistics were performed on carriage rates of *E. coli* and their resistance patterns among different animal species. *E. coli* prevalence

among different meat types and slaughter sites were compared using the Chi-squared test. Differences in the level of MDR bacteria between study sites and livestock types were tested for statistical significance using the Chi-squared test. Statistical significance was tested at a p value of 0.05. Data were analyzed using STATA version 15 and the results presented in tables, graphs and charts.

3.15. Ethical Considerations

Approval for this study was obtained from the Institutional Review Board (IRB) at Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana. Permission was obtained from relevant authorities in the animal health sector to enable sampling from slaughter sites and access to slaughter records. Slaughterhouse authorities were free to decide not to participate if they were not comfortable with the sampling procedure.

Participation in this study was voluntary for butchers and staff at the selected slaughter sites. Sample collection from carcasses in this study was carried out with consent from butchers and livestock owners present at slaughter. The sampling methods and periods adopted in this study did not disrupt the slaughter process at all selected sites. Data were only used for the purpose of this study and the names and origin of animals for slaughter were kept confidential.

CHAPTER FOUR

4. RESULTS

4.1. Descriptive characteristics of livestock slaughtered

Of the two hundred and five (205) samples collected, 108 (52.7%) were collected from goats, 81(39.5%) from cattle and 16 (7.8%) from sheep. Cattle, sheep and goats for slaughter were received from parts of the Greater Accra, Upper East and Northern regions. Occasionally, goats and sheep for slaughter came from backyard farms in Tema, Ashaiman and Madina in the Greater Accra region.

Table 3. Number and type of livestock sampled from each slaughter site

Livestock type	Slaughterhouses			Total N=205
	TSH n=63, (30.7%)	TSS n=79 (38.6%)	JFC n=63 (30.7%)	
Cattle	54	24	3	81
Sheep	-	9	7	16
Goats	9	46	53	108
Total	63	79	63	205

TSS- Tema slaughterhouse, TSS- Tulaku slaughter slab, JFC- Private slaughter facility

The West African Short Horn (WASH) and Sanga breeds were the most predominant breeds of cattle slaughtered. The Sahelian and West African Dwarf (WAD) breeds were the most predominant breeds of sheep and goats slaughtered. Most of the samples (187/205, 91%) were obtained from singed skin-on-meat with only a few (18/205, 9%) samples obtained from meat without the skin (flayed carcasses). All three sites were found to be points of meat sale although meat was mostly conveyed to markets and cold stores in the Greater Accra Region.

4.2. Enumeration of microorganisms in surface swab samples from meat

Table 4. Microbiological quality of the raw meat samples from selected slaughterhouses

Slaughterhouse	Mean count (log CFU/cm ² ±SD)	
	Viable count (total)	Coliform count (total)
TSH	5.20 ± 0.77	3.12±0.2
TSS	5.14 ± 1.0	3.87±0.7
JFC	4.79 ± 1.4	0.88±1.68
Total (all sites)	5.03±1.06	1.93±2.0

TSS- Tema slaughterhouse, TSS- Tulaku slaughter slab, JFC- Private slaughter facility

Of the 205 surface swab samples analysed from raw meat, the mean total viable count was 5.03 (±1.06) log CFU/cm². This value exceeds the limit of 5.0 log CFU/cm², as determined by International Standards Organization (ISO) guidelines for total viable microbial counts for cattle, sheep and goat carcasses. High total viable counts were observed in samples from TSH and TSS (5.20 ± 0.77 and 5.14 ± 1.0). The mean total viable count was the lowest for samples from JFC (4.79 ± 1.4). Overall, 124 (60%) surface swab samples from raw meat in this study exceeded the limit of 5.0 log CFU/cm², for total viable counts for cattle, sheep and goat carcasses. Most of the samples from TSH (52/63, 82.5%) exceeded the limits for the total viable counts. More than half of the samples from TSS (46/79, 58.2%) exceeded the limit for the total viable counts. Close to half of the samples from JFC (26/63, 41.2%) exceeded the limit for the total viable counts.

The mean total coliform count in this study was 1.93±2.0 log CFU/cm². While this value did not exceed the limit of 2.5 log CFU/cm², as determined by ISO guidelines for Enterobacteriaceae, high counts were reported at TSH and TSS (3.12±0.2 and 3.87±0.7). The mean total coliform count in this study was lowest in samples from JFC (0.88±1.68). Overall, 145 (70.7%) surface swab samples from raw meat in this study exceeded the limit of 2.5 log CFU/cm², for Enterobacteriaceae counts for cattle, sheep and goat carcasses. Most of the samples from TSS (76/79, 96.2%) exceeded

the limit for enterobacteriaceae counts. Similarly, most of the samples from TSH (55/63, 87.3%) exceeded the limits for enterobacteriaceae counts. One in five samples from JFC (14/63, 22.2%) exceeded the limit for the total viable counts.

Table 5. Microbiological quality of the different types of meat

Meat type	Mean count (log CFU/cm ² ±SD)	
	Viable count (total)	Coliform count (total)
Beef	5.08 ± 0.74	3.06±1.02
Chevon	5.01 ± 1.16	4.02±0.49
Mutton	5.02 ± 0.06	3.09±0.67
Total (all sites)	5.03±1.06	1.93±2.0

Note: Counts for samples from mutton are not included

High total viable counts, exceeding the limit of 5.0 log CFU/cm², as determined by ISO guidelines were observed for beef and chevon in this study (5.08 ± 0.74 and 5.01 ± 1.16). Similarly, counts for Enterobacteriaceae in beef and chevon, exceeded the limit of 2.5 log CFU/cm², determined by ISO guidelines.

4.3. Distribution of *E. coli* in meat samples

Table 6. Frequency of *E. coli* from raw meat

Slaughter sites	TSH (n=63)	TSS (n=79)	JFC (n=63)	Total (N=205)
No. of <i>E. coli</i> isolates	50 (79%)	42 (53%)	6 (9.5%)	98 (48%)
Cattle	42 (84%)	8 (19%)	-	50 (51%)
Sheep	-	3 (7%)	1 (17%)	4 (4%)
Goat	8 (16%)	31 (74%)	5 (83%)	44 (45%)

TSS- Tema slaughterhouse, TSS- Tulaku slaughter slab, JFC- Private slaughter facility

The overall prevalence of *E. coli* in raw meat was 48% (98/205). The highest level of contamination was detected at TSH and was found to be 79% (50/63). We recovered *E. coli* from more than half of raw meat from TSS (53%, 42/79). Only one in ten samples from JFC (9.5%, 6/63) had *E.coli* contamination. Of the livestock sampled, a high proportion of *E. coli* was found in raw meat from cattle (51%, 50/98). A lower proportion of *E. coli* was obtained from raw meat from goat (45%, 44/98) and sheep (4%, 4/98).

Significant differences were observed for *E. coli* prevalence by slaughter facility ($p<0.05$). The frequency of *E. coli* recovered from TSH samples was found to be higher in comparison to TSS and PSH. The frequency of *E. coli* recovered from samples obtained from cattle was found to differ significantly among sheep and goats ($p<0.05$).

Table 7. Frequency of other bacterial species recovered from the raw meat samples

Microorganisms	Number of isolates			
	TSH	TSS	JFC	Total
<i>Acinetobacter baumannii</i>	2	17	-	17
<i>Klebsiella pneumonia</i>	3	-	-	3
<i>Plesiomonas shigelloides</i>	1	-	-	1
<i>Enterobacter aerogenes</i>	-	1	-	1
<i>Pseudomonas aeruginosa</i>	1	-	-	1
<i>Serratia marcescens</i>	2	-	-	2
<i>Citrobacter freundii</i>	8	1	-	9
<i>Citrobacter braakii</i>	2	-	-	2
<i>Citrobacter youngae</i>	1	-	-	1
<i>Aeromonas caviae</i>	3	-	-	3
Total	23	19	-	40

TSS- Tema slaughterhouse, TSS- Tulaku slaughter slab, JFC- Private slaughter facility

A proportion of samples without *E. coli* contamination (40/205, (19.5%)) were found to have other bacterial contaminants such as *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Plesiomonas shigelloides*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Citrobacter spp.* Of this number, more than half (23/40, 57.5%) were found in samples from TSH. Similarly, 19 (47.5%) of such isolates were found in samples from TSS. *Acinetobacter baumannii*

was the most common pathogen found in samples from TSS contaminated with bacteria other than *E. coli*.

4.4. Antimicrobial susceptibility of *E. coli* isolates

4.4.1. Distribution of antimicrobial resistance among *E. coli* isolates

E. coli isolates showed resistance mainly to the penicillin, tetracycline, second- generation cephalosporin class of antibiotics and folate pathway inhibitors.

Table 8. Antimicrobial resistance of *E. coli* isolates from raw meat at slaughterhouses

Antimicrobial agent	TSH n=50 (%)	TSS n=42, (%)	JFC n=6 (%)	Total N=98, (%)
Ampicillin	23 (46)	33 (79)	0(0)	56 (57)
Tetracycline	26 (52)	18 (43)	0(0)	44 (45)
Cefuroxime	8 (16)	13 (30)	0(0)	21 (21)
Sulphamethoxazole/Trimethoprim	10 (20)	7 (16)	0(0)	17 (17)
Amikacin	2 (4)	6 (14)	0(0)	8 (8)
Ciprofloxacin	4 (8)	3 (7)	1(16)	8 (8)
Cefotaxime	1(2)	1(2)	0(0)	2 (2.1)
Gentamycin	2(4)	1(2)	0(0)	3 (3.1)
Chloramphenicol	2(4)	1(2)	0(0)	3 (3.1)
Ceftriaxone	0(0)	1(2)	0(0)	1 (1)
Meropenem	0(0)	0(0)	0(0)	0 (0)

n= No. of isolates, TSS- Tema slaughterhouse, TSS- Tulaku slaughter slab, JFC- Private slaughter facility

Resistance to ampicillin was most common in 57% of the isolates tested. The isolates also showed resistance to tetracycline (44%), cefuroxime (21%) and sulphamethoxazole /Trimethoprim (17%).

High susceptibility was observed for the carbapenem, third-generation cephalosporin,

aminoglycoside and quinolone class of antibiotics. High susceptibility was observed for meropenem (100%). High rates of susceptibility were observed for ceftriaxone (99%), cefotaxime (98%), chloramphenicol (97%), gentamycin (97%), ciprofloxacin (92%) and amikacin (92%).

Isolates from TSH showed resistance for nine out of the eleven antimicrobial agents. For these isolates, high rates were observed for tetracycline (52%), ampicillin (46%) and sulphamethoxazole/trimethoprim (20%). Resistance to ten out of eleven antimicrobial agents tested was observed for *E. coli* isolates from TSS. The highest rate of resistance in isolates from TSS was observed for ampicillin (79%), followed by tetracycline (43%) and cefuroxime (30%). Resistance was observed only to ciprofloxacin (16%) for *E. coli* isolates from JFC. The proportion of isolates with resistance to ampicillin was significantly greater in meat from TSS in comparison to meat from TSH ($p < 0.05$). The proportion of *E. coli* showing resistance to tetracycline between the study sites did not differ significantly. Similarly, the differences in the rates of resistance to sulphamethoxazole/trimethoprim and cefuroxime for all study sites were not significant. *E. coli* isolates from all study sites were highly susceptible to meropenem, ceftriazone, cefotaxime, gentamycin and chloramphenicol.

E. coli isolates obtained from beef were resistant to ten out of the eleven antimicrobial agents tested. The highest rate of resistance in isolates from beef was observed for ampicillin (56%), followed by tetracycline (50%) and cefuroxime (26%). Resistance to eight out of eleven antimicrobial agents tested was observed for *E. coli* recovered from chevon.

4.4.2. Distribution of resistance in *E. coli* isolates by meat type**Table 9. Antimicrobial resistance profile of *E. coli* isolates from beef, mutton and chevon**

Antimicrobial agent	Beef	Mutton	Chevon	Total
	n=50, (%)	n=4, (%)	(n=44, (%)	N=98, (%)
Ampicillin	28 (56)	2(50)	26(59)	56(57)
Tetracycline	25 (50)	2(50)	17 (39)	44(45)
Cefuroxime	13 (26)	2(50)	6 (14)	21(21)
Sulphamethoxazole/Trimethoprim	9 (18)	1(25)	7 (16)	17(17)
Amikacin	6 (12)	0(0)	2 (5)	8 (8)
Ciprofloxacin	4 (8)	0(0)	4 (9)	8(8)
Cefotaxime	2 (4)	0(0)	0(0)	2(2)
Gentamycin	2 (4)	0(0)	1 (2)	3(3)
Chloramphenicol	2 (4)	0(0)	1 (2)	3(3)
Ceftriazone	1 (2)	0(0)	0(0)	1(1)
Meropenem	0(0)	0(0)	0(0)	0(0)

n= No. of *E. coli* isolates,

E. coli from chevon was observed to show resistance to ampicillin (59%), followed by tetracycline (39%), cefuroxime (14%) and sulphamethoxazole/trimethoprim (16%). Similarly, isolates from mutton exhibited resistance to ampicillin (50%), tetracycline (50%), cefuroxime (50%) and sulphamethoxazole/trimethoprim (25%). The differences observed in the proportion of *E. coli* resistant to all antimicrobial agents tested among the different meat types were found not to be significant.

4.4.3. Multidrug resistance rates of *E. coli* recovered from raw meat

Of the ninety-nine *E. coli* isolates tested, 84% (82/98) showed resistance to at least one out of the eleven antimicrobial agents. Of this number, 42% (41/98) showed resistance primarily to one antimicrobial agent only, 19% (19/98) were resistant to two antimicrobial agents, 13% (13/98) were resistant to three antimicrobial agents, 5% (5/98) were resistant to four antimicrobial agents and 4% (4/98) were resistant to five antimicrobial agents. In all, 22% (22/98) of the isolates were multidrug resistant (MDR) as they were resistant to three or more antimicrobial agents. The highest frequency of MDR was found in isolates from beef (13 isolates) and was followed by chevon (7 isolates) and mutton (2 isolates).

Table 10. Distribution of multi-resistance among *E. coli* by meat type

No. of antibiotics resistant	Beef (n=50, %)	Mutton (n=4, %)	Chevon (n=44, %)	Total (N=98, %)
0	9(18)	1(25)	6(14)	16(16)
1	17(34)	1(25)	23(52)	41(41)
2	11(22)	0(0)	8(18)	19(19)
3	4(8)	2(50)	7(16)	13(13)
4	5(10)	0(0)	0(0)	5(5)
≥5	4(8)	0(0)	0(0)	4(4)
MDR	13(26)	2(50)	7(16)	22(22)

n= No. of *E. coli* isolates

MDR was high in isolates from TSS (13 isolates) and was followed by isolates from TSH 9 (7 isolates). No MDR was seen in isolates obtained in raw meat from JFC in this study. The differences in the rate of MDR in *E. coli* from was not statistically significant for slaughter sites and livestock type sampled.

Table 11. Distribution of multi-resistant *E. coli* from raw meat from slaughter facilities

No. of antibiotics resistant	TSH (n=50, %)	TSS (n=42, %)	JFC (n=6, %)	Total (N=98, %)
0	11(22)	1(2)	4(67)	16(16)
1	20(40)	19(45)	2(33)	41(41)
2	8(16)	11(26)	0(0)	19(19)
3	3(6)	10(24)	0(0)	13(13)
4	3(6)	1(2)	0(0)	4(4)
≥5	3(6)	2(5)	0(0)	5(5)
MDR	9(18)	13(31)	0(0)	22(22)

n= Number of *E. coli* isolates, TSS- Tema slaughterhouse, TSS- Tulaku slaughter slab, JFC- Private slaughter facility

4.4.4. Pattern of multidrug resistance in *E. coli* isolates from raw meat

The resistance pattern comprising the penicillin, tetracycline and sulphonamide/trimethoprim class of antibiotics (32%) was most common. Other patterns seen were resistance to penicillin, tetracycline, SXT, and fluoroquinolone class of antibiotics (14%), penicillin, tetracycline and cephalosporin class of antibiotics (14%), penicillin, tetracycline and amphenicols (10%) and penicillin, aminoglycoside, SXT, tetracycline and cephalosporin (10%). Most of the MDR patterns observed in the *E. coli* isolates (95%) showed resistance to penicillin and tetracycline class of antibiotics in addition to other antimicrobial classes. A high number of MDR isolates also showed resistance to penicillin, tetracycline and SXT class of antibiotics (61%) in addition to other antimicrobial classes.

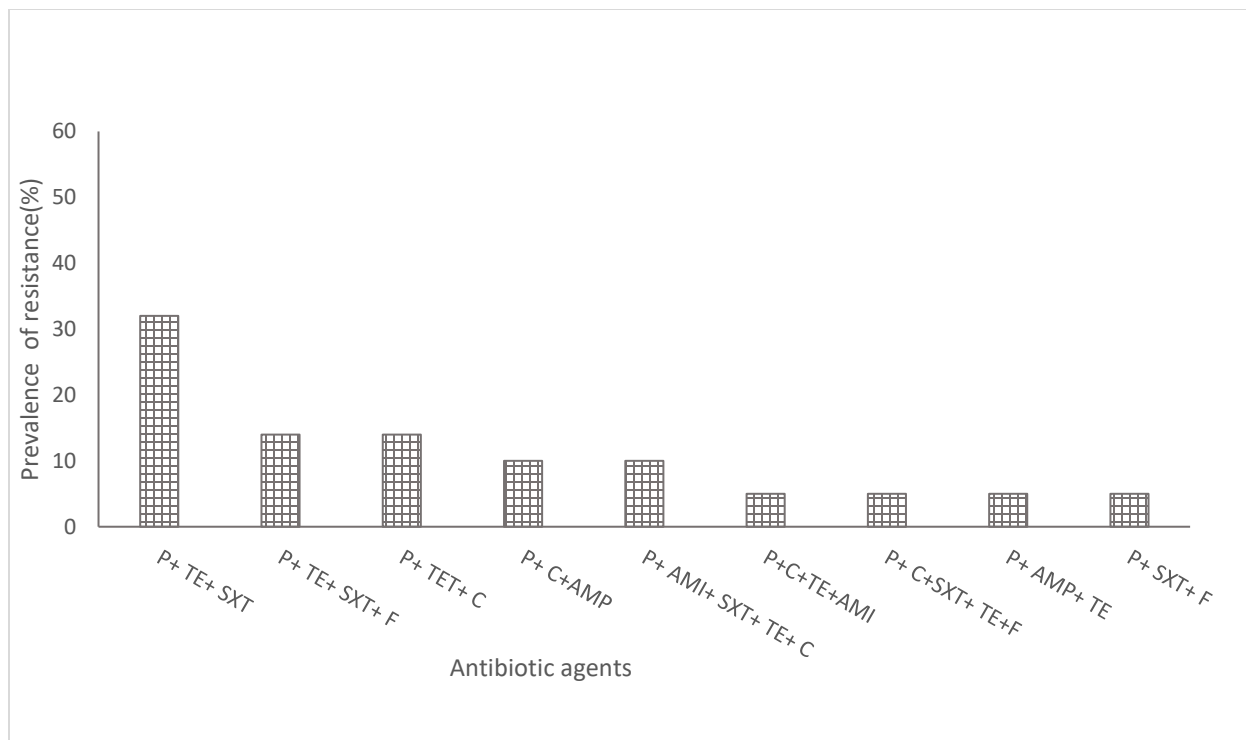


Figure 3. Distribution of resistant patterns of *E. coli* isolates against ≥ 3 antibiotic classes

P=Penicillin, TE= Tetracycline, SXT=Sulphamethoxazole/ Trimethoprim, AMI= Aminoglycoside, C=Cephalosporin, F=Quinolones/Fluoroquinolones, AMP=Amphenicol).

E. coli isolates in this study showed variability in their resistance patterns to the 11 antimicrobial drugs used for susceptibility testing for each slaughter facility. Resistance to only one antimicrobial drug occurred mostly to ampicillin (56%), tetracycline (22%), cefuroxime (17%) and ciprofloxacin (5%). Resistance to ampicillin and cefuroxime was common in isolates from both TSH and TSS. Resistance of *E. coli* isolates to tetracycline only was found primarily in isolates from TSH.

While a proportion of the resistance pattern to two antibiotics were common among isolates from both TSH and TSS, some patterns were found only at one slaughter site. Resistance of a single isolate to ampicillin, tetracycline, SXT and ciprofloxacin was prevalent only at TSH. Similarly,

resistance of a single isolate to ampicillin, amikacin, SXT, Tetracycline and cefuroxime was prevalent only at TSS.

Table 12. Distribution of *E. coli* antibiotypes by slaughter facility

Antibiotypes (Resistance patterns)	Number of isolates for each slaughter site		
	TSH	TSS	JFC
Ampicillin	11	12	-
Tetracycline	9		
Cefuroxime	3	4	-
Ciprofloxacin		1	1
Ampicillin+ cefuroxime	2		
Ampicillin+ tetracycline	3	5	-
Ampicillin+ SXT		3	
Ampicillin+ ciprofloxacin		1	-
Tetracycline + cefuroxime	1	1	-
Tetracycline + SXT	1		
Gentamycin+ cefuroxime		1	-
Amikacin+ cefuroxime		1	-
Ampicillin+ tetracycline+ SXT	3	5	-
Ampicillin+ tetracycline + chloramphenicol		1	-
Ampicillin+ tetracycline+ cefuroxime		3	-
Ampicillin+ SXT+ ciprofloxacin		1	-
Ampicillin+ cefuroxime+ chloramphenicol+ cefotaxime	1		-
Ampicillin+ tetracycline+ SXT+ ciprofloxacin	3		-
Ampicillin+ chloramphenicol+ cefuroxime+ ceftriaxone		1	-
Ampicillin+ cefuroxime+ amikacin+ gentamycin+ tetracycline	1		-
Ampicillin+ cefuroxime +SXT+ tetracycline+ ciprofloxacin	1		-
Ampicillin+ amikacin+ SXT+ tetracycline+ cefuroxime		2	-

TSS- Tema slaughterhouse, TSS- Tulaku slaughter slab, JFC- Private slaughter facility

4.5. Prevalence of ESBL producing bacteria and resistance genes

Out of the 98 *E. coli* isolates recovered from raw meat in this study, 14 isolates were suspected to have ESBL genes based on their phenotypic resistance patterns and results of the combination disk test. Out of these, four (4) *E. coli* isolates were found to have the *TEM* gene. These four isolates were multidrug resistant with most of them (3/4) showing phenotypic resistance to ampicillin, amikacin, tetracycline, SXT and cefuroxime. *CTX-M* and *SHV* genes were not found in any of the isolates found to be ESBL producers.

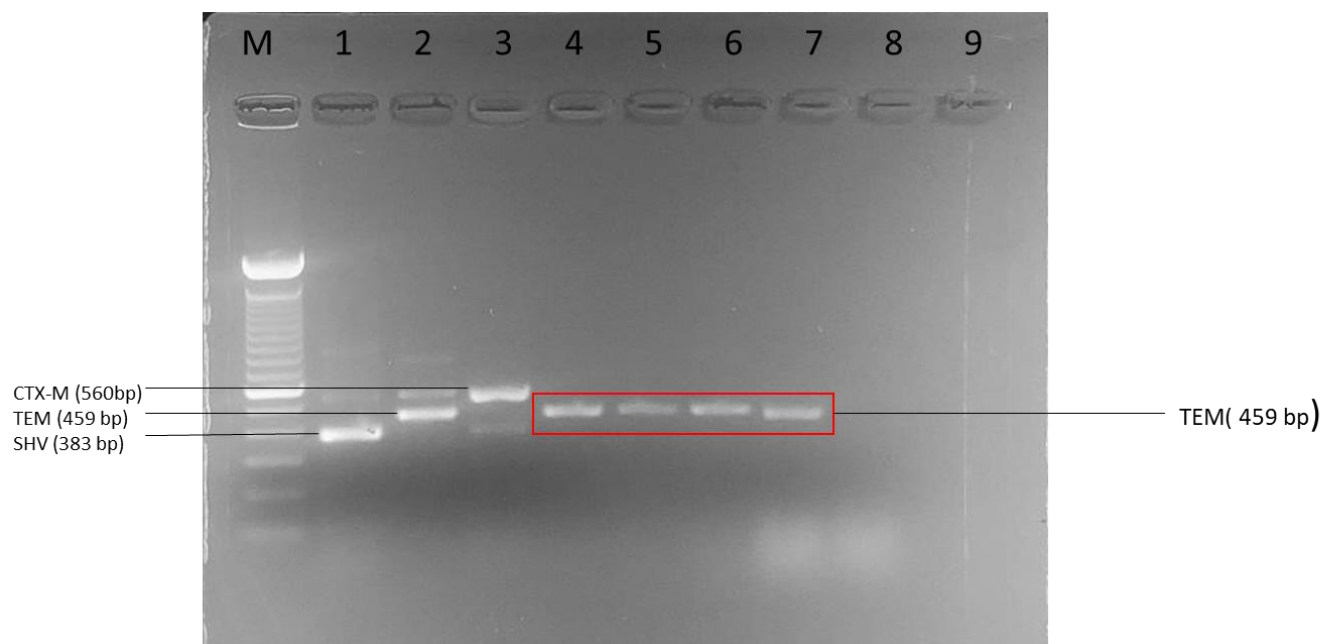


Figure 4. Gel electrophoresis image showing bands of PCR product (target genes)

M: Molecular marker

No. 1-3: Positive controls for *CTX-M*, *TEM* and *SHV* genes

No. 4-7: *E. coli* isolates suspected to be ESBL producers

TEM, CTX, SHV- Beta lactamase genes

4.6. Assessment of slaughterhouse structure, practices and environment

The observational assessment of slaughter facilities showed that TSH and JFC had better slaughter infrastructure as compared to TSS. TSH was found to have good fencing and an ample supply of portable water sourced through pipes available at the facility. Lighting and ventilation mechanisms at TSH met the standards set for slaughter facilities by the Ghana Food and Drugs Authority (FDA). TSH has an efficient waste disposal system for both solid and waste. The internal design of TSH including the walls, floors and ceiling were also suited for easy cleaning and disinfection. There was no clear demarcation between clean and dirty areas within TSH. Livestock presented for slaughter were kept temporarily in a holding area made up of a fenced area on the premises with no flooring or roofing. Examination of carcasses and offal for pathological lesions was conducted routinely at TSH. Most of the livestock for slaughter at JFC were transported from a holding farm in close proximity to the slaughterhouse. JFC had adequate lighting and ventilation, an ample supply of portable water and efficient waste disposal system. It had a clear demarcation between clean and dirty areas. Records on slaughter activities were available at this facility

TSS was found to have no appropriate structure for slaughter activities. Located close to the Tulaku livestock market, large numbers of people were seen moving through the premises during the observational assessment. Livestock were slaughtered, dressed and sold by different groups of butchers concurrently. Slaughter of cattle, sheep and goats was done in the open, and under wooden sheds available on the premises. Portable water supply for slaughter activities was not adequate and no drainage systems were seen at this site.

All three slaughter sites had no carcass carriers for moving carcasses between the different sections of the facility. Staff were seen carrying carcasses, including large cuts of beef on their bodies, or using uncleaned wheelbarrows. At all three facilities, cattle were slaughtered and dressed on the

floor. Small ruminants such as sheep and goats were dressed on tables and had minimal contact with the slaughter environment as compared to cattle. Butchers at all three slaughter sites were seen working without PPE's during the entire study period. Records on livestock for slaughter and daily slaughter process at TSS and TSH were not available. There were no reports of regular training for butchers on slaughter hygiene at all three slaughter sites.

CHAPTER FIVE

5. DISCUSSION

Microbial quality of meat and contamination with *E. coli*

In this study, 60% (124/205) of raw meat samples had total viable counts exceeding the limit of 5.0 log colony forming units(CFU) per cm², for cattle, sheep and goat carcasses set by the Ghana Standards Authority based on Codex guidelines (Ghana Standards Authority, 2018). Total viable bacterial counts were found to be particularly high at TSH and TSS, and for all meat types. This is significantly higher than counts observed in a similar study on meat from retail shops in India in the year 2013(Kumar et al., 2014). Similarly, high counts were reported at TSH and TSS (3.12±0.2 and 3.87±0.7) for Enterobacteriaceae with the lowest counts recorded in samples from JFC (0.88±1.68). The high counts at TSH and TSS may be explained by poor lairage (holding) conditions and slaughter hygiene at these slaughterhouses. JFC premises was found to have a better sanitary status, which may explain the low counts observed. High microbial counts in foods pose a public health threat to consumers as they may become exposed to pathogens that can cause disease in humans.

The proportion of raw meat found to have *E. coli* in this study (48%) was greater than that observed in a similar study in Ethiopia where a prevalence of 16% was observed for *E. coli* in meat (Feyera, 2014). The proportion of raw meat contaminated with *E. coli* from the selected slaughter sites varied in this study (9.5-79%). According to previous reports from northern Ghana, the proportion of *E. coli* in beef at different locations ranged from zero to 100% (Adzitey, 2015). The detection of *E. coli* in raw meat is widely attributed to contamination of with faeces from livestock due improper handling during the slaughter process. The differences observed in the proportion of *E. coli* in raw meat among slaughter facilities may be attributed to poor sanitary conditions at

slaughter sites. TSS samples had a lower proportion of *E. coli* than TSH due to the presence of a high number of other contaminants such as *Acinetobacter baumannii*. This finding is of critical concern to human health as *Acinetobacter* spp. have been reported in healthcare settings as a causative pathogen for nosocomial infections, causing significant morbidity and mortality (Scales, 2006). The proportion of *E. coli* in meat poses a challenge for consumers as they may cause major foodborne outbreaks, with increasing mortality rates (CDPH, 2015).

Antimicrobial resistance patterns of *E. coli* isolates

E. coli is widely used as an indicator of antimicrobial resistance of bacteria in a community (Pessoa-Silva, 2018). Most of the *E. coli* isolates (57%) in this study were resistant to ampicillin. This was found to concur with the level of ampicillin resistance of *E. coli* isolated from humans, different livestock species and food products globally. High levels of ampicillin resistance in *E. coli* isolates were reported in chicken meat (78%) in Saudi Arabia (Gherbawy et al, 2009), fermented milk (100%) in Nigeria (Reuben et al, 2013) and in pig carcasses (91%) in Thailand and Cambodian provinces (Trongjit et al, 2016). In Ghana, high levels of resistance of *E. coli* isolates to ampicillin have been found in food products such as cabbage (Adzitey, 2018). Ampicillin resistance was reported to be 96.7% in healthy cattle in Nigeria (Okunlade et al., 2013). High rates of ampicillin have been observed for *E. coli* isolates from humans. Isolates from healthy adult patients in a psychiatric hospital in Ghana were found to be 100% resistant to ampicillin.

The high levels of resistance to ampicillin observed may indicate a general pattern of its abuse across different sectors. Ampicillin has a broad spectrum of activity and is commonly used in combination with aminoglycosides for the treatment of meningitis and sepsis. The rates of ampicillin resistance observed for *E. coli* isolates in this study have implications for the treatment of infections such as pneumonia, gonorrhoea and meningitis (Scales, 2006) .

In this study, resistance were observed similarly for tetracycline (44%), cefuroxime (21%) and found to be 17% for sulphamethoxazole/trimethoprim (SXT). *E. coli* isolates from beef in Techiman have been reported to be resistant to tetracycline (44%) and SXT (18%)(Adzitey, 2015). Similar isolates from livestock in northern Ghana were found to have high levels of resistance to tetracycline (16-74%) depending on the livestock type (Saba, 2019). Blake et al, found that tetracycline exposure in the livestock production chain was a major determinant of the expression of tetracycline resistant genes in *E. coli* recovered from healthy livestock (Blake et al., 2003). Tetracycline is commonly used in the livestock production chain in Ghana for treatment of systemic and local infections. Suboptimal doses of tetracycline are used in intensive farming systems for growth promotion. Livestock farmers in Ghana have reported overuse of injectable tetracycline for the management of skin conditions and respiratory infections (Sekyere, 2015). The levels of cefuroxime resistance observed for *E. coli* in this study (21%) has similarly been reported in patients with urinary tract infections (15.3%) in Malaysia (Shafina et al., 2015), but differed from isolates recovered from patients at the Komfo Anokye Teaching Hospital (79%) in Ghana (Feglo et al, 2016). The rates of cefuroxime resistance observed have implications for the treatment of upper and lower respiratory tract infections, urinary tract infections and uncomplicated skin and soft tissue infections (Scales, 2006).

The rates of SXT resistance observed in this study (17%) were higher than the level of SXT resistance reported in livestock populations in Uganda (9%). They were however lower than the prevalence in livestock attendants in the same study (Madoshi et al., 2016).

The occurrence of SXT resistance in commensal *E. coli* poses a challenge for clinical therapy in the case of urinary tract infections. The higher levels of resistance in meat products as compared to healthy livestock may highlight the need to investigate other sources of meat contamination

other than fecal coliforms from the gut of livestock. While the rates of resistance of *E. coli* from meat from different slaughterhouses did not differ significantly for tetracycline, cefuroxime and SXT, the higher prevalence of ampicillin resistance at TSS may point to diversity of *E. coli* strains and their tendency to persistence at slaughter sites due to poor sanitary conditions.

High susceptibility rates were observed for meropenem, ceftriaxone, chloramphenicol and gentamycin in this study. Susceptibility of the isolates to carbapenems reinforces their use for therapy in the case of life -threatening infections. Third generation cephalosporins and phenicols are not commonly used for livestock in Ghana (Sekyere, 2014).

Majority of the *E. coli* isolates (84%) showed phenotypic resistance to at least one antimicrobial drug, while 4% of the isolates were resistant to five or more antimicrobial drugs. The MDR rate of 22% observed in this study was less than MDR rates reported in *E. coli* in beef and clinical samples in Ghana (Adzitey, 2015). This differs from studies conducted in Egypt where MDR rates in food products were significantly higher than MDR rates from clinical specimen in humans (Aly et al., 2012) . The differences in the rates of MDR observed were not significant for livestock type or slaughter facility in this study. The development of MDR in commensal *E. coli* may not vary much across different geographic areas in Ghana, as antibiotic misuse in livestock and humans is widespread (Newman et al., 2015). Furthermore, unhygienic slaughter and sale conditions exacerbate the occurrence of MDR in at these sites as *E. coli* has the ability to exchange genetic material readily with other bacteria.

The most common pattern of MDR found in this study was ampicillin, tetracycline and SXT. This pattern has been reported in previous studies on MDR in *E. coli* (Sanchez et al., 2002). Among SXT resistant *E. coli* isolates of animal origin, ampicillin and tetracycline have been identified in previous studies as common co-transferred resistant phenotypes (Tadesse et al., 2012). Some of

the patterns observed among isolates were unique for the each slaughter site. This highlights the role that slaughter site-specific factors play in the development of resistance in bacteria.

Prevalence of ESBL-producing *E.coli* and presence of resistance genes

The number *E. coli* reported to produce ESBL in this study (4%. 4/98) is lower than the prevalence reported in studies on ESBL producing bacteria from clinical isolates in Ghana (Tetteh, 2015). High rates of ESBL found in these studies were attributed to the persistence of ESBL producing bacteria in health care settings, causing them to become multidrug resistant over time. The proportion of ESBL producing *E. coli* found, was also lower than the prevalence reported in livestock in Madagascar. In this study, four isolates were found to have the *TEM* gene. This differs from previous studies in Ghana and India where *E. coli* that were ESBL producers had the CTX gene (Warjri et al, 2013).

Risk factors for occurrence of multidrug resistant strains of bacteria in meat

In Ghana, laws guiding the access and use of antimicrobial drugs in the livestock sector are not well established. Antibiotics are dispensed to farmers and other livestock care givers without any form of regulation (Yevutsey et al., 2017). Poor prescribing practices in both human and animal health systems further compound the problem of antibiotic use and abuse (Lowe, 2016). The ongoing trend of misuse in livestock production systems contributes immensely to antibiotic selective pressure and development of resistant mechanisms by commensal bacteria in livestock.

Two out of three slaughter sites in this study are lacking in slaughterhouse design, structure and practices necessary to maintain food safety. These finding are similar to assessment of slaughter sites conducted in Nigeria (Andrea et al, 2012). Locations with better structure, hygiene standards and good slaughter practices had a lower levels of *E. coli* and this was previously reported in

studies in Ghana (Adzitey, 2015). The incidence of MDR was more prevalent in sites with a high prevalence of bacteria of clinical importance such as *E. coli*, *Klebsiella Pneumonia* and *Acinetobacter* species. JFC had better slaughter structures and better hygiene as compared to TSS and TSH. Consequently, a low proportion of samples with *E. coli* and no MDR was detected among isolates from this site. This study shows that sanitary conditions at slaughter sites must be monitored routinely, to minimize bacterial contaminants, and more importantly, prevent the exposure of consumers to multidrug resistant strains of bacteria.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The study findings showed that raw meat samples from selected slaughterhouses in the Greater Accra Region had microbial loads exceeding the limits set by both local and international food safety organizations. This study detected *E. coli* in 48% of raw meat from the selected slaughterhouses. It also showed that, *E. coli* in meat from the different slaughterhouses differed significantly and ranged from 9.5%-79%. Multidrug resistance was seen in 22% of the isolates in this study. Resistance to ampicillin, tetracycline and sulphamethoxazole/trimethoprim was found to be the most common MDR pattern observed. A total of four ESBL producing-*E. coli* were found to have the *TEM* gene. Poor sanitary conditions and slaughter practices at some slaughter sites in this study may have contributed to the levels of MDR *E. coli* observed.

These findings highlight the urgent need to adopt practices tailored at reducing the transfer of AMR bacteria through food.

6.2. RECOMMENDATIONS

Food safety authorities should:

1. Ensure that slaughterhouses comply with standards prescribed by the FDA in Ghana for slaughterhouse design, equipment and operations.
2. Conduct regular training for butchers, and other slaughter site workers on proper meat hygiene, zoonosis and sanitary practices.
3. Conduct rigorous epidemiological studies to determine the full scope of meat contamination at slaughter sites

Animal health authorities should:

4. Educate middlemen involved in the transportation of livestock for slaughter on good practices for reducing microbial shedding during transport

REFERENCES

- Adesokan, H. K. (2014). Safe meat-handling knowledge , attitudes and practices of private and government meat processing plants ' workers : implications for future policy, 10–16.
- Adzitey, F. (2015a). Antibiotic Resistance of Escherichia coli Isolated from Beef and its Related Samples in Techiman Municipality of Ghana. *Asian Journal of Animal Sciences*, 9(5), 233–240. <https://doi.org/10.3923/ajas.2015.233.240>
- Adzitey, F. (2015b). Prevalence of Escherichia coli and Salmonella spp . in Beef Samples Sold at Tamale Metropolis , Ghana. *International Journal of Meat Science*, 5(1), 8–13. <https://doi.org/10.3923/ijmeat.2015.8.13>
- Adzitey, F. (2018). Antibiotic resistance of Escherichia coli and Salmonella enterica isolated from cabbage and lettuce samples in Tamale metropolis of Ghana.
- Aidarose, H. (2015). Contamination of beef carcasses during slaughtering in two Egyptian Slaughterhouses.
- Ali, N. H., Farooqui, A., Khan, A., Khan, A. Y., & Shahana, U. (2010). Original Article Microbial contamination of raw meat and its environment in retail shops in.
- Alum. (2016). Microbiological Contamination Of Food: The Mechanisms, Impacts And Prevention. *International Journal of Scientific & Technology Research*, 5(3), 65–78.
- Aly, M. E. A., Essam, T. M., & Amin, M. A. (2012). Antibiotic Resistance Profile of E . coli Strains Isolated from Clinical Specimens and Food Samples in Egypt, 3(3), 176–182. <https://doi.org/10.5829/idosi.ijmr.2012.3.3.663>
- Andoh, L. A., Dalsgaard, A., & Newman, M. J. (2016). Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry in Ghana, 3288–3299. <https://doi.org/10.1017/S0950268816001126>
- Andrea, P., & Aburi, S. (2012). Assessment of Hygiene practices used by Small Butchers and Slaughter Slabs in beef value chain in Juba town-South Sudan. Research Project. Van Hall Larenstein University of Applied Science.
- Bassiri, E. (2016). Microbiology BIOL 275, Enumeration of bacterial pathogens. 1–10.

- Blake, D. P., Humphry, R. W., Scott, K. P., Hillman, K., Fenlon, D. R., & Low, J. C. (2003). Influence of tetracycline exposure on tetracycline resistance and the carriage of tetracycline resistance genes within commensal *Escherichia coli* populations, (Acmsf 1999), 1087–1097.
- Boamah, V., & Agyare, C. (2016). Antibiotic Practices and Factors Influencing the Use of Antibiotics in Selected Poultry Farms in Ghana. *Journal of Antimicrobial Agents*, 2(2). <https://doi.org/10.4172/2472-1212.1000120>
- Boyer, R. (2015). Common Foodborne Pathogens : *E . coli* O157 : H7, 2015.
- Camargo, A., Cossi, M., Silva, W., Bersot, L., Landgraf, M., Baranyi, J., ... Luís Augusto, N. (2019). Microbiological Testing for the Proper Assessment of the Hygiene Status of Beef Carcasses. *Microorganisms*, 7(3), 86. <https://doi.org/10.3390/microorganisms7030086>
- CDC. (2011). Pathogens causing the most illnesses, hospitalizations, and deaths each year, 3–4. Retrieved from www.cdc.gov/foodborneburden
- CDC. (2019). *Outbreak of E. coli Infections Linked to Ground Beef*.
- CDPH. (2015). Foodborne pathogens and illnesses. *California Department of Public Health Report*.
- Cecchini, M., Langer, J., & Slawomirski, L. (2015). Resistance In G7 Countries And Beyond : Economic Issues , Policies. Organisation for Economic Co-operation and Development (OECD), (September).
- Center for Food Safety. (2014). Microbiological Guidelines for Food. *Food and Environment Hygiene Department, 2014*(August).
- Chantziaras, I. (2017). Epidemiology of antimicrobial resistance in commensal *E . coli*, 2016–2017.
- Commission, C. A. (2003). GENERAL PRINCIPLES OF FOOD HYGIENE, 1–31.
- Commission, C. A. (2016). *Codex Alimentarius, Understanding codex*.
- Cooke, B. Y. E. M., & Cooke, E. M. (1985). *Escherichia coli* - an overview, 523–530.
- Dashti, A. A., Jadaon, M. M., & Dashti, H. (2009). Heat Treatment of Bacteria : A Simple

Method of DNA Extraction for Molecular Heat Treatment of Bacteria : A Simple Method of DNA Extraction for Molecular Techniques, (June).

Doyle, M. E. (2015). Multidrug-Resistant Pathogens in the Food Supply. *Foodborne Pathogens and Disease*, 12(4), 261–279. <https://doi.org/10.1089/fpd.2014.1865>

Eltai, N. O., Yassine, H. M., Thani, A. A. Al, Madi, M. A. A., Ismail, A., Ibrahim, E., & Alali, W. Q. (2018). Prevalence of antibiotic resistant Escherichia coli isolates from fecal samples of food handlers in Qatar, 1–7.

FAO. (2016). *Drivers, Dynamics and Epidemiology of Antimicrobial Resistance in Animal Production*. Food and Agriculture Organization of the United Nations. <https://doi.org/10.1128/9781555818463.ch2>

FAO. (2017). Call for data on foodborne antimicrobial resistance, (December), 1–6.

FDA. (2016). Manual for Foodborne Disease Surveillance in Ghana, (April).

Feglo, P. K., & Adu-sarkodie, Y. (2016). Antimicrobial Resistance Patterns of Extended Spectrum B -Lactamase Producing Klebsiellae and E . coli Isolates from a Tertiary Hospital in Ghana, (October). <https://doi.org/10.19044/esj.2016.v12n30p174>

Feyera, T. (2014). Prevalence and Antimicrobial Susceptibility Pattern of E . Coli Isolates from Raw Meat Samples Obtained from Abattoirs in Dire Dawa City , Eastern Ethiopia, (January). <https://doi.org/10.5829/idosi.ijmr.2014.5.1.82306>

Food and Drugs Authority, G. (2013). Food and Drugs Authority Code of Practice for Slaughter (February), 1–13.

Galland, J. C. (1997). Risks and prevention of contamination of beef carcasses during the slaughter process in the United States of America Slaughter process in the United States of America, 16(January 1993), 395–404.

Gay, N., Leclaire, A., Laval, M., Miltgen, G., Jégo, M., Stéphane, R., ... Cardinale, E. (2018). Risk Factors of Extended-Spectrum β -Lactamase Producing Enterobacteriaceae Occurrence in Farms in Reunion, Madagascar and Mayotte Islands, 2016–2017. *Veterinary Sciences*, 5(1), 22. <https://doi.org/10.3390/vetsci5010022>

- Gelting, R. (2007). Investigation of an Escherichia coli O157:H7 Outbreak Associated with Dole Pre-Packaged Spinach. *Centers for Disease Control and Prevention (CDC) National Center for Environmental Health/Environmental Health Services Branch*, 95899(997435), 1–51.
- Ghana Standards Authority. (2018). *GHANA STANDARD CDGS 955-2018*.
- Gill, C. O., Badoni, M., & Ginnis, J. C. M. C. (2001). Microbiological Sampling of Meat Cuts and Manufacturing Beef by Excision or Swabbing †, *64*(3), 325–334.
- Herenda, D., Chambers, P. G., Ettriqui, A., & Seneviratna, P. (2000). *Manual on meat inspection for developing countries*.
- Hernández Rodríguez, A. (2014). Los contratos internacionales de construcción “llave en mano.” *Cuadernos de Derecho Transnacional*, *6*(1), 161–235. <https://doi.org/10.1128/mBio.02227-15>. Editor
- Hesp, A., Veldman, K., Goot, J. Van Der, Mevius, D., & Schaik, G. Van. (2019). Monitoring antimicrobial resistance trends in commensal Escherichia coli from livestock , the Netherlands , 1998 to, 1–13.
- Hudzicki, J. (2016). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, (December 2009), 1–23.
- Kibret, M., & Abera, B. (2011). Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. *African Health Sciences*, *11*(SPEC. ISSUE). <https://doi.org/10.4314/ahs.v11i3.70069>
- KORNACKI, J. L., & MARTH, E. H. (2016). Foodborne Illness Caused by Escherichia coli: A Review. *Journal of Food Protection*, *45*(11), 1051–1067. <https://doi.org/10.4315/0362-028x-45.11.1051>
- Kumar, P., Rao, J., Haribabu, Y., & Manjunath. (2014). Microbiological Quality of Meat Collected from Municipal Slaughter Houses and Retail Meat Shops from Hyderabad Karnataka Region, India. *APCBEE Procedia*, *8*(Caas 2013), 364–369. <https://doi.org/10.1016/j.apcbee.2014.09.001>
- Lawrence, J. G. (2002). Gene Transfer in Bacteria : Speciation without Species ?, *460*, 449–460.

<https://doi.org/10.1006/tpbi.2002.1587>

- Lowe, D. (2016). A plan for new antibiotics. *Website*. Retrieved from <http://blogs.sciencemag.org/pipeline/archives/2016/05/13/a-plan-for-new-antibiotics>
- Ma, Y., Zhao, Y., Tang, J., Tang, C., Chen, J., & Liu, J. (2018). Antimicrobial susceptibility and presence of resistance & enterotoxins / enterotoxin-like genes in *Staphylococcus aureus* from food. *CyTA - Journal of Food*, 16(1), 76–84. <https://doi.org/10.1080/19476337.2017.1340341>
- Madoshi, B. P., Kudirkiene, E., Mtambo, M. M. A., Muhairwa, A. P., Lupindu, A. M., & Olsen, J. E. (2016). Characterisation of Commensal *Escherichia coli* Isolated from Apparently Healthy Cattle and Their Attendants in Tanzania, 1–19. <https://doi.org/10.1371/journal.pone.0168160>
- Mainda, G., Bessell, P. R., Muma, J. B., Mcateer, S. P., Chase, M. E., Gibbons, J., ... Bronsvort, B. M. (2015). Prevalence and patterns of antimicrobial resistance among *Escherichia coli* isolated from Zambian dairy cattle across different production systems. *Nature Publishing Group*, (June), 1–11. <https://doi.org/10.1038/srep12439>
- Messele, Y. E., Abdi, R. D., Yalew, S. T., & Tegegne, D. T. (2017). Molecular determination of antimicrobial resistance in *Escherichia coli* isolated from raw meat in Addis Ababa. *Annals of Clinical Microbiology and Antimicrobials*, 1–9. <https://doi.org/10.1186/s12941-017-0233-x>
- Mohammed, J., Hounmanou, Y. M. G., & Thomsen, L. E. (2018). Antimicrobial resistance among clinically relevant bacterial isolates in Accra: a retrospective study. *BMC Research Notes*, 11(1), 254. <https://doi.org/10.1186/s13104-018-3377-7>
- Moore, L. S. P., Regmi, S., Piddock, L. J. V., Karkey, A., Guerin, P. J., Sundsfjord, A., ... Holmes, A. H. (2015). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, 387(10014), 176–187. [https://doi.org/10.1016/s0140-6736\(15\)00473-0](https://doi.org/10.1016/s0140-6736(15)00473-0)
- Newman, M. J., Opintan, J. A., & Sampane-Donkor, E. (2015). *Surveillance of Antimicrobial Drug Resistance in Ghana*.

- Newman, P. M. J., & Opintan, J. A. (2015). Surveillance of Antimicrobial Drug Resistance in Ghana, (February).
- O'Neill, J. (2014). Antimicrobial Resistance : Tackling a crisis for the health and wealth of nations, (December).
- O'Neill, J. (2015). Antimicrobials in agriculture and the environment: Reducing unnecessary use and waste. *The Review on Antimicrobial Resistance*, (December), 1–28.
<https://doi.org/Medium>
- O'Neill, J. (2016). The review on antimicrobial resistance, (March).
- Ogunbanjo, G. A. (2018). Listeriosis outbreak in South Africa : Are we winning the battle ?, (May), 3–4.
- Okunlade, A. O., & Jemilehin, F. O. (2013). Antibiotic resistance in Escherichia coli isolated from healthy cattle at a major cattle market in Ibadan , Oyo State , South Western , Nigeria, (October 2016). <https://doi.org/10.5897/AJMR2013.6028>
- Overdeest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., ... Kluytmans, J. (2011). Extended-spectrum β -lactamase genes of Escherichia coli in chicken meat and humans, the Netherlands. *Emerging Infectious Diseases*, 17(7), 1216–1222.
<https://doi.org/10.3201/eid1707.110209>
- Gherbawy, Y., & Hassan, S. (2009). Antibiotic Resistance in Escherichia coli Isolated from Retail Raw Chicken Meat in Taif , Saudi Arabia, (June 2014).
<https://doi.org/10.1089/fpd.2009.0365>
- Pessoa-Silva, C. (2018). Global AMR Surveillance System Objectives of GLASS Foster national AMR surveillance, (October).
- Petar, K. (2008). Antimicrobial resistance of commensal Escherichia coli of food-producing animals from three Vojvodinian farms, Serbia. *International Journal of Antimicrobial Agents*.
- Rather, T. A., Hussain, S. A., Bhat, S. A., Shah, S. N., Arshid, S., & Shahnawaz, M. (2013). Antibiotic sensitivity of E. coli and Salmonella isolated from different water sources in

- Kashmir, India. *Comparative Clinical Pathology*, 22(4), 729–731.
<https://doi.org/10.1007/s00580-012-1474-x>
- Reuben, R. C., & Owuna, G. (2013). Antimicrobial Resistance Patterns Of Escherichia Coli O157 : H7 From Nigerian Fermented Milk Samples In Nasarawa State , 2(3), 38–44.
- Robinson, T. P., Bu, D. P., Carrique-Mas, J., Fèvre, E. M., Gilbert, M., Grace, D., ... Woolhouse, M. E. J. (2016). Antibiotic resistance is the quintessential One Health issue. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 110(7), 377–380.
<https://doi.org/10.1093/trstmh/trw048>
- Rupp, M. E., & Fey, P. D. (2003). 2003 Mark E. Rupp and Paul D. Fey - Extended Spectrum β -Lactamase (ESBL)-Producing Enterobacteriaceae.pdf. *Springer*, 63(4), 353–365. Retrieved from <https://link.springer.com/article/10.2165/00003495-200363040-00002>
- Saba, C. K. S. (2019). Antimicrobial Resistance Pattern in Food-Producing Animals in the Northern Region of Ghana Courage Kosi Setsoafia Saba , PhD University for Development Studies , Tamale , Ghana, (March 2017). <https://doi.org/10.13140/RG.2.2.19800.55044>
- Salihu, M. (2013). Bacteriological quality of raw meat displayed for sale at Sokoto, Sokoto state, Nigeria. *Scientific Journal of Microbiology*, 2(7), 134–139.
<https://doi.org/10.14196/sjmi.v2i7.863>
- Sanchez, S., Stevenson, M. A. M., Hudson, C. R., Maier, M., Buffington, T., Dam, Q., & Maurer, J. J. (2002). Characterization of Multidrug-Resistant Escherichia coli Isolates Associated with Nosocomial Infections in Dogs, 40(10), 3586–3595.
<https://doi.org/10.1128/JCM.40.10.3586>
- Sarkar, D. J., Mukherjee, I., Shakil, N. A., & Rana, V. S. (2018). Antibiotics in Agriculture : Use and Impact ISSN : 2455-5533 Guest Editorial Column Antibiotics in Agriculture : Use and Impact * Corresponding author :, (May).
- Scales, L. (2006). Guidelines for the Use of. *Leadership*, (July), 1–5.
- Sekyere, J. O. (2014). Antibiotic types and Hnadling Practices in Disease Management among Pig Farms in Ashanti Region, Ghana. *Journal of Veterinary Medicine*.

- Shafina, N., Nor, M., Ireland, M. P., Abu, N. A., Uk, M., Rashid, M. A., ... Uk, M. (2015). Bacterial pathogens and antibiotic resistance patterns in children with urinary tract infection in a Malaysian tertiary, *70*(3), 153–157.
- Singh, A. K., Das, S., Singh, S., Gajamer, V. R., Pradhan, N., Lepcha, Y. D., & Tiwari, H. K. (2018). Prevalence of antibiotic resistance in commensal *Escherichia coli* among the children in rural hill communities of Northeast India, 1–15.
- Small, A., & Reid, A. (2003). Conditions in lairages at Abattoirs for Ruminants in Southwest England and In Vitro Survival of *Escherichia coli* O157, *Salmonella* Kedougou, and *Campylobacter* Jejuni on Lairage related substrates.
- Stadler, T., Meinel, D., Aguilar-Bultet, L., Huisman, J. S., Schindler, R., Egli, A., ... Tschudin-Sutter, S. (2018). Transmission of ESBL-producing Enterobacteriaceae and their mobile genetic elements - Identification of sources by whole genome sequencing: Study protocol for an observational study in Switzerland. *BMJ Open*, *8*(2), 1–6.
<https://doi.org/10.1136/bmjopen-2018-021823>
- Strommenger, B., Kettlitz, C., Werner, G., & Witte, W. (2003). Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *Journal of Clinical Microbiology*, *41*(9), 4089–4094.
<https://doi.org/10.1128/JCM.41.9.4089-4094.2003>
- Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & Mcdermott, P. F. (2012). Antimicrobial Drug Resistance in *Escherichia coli* from Humans, *18*(5), 741–749.
- Tam, C., Viviani, L., Adak, B., Bolton, E., Dodds, J., Cowden, J., ... O'Brien, S. (2011). the Second Study of Infectious Intestinal Disease in the Community (Iid2 Study). *Food.Gov.Uk*, 1–250. Retrieved from
<http://www.food.gov.uk/multimedia/pdfs/board/info111101.pdf%5Cnwww.gutfeelings.org.uk>
- Tang, K. L., Caffrey, N. P., Nóbrega, D. B., Cork, S. C., Ronksley, P. E., Barkema, H. W., ... Ghali, W. A. (2017). Restricting the use of antibiotics in food-producing animals and its

associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *The Lancet Planetary Health*, 1(8), e316–e327. [https://doi.org/10.1016/S2542-5196\(17\)30141-9](https://doi.org/10.1016/S2542-5196(17)30141-9)

Tauxe, R. (2008). Estimated annual number of E . coli O157 infections , United States.

Tetteh, M. (2015). University of Ghana <http://ugspace.ug.edu.gh> EXTENDED SPECTRUM BETA-LACTAMASE IN CLINICAL ISOLATES OF, (10207184).

Trongjit, S., Angkittittrakul, S., & Chuanchuen, R. (2016). Occurrence and molecular characteristics of antimicrobial resistance of Escherichia coli from broilers , pigs and meat products in Thailand and Cambodia provinces, (April), 575–585. <https://doi.org/10.1111/1348-0421.12407>

Urahn, S. K., Eskin, S., & Hoelzer, K. (2016). Emerging Pathogens in Meat and Poultry The Pew Charitable Trusts Safe food project. Retrieved from <http://www.pewtrusts.org/~media/assets/2016/09/emergingpathogensinmeatandpoultry.pdf>

Valenza, G., Nickel, S., Pfeifer, Y., Eller, C., Krupa, E., Lehner-Reindl, V., & Höller, C. (2014). Extended-spectrum- β -lactamase-producing escherichia coli as intestinal colonizers in the German community. *Antimicrobial Agents and Chemotherapy*, 58(2), 1228–1230. <https://doi.org/10.1128/AAC.01993-13>

Veterinary Services Department. (2018). Annual Report of Veterinary Activities. *Annual Report*.

Warjri, I., & Chandra, R. (2013). PCR-Based Detection of Extended-Spectrum β -Lactamases (blaCTX-M-1and blaTEM) in Escherichia coli, Salmonella spp. and Klebsiella pneumoniae Isolated from Pigs in North Eastern India (Mizoram). *Indian Journal of Microbiology*, 53(3), 291–296. <https://doi.org/10.1007/s12088-013-0378-z>

WHO. (2010). WHO Estimates of The Global Burden of Food Borne Diseases, (Foodborne diseases epidemiology reference group).

WHO. (2017). Antimicrobial Resistance Fact sheets on sustainable development goals: health targets. Retrieved from http://www.euro.who.int/__data/assets/pdf_file/0005/348224/Fact-sheet-SDG-AMR-FINAL-07-09-2017.pdf

- WHO Regional Office for Africa. (2012). Manual for Integrated Foodborne Disease Surveillance in the.
- Yafetto, L., Adator, E. H., Ebuako, A. A., Ekloh, E., & Afeti, F. Y. (2019). Microbial Quality of Raw Beef and Chevron From Selected Markets in Cape Coast, Ghana. *Journal of Biology and Life Science*, *10*(1), 78. <https://doi.org/10.5296/jbls.v10i1.14022>
- Yevutsey, S. K., Buabeng, K. O., Aikins, M., Anto, B. P., Biritwum, R. B., Frimodt-møller, N., & Gyansa-lutterodt, M. (2017). Situational analysis of antibiotic use and resistance in Ghana : policy and regulation, 1–7. <https://doi.org/10.1186/s12889-017-4910-7>
- Yousefipour, M. (2018). Bacteria Producing Extended Spectrum β -lactamases (ESBLs) in Hospitalized Patients: Prevalence, Antimicrobial Resistance Pattern and its Main Determinants. *Iranian Journal of Pathology*, *14*(1), 61–67. <https://doi.org/10.30699/ijp.14.1.61>
- Zein, A., & Kassem, E. (2018). Cost of Foodborne Illnesses: A literature Review, (August).
- Zimbardo, J. M., Power, D. A., & Miller, S. M. (2009). *Difco and BBL Manual of Microbial Culture Media* (Second Edi).

APPENDICES

A. LABORATORY PROTOCOLS

a. Procedure for the collection, transport and storage of samples

Purpose

This document provides guidelines for collection of surface swabs from beef, mutton and chevon at selected slaughterhouses in the Greater Accra Region.

Materials: Sterile cotton swabs, falcon tubes, 100cm² template, 25cm² template, ice chest, sterile disposable gloves, sample record sheet, buffered peptone water, field coat

Procedure

Instructions for sample collection

1. Trained laboratory personnel, food veterinarians or meat hygiene inspectors should collect samples for this study.
2. Carcasses should be selected randomly from livestock available for sampling.
3. All samples should be collected following evisceration of carcasses and removal of the offal.
4. Samples should be collected from from cattle, sheep and goats after they are slaughtered and dressed ..
5. Samples should be collected using sterile cotton swabs
6. A sterile template must be used for all samples to determine the area to be swabbed. A new template should be used for each carcass.
7. Surface swabs from meat should be placed into sterile falcon tubes with tight fitting lids.
8. The tubes must contain buffered peptone water or other transport media as required for organisms of interest.

9. Samples collected should be transported to the microbiological laboratory (NMIMR) within 3-4 hrs of collection.

Large ruminant samples

1. Three (3) sites (flank, brisket and upper thigh) each of 100cm² must be sampled for all bovine carcasses.
2. To sample the flank area, place the template on the cutaneous flank muscle. Move along the medial border of the muscle anteriorly to reach the area 8cm from the midline. Position the template and then proceed to swab.
3. For brisket sample, find the elbow area of the carcass. Draw an imaginary line perpendicular to the midline cut.
4. For upper thigh sample, situate the posterior portion of the aitchbone. Create an imaginary line to the Achilles tendon. Measure 10cm up the line leading to the Achilles tendon. Then 10cm over (laterally), then 10cm back to the cut surface on the round, then 10cm along the cut surface to form the 10cm square area.

Small ruminant carcasses

1. For goat and sheep carcasses, samples must be obtained from three(3) sites (flank, brisket and mid-loin).
2. Each sampling site must be 25 cm² in area.
3. To obtain a brisket sample, find the elbow area of the carcass. Create a line from the angle of the elbow dorsal towards the midline.
4. For flank sample, find the caudal edge on the 13th rib. Position your template 8cm above that area.

5. To obtain a mid-loin sample, find the area approximately 8cm below the base of the tail and sample.

Sampling procedure

1. Label all tubes prior to sampling.
2. Locate sampling site using the guidelines stated above.
3. Put on a pair of sterile gloves.
4. Carefully open a swab pack and remove a sterile swab in one hand.
5. Remove the template by holding outer edge
6. Make sure not to contaminate the inner edges of the template or area to be swabbed
7. Place the template over the sampling site.
8. Hold the template in place with one gloves hand, making sure not to touch the sampling site.
9. With the other hand, swab the sampling area in the vertical and horizontal direction approximately 10 times each. Place moderate pressure on the swab. Care should be taken not to destroy the swab in the process.
10. Repeat the above steps for all sampling sites
11. Use a sterile swab for each sampling site
12. Place all swab into buffered peptone water or other transport media immediately after sampling.

Sample Handling and Transport

1. All samples should be well arranged to maintain a temperature between 0 and 5°C during transport to the laboratory.
2. Samples should be processed in the laboratory immediately.

Inclusion and Exclusion criteria

Samples were obtained from livestock (cattle, sheep and goats) that are clinically healthy and have been passed for slaughter as determined by the Meat Inspection Officer. Carcasses that were found not to be wholesome for consumption post evisceration were excluded from the study.

Laboratory Sample Labelling Protocol

1. All sample containers should be labelled.
2. Label the container NOT the lid or biohazard bag.
3. Label the containers with waterproof marker/pencils.
4. Label specimen with: Unique ID number, date, time and place of collection, specimen type.

Samples were rejected based on the following criteria:

1. Inappropriate storage and transport temperature
2. Specimens with no labels
3. Leaking/damaged tubes
4. Frozen samples

Samples that could not be processed within 4 hours were stored immediately at 4°C for later testing

b. Study specific procedure for enumeration of bacteria, culture and identification

Purpose

These guidelines were used for the enumeration of bacteria, culture and identification of bacteria from surface swab samples obtained from raw meat at selected slaughter sites in the Greater Accra Region.

Materials and equipment: Vortex, Incubator, Laminar-flow Class II biosafety cabinet (BSC)

Reagents/supplies: MacConkey Agar plates ,70% ethanol, gloves and lab coat, disposable inoculation loops (1 µl) , disposable inoculation loops (10 µl) , MALDI -TOF target plate , tubes, Plate count agar, *E. coli*/ Coliform agar , petri dishes.

Procedures

Enumeration of bacteria

1. Serial tenfold dilutions of each sample were plated on Plate Count Agar (OXOID) using the pour plate method.
2. Samples were incubated at 37°C for a period of 24 hours.
3. Following incubation, plates with 30-300 colony forming units were counted, with the aid of a colony counter.
4. The total coliform count was performed in the same way using Brilliance *E. coli*/ Coliform selective agar (Oxoid).
5. The total count in 1ml of the original sample (CFU/ml) was calculated by multiplying the number of colony forming units on a dilution plate by the corresponding dilution factor and dividing it by the volume plated.

6. CFU/cm² was calculated by multiplying the CFU/ml by the volume of original sample, and further dividing it by the area sampled.

Culture of surface swabs from meat

1. Incubate samples pre enriched with brain heart infusion from slaughter sites at 37°C for 24 hours.
2. Inoculate ten (10) µl of each sample fluid on MacConkey agar.
3. Vortex all samples prior to inoculation on MacConkey agar.
4. Identification of *E Coli* was done by colonial morphology.

Procedure for MALDI-TOF MS

1. A few colonies of fresh overnight cultures were selected and potted on a target plate
2. Each sample spot was overlaid with 1µl of formic acid and left to dry for 15 minutes
3. Each sample spot was then overlaid with 1µl of matrix solution and allowed to dry for 15 min.
4. MALDI-TOF MS will then performed .

Quality Control and Quality Assurance

Media prepared for culture was incubated overnight to check for sterility before it was used for sample culturing the next day.

c. Study specific procedure for antimicrobial susceptibility testing, ESBL screening and PCR

Purpose

These guidelines were used for determining the antimicrobial susceptibility profile of *E. coli* isolated from raw meat. It details the steps required for performing antimicrobial sensitivity, ESBL detection and PCR screening for resistant genes from bacteria isolated from meat samples received from the study sites.

Materials: Inoculating loops, Mueller Hinton agar, antimicrobial discs, McFarland Standard, Quality control standard bacteria strains, normal saline, autoclaved distilled water, petri-dish, ruler/Measuring caliper, glass tubes, cotton swabs, Nephelometer, PCR tubes, PCR reagents, thermal cycler, heat block

Antimicrobial susceptibility testing

Procedure

Day 1

Preparing the inoculum:

1. We observed the agar plates to make sure that the cultures were pure.
2. Using a sterile loop, we selected 2 to 3 well isolated colonies. Transfer the growth to the tube of saline.
3. We placed the selected colonies into the tubes containing saline solution and prepared a uniform mixture.

4. Using a nephelometer we ensured that the turbidity of the prepared solution was equivalent to 0.5 McFarland:

Inoculating the Mueller-Hinton plate:

1. We observed all the prepared plates before inoculation to check for contamination.
2. We dipped a cotton swab in the prepared inoculum suspended in saline
3. The swab was rotated several times to ensure that much of the inoculum had soaked in. To remove the excess fluid, we pressed the swab firmly to the side of the tube.
4. We then streaked the surface of the plates making sure to move the plate in a 90 degree direction after the plate was fully streaked each time.
5. After the final lap of streaking, we swabbed the entire circumference of the entire plate.
6. We waited for 5 minutes to allow excess moisture on the agar plates to dry before adding the antimicrobial disks.

Applying the antimicrobial disks:

1. Antimicrobial disks were added with the aid of an automated disk dispenser.
2. Care was taken not to move disks once they are applied to the agar.
3. To ensure that all disks had sufficient contact with the agar, the tip of a forceps was used to press the disks gently on the agar.

Day 2

Reading Results:

1. Before reading the results, both control and sample plates were observed for evidence of contamination or mixed growth.
2. Next, the plates were also observed for uniformity of growth on the agar plates.
3. With the aid of calipers, we measured the zone sizes around for each disk.
4. We interpreted all the zone diameters based on the standard guidelines available at <http://www.eucast.org>

Quality Control

E. coli ATCC 25922 was used to monitor the performance of the test. Control strains were stored under conditions that will maintain viability and organism New batches of Mueller-Hinton agar were tested to ensure that all zones were within range.

ESBL detection

1. The inoculum was standardized to 0.5 McFarland prior to inoculation.
2. Ceftazidime disks and ceftazidime combined with clavulanic acid disks were positioned 30 mm apart on the MH plates, and left to incubate overnight at a temperature of 37°C.
3. The diameter of the zone of inhibition for the disk with ceftazidime was compared to the diameter zone of inhibition for the disk with ceftazidime and clavulanic acid combined.

4. Isolates were classified as ESBL positive if the difference in the inhibition zone diameter of ceftazidime combined with clavulanic acid was ≥ 5 mm greater than the diameter for the zone of inhibition of the ceftazidime-only disk.
5. Cefotaxime disks and cefotaxime combined with clavulanic acid disks were used concurrently with ceftazidime for confirmation of ESBL production.

PCR screening for resistance genes

1. Bacterial DNA extraction consisted of placing two colonies of overnight growth in a tube containing 1ml of distilled water.
2. The mixture was boiled for 10 minutes and centrifuged for 5 minutes at 1000 rotations per minute.
3. The supernatant was collected and used for molecular analysis (Dashti et al., 2009).
4. PCR was conducted using 2 μ l of the bacterial lysate (template DNA) in a final volume of 25 μ l
5. 10mM of each primer was used together with PCR grade water and multiplex PCR master mix (QIAGEN).
6. Multiplex PCR was conducted in a thermal cycler.
7. The first denaturation step was set to a temperature of 95°C for 5 minutes.
8. 35 cycles followed, consisting of amplification with denaturation at a set temperature of 95°C for 30 seconds, an annealing step at a temperature 60°C for 30 seconds, and an extension step at a temperature of 72°C for 2 minutes, ending with a final extension step at a temperature of 72°C for 10 minutes.
9. Gel electrophoresis was conducted.
10. We viewed the PCR products using a trans- illuminator.

B. SAMPLE RECORD SHEET

Title of Study: Antimicrobial resistance of food borne pathogens in raw meat in Ghana

To be filled out for each meat sample collected

1. Sample No. _____
2. Location of slaughter house _____
3. Slaughter level
Abattoir Slaughter house Slaughter slab
4. Date and time of slaughter _____
5. Type of animal species slaughtered
Cattle Sheep Goat Pigs Other _____
6. Sex of animal species slaughtered
Female Male
7. Age of animal species slaughtered (where available) _____
8. Breed of animal species slaughtered _____
9. Origin of animal species slaughtered _____
10. What is the source of feed for animals on farm of origin? (Please select all that apply)
Pasture Commercially prepared feed Self-prepared feed
Free-range Other _____