UNIVERSITY OF GHANA

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ANTIBIOTIC RESIDUES AND MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA CONTAMINATING RAW MEAT SOLD IN ACCRA, GHANA

BY

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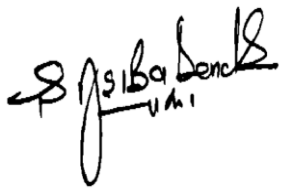
DECLARATION

I, Deric Asinor Baah, hereby declare that except for references to the literature and works of other researchers which have been duly acknowledged, the work in this dissertation is the result of my work put together and that I have never before submitted it to any other tertiary institution for a degree.



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DEDICATION

I dedicate this piece of work to my lovely dad and mom for sacrificing their comfort for my education.

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ABSTRACT

Background: Antimicrobial resistance (AMR) is considered by the World Health Organization as one of the greatest threats to health and economies in recent times. Multidrug resistance limits treatment options and has serious implications for human and animal health. Efforts to combat AMR should be based on the One Health approach and involve human health, animal health, and the environment. In Ghana, previous studies on AMR have given little attention to animal source food, which is a major route of transmission of antibiotic-resistant zoonotic pathogens.

Aim: The aim of the study was to investigate the occurrence of antibiotic residues and multidrug-resistant bacteria in meat sold in Accra.

Methods: This was a cross-sectional study in which 270 meat samples were collected. The presence of antibiotic residues in the meat samples was detected using microbiological inhibition assays. Standard microbiological methods were employed in cultural isolation and identification of bacterial pathogens present in the meat samples. Bacteria isolated from the samples were subjected to antimicrobial susceptibility testing (using the Kirby-Bauer method) against the following antimicrobials: amikacin (30 µg), ampicillin (10 µg), amoxicillinclavulanate (20/10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ertapenem (10 µg), meropenem (10 µg), imipenem (10 µg), tigecycline (15 µg), and gentamicin (10 µg).

Results: The prevalence of antibiotic residues among the meat samples was 7.7% [beef (0.0%), goat meat (0.0%), and chicken (23.3%, n = 21)]. Furthermore, thirty-two (32) different types of bacterial agents, totaling 588, were isolated from the samples. The predominant ones were *Escherichia coli* [262; Beef = 30.5%, *n* = 80; Goat meat = 30.5%, *n* = 80; Chicken = 38.9%, *n* = 102], *Aeromonas hydrophila* [117; Beef = 35.9%, *n* = 42; Goat meat = 53.0%, *n* = 62;

Chicken = 11.1%, *n* = 13], *Vibrio cholerae* [20; Beef = 50.0%, *n* = 10; Goat meat = 50.0%, *n* = 10; Chicken = 0.0%, *n* = 0], *Aeromonas veronii* [19; Beef = 63.1%, *n* = 12; Goat meat = 36.8%, *n* = 7; Chicken = 0.0%, *n* = 0], and *Klebsiella pneumoniae* [18; Beef = 22.2%, *n* = 4; Goat meat = 16.7%, *n* = 3; Chicken = 61.1%, *n* = 11]. The prevalence of MDR among the contaminating bacteria was 14.9% (*n* = 83), and the distribution was beef (3.8%, *n* = 21), goat meat = (5.0%, *n* = 28), and chicken (6.1%, *n* = 34). Also, the MDR distribution among the predominant bacteria was *E. coli* (Overall = 18.7%, *n* = 49; Beef = 5.7%, *n* = 15; Goat meat = 5.7%, *n* = 15; Chicken = 7.3%, *n* = 19), *A. hydrophila* (Overall = 11.1%, *n* =13; Beef = 2.3%, *n* = 3; Goat meat = 7.7%, *n* = 9; Chicken = 0.9%, *n* = 1), *V. cholerae* and *A. veronii* (0.0% each), and *K. pneumoniae* (Overall = 5.6%, *n* = 1; Beef = 0.0%, *n* = 0; Goat meat = 0.0%, *n* = 0; Chicken = 5.6%, *n* = 1). Moreover, 2.0% (*n* = 11) of the contaminating bacteria were ESBL producers, all of which occurred in 11 of the chicken samples, and their distribution was: *E. coli* (1.3%, *n* = 7), *K. pneumoniae*, *Pantoea spp.*, *E. cloacae*, and *Serratia plymuthica* (0.2% each, *n* = 1).

Conclusion: The prevalence of antibiotic residues in the meat samples was low, and the occurrence was restricted to chicken. The major bacterial contaminants were E. *coli, A. hydrophila, V. cholerae, A. veronii*, and *K. pneumoniae*. The prevalence of multidrug resistance was moderate, while that of ESBL producers was low.

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LIST OF ABBREVIATIONS

MDR: Multidrug-resistant organisms

AST: Antibiotic susceptibility testing

WHO: World Health Organisation

CDC: Centre for Disease Control

UTI: Urinary tract infection

STC: Shigella toxin-producing *E. coli*

CLSI: Clinical Laboratory Standards Institute

EUCAST: European committee on Antimicrobial susceptibility testing

LA-MRSA: Livestock Associated- Methicillin Resistant *Staphylococcus aureus*

MIC: Minimum inhibitory Concentration

ESBL: Extended spectrum beta-lactamase

LAMP: Loop-mediated Isothermal Amplification

VRE: Vancomycin resistant enterococcus

MRSA: Methicillin-Resistant *Staphylococcus aureus*

DNA: Deoxyribonucleic acid

PCR: Polymerase Chain Reaction

XLD: Xylose Lysine Deoxycholate

TCBS: Thiosulphate-Citrate-Bile Salt-Sucrose agar

MAR: Multiple Antibiotic Resistance index

GDP: Gross Domestic Product

USDA: United States Department of Agriculture

VIS: Veterinary Services

TLC: Thin Layer Chromatographer

ELISA: Enzyme-Linked Immunosorbent Assay

1 Contents

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The quality of food is generally affected by the presence of chemicals and infectious agents such as aflatoxins, antibiotic residues, pesticides, and pathogenic microorganisms. Food-borne diseases which result from the intake of food contaminated with bacteria, viruses, parasites, and or chemicals are major causes of mortality and morbidity due to their broad spectrum and quick rate of transmission (Smith, M'ikanatha, & Read, 2015). Animal-source foods are protein-dense foods of animal origin such as meat, yogurt, cheese, egg, and honey. Although they have a high nutritional content, the increased occurrence of food-borne diseases has been associated with their consumption (Todd, 2014).

Globally, the emergence of antibiotic resistance among isolates of bacteria has led to discussions on the appropriate and judicious use of antibiotics, not only in human medicine but also in agriculture and veterinary medicine. The use of antibiotics in the production of food from animal sources is not only limited to the treatment and prevention of diseases among farm animals but also has non-therapeutic purposes of increasing yield by serving as feed proficiency enhancers and growth promoters. Moreover, the classes of antibiotics used in sustaining the health and productivity of animals are similar to those used clinically to treat human infections. Although antibiotic use in livestock production has profound economic gains (Durso & Cook, 2014), the negative effects of exposing consumers to antibiotic residues and the emergence of antibiotic resistance cannot be overemphasized. This can worsen the public health concerns of antibiotic resistance as zoonotic pathogens can be potential carriers of resistance genes. Moreover, it could lead to the presence of antibiotic residues, which could occur due to a myriad of factors, such as non-observance of withdrawal time, little veterinarian supervision, and inappropriate use of antibiotics (Agmas & Adugna, 2018). Exposure of consumers to unacceptable levels of antibiotic residues in animal-source foods poses a serious public health

concern due to the potential health risk of eliciting unwanted immunological responses such as allergies, toxicity, and the emergence of antibiotic-resistant strains (Bacanlı & Başaran, 2019). Foodstuffs of animal origin are usually contaminated with bacteria, hence can be a major route of transmission of multidrug-resistant bacteria and resistance genes to humans (Djordjevic, Stokes, & Chowdhury, 2013; Exner et al., 2017; Marshall & Levy, 2011). Thus, the widespread applications of antibiotics in the rearing of farm animals can encourage the emergence of novel antibiotic-resistant bacteria strains in humans (Koch, Hungate, & Price, 2017).

Besides the issue of antibiotic residues, several studies conducted globally have identified multidrug-resistant bacteria on meat products, some of which include methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, multidrug-resistant *Salmonella*, and multidrug-resistant *E. coli* 0157 (Djeffal, Mamache, Elgroud, Hireche, & Bouaziz, 2018). Most of these bacteria are important pathogenic bacteria isolated in a wide array of human infections, and their presence in meat highlights the fact that the challenge of ensuring the safety of meats is multifaceted, as is the largely unending problem of antimicrobial resistance.

In Ghana, recent studies on AMR have given less attention to food from animal sources, which cannot be underestimated as a major route of spread of antibiotic-resistant zoonotic pathogens (Addae-Nuku et al., 2022; Afum et al., 2022; Agyepong, Govinden, Owusu-Ofori, & Essack, 2018; Ahmed et al., 2022; Anning et al., 2022; Dayie, Bannah, Dwomoh, Kotey, & Donkor, 2022; Donkor et al., 2019). Furthermore, the large application of antibiotics in animal husbandry and the paradoxical paucity of AMR surveillance data regarding animal-source foods suggest that data generated on AMR are far from robust. (Ellen De Leener et al., 2005; Vishnuraj, Kandeepan, Rao, Chand, & Kumbhar, 2016)

Routine surveillance of antimicrobial resistance and antibiotic residues in animal source foods will serve as a "double-edged sword" that helps to address microbial food safety issues as well as the antimicrobial resistance menace.

1.2 Problem statement

The safety of food is under immense threat globally, but this threat is disproportionately higher in poor-resource settings. To illustrate, the World Health Organization (WHO) estimated that globally, 600 million people are affected annually by infections due to food-borne diseases, leading to annual mortality of 420,000 and economic loss of US$110 billion in low- and middle-income countries (World Health Organisation, 2018). Meat is an important food item whose safety is in peril. Among other things, it is highly vulnerable to contamination with microbes that cause infections and spoilage as it is an ideal medium for their growth due to the high content of moisture, vitamins, proteins, minerals, and other growth factors (Bhaisare, Thyagarajan, Churchil, & Punniamurthy, 2014). Contaminated meats are one of the major causes of food-borne illnesses and an important conduit of zoonotic diseases (Ali, Farooqui, Khan, Khan, & Kazmi, 2010). Besides microbial contamination, the non-observance of withdrawal periods and inappropriate use of antibiotics for the treatment and prevention of infectious diseases, as well as growth promotion in animal production may lead to antibiotic residues in meats (Muaz, Riaz, Akhtar, Park, & Ismail, 2018; Shareef, Jamel, & Yonis, 2009). Of concern, the presence of antibiotics in human diets has been linked to adverse effects on human health comprising direct toxicity, allergic reactions, neurological disorders, gastrointestinal disturbance, tissue damage, and disruption of the intestinal microbiome (E De Leener, 2005; Mund, Khan, Tahir, Mustafa, & Fayyaz, 2017; Vishnuraj et al., 2016). Most importantly, it could further exacerbate the already huge menace of antimicrobial resistance (E De Leener, 2005; Mund et al., 2017; Vishnuraj et al., 2016). Meanwhile, it is estimated that by 2050, the lack of effective planned actions against antibiotic resistance will culminate in an

annual 10 million mortality rate and a loss of US$ 100 trillion (Jonas, Irwin, Berthe, Le Gall, & Marquez, 2017). Moreover, it is expected from the projections that low-income countries will be largely affected in terms of lowering economic growth than wealthy countries.

In Ghana, according to a comprehensive review report, bacterial isolates of clinical relevance from human, food, and environmental samples have been shown to have a high level of resistance to *E. coli* (62.2%), *Klebsiella* spp. (60.4%), and *Pseudomonas* spp. (52.1%) (García-Vello, González-Zorn, & Saba, 2020). According to their report, most of the isolates showed a greater level of resistance to clinically useful antibiotics such as ampicillin, cefadroxil, cefotiam, cloxacillin, cotrimoxazole, erythromycin, penicillin, and trimethoprim. To help tackle the antimicrobial resistance menace and improve upon public health interventions, it is important to conduct continuous surveillance on the occurrence of antibiotic residues and multidrug-resistant pathogens in animal-source foods.

1.3 Justification

The enormity of the antimicrobial resistance menace requires that remedial efforts be based on the One Health approach and involve human health, animal health, and the environment. In Ghana, previous studies on AMR have given little attention to animal-source food, which is a major route of transmission of antibiotic-resistant zoonotic pathogens (Asafo-Adjei, Mensah, Labi, Dayie, & Donkor, 2018; Borquaye et al., 2019; Labi et al., 2021; Opintan et al., 2015). Furthermore, very few of the studies conducted on animal-source foods in the country have evaluated them for the occurrence of antibiotic residues (Addo, Adjei, Mensah, & Jackson-Sillah, 2015; Donkor et al., 2011; Ekli, 2019; Mingle et al., 2021). Moreover, the wide usage of antibiotics in animal husbandry and the paradoxical paucity of AMR surveillance data regarding animal source foods means that data generated on antimicrobial resistance are far from robust (E De Leener, 2005; Mund et al., 2017; Vishnuraj et al., 2016). This study is

designed to help fill these knowledge gaps by determining the prevalence of antibiotic residues, a spectrum of bacterial pathogens contaminating three types of meat sold in Accra – beef, goat meat, and chicken – as well as the occurrence of multidrug-resistant bacteria in them. This is especially important, as antimicrobial resistance in animal-source foods needs continuous monitoring, just as is being done in humans.

1.4 Aim

To investigate the occurrence of antibiotic residues and multidrug-resistant bacteria in meats (beef, goat meat, and chicken) sold in Accra

1.5 Specific Objectives

The specific objectives were:

- To determine the prevalence of antibiotic residues in the meats
- To determine the spectrum of bacterial pathogens contaminating the meats
- To determine the prevalence of multidrug resistance among the contaminating bacteria and the antimicrobial resistance patterns of the bacteria

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Antibiotics: Their history and use in food production

Antibiotics are an important class of drugs that act by killing or inhibiting the growth of bacteria. They are used both in animals and humans for the treatment and prevention of infections. The discovery of penicillin in 1928 by Sir Alexander Fleming was a major transformational event in the treatment of infectious diseases which has saved the lives of millions of patients with sepsis, pneumonia, severe wound infections, and other life-threatening diseases caused by bacteria. Apart from antibiotics being used to cure diseases caused by pathogenic bacteria, they have contributed to the success and significant risk reduction of important clinical interventions and procedures, such as organ transplantation, cancer treatment, and open-heart surgery (Laxminarayan et al., 2013). Antibiotics have not only been useful in humans – they have had significant usage in agricultural settings, and in crop and animal production. The focus of current Agribusiness aimed at increasing cost efficiency has led to the proliferation of antibiotic use in animal husbandry. Apart from antibiotics being used therapeutically for treatment and disease prevention, their application as production tools for growth promotion has gained significant prominence among farmers across the world including Ghana (Graham, Boland, & Silbergeld, 2007; Paintsil et al., 2021; Phares, Danquah, Atiah, Agyei, & Michael, 2020).

The positive relationship between the introduction of sub-therapeutic levels of antibiotics and weight gain of farm animals such as poultry and beef date back to the 1950s (Libby & Schaible, 1955). This led to the commercial application of antibiotics to enhance the market weight of farm animals. Although several justifiable concerns are being raised by WHO on the use of antibiotics in animal husbandry, the economic gains enjoyed by farmers involved in animal husbandry in terms of cost reduction are encouraging the use of antibiotics (Casewell, Friis,

Marco, McMullin, & Phillips, 2003). Globally, 73% of antibiotics used in the universe are applied in the rearing of farm animals (Van Boeckel et al., 2019). In the US, more than 50% of antibiotics are used in livestock (Center for disease control, 2013). The use of antibiotics among farmers in Ghana is also widespread. According to a recent study conducted in Ghana, it was observed that 94.7% of farmers in Ghana applied antibiotics in livestock production, with a significant proportion (86.3%) of the farmers administering antibiotics to prevent and treat diseases, whiles 13% of them used antibiotics to enhance the growth of the farm animals (Phares et al., 2020). Although antibiotics are used in Agricultural settings in Ghana, relatively less attention has been given to how antibiotic application in farm animals is contributing to the menace of antibiotic resistance.

Frequently used antimicrobials in animal rearing include β-lactams, tetracyclines, aminoglycosides, lincosamines, macrolides, pleuromutilins, and sulfonamides (Lee *et al.*, 2020). These same classes of antibiotics are used clinically to treat infections in humans. In Africa, large amounts of antibiotics, particularly tetracycline, aminoglycoside, and penicillin, are applied in the rearing of farm animals (Kimera, Mshana, Rweyemamu, Mboera, & Matee, 2020). The animal carcasses and the resulting foodstuffs after the application of antibiotics to farm animals may contain traces of antibiotics (Wassenaar, 2005). Consumption of such foods could result in the accumulation of these sub-therapeutic levels of antibiotics among consumers, and render these drugs less effective when subsequently used in therapy. This has been a key underlying factor via which the antimicrobial resistance menace has exacerbated and become entrenched.

2.2 Nutritional value and consumption of animal source foods in Africa

Animal source foods are foodstuffs derived from animal origin and comprise fish, milk, meat, eggs, honey, cheese, and yogurt. Meats are the comestible part of domestic, farmed, and wild animals, including cattle, sheep, goats, pigs, and poultry. They are rich sources of protein, different types of fats comprising omega-3 polyunsaturated fatty acids, zinc, iron, selenium, potassium, magnesium, sodium, vitamin A, B-complex vitamins, and folic acid. Animal-source foods such as milk have a significant impact. The significant impact of the consumption of meals containing animal-source foods is enormous. In malnourished children, nutrients from animal-source foods like protein have been shown to enhance cognitive function, and anthropometric indicators, and consequently reduced death and morbidity (Dror & Allen, 2011). Some negative health outcomes associated with the intake of diets lacking animal-source foods include rickets, anemia, impaired cognitive abilities, poor growth, neuromuscular deficits, and even death (Murphy & Allen, 2003).

For the last 50 years, the global production of meats has tripled, and in 2018, the production of meats was about 340 million tons, with about 80 billion animals killed every day for consumption (Ritchie & Roser, 2019). This has led to an increase in the commercialization of animal husbandry to balance the high demand for meat consumption. In developing countries, such as countries of Sub-Saharan Africa, where there is significant growth of populations and economies, the per capita meat and fish consumption is expected to rise by 54–69% if the GDP of Sub-Saharan Africa doubles (Desiere, Hung, Verbeke, & D'Haese, 2018). Thus, there will be a concomitant increase in livestock rearing to meet demands.

2.3 Food safety and antibiotic residues in animal source foods

Application of antibiotics among animals may lead to the presence of antibiotic residues in foods of animal origin and raises concerns regarding the safety of animal-source foods (Bacanlı & Başaran, 2019). Food safety encompasses zoonotic diseases and acute and chronic ingestion

of natural and synthetic xenobiotic which greatly affect the quality of food concerning its detrimental effects on human health. Some of the concerns of end users of food derived from animal sources are the presence of antibiotic residues, which can be hazardous to human health. In developing countries, it has been suggested that the presence of antibiotic residues in meats above the threshold concentrations is due to a lack of knowledge and information among farmers, coupled with negligence among regulating authorities (Muaz *et al*., 2018).

A withdrawal period is usually set to prevent exposure of humans to antibiotics used during animal rearing. The withdrawal time refers to the interval between the stoppage of antibiotic usage on the farm animal and the defined time of slaughter, with the sole aim of reaching safe and tolerable concentrations in the animal carcasses (Nisha, 2008). The application of heat during cooking can significantly reduce the risks of exposure to residues of antibiotics like sulphonamides, tetracyclines, and fluoroquinolones, but does not assure the entire destruction of the residues of the antibiotics in meats (Muaz et al., 2018).

Adverse effects posed by the presence of antibiotic residues in foods of animal origin are numerous and include allergic reactions, transfer of antibiotic resistance genes, nephrotoxicity (gentamicin), bone marrow toxicity (chloramphenicol), carcinogenicity, mutagenicity, and hepatotoxicity (Nisha, 2008). Also, the misapplication of antibiotics may result in the emergence of resistant strains of bacteria which reduces the efficiency of antibiotics and this is likely to culminate in the treatment failure of infected animals (Laxminarayan et al., 2013).

Owing to the dangers associated with the presence of antimicrobial residues in foods and other food-safety-related threats, in most developed countries around the world, surveillance systems are institutionalized to monitor the presence of veterinary drugs in foodstuffs from animals to ensure the safety of their livestock and their products. In the USA for instance, the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), and Veterinary Services (VIS) are responsible institutions to undertake these monitoring works

(Animal and Plant Health Inspection Service, 2020). In the UK, operation of residue monitoring is being conducted by Veterinary Medicine Directorates. In Ghana, there seem to be lapses in monitoring and surveillance of antibiotic residues in foods among regulatory institutions, and this is a major drawback to the insurance of the safety of foods marketed in the country.

2.4 Analytical methods for determination of antibiotic residues in meats

The detection of antibiotic residues in meats falls under three (3) broad analytical procedures, which include qualitative, quantitative, and semi-quantitative (Chen, Ying, & Deng, 2019). The qualitative methods involve the categorization of samples as positive or negative based on a threshold reference value relative to specific antibiotic concentrations, whiles the quantitative determination requires the response of specific antibiotics taking into consideration extrapolation from a standard curve of a standard antibiotic substance which serves as a positive control. The quantitative methods require instruments with hypersensitivity and response values. The semi-quantitative methods are similar to the quantitative analysis, but the test results are interpreted about a range of drug concentrations (e.g., negative, low positive, and high positive).

Before the 21st century, some of the detection methods applied for the determination of antibiotic residues in foodstuffs of animal origin include microbial inhibition assays, microbial receptor assays, enzymatic colorimetric assays, receptor binding assays, chromatographic methods, and immunoassays (Pikkemaat, 2009). Most of these methods fall under the qualitative and semi-quantitative methods. Recent advancements in detection employ qualitative and sophisticated hypersensitive chemistry analyzers, such as liquid chromatography-mass spectrophotometry for detection, which reduces the number of false positives and targets a wide range of antibiotics (Dasenaki & Thomaidis, 2015). In a study

conducted in South Africa comparing thin layer chromatography (TLC), enzyme-linked immunosorbent assay (ELISA), and high-performance liquid chromatography (HPLC) for antibiotic detection, immunoassays were affirmed sensitive and selective method for monitoring antibiotic residues in foodstuffs of animal origin (Ramatla, Ngoma, Adetunji, & Mwanza, 2017).

2.5 Emergence of antibiotic resistance as a global threat

Antibiotic resistance is present throughout the world and is one of the major public health crises in this current dispensation. In fact, due to the emergence of antibiotic resistance, the likelihood of the vast majority of infectious diseases lacking effective antibiotic therapy is fast approaching normalcy (Morehead & Scarbrough, 2018).

The concept of antibiotic resistance came to light immediately after the discovery of penicillin, and resistance of *Staphylococcus aureus* to penicillin developed even before the mass production of antibiotics in 1943 (Hwang & Gums, 2016). Since then, over 20 000 likely genes conferring resistance to antibiotics have been found in about 400 different types of bacteria (Davies & Davies, 2010). Concerning antibiotic resistance, infections caused by organisms that were susceptible to antibiotics are becoming difficult and almost impossible to treat and cure. Thus, healthcare is fast approaching post-antibiotic times where simple infections that were easily treated will lead to significant morbidity and mortality. Antibiotic resistance is also posing a damaging reality to people, healthcare and veterinary services as well as Agro-businesses.

In the US, it is suggested that the economic impacts of antibiotic resistance in terms of direct cost to healthcare and loss of productivity will be higher than $20 billion and $35 billion respectively (Center for disease control, 2013). Moreover, it is suggested that by 2050, the

lack of effective planned actions against antibiotic resistance will culminate in an annual death toll of 10 million and a loss of US$ 100 trillion (O'Neill *et al*., 2014). A recent study by the World Bank presumes antibiotic resistance can potentially affect the economic fortunes of developing countries by increasing the rate of poverty(The world Bank, September 20, 2016).

Antibiotic-resistant organisms have been implicated in a myriad of infections such as UTIs, sepsis, upper and lower respiratory infections, catheter-associated infections, eye infections, ear infections, and wound infections. The impacts of infections caused by antibiotic-resistant organisms on patients include increased costs of healthcare, prolonged hospitalization, higher mortality, and treatment failures (Aslam et al., 2018; Prestinaci, Pezzotti, & Pantosti, 2015).

2.6 Factors contributing to the emergence of antibiotic resistance

Several factors have been enumerated as the cause of the rapid emergence of antibiotic resistance in both developed and developing countries. One of the contributing factors accelerating the emergence of antibiotic resistance is the overuse and inappropriate use (in choice, dose, patient adherence, etc.) of antibiotics. In developing countries, the less restriction and almost no regulation on the availability and usage of antibiotics are leading to self-medication (Morgan, Okeke, Laxminarayan, Perencevich, & Weisenberg, 2011). This situation is worse in Africa, as it has been suggested that in most countries on the continent, the majority of antibiotics used are not prescribed (Prestinaci *et al*., 2015) This causes less adherence to dosing and duration, which compounds the problem of unnecessary usage.

Many pharmaceutical companies involved in drug development, research, and production have collapsed their antibiotic department due to business financial losses in antibiotic production (Fair & Tor, 2014). This has led to a slower rate of research conducted on the development of antibiotic resistance.

One of the main areas for the large and inappropriate use of antibiotics, which may be an important contributing factor to the spread of antibiotic resistance is the modernization of agribusiness which aids in the large-scale production of meats, eggs, and dairy products. This has been noted as a major contributing factor for the spread of antibiotic resistance. As recommended by WHO, farmers and food industries have been cautioned to stop using antibiotics routinely to enhance growth and also prevent growth in health animals (World Health Organisation, 2017). The harrowing fact that most of the classes of antibiotics used in agricultural settings and veterinary medicine are of similar types, applications, and modes of action as those prescribed for human consumption aggravates the problem of antibiotic resistance (Islam, Shiraj-Um-Mahmuda, & Hazzaz-Bin-Kabir, 2016). The close linkage of the use of antibiotics in Agriculture leading to the emergence of life-threatening infections caused by multidrug-resistant organisms has been suggested (Landers, Cohen, Wittum, & Larson, 2012). With much focus on the development of antibiotic resistance in developing countries, It has been suggested that immoderate antibiotic usage in food-producing animals plays a key role in the emergence of antibiotic-resistant organisms and should be given similar consideration as poor regulation of antibiotic use in healthcare centers (Chokshi, Sifri, Cennimo, & Horng, 2019).

2.7 Multidrug-resistant organisms of public health importance isolated in animal-source foods

Some bacteria, such as *Salmonella* spp. and *Campylobacter* spp., are important zoonotic pathogens that can be transferred to humans through the food chain, and resistance of these organisms to antibiotics can challenge their treatment. In the case of *Salmonella*, there are many serovars of *Salmonella spp. enterica*. The non-typhoidal serovars are transmitted to humans through the food chain whiles the typhoidal serovar which causes typhoid fever is limited to human-to-human transmission (Boyen *et al*., 2008). The majority of non-typhoidal infections

from foodstuffs are self-limiting, but others can lead to life-threatening systemic infections and demonstrate resistance against drugs of choice such as fluoroquinolones and second-generation cephalosporins will exacerbate the infections. There are suggestions that multidrug-resistant *Salmonella spp.* could have adverse effects on human health via the food chain (Lai *et al*., 2014; L. Zhang et al., 2018). Across the globe, extended-spectrum beta-lactamase-producing *Salmonella* has been identified in animal-source foods (Djeffal et al., 2017; Guo et al., 2021; Ibrahim, Dodd, Stekel, Ramsden, & Hobman, 2016). Also, several studies have isolated fluoroquinolone-resistant *Salmonella spp.* in meats (Cui et al., 2019; Tadesse, Tessema, Beyene, & Aseffa, 2018).

The genus *Campylobacter* which comprises two species – *Campylobacter jejuni* and *Campylobacter coli* – is found in the gastrointestinal tracts of poultry (Bolton, 2015). They cause campylobacteriosis, which is the leading cause of gastroenteritis in humans. Although the majority of infections are self-limiting and mild, antibiotics such as macrolides and fluoroquinolones are warranted in prolonged and severe infections. *Campylobacter* spp. has long become increasing resistant to antibiotics, thereby limiting the effectiveness of medical therapy (Engberg, Aarestrup, Taylor, Gerner-Smidt, & Nachamkin, 2001).

Some antibiotic-resistant organisms contaminating ready-to-eat dairy products such as cheese and milk can colonize the gastrointestinal tracts of humans and transfer genes to the numerous microbiota present in the gut (Giraffa, Carminati, & Neviani, 1997). One classical example of such contaminants capable of transferring resistance genes is *Enterococcus* spp. Studies have suggested that multidrug-resistant *Enterococcus* spp. which are implicated organisms for urinary tract infections and endocarditis can harbor genes conferring resistance to linezolid and vancomycin, and can transfer these genes to receptive human microbiota (Chajęcka-Wierzchowska, Zarzecka, & Zadernowska, 2021).

Some subtypes of *Escherichia coli* are part of the organisms causing food-borne illnesses. In the USA, the Centers for Disease Control and Prevention, 2019, reported a sudden occurrence of *Shigella* toxin-producing *E. coli* strain (STEC) 0103 infections linked to the consumption of ground beef (Center for Disease Control, 2019). Usually, the presence of *E. coli* as a contaminant in meat is mainly due to the delay in the slaughtering, transportation, and selling of meats to potential buyers (Ritchie & Roser, 2019).

*Staphylococcus aureus i*s also one of the organisms causing food poisoning and is an important consideration of major public health programs globally (Hennekinne, De Buyser, & Dragacci, 2012). One of the frequent causes of outbreaks of food-borne diseases caused by Staphylococci is the improper handling of foods in the retail end of the food chain (Lues & Van Tonder, 2007). Staphylococcal food-borne disease is caused by eating food containing pre-formed staphylococcal enterotoxins. The determinant of the severity of this disease is directly associated with the number of toxins ingested and the health status of the patient (Schelin, Wallin-Carlquist, Thorup Cohn, Lindqvist, & Barker, 2011). The majority of the patients suffering from food poisoning caused by *Staphylococcus aureus* include vomiting, diarrhea, abdominal pain, and nausea with a rapid onset. The loss of large amounts of fluids leads to physical signs of dehydration and hypotension (Chaibenjawong & Foster, 2011). Several kinds of foods, such as meat, unpasteurized milk, poultry, and egg products are foodstuffs that provide conducive environments for the growth of *Staphylococcus aureus* (Le Loir, Baron, & Gautier, 2003). Currently, a newer strain, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is contributing to the emergence of human infections (Price *et al.*, 2012). Several studies across the globe have isolated MRSA in meat products

across the globe, which poses potential threats to consumers and meat handlers (De Boer et al., 2009; Mkize, Zishiri, & Mukaratirwa, 2017; Syed et al., 2018).

2.8 Mechanisms of antibiotic resistance

The resistance of bacteria to antibiotics can be naturally inherent as well as via the acquisition of antibiotics resistance through mutations in the chromosomal genes and by horizontal transfer. The structural and functional features of some bacterial organisms make them inhibit the bacteriostatic and or bactericidal action of antibiotics. One classical example is daptomycin, which is a lipopeptide that has significant action against only Gram-positive bacterial organisms but does not have any effectiveness against Gram-negative organisms. This can be attributed to differences in the composition of the cytoplasmic membrane in Gram-positive and Gram-negative organisms. Invariably, the action of daptomycin is mediated by Ca^{2+}, which facilitates its uptake into the cytoplasmic membrane. The lower amount of anionic phospholipids in Gram-negative organisms relative to Gram-positive organisms reduces the insertion of daptomycin into the cytoplasm.

Another mechanistic means of bacterial resistance apart from intrinsic resistance is the acquisition of resistance which can be broadly divided into three main phenomena: first, reduction in the concentration of antibiotics due to poor penetration; second, modification of the antibiotic target by genetic mutation or post-translational modification of the target; third, inactivation of the antibiotic by hydrolysis or modification.

2.8.1 Inhibition of antibiotic penetration and efflux

Several antibiotics have their target situated inside bacterial cells or found in the cytoplasmic membrane (usually among Gram-negative bacteria). Thus, the antibiotic must penetrate the outer and or cytoplasmic membrane of bacterial cells to exhibit its antibiotic action. Generally,

relative to Gram-positive bacteria, Gram-negative bacteria have poor penetrability to several antibiotics due to the structural presence of the outer membrane which forms a permeability barrier (Kojima & Nikaido, 2013). Hydrophilic antibiotics such as tetracycline, β-lactams, and some fluoroquinolones transverse the outer membranes via water-filled diffusion channels called outer membrane porin proteins (Pagès, James, & Winterhalter, 2008). Hence, the down regulation of porins or the substitution of porins with more selective channels will limit the penetrability of the outer membrane which will culminate in the reduction of antibiotic entry into Gram-negative bacterial cells ((Blair, Webber, Baylay, Ogbolu, & Piddock, 2015). Apart from the commonest way of resistance to carbapenems due to enzymatic degradation, *Enterobacteriaceae, Acinetobacter spp., and Pseudomonas* spp. can be resistance to carbapenems due to the mutations in porin genes or genes that regulate porin expressions (Tamber & Hancock, 2003; Tängdén, Adler, Cars, Sandegren, & Löwdin, 2013). Some of the best-characterized porins include the *Pseudomonas aeruginosa* OprD and three major proteins produced by *E. coli* which are ompF, ompC, and PhoE. Reduction in the amount of porins expressions and faulty porin functions can lead to resistance. Studies suggest that treatment of infections caused by *Pseudomonas aeruginosa* has resulted in mutations in the oprD gene during therapy (Quinn, Dudek, DiVincenzo, Lucks, & Lerner, 1986).

One of the ways access to antibiotics in bacterial cells is denied is through the action of efflux pumps which actively extrude antibiotics out of the cell. Some efflux pumps may have fewer substrate specificity such as *tet* determinants for tetracyclines and *mef* genes for macrolides in pneumococci. Other efflux pumps can transport a plethora of structurally different substrates and are known as multidrug resistance efflux pumps. One important example of multidrug resistance efflux pumps is LmrS in *Staphylococcus aureus* (Kim *et al*., 2013). Heightened expression of efflux pumps observed in multidrug-resistant organisms can be due to mutations

in the regulatory arms influencing efflux pumps production and inducement from environmental signals. Multidrug resistance efflux pumps such as acrAB genes in *E. coli* and *Salmonella* spp. can be triggered by indole and bile produced during infections (Baucheron *et al*., 2014).

2.8.2 Modification of the antibiotic target

The successful action of most antibiotics upon entry into bacterial cells requires specific high-affinity interaction with their target which is usually an important enzyme or ribosomal site. Alteration in the target structure will reduce the inhibitory effects of the antibiotic by preventing effective antibiotic binding whiles the functionality of the antibiotic is maintained. Substitution of a single amino acid at a specific point of the amino acid sequence of a protein target can change its binding affinity to its corresponding antibiotic without affecting the action of the target. This alteration of the target comprises (a) point mutation in the genes encoding the target site; (b) changes of the binding site due to the action of enzymes; (c) substitution of the original target.

One of the examples of resistance due to mutation in the genes involves mechanisms of resistance to fluoroquinolone. The intracellular target of fluoroquinolones such as ciprofloxacin and nalidixic acid is two important enzymes: DNA gyrase and topoisomerase IV involved in replication (Gibson, Ashley, Kerns, & Osheroff, 2018). These enzymes are important in the removal of positive and negative super helical twists and knots in DNA which is crucial in DNA replication. Mutations in the gyrase genes (gyr A, gyr B) and topoisomerase IV genes (par C, par D) can lead to lower sensitivity to fluoroquinolones.

Enzymatic modification of the target sites of antibiotics is responsible for resistance observed among certain antibiotics. The addition of one or two methyl groups of an adenine residue in position A2058 of domain V of 23rRNA of the 50s ribosomal subunits is mediated by the catalytic action of enzymes encoded by erm genes (erythromycin ribosomal methylation). Due to this modification in the structure of the target sites, the interaction of the antibiotic with its target is compromised. Since macrolides, lincosamides, and streptogramins share the same binding sites in the 23sRNA, the expression of these genes causes resistance to antibiotics in the MLS_B group. An example of this mechanism is seen in MRSA, in which resistance occurs by the acquisition of the staphylococcal cassette chromosome *mec* (SCCmec) element. This carries the *mec*A gene which encodes PBP 2a which is different in structure from the wild-type peptidoglycan transpeptidase penicillin-binding proteins (PBPs). This enables cell wall synthesis to occur whiles native PBPs are destroyed by β-lactams antibiotics (Katayama, Ito, & Hiramatsu, 2000). It is worth noting that PBP2a harbors a transpeptidase domain, but it does not function as a transglycosylase (class B PBP), so the action of other native PBPs to perform transglycosylation is important for robust peptidoglycan (Munita & Arias, 2016).

2.8.3 Inactivation of the antibiotic molecule

One of the effective ways bacteria renders antibiotic ineffective is by producing enzymes that inactivate the antibiotic by breaking down its active component or chemically modifying and or disrupting the structure of the drug. The resultant antibiotic, thus, is unable to bind to its target leading to resistance.

2.8.4 Inactivation of antibiotics by hydrolysis

After the discovery of penicillin in 1940, thousands of enzymes have been isolated that can break open and change antibiotics of different groups including β-lactams, aminoglycosides,

phenicols, and macrolides. β-Lactamase is a principal term given to bacterial enzymes that can disintegrate the amide bond of β -lactam ring. The various subclasses of the β -lactam antibiotics such as penicillin, cephalosporins, carbapenems, and monobactams are destroyed by a wide array of β-Lactamases (Woodford, Turton, & Livermore, 2011). After the discovery of penicillin, the first β-Lactamase to be discovered when penicillin-resistant strains of *S. aureus* became widespread was penicillinase which was able to inactivate penicillin in vitro.

Over the years, the extension of antibiotics of the β -lactam class with improved antimicrobial properties has led to the concomitant emergence of β-Lactamases that have effective action against novel antibiotics discovered and introduced for usage (Munita & Arias, 2016). Thus, the substrate specificity and physical properties of every β-Lactamases are unique. The development of extended-spectrum β-Lactamases (ESBLs) that have wider spectra of activities came after following β-Lactamases that were limited in their activity against first-generation β-Lactams. ESBLs can hydrolyze penicillin, third-generation cephalosporins, and monobactams but have no activity against cephamycins and carbapenems. Carbapenems which are essentially the last line of drugs for the treatment of infections caused by ESBL-producing bacteria are progressively being rendered useless due to the development of carbapenemases. Carbapenemases which have "versatile potential" can cleave penicillin, cephalosporins, monobactams, and carbapenems, and organisms harboring carbapenemases can render almost all the classes of β-Lactams ineffective leading to serious infections (Queenan & Bush, 2007). The harboring of wide array ESBLs and carbapenemases such as IMP (imipenemase), VIM (Verona integron encoded metallo β-lactamase), K. pneumoniae carbapenemase (KPC), OXA (oxacillinase) and NDM enzymes in organisms including *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *A. baumannii* has negative consequences in the treatment of severe infections in the hospital settings (Lynch III, Clark, & Zhanel, 2013).

2.8.5 Chemical alterations of the antibiotic molecule

One common means bacteria acquire resistance to antibiotics is the production of modifying enzymes that alters the chemical structure of the antibiotic by the addition of chemical moieties to susceptible sites. This causes drug resistance by inhibiting the antibiotic from interacting with its target as a result of steric hindrance. The most frequent biochemical reactions leading to alteration in the structure of antibiotics include acetylation which causes associated with aminoglycosides, streptogramin, and chloramphenicol resistance; phosphorylation, which leads to resistance in aminoglycosides and chloramphenicol and adenylation which causes resistance in aminoglycosides and lincosamines. The functional groups that are transferred by these modifying enzymes include acyl, phosphate, nucleotidyl, and ribotoyl groups.

One of the antibiotics that are vulnerable to alteration is aminoglycosides such as amikacin, gentamycin, and tobramycin which have numerous hydroxyl and amide groups exposed. They are modified by three principal groups of enzymes such as acetyltransferase, phosphotransferase, and nucleotidyltransferase. These enzymes can modify the structure of aminoglycosides since their active sites have a resemblance to the target area of the ribosomal binding clefts (Romanowska, Reuter, & Trylska, 2013). Also, genomic islands, which harbor multiple aminoglycosides resistance genes that encode aminoglycosides modifying enzymes have been identified in *Campylobacter coli* strains in broiler Chickens in China (Qin *et al*., 2012).

2.9 Techniques for determining the susceptibility of antibiotics

One of the ways the evolutionary potentials of bacterial resistance to antibiotics can be suppressed in favor of humans is by performing Antibiotic Susceptibility Testing (AST). AST is broadly laboratory based in vitro activity that evaluates the antibiotic resistance profiles of bacterial organisms. Thus, it determines whether a bacterial isolate is susceptible, intermediate,

or resistant to an antibiotic. AST which are used to determine the minimum inhibitory concentration (MIC) is usually a standardized method requiring measured inoculum of the bacteria and appropriate growth condition in the area of appropriate medium, incubation temperature, and length of time of incubation.

Any antibiotic approved for medical application has been shown in vitro to inhibit some particular group of bacteria at a concentration within the acceptable limit of toxicity. MIC is the lowest concentration of antibiotic that prevents the growth of an organism and it is used to evaluate whether a bacterial isolate is susceptible or resistant to an antibiotic (Bauer, Perez, Forrest, & Goff, 2014).

Susceptibility to an antibiotic denotes the antibiotic is effective against the bacteria isolates whiles resistant means the bacteria can grow in the presence of the antibiotic and thus ineffective. The borderline between resistance and susceptibility is termed "intermediate" which implies higher concentration is required to inhibit the growth of the bacteria. A breakpoint refers to the concentration of an antibiotic that helps categorize the results of AST concerning bacterial organisms as susceptible, intermediate, or resistant (Wiegand, Hilpert, & Hancock, 2008). Clinical breakpoints for a wide array of antibiotics and bacteria isolates are yearly assessed and updated by institutions including the Clinical Laboratory Standards Institute (CLSI) in the USA and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) which serve as useful references and guides for antibiotic reporting. The usefulness of MIC is not only limited to selecting appropriate antibiotics for the treatment of patients but can also be applied in epidemiological monitoring of the evolution of multidrug-resistant organisms, evaluating the effectiveness of novel antibiotics, and standardizing new techniques for AST (Syal *et al*., 2017).

The laboratory techniques involved in determining MIC are usually based on determining bacterial growth in the presence of antibiotics using manual methods such as agar dilution assays (E-test and disc diffusion), broth dilution assays, and automated systems.

2.9.1 Manual methods for determination of antibiotic susceptibility

Most clinical laboratories employ the use of disc diffusion for determining the susceptibility of rapidly growing organisms (Reller, Weinstein, Jorgensen, & Ferraro, 2009). In this conventional method, a standardized suspension of the test bacteria is evenly spread on a sterile agar surface by swabbing, and filter paper discs impregnated with a predetermined concentration of the antibiotic are placed on an agar surface. The plate is then incubated at 37^{o}C for a period of 16 to 24 hours to allow the antibiotic-impregnated disc to diffuse through the agar. The inhibitory effect of the antibiotic leads to a clear, visible circular area around the disc. The zone of clearance around the disc is a direct measure of the susceptibility of the bacteria to the antibiotic. The measured diameter is compared to the CLSI reference table to determine whether the test isolate is susceptible, intermediate, or resistant to the antibiotic tested. The merits associated with disc diffusion in estimating AST are: multiple antibiotics can be tested on one plate, are relatively simple to perform and interpret, and are less costly. The demerit includes providing qualitative results, not MIC values which are essential in inpatient treatment.

Dilution methods measure MIC instantly by utilizing serial dilution of the antibiotic in broth or agar within the range of medical relevant concentration spectrum. In the broth dilution method, antibiotics are diluted two folds in a liquid growth medium (broth) and incubated after inoculation with a standard bacterial suspension. The method employs multiple tubes or microdilution wells and dilution in these tubes or wells is repeated by applying a base of 1

µg/mL (0.25, 0.5, 1, 2, 4, 8, and so on). . Thus, they are broadly categorized into broth macro-dilution method and broth micro-dilution method. For the broth macro-dilution method, the volume used per tube is usually 1ml or higher whiles for the broth micro-dilution method, the volume utilized is in the microliter scale (~100 µl) (Ferraro, 2000). Pure colonies of the test bacteria are suspended in the media, diluted appropriately, and placed in each tube to obtain a concentration of ~5*10^5 CFU/ml based on CLSI guidelines. After 24 hours of incubation at 37^{o}C, the tubes containing serial dilutions of antibiotics and test isolates are examined for turbidity produced by the growth of bacteria. The first tube or microwell at which no visible growth is seen infers the lowest concentration of each antibiotic and equates to MIC. The advantages accompanying this method include reproducibility and cost-effectiveness however it is labor-intensive and time-consuming.

Another well-characterized method that utilizes the principle of dilution is the agar dilution method. This method involves preparing a sequence of agar plates containing antibiotics in increasing concentrations frequently in doubling dilutions (i.e., 1, 2, 4, 8, 16, 32 µg ml^{-1}, etc.)(Schmidt, 2019). 0.5 McFarland standard of the tested bacterial isolate is prepared and 1–5 µl suspension is placed on each of the series of the plate with increasing concentration of the antibiotics. The area on the agar plate where bacterial suspensions are placed is marked as a spot on the agar surface typically 5-8mm in diameter. After an incubation period of 16-20 hours, the lowest concentration of antibiotic in which no bacterial growth is observed is the MIC value. In this method, several organisms can be tested on a single plate but only one antibiotic concentration can be tested on a single plate.

Another method that utilizes the combined principle of dilution and diffusion method is the antibiotic gradient method also known as E-test to determine MIC. (Van Belkum & Dunne, 2013). The E-test comprises a thin plastic reagent strip impregnated with a continuous

lowering concentration of antibiotic. The procedure involves initially making a confluent growth of the adjusted bacterial suspension on an agar surface. After overnight incubation, the aftermath interaction of the antibiotic gradient and the test organism leads to the formation of elliptical-shaped inhibitory zones. the intersection of the growth inhibition ellipse and the strip is determined as the MIC value of the antibiotic relative to the tested bacterial isolate (Balouiri, Sadiki, & Ibnsouda, 2016).

2.9.2 Automated and newer methods of antibiotic susceptibility testing

Most of the automation in antibiotic susceptibility testing are technologies adapted from micro-dilution assays which provide relatively precise, reliable, and quantitative MIC values. The commercially available fully automated and semi-automated instruments are Microscan walkaway, Vitek-2, BD phoenix and Sensititre, Micronaut (Merlin, Berlin, Germany) (1990), the advantage test (Abbott Laboratories, Irving, Texas, USA) (1980) (Khan, Siddiqui, & Park, 2019; Syal et al., 2017). Another newest instrument reducing time for AST is the MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA) used for MIC determination (Zimmermann & Burckhardt, 2017). Apart from the MBT-ASTRA, this automated instrument utilizes either the principle of fluorescent emission or turbidity to determine the susceptibility pattern of the antibiotics

Molecular and genotypic assays are also highly sensitive and specific methods that reduce laborious procedures of bacterial cultures and the long incubation involved in the performance of antibiotic susceptibility. These methods include polymerase chain reaction (PCR), DNA microarray and DNA chips, and loop-mediated isothermal amplification (LAMP). Assessment of the genetic changes associated with Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococcus*, Extended Spectrum Beta Lactamase (ESBL),

Carbapenem resistance Enterobacteriaceae, Multidrug-Resistance *Mycobacterium tuberculosis* has all been done using molecular methods.

PCR seems to be the widely used molecular method used for the identification of resistance genes. The overall procedures of PCR include cycles of denaturation, annealing of the primers, and elongation of the primers by a heat-stable DNA polymerase called taq polymerase in a buffered pH environment containing nucleotides, ions, and others. Each cycle of amplification doubles the target DNA molecule. The amplified target can be confirmed for the presence of resistance genes through electrophoresis, southern blotting, restriction fragment-length polymorphism, single-strand conformation polymorphism (SSCP), DNA fingerprinting, molecular beacons, and other DNA sequencing analysis methods (Miller & Tang, 2009)

To address the demerits associated with conventional methods of ASTs which includes labor intensiveness, imprecision, and complexity, microfluidics has been created for rapid phenotypic and molecular analysis of antibiotic resistance (K. Zhang, Qin, Wu, Liang, & Li, 2020). This method reduces the time of ASTs to 1-3 hours and is less labor-intensive.

2.10 A review of some studies conducted on the safety of animal-source foods in Ghana

Several studies have been conducted on the microbial safety of animal-source foods in the country, and have employed a variety of methodological approaches, such as determining microbial contamination or load with or without evaluation of the presence of antibiotic residues. A variety of animal-source foods, spanning different meat types, as well as egg and milk, have been the targets of these studies. For example, in the study of Aning *et al*. (2007), the researchers investigated antibiotic residue occurrence in 394 milk samples and reported the prevalence to be 35.5% (Aning et al., 2007). In another study conducted in 2011 which

investigated the occurrence of antibiotic residues in a variety of animal source foods – egg, pork, mutton, chevon, and beef – in a composite of 634 samples, the prevalence of antibiotic residues in the meat samples was reported to be 21%, with the distribution being egg (6.8%), pork (28.6%), mutton (24%), chevon (29.3%), and beef (30.8%) (Donkor et al., 2011).

In a similar study, but which additionally quantified the occurring antimicrobial residues using HPLC in association with a triple quadrupole mass spectrometer, 63% of the 144 samples studied had antibiotic residues occurring in them (Mingle et al., 2021). The researchers further reported that the mean amounts of antibiotic residues were chlortetracycline (234.43 µg/kg), oxytetracycline (76.94 µg/kg), tetracycline (81.35 µg/kg), penicillin G (41.02 µg/kg), cefazolin (47.02 µg/kg), amoxicillin (35.76 µg/kg), sulfathiazole (68.63 µg/kg), sulfadoxine (46.05 µg/kg), sulfamethoxazole (103.98 µg/kg), enrofloxacin (30.19 µg/kg), haloperidol (9.62 µg/kg), ketoprofen (14.94 µg/kg), prednisone (23.66 µg/kg), erythromycin (77.18 µg/kg), and salbutamol (6.32 µg/kg). The researchers aptly concluded that consumers of these animal-source foods were at risk of compromising their well-being.

Furthermore, in the study involving sampling 200 cattle slaughtered from the University of Ghana (UG) Farms and slaughterhouses at Amasaman, Accra, Tema (GIHOC), and Madina, the researchers reported an antibiotic residue prevalence of 18%, the distribution being 12% and 6% for the kidney and liver samples, respectively (Addo et al., 2015). Moreover, they recovered 43 different pathogens, with the distribution of the key ones being *E. coli* (69.76%), *S. aureus* (18.69%), *Listeria monocytogenes* (4.65%), *Salmonella typhimurium* (4.65%), and *Yersinia enterocolitica* (2.3%). Other organisms recovered were *Klebsiella* spp., *Aeromonas* spp., *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Bacillus* spp..

In a recent study conducted in Accra by Dsani *et al*. (2020), but on a mixture of 205 carcasses of mutton ($n = 16$), chevon ($n = 108$), and beef ($n = 81$), and focused on *E. coli*, the researchers

reported an *E. coli* prevalence of 48%. The resistance rates of the organism increased across sulphamethoxazole/trimethoprim (SXT) (17%), cefuroxime (21%), tetracycline (45%), and ampicillin (57%), and the susceptibility rates increased across amikacin (92%), ciprofloxacin (92%), gentamicin (97%), chloramphenicol (97%), cefotaxime (98%), and ceftriaxone (99%), and no meropenem resistance was recorded. Also, the MDR prevalence was 22.4% (n = 22), and that of ESBL producers was 14.3% (n = 14), with four of these harboring the bla_{TEM} gene. Similarly, in another similar study conducted to evaluate the microbial quality of raw beef and chevon from selected markets in Cape Coast, Ghana – Abura, Kotokuraba, and Science, the outcome of the study revealed the Science Market recorded the highest contamination of chevon, with mean highest bacterial counts of 1.67 x 10^8 and 7.10 x 10^7 CFU/ml in nutrient agar and MacConkey agar media, respectively, the Kotokuraba Market recorded the highest contamination of beef, at respective mean highest bacterial counts of 1.15 x 10^8 and 9.40 x 10^7 CFU/ml for nutrient agar and MacConkey agar (Yafetto, Adator, Ebuako, Ekloh, & Afeti, 2019). Moreover, the fungal counts in potato dextrose agar were the least recorded for the two meat types at the various markets sampled.

In a similar study conducted in the Wa Municipality of Ghana, the researchers, with the aid of the USA-FDA Bacteriological Analytical Manual, evaluated the prevalence of resistant *Salmonella* spp. in cattle liver and beef, as well as antibiotic residues present in the meats (using the Premi®Test Kit) (Ekli, 2019). The antibiotic-resistant pattern of the various organisms was determined by the CLSI guidelines via disc diffusion. The researchers reported a higher prevalence of *Salmonella* in the liver (32%, n = 16) than in the beef (30%, n = 15). The organism demonstrated a high resistance towards teicoplanin (96.77%), but high susceptibility towards sulphamethoxazole/trimethoprim (100%), tetracycline (100%), ciprofloxacin (100%), chloramphenicol (100%), ceftriaxone (93.55%), Amoxycillin/clavulanic acid (93.55%), and

gentamicin (83.87%). Moreover, antibiotic residues were detected in 20% of the meat samples – 17% in the liver and 3% in the beef.

Moreover, in the study conducted in the Tamale Metropolis which determined the prevalence of *E. coli* and antimicrobial resistance patterns in a variety of meats (chevon, local chicken, beef, guinea fowl, and mutton), the researchers reported the prevalence among the meats to be 84%, and the distribution across the meat types was chevon (75.56%), local chicken (80.00%), beef (86.67%), guinea fowl (88.89%), and mutton (88.89%) (Adzitey et al., 2020). The chicken samples were reported to have the least mean coliform count (3.23 log CFU/cm^2), whereas guinea fowl recorded the highest (4.94 log CFU/cm^2). The commonest resistance pattern was ampicillin-tetracycline-erythromycin, and the rates recorded against these antibiotics were ampicillin (71.67%), tetracycline (73.33%), and erythromycin (85.00%). Moreover, the MDR prevalence was 68.33% (n = 41).

In another study that evaluated the microbiological quality of chicken imported from Brazil, the Netherlands, and the USA and sold in the Kumasi Metropolis, the researchers reported the distribution of the contaminating bacteria and fungi as follows: bacteria (*Klebsiella* spp. [13.8%], *Salmonella* spp. [24.6%], *E. coli* [26.2%], and *Staphylococcus* spp. [35.4%]) and fungi (*Cladosporium* spp. [15.2%], *Penicillium* spp. [24.2%], *Rhizopus* spp. [27.3%], *Aspergillus* spp. [33.3%]) (Yar et al., 2020).

The findings of these studies emphasize the value of continuous monitoring of the microbial safety of animal-source foods in the country to inform policies and public health interventions.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design and site

This study was a cross-sectional study involving the collection of meat samples from selected vending shops in Accra which is the capital of Ghana with a total land area covering 225.67km^2 and an estimated population of 1,665,08 (Ghana Statistical Service, 2012). In Accra, a greater number of butchers handle and process meats inconsistent with microbiological food safety standards according to Ghana Standards Authority and Food and Drugs Authority (Soriyi, Agbogli, & Dongdem, 2008). This predisposes consumers to unacceptable bacterial load and multidrug-resistant bacteria. Selected areas for the collection of the meat sample included meat vending shops at East Legon, Agblobloshie Market, and Madina where carcasses from these areas are consumed in the homes and eateries and also serve a greater population of the populace in Accra

3.2 Sample size determination and sample collection

A sample size of 270 meat was utilized taking into consideration the survey formula by (Kish, 1965); $n=z^2\, p(1-p)/d^{\,2}$, where z=Z score for 95% confidence interval which is equal to 1.96, p= prevalence, and d=acceptable error (5%). The prevalence rate of 22% was used according to a previous study (Dsani *et al.*, 2020). The meat samples were evenly distributed across the three meat types - 90 each of goat, beef, and chicken were collected usually in the afternoon from 12 pm to 4 pm which is the time most consumers and customers buy their meat samples from the meat vendors. The meat vending shops in Accra were selected by simple random sampling. All collected meat samples were tightly sealed with sterile plastic wrap and placed

in a cold box at 4°C and then transported to the research laboratory of department of Microbiology at the University of Ghana Medical School for microbiological analysis within 24 hours.

Figure 1. A map showing the study sites.

3.3 Laboratory processing

3.3.1 Sample preparation

Five to ten grams of the meat was mixed with 10ml of peptone water (0.1%) and then the homogenized suspension was prepared using a sterilized pestle and mortar.

3.3.2 Isolation and Identification of bacteria in the meat samples

Homogenized samples were plated on MacConkey with crystal violet agar (Oxoid Ltd., Basingstoke, UK), blood agar, (Oxoid Ltd., Basingstoke, UK), and Xylose Lysine Deoxycholate Ltd agar (Oxoid., Basingstoke, UK), and incubated for 16-18 hours at 37°C. The plates were subsequently inspected for growth, lactose fermentation, hydrogen sulfide production, and characteristic colony morphology. Sub-culturing was done, following which the resultant pure cultures were identified based on Gram stain, oxidase test, indole test, and

API 20E (Biomerieux SA, Marcy-l'Étoile, France) and MALDI-TOF (Bruker Daltonics GmbH & Co., Bremen, Germany, MBT Compass IVD Ver. 4.2.100).

For *Salmonella* isolates, serological identification was also employed, using the slide agglutination test. During the test, two separate drops (40 µl each) of saline were placed on a glass slide. Portions of the isolate were emulsified with a loop to give a smooth, fairly dense suspension. One drop (40 µl) of saline was added to saline and mixed and one drop of polyvalent O serum (Thermo Fisher Diagnostics BV, Landsmeer, The Netherlands) was added to the other slide and it was repeated for polyvalent H serum(Thermo Fisher Diagnostics BV, Landsmeer, The Netherlands) and polyvalent Vi serum (Thermo Fisher Diagnostics BV, Landsmeer, The Netherlands). *Salmonella typhimurium* 4,512:1:1, 2 NCTC 3048 was used as a positive control, and *Hafnia alvei* NCTC 8535 was also used as a negative control.

In the case of *Vibrio cholera*, homogenized samples were cultured on Thiosulphate citrate bile salt agar (TCBS) (Mast Group Ltd., Liverpool, Merseyside, UK) and inoculated in alkaline peptone water. TCBS culture plates were incubated at 16-18 hours at 37°C and alkaline peptone water was incubated for 4 hours at 37°C and sub-cultured at 37°C for 16-18 hours. Identification of *Vibrio cholerae* was done via Gram stain, indole and oxidase tests, and API 20E (Biomerieux SA, Marcy-l'Étoile, France). For serological identification, two separate drops (40 µl each) were placed on the glass slide. The isolate was emulsified in each drop of saline to give a smooth, fairly dense suspension. To one suspension as a control, one drop (40 µl) of saline was added and one drop (40 µl) of undiluted serum of *Vibrio cholerae* O1 (*Vibrio cholerae* Remel ™, Dartford, Kent, UK) was added and mixed. The two slides were rocked for one minute and observed for agglutination by viewing against a dark background. Known positive and known negative cultures were used as quality control checks

Serological identification of species of Shigella was done as follows: about 200 µl of saline was placed into a tube. Two colonies from an overnight culture were emulsified in the saline

to produce a homogenized suspension. The latex reagents (Wellcolex^{TM} Colour Shigella, Thermo Fisher Diagnostics BV, Landsmeer, The Netherlands) were shaken intensely for some seconds and the latex reagent was dispensed into a separate circle on a flat reaction card. Using an applicator stick, the contents of each circle were mixed and spread to cover the whole area of the circle. The card was placed on a rotator and shaken at 150 rpm for 2 minutes. The results were read using the Wellcolex* Colour Shigella Reading Guide.

3.3.3 Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed according to guidelines set by the Clinical and Laboratory Standards Institute (CLSI, 2021)using Kirby Bauer's disc diffusion method on Muller-Hilton agar (Oxoid, Basingstoke, UK). Antibiotics discs, such as amikacin (30 µg), ampicillin (10 µg), ceftriaxone (30 µg), ciprofloxacin (10 µg), cotrimoxazole (trimethoprim/ sulfamethoxazole, 1.25 µg/23.75 µg), meropenem (10 µg), imipenem (10 µg), ertapenem (10 µg), gentamycin (10 µg), tetracycline (15 µg), and amoxicillin-clavulanic acid (25 µg) were selected for testing.

Pure colonies of isolates were inoculated in peptone water (Oxoid Limited, Basingstoke, UK). The turbidity was adjusted to 0.5 McFarland standard using sterile peptone water and swabbed on Müller Hinton Agar (Oxoid, Basingstoke, UK) in a manner allowing for semi-confluent growth post-incubation. Incubation was carried out at 37°C for 24 hours. After incubation, the inhibition zones were measured, and the results were interpreted using the CLSI (2021) guidelines. Isolates showing resistance to three or more antibiotic classes were considered multidrug-resistant (MDR) (Magiorakos et al., 2012). Moreover, the multiple antibiotic resistance index (MAR) was computed for each bacterial isolate as the fraction of the number of antibiotics to which an isolate displayed resistance out of the total number of antibiotics against which the susceptibility of the isolate was evaluated (Krumperman, 1983).

3.3.4 Phenotypic detection of ESBL-producing Enterobacteriaceae

Mueller Hinton agar (MHA) (Oxoid, Basingstoke, UK) was inoculated with standard inoculum (0.5 McFarland) of the test isolate, similar to the description in the preceding subsection "Antibiotic susceptibility testing". Ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/10 µg) discs were placed on the surface of the agar and incubated at 37°C for 16–18 hours. An increase in zone diameter of ≥5 mm after incubation in the presence of clavulanic acid than ceftazidime alone was interpreted as an ESBL producer. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control strains, respectively.

3.3.5 Microbial inhibition plate test for determination of antibiotic residues

The meat samples were screened for the presence of antibiotic residues using procedures outlined by Koenen-Dierick *et al*. (1995), but with some modifications using saline and antibiotics as controls (Koenen-Dierick et al., 1995). A 0.5 McFarland standard of *Bacillus subtilis* ATCC 65313 was prepared and swabbed on Mueller Hinton agar plates. A sterile 8 mm diameter cork borer was utilized to make a circular-shaped meat sample of the thickness of 2mm and placed on the surface of the Mueller Hinton agar (Oxoid, Basingstoke, UK) and incubated at 18 to 24 hours at 37°C. After incubation, samples whose zones of inhibition were greater than or equal to 10 mm were considered positive for the microbial inhibition assay, and those with zones of inhibition less than 10 mm were interpreted as negative for the assay. A 10 µg ciprofloxacin disc and a filter paper impregnated with distilled water were utilized as positive and negative controls respectively.

3.4 Statistical analysis

The laboratory data collected were entered into STATA 14 (Strata Corp, College Station, TX, USA) for analysis. Descriptive statistics were used to summarize the data on the spectrum of bacterial pathogens contaminating the meat samples and their AMR rates, MDR prevalence, and MAR indices.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of antibiotic residues in the meat samples

Of the 270 meat samples evaluated for the presence of antibiotic residues, 21 (7.7%), all being chicken, showed positive results. No antibiotic residues were detected in the other meat types. Hence the distribution of the prevalence of antibiotic residues among the meat samples was beef (0.0%), goat meat (0.0%), and chicken (23.3%, *n* = 21).

4.2 Spectrum of bacterial pathogens contaminating the meats

All the individual meat samples had bacterial contamination—5.9% (*n* = 16) with one bacterium, 83.3% (*n* = 225) with two bacteria, 8.9% (*n* = 24) with three bacteria, and 1.9% (*n* = 5) with four bacteria. A summary of the number of individual bacteria isolated per sample is presented in Table 1.

Table 1. A summary of the number of individual bacteria isolated per sample.

Number of Individual Bacterial Contaminants Per Sample	Number of Samples			
	All Meat Types	Beef	Goat	Chicken
One	16 (5.9%)	0 (0.0%)	1 (1.1%)	15 (16.7%)
Two	225 (83.3%)	85 (94.4%)	77 (85.6%)	63 (70.0%)
Three	24 (8.9%)	4 (4.4%)	12 (13.3%)	8 (8.9%)
Four	5 (1.9%)	1 (1.1%)	0 (0.0%)	4 (4.4%)

The spectrum of the bacterial contaminants was broad, involving 32 different types of bacteria totaling 558. The predominant ones were *E. coli* [262; beef = 30.5%, *n* = 80; goat meat = 30.5%, *n* = 80; chicken = 38.9%, *n* = 102], *Aeromonas hydrophila* (*A. hydrophila*) [117; beef = 35.9%, *n* = 42; goat meat = 53.0%, *n* = 62; chicken = 11.1%, *n* = 13], *Vibrio cholerae* (*V. cholerae*) [20; beef = 50.0%, *n* = 10; goat meat = 50.0%, *n* = 10; chicken = 0.0%, *n* = 0],

Aeromonas veronii (*A. veronii*) [19; beef = 63.1%, *n* = 12; goat meat = 36.8%, *n* = 7; chicken = 0.0%, *n* = 0], and *K. pneumoniae* [18; beef = 22.2%, *n* = 4; goat meat = 16.7%, *n* = 3; chicken = 61.1%, *n* = 11]. The distribution of the bacterial contaminants is presented in Table 2.

Table 2. Distribution of the proportion of bacteria contaminating the meat sold in the markets.

Isolated Bacterium	Total Number (*n*, %) *	Distribution of the Bacterium Across the Meat Types #		
		Beef (*n*, %)	Goat (*n*, %)	Chicken (*n*, %)
Escherichia coli	262 (47.0%)	80 (30.5%)	80 (30.5%)	102 (38.9%)
Aeromonas hydrophila	117 (21.0%)	42 (35.9%)	62 (53.0%)	13 (11.1%)
Vibrio cholera	20 (3.6%)	10 (50.0%)	10 (50.0%)	0 (0.0%)
Aeromonas veronii	19 (3.4%)	12 (63.1%)	7 (36.8%)	0 (0.0%)
Klebsiella pneumonia	18 (3.2%)	4 (22.2%)	3 (16.7%)	11 (61.1%)
Serratia plymuthica	14 (2.5%)	1 (7.1%)	2 (14.3%)	11 (78.6%)
Pantoea spp.	13 (2.3%)	1 (7.7%)	1 (7.7%)	11 (84.6%)
Moellerella wisconsensis	10 (1.8%)	7 (70.0%)	3 (30.0%)	0 (0.0%)
Acinetobacter baumannii	9 (1.6%)	1 (11.1%)	1 (11.1%)	7 (77.8%)
Vibrio spp.	9 (1.6%)	4 (44.4%)	4 (44.4%)	1 (11.1%)
Enterobacter cloacae	7 (1.3%)	2 (28.6%)	0 (0.0%)	5 (71.4%)
Vibrio alginolyticus	6 (1.1%)	1 (16.7%)	3 (50.0%)	2 (33.3%)
Pseudomonas luteola	6 (1.1%)	3 (50.0%)	3 (50.0%)	0 (0.0%)
Proteus mirabilis	6 (1.1%)	4 (66.7%)	0 (0.0%)	2 (33.3%)
Salmonella enteritidis	6 (1.1%)	2 (33.3%)	2 (33.3%)	2 (33.3%)

Citrobacter koseri	5 (0.9%)	3 (60.0%)	1 (20.0%)	1 (20.0%)
Yersinia enterolytica	5 (0.9%)	0 (0.0%)	0 (0.0%)	5 (100.0%)
Shigella flexneri	3 (0.5%)	0 (0.0%)	0 (0.0%)	3 (100.0%)
Enterobacter aerogenes	3 (0.5%)	2 (66.7%)	1 (33.3%)	0 (0.0%)
Citrobacter freundii	3 (0.5%)	2 (66.7%)	1 (33.3%)	0 (0.0%)
Rahnella aqualitis	2 (0.4%)	0 (0.0%)	0 (0.0%)	2 (100.0%)
Serratia odorifera	2 (0.4%)	0 (0.0%)	0 (0.0%)	2 (100.0%)
Citrobacter youngae	2 (0.4%)	1 (50.0%)	1 (50.0%)	0 (0.0%)
Klebsiella oxytoca	2 (0.4%)	2 (100.0%)	0 (0.0%)	0 (0.0%)
Providencia rettgeri	2 (0.4%)	1 (50.0%)	1 (50.0%)	0 (0.0%)
Acinetobacter iwoffi	1 (0.2%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
Serratia rubidaea	1 (0.2%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
Kluyvera spp.	1 (0.2%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Pasteurella multocida	1 (0.2%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Yersinia ruckeri	1 (0.2%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Stenotrophomonas maltophilia	1 (0.2%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Pasteurella pneumotropica	1 (0.2%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
Total	558 (100%)	186 (33.3%)	191 (34.2%)	181 (32.4%)

* The proportions were computed using the total number of bacteria as the denominator; # the proportions were computed using the total number of each bacterium as the denominator.

4.3 Antimicrobial resistance rates among the bacterial Contaminants of the Meats

Almost all the meat samples (96.7%; *n* = 261) were contaminated with antibiotic-resistant bacteria—beef (97.8%; *n* = 88), goat (97.8%; *n* = 88), and chicken (94.4%; *n* = 85). When the

resistance data for all the bacteria are put together, the highest resistance rate was recorded against ampicillin (83.3%), followed by amoxicillin-clavulanate (36%). The rates of cefuroxime, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, trimethoprim-sulphamethoxazole, ertapenem, meropenem, and tetracycline ranged between 1.3% and 19.5%, whereas no resistance was recorded against either of amikacin, imipenem, and gentamicin for any of the bacterial contaminants. In *E. coli*, the antibiotics whose resistance rates were the highest were ampicillin (81.3%), trimethoprim-sulphamethoxazole (26.3%), and amoxicillin-clavulanate (25.2%). Concerning *A. hydrophila*, the antibiotics whose resistance rates were the highest were ampicillin (94%), amoxicillin-clavulanate (62.4%), and cefuroxime (10.3%). Concerning *V. cholerae*, the antibiotics whose resistance rates were the highest were ampicillin (90%) and trimethoprim-sulphamethoxazole (25%), with no resistance recorded against any of the remaining antibiotics that were tested. In *A. veronii*, all the organisms were resistant to ampicillin and amoxicillin-clavulanate but not to any of the remaining antibiotics that were tested; these rates were identical, regardless of whether the meat type was beef, goat, or chicken. For *K. pneumoniae*, the antibiotics whose resistance rates were the highest were ampicillin (94.4%) and trimethoprim-sulphamethoxazole (27.8%). Details of the AMR rates of the bacterial contaminants are presented in Table 3.

Table 3. Antimicrobial resistance rates of the bacterial species contaminating the meat samples.

Organisms/Antibiotics	AMP	AMC	CEFU	CEFT	CFTZ	CEFP	CIP	TMS	ERT	MEM
All Bacteria (*n* = 558)	83.3	36	9.1	2.2	2.2	2.3	5.7	19.5	1.3	1.3
All *E. coli* (*n* = 262)	81.3	25.2	7.6	2.7	2.7	3.1	9.5	26.3	1.1	1.1
Beef *E. coli* (*n* = 80)	78.8	42.5	3.8	0	0	1.3	10	28.8	1.3	1.3
Goat *E. coli* (*n* = 80)	77.5	23.8	6.3	0	0	0	11.3	12.5	0	0
Chicken *E. coli* (*n* = 102)	86.3	12.7	11.8	6.9	6.9	6.9	7.8	26.5	2	2

All *A. hydrophila* (*n* = 117)	94	62.4	10.3	0	0	0	3.4	6	0	0
Beef *A. hydrophila* (*n* = 42)	100	88.1	2.4	0	0	0	3.2	1.6	0	0
Goat *A. hydrophila* (*n* = 62)	88.7	53.2	17.7	0	0	0	3.2	3.2	0	0
Beef *A. hydrophila* (*n* = 13)	100	23.1	0	0	0	0	0	30.8	0	0
All *V. cholerae* (*n* = 20)	90	0	0	0	0	0	0	25	0	0
Beef *V. cholerae* (*n* = 10)	100	0	0	0	0	0	0	0	0	0
Goat *V. cholerae* (*n* = 10)	80	0	0	0	0	0	0	50	0	0
All *A. veronii* (*n* = 19)	100	100	0	0	0	0	0	0	0	0
Beef *A. veronii* (*n* = 12)	100	100	0	0	0	0	0	0	0	0
Goat *A. veronii* (*n* = 7)	100	100	0	0	0	0	0	0	0	0
All *K. pneumoniae* (*n* = 18)	94.4	11.1	5.6	5.6	5.6	5.6	0	27.8	0	0
Beef *K. pneumoniae* (*n* = 4)	75	0	0	0	0	0	0	25	0	0
Goat *K. pneumoniae* (*n* = 3)	100	0	0	0	0	0	0	66.7	0	0
Chicken *K. pneumoniae* (*n* = 11)	100	18.2	9.1	9.1	9.1	9.1	0	18.2	0	0

Amikacin, imipenem, tigecycline, and gentamicin are not included in the table, due to absence of resistance to any of them; AMP = Ampicillin; AMC = Amoxicillin-clavulanate; CEFU = Cefuroxime; CEFT = Ceftriaxone; CFTZ = Ceftazidime; CEFP = Cefepime; CIP = Ciprofloxacin; TMS= Trimethoprim-sulphamethoxazole; ERT = Ertapenem; MEM = Meropenem.

As observed in Table 4, the prevalence of MDR among the contaminating bacteria was 14.9% (*n* = 83)—11.3% (*n* = 21) in beef, 14.7% (*n* = 28) in goat meat, and 18.8% (*n* = 34) in chicken. Additionally, the MDR distribution among the predominant bacteria was *E. coli* (18.7%, *n* = 49), *A. hydrophila* (11.1%, *n* = 13), *V. cholerae* and *A. veronii* (0.0% each), and *K. pneumoniae*

(5.6%, *n* = 1) (Table 4). Moreover, the mean MAR index, as a composite, was 0.12 ± 0.09 (beef = 0.11 ± 0.08; goat meat = 0.11 ± 0.07; chicken = 0.13 ± 0.12), with 15.23% (*n* = 85) (beef = 10.8% [*n* = 20]; goat meat = 15.71% [*n* = 30]; chicken = 19.33% [*n* = 35]) of the bacteria recording a MAR index greater than 0.2 (Table 4). Additionally, 2.0% (*n* = 11) of the contaminating bacteria were ESBL producers, all of which occurred in 11 of the chicken samples, and their distribution was: *E. coli* (1.3%, *n* = 7), *K. pneumoniae*, *Pantoea* spp., *Enterobacter cloacae*, and *Serratia plymuthica* (0.2% each, *n* = 1).

Table 4. Multidrug resistance rates among the bacterial contaminants.

Bacteria/Meat Types	MDR Prevalence	MAR Index
All Bacteria (from all meat types) (*n* = 558)	14.9% (*n* = 83)	0.12 ± 0.09
All Bacteria from Beef (*n* = 186)	11.3% (*n* = 21)	0.11 ± 0.08
All Bacteria from Goat (*n* = 191)	14.7% (*n* = 28)	0.11 ± 0.07
All Bacteria from Chicken (*n* = 181)	18.8% (*n* = 34)	0.13 ± 0.12
All *E. coli* (*n* = 262)	18.7% (*n* = 49)	0.11 ± 0.10
Beef *E. coli* (*n* = 80)	18.8% (*n* = 15)	0.12 ± 0.09
Goat *E. coli* (*n* = 80)	18.8% (*n* = 15)	0.10 ± 0.08
Chicken *E. coli* (*n* = 102)	18.6% (*n* = 19)	0.12 ± 0.12
All *A. hydrophilia* (*n* = 117)	11.1% (*n* = 13)	0.13 ± 0.05
Beef *A. hydrophilia* (*n* = 42)	7.1% (*n* = 3)	0.14 ± 0.03
Goat *A. hydrophilia* (*n* = 62)	14.5% (*n* = 9)	0.12 ± 0.06
Chicken *A. hydrophilia* (*n* = 13)	7.7% (*n* = 1)	0.11 ± 0.05
All *V. cholerae* (*n* = 20)	0.0% (*n* = 0)	0.08 ± 0.04
Beef *V. cholerae* (*n* = 10)	0.0% (*n* = 0)	0.09 ± 0.03
Goat *V. cholerae* (*n* = 10)	0.0% (*n* = 0)	0.08 ± 0.05

All *A. veronii* (*n* = 19)	0.0% (*n* = 0)	0.14 ± 0.00
Beef *A. veronii* (*n* = 12)	0.0% (*n* = 0)	0.14 ± 0.00
Goat *A. veronii* (*n* = 7)	0.0% (*n* = 0)	0.14 ± 0.00
All *K. pneumoniae* (*n* = 18)	5.6% (*n* = 1)	0.12 ± 0.10
Beef *K. pneumoniae* (*n* = 4)	0.0% (*n* = 0)	0.11 ± 0.04
Goat *K. pneumoniae* (*n* = 3)	0.0% (*n* = 0)	0.12 ± 0.04
Chicken *K. pneumoniae* (*n* = 11)	9.1% (*n* = 1)	0.12 ± 0.13

MDR= Multidrug resistance; MAR = Multiple antibiotic resistance index.

CHAPTER FIVE

5.0 DISCUSSION

The purpose of the current study was to investigate the occurrence of antibiotic residues and multidrug-resistant bacteria in meats (beef, goat meat, and chicken) sold in Accra. One major focus of it was to determine the prevalence of antibiotic residues in the meat samples. Very few of the studies conducted on animal-source foods in the country have evaluated them for the occunce of antibiotic residues (Addo et al., 2015; Aning et al., 2007; Donkor et al., 2011; Mingle et al., 2021). This study thus fills an important knowledge gap. As observed, the prevalence of antibiotic residues in the meat samples was 7.7%, and these occurred in chicken exclusively (23.3%). This prevalence in chicken is comparable to those reported in mutton (24%), pork (28.6%), chevon (29.3%), and beef (30.8%), but higher than that reported in eggs (6.8%) in the study conducted by Donkor *et al*. (2011) that evaluated the risk of exposure to egg- and meat-contained antibiotic residues in the country. It is also comparable to the prevalence of 35.5% reported by Aning *et al*. (2007) in marketed milk in the country. Addo *et al*. (2014) also reported a prevalence of 18% in raw beef, and Mingle *et al*. (2021) reported a prevalence of 63% in beef, chicken, and eggs. Moreover, in the study of Ekli *et al*. (2019), antibiotic residues were detected in 20% of the meat samples – 17% in the liver and 3% in the beef.

In many other countries particularly developing ones, a high prevalence of antibiotic residues has been reported in a variety of animal-source foods. For example, a study conducted in 2005 in Kenya reported the occurrence of antibiotic residues in meats to be 16% (Kang'ethe et al., 2005). Another similar study conducted in Tanzania reported a high prevalence of antibiotic residues to be 36% in the same sample types (Kurwijila, Omore, Staal, & Mdoe, 2006).

The exclusive occurrence of antibiotic residues in chicken, but not beef and goat meat, suggests that generally, withdrawal periods are probably observed by cattle and goat farmers in the country. It is difficult to ascribe the occurrence of antibiotic residues in the chicken to non-observance of withdrawal periods by poultry farmers in the country, as most of the chicken seems to be imported. That notwithstanding, given that the chicken samples, like the other meat types, were obtained from markets that receive high patronage, the high prevalence of antibiotic residues in them raises several safety concerns. First, consumers may be at a high risk of exposure to the residues, and this risk could further increase with repeated patronage and antibiotic residue accumulation (Hosain, Kabir, & Kamal, 2021; Ngangom, Tamunjoh, & Boyom, 2019). Health impacts that the consumers could be exposed to include direct toxicity, allergic reactions, neurological disorders, gastrointestinal disturbance, tissue damage, and disruption of the intestinal microbiome (E De Leener, 2005; Mund et al., 2017; Vishnuraj et al., 2016). It is, however, noted that the microbial inhibition assay employed in the screening for the antibiotic residues, although capable of detecting the presence of a broad range of antibiotics beyond allowable limits, is unable to quantify and differentiate the residues. Hence although it is logical to conclude that consumers of chicken obtained from the pool available at the markets studied could be at a higher risk of exposure to antibiotic residues than would consumers of beef and goat meat, it would be arduous to quantify the consumers' risk of exposure to the antibiotic residues and their development of the health conditions highlighted. Admittedly, that was beyond the scope of this study, but it could be included in the design of future studies conducted on animal source foods. The need for follow-up studies cannot be overemphasized, not just because the surveillance needs to be continuously done owing to the dynamic nature of the problem, but also because of the paucity of information on antibiotic residue occurrence in animal source food in the country. This would help generate more robust data to effectively tackle the ever-growing antibiotic resistance menace.

Another objective of the study was to determine the spectrum of bacteria contaminating the meat samples. The bacterial contaminants were observed to be of a broad range, involving 32 different types totaling 558, with the predominant ones being *Escherichia coli* (97%), *Aeromonas hydrophila* (43.3%), *Vibrio cholerae* (7.4%), *Aeromonas veronii* (7%), and *Klebsiella pneumoniae* (6.7%). The other contaminants occurred at a prevalence of about 5% or lower, and some of them include *Serratia plymuthica*, *Pantoea* spp., *Moellerella wisconsensis*, *Acinetobacter baumannii*, *Vibrio* spp., *Enterobacter cloacae*, *Vibrio alginolyticus*, *Pseudomonas luteola*, *Proteus mirabilis*, *Salmonella* spp., *Citrobacter koseri*, *Yersinia enterolytica*, and *Shigella flexneri*.

In the study of Addo *et al*. (2014) too, the predominant bacterial contaminant was *E. coli* (69.76%), and other recovered bacterial contaminants comprised *Klebsiella* spp., *Aeromonas* spp., *Pseudomonas aeruginosa*, *Enterobacter* spp., *Bacillus* spp., *Y. enterocolitica*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *S. aureus*. Adzitey *et al*. (2015) and Adzitey *et al*. (2020), whose studies were conducted on various meat types in the Tamale Metropolis, also reported *E. coli* at rates of 84–100%. In the study by Yar *et al*. (2020) that evaluated the microbiological quality of chicken imported from Brazil, the Netherlands, and the USA and sold in the Kumasi Metropolis as well, the researchers reported a mixture of bacteria and fungi contaminating the meat with the following distribution: bacteria (*Klebsiella* spp. [13.8%], *Salmonella* spp. [24.6%], *E. coli* [26.2%], and *Staphylococcus* spp. [35.4%]) and fungi (*Cladosporium* spp. [15.2%], *Penicillium* spp. [24.2%], *Rhizopus* spp. [27.3%], *Aspergillus* spp. [33.3%]). Contrastingly, Dsani *et al*. (2020), whose study was on a mixture of 205 carcasses of mutton ($n = 16$), chevon ($n = 108$), and beef ($n = 81$), and focused on *E. coli* reported an *E. coli* prevalence of 48%.

The high diversity of the bacterial contaminants is a reflection of the wide range of infections to which consumers of the meats predispose themselves, especially, if those meats are not well-cooked before their consumption. Contamination of the meats with zoonotic pathogens like *Salmonella* spp., *Yersinia enterolitica*, and *Shigella flexneri* also indicates a potential for zoonotic transmission of these pathogens to the consumers of the meats, although this risk cannot be accurately quantified with the available data. Moreover, the high bacterial contamination rate of the various meat types, particularly, with *E. coli*, is of grave concern, as *E. coli* presence indicates fecal contamination of the meats, and could potentially result in gastroenteritis among the consumers (Çakır, 2018; Ekici & Dümen, 2019).

That said, the high bacterial contamination rate is not surprising, as the hygienic practices of the vendors were generally poor. For example, the following were common practices among the vendors: chatting with each other, open display of carcasses without covers, use of unsterilized knives and other cutting edges for butchering the meats, inadequate control of insects like houseflies, and sneezing or coughing during meat handling, situations prevalent in many market settings in the country. Adzitey *et al*. (2010) whose study was conducted in the Tamale Metropolis on mutton and chevon made similar observations. Interestingly, Koffi-Nevry *et al*. (2011), who conducted their study in Cote D'Ivoire echoed similar observations, which suggests that poor meat handling practices may be a widespread problem (Nevry, Koussemon, & Coulibaly, 2011). Consequently, it would be necessary for regulatory bodies in the country to increase the robustness with which they monitor and enforce the microbial safety of meats and other foods as well as strict adherence of vendors to good food handling practices. It would also be necessary to concurrently sensitize consumers on the need to ensure that they cook their meats well to get rid of contaminating bacteria before they consume them.

The final goal of the study was to determine the antimicrobial resistance and prevalence of multidrug resistance among the bacterial contaminants of the meats. Antibiotic resistance has become a growing public health burden with a global dimension that requires quick intervention. Most of the monitoring studies have focused on bacterial isolates from humans with little information about antibiotic resistance profiles in isolates from animals. In this study, the bacterial isolates from the meat samples showed high levels of resistance to ampicillin (83.3%), augmentin (36%), and cotrimoxazole (19%). The high rates of resistance of greater than 50% to penicillin and its derivatives from meat have been reported by several studies in Ghana. In a study conducted in Accra, 57% of the bacterial isolates from animal-source foods showed resistance to ampicillin (Dsani *et al*., 2020). Another study performed in Ghana showed a 70.97% resistance to amoxicillin-clavulinic acid combination (Abass, Adzitey, & Huda, 2020). The high level of resistance to ampicillin (a derivative of penicillin) may be due to easy access to penicillin and its frequent use in animal husbandry. The study showed high rates of susceptibility of the bacterial isolates to meropenem (100%), ertapenem (98.72), imipenem (100%), amikacin (100%) and tigecycline (98.72%), and this could be explained by their non-routine use in animal husbandry. Similar to this, in the study of Dsani *et al*. (2020), the reported *E. coli* resistance rates increased across sulphamethoxazole/trimethoprim (SXT) (17%), cefuroxime (21%), tetracycline (45%), and ampicillin (57%), and the susceptibility rates increased across amikacin (92%), ciprofloxacin (92%), gentamicin (97%), chloramphenicol (97%), cefotaxime (98%), and ceftriaxone (99%), and no meropenem resistance was recorded. Moreover, Adzitey *et al*. (2020) reported *E. coli* resistance rates of ampicillin (71.67%), tetracycline (73.33%), and erythromycin (85.00%).

In this study, the prevalence of MDR and ESBLs among the contaminating bacteria was 14.9% and 2% respectively, with a good proportion of the contaminants (15.23) recording a MAR index of greater than 0.2. The highest occurrence of MDR organisms was observed in chicken,

with the highest with a good proportion of the contaminants (15.23) recording a MAR index of greater than 0.2 magnitudes of occurrence seen in isolates of *E. coli.* Dsani *et al.* (2020), cited earlier, reported their MDR prevalence to be 22.4%, and that of ESBL producers to be 14.3%. Adzitey *et al.* (2020) reported a higher MDR prevalence in their study (68.33%), but ESBL production was not reported. It is noted that many studies of this nature on occurrence have yielded different results but higher rates of resistance are observed in chicken. In a recent study involving only *E. coli* isolates, the prevalence of MDR in meat samples was reported to be 22% and the highest occurrence was seen in isolates from chicken. In a study conducted in Nepal, the prevalence of MDR organisms in the meat samples was reported to be 32.7%, with the highest occurrence observed in bacteria isolates from chicken relative to buffalo meat (Saud *et al*., 2019). The high occurrence of MDR organisms in chickens can be due to the routine use of antibiotics in poultry, not only therapeutically, but also, in growth promotion (Elmonir et al., 2021). Generally, the presence of antibiotic-resistant organisms in meats usually demonstrates the resistant situations in the gut of the animals and environments in which the animals are slaughtered and handled (Kirbis & Krizman, 2015).

MDR is a major public health and economic concern across the globe. The presence of MDR organisms in meat-related bacteria observed in the current study is cause for worry, as poor cooking of such meats could predispose consumers to not just colonization with these MDR pathogens, but also, infections with them. Under such circumstances, the potential for dissemination of the resistance traits from the MDR organisms to the microflora with whom they co-colonize the gut cannot be overlooked (Duedu, Offei, Codjoe, & Donkor, 2017). In like manner, the resistance traits can be transferred to other organisms in circulation when colonized persons shed them in fecal matter. Additionally, it will not be implausible to suggest the possibility of MDR bacteria transmitting to the hospital environments through rodents,

cockroaches and other insects, and other vehicles of transmission (Futagbi et al., 2017; S. Donkor, 2019; Tetteh-Quarcoo et al., 2013).

One limitation of the study is that the colony count of the bacteria isolates in the meat samples was not determined. Moreover, the choice of media and incubation conditions employed makes it likely to lose microaerophilic, fastidious and anaerobic bacteria that may be present in the meat samples.

CHAPTER SIX

6.0 CONCLUSIONS, RECOMMENDATIONS, AND LIMITATIONS

6.1 Conclusions

The prevalence of antibiotic residues in the meat samples was low (7.7%), and the occurrence was restricted to chicken (21%). Also, the major bacterial contaminants were *E. coli* (97.0%), *Aermonas hydrophilia* (43.3%), *Vibrio cholerae* (7.4%), and *Klebsiella pneumoniae* (7.0%). The prevalence of multidrug-resistant bacteria was moderate (14.9%), while that of ESBL producers was low (2%).

6.2 Recommendation

The high prevalence of *E. coli*, a key indicator of fecal contamination, in the meats, suggests that the slaughterers and meat vendors need to be educated on good food handling practices. The presence of antibiotic residues in the chicken indicates non-observance of withdrawal periods by some poultry farmers and suggests the need for the provision of education on its implication to poultry farmers, poultry product importers, and the general public. Also, studies of this nature need to be routinely conducted to fill gaps in antimicrobial resistance surveillance. Also, future studies should consider employing media and incubation conditions that will enable the isolation of fastidious, microaerophilic, and anaerobic organisms in the meat samples to enable assess the distribution of such organisms in meat.

6.3 Limitations

The microbial inhibition assay used for determining the presence of antibiotic residues did not quantify and differentiate the types of antibiotics present. Also, due to the choice of

media and incubation conditions used, it is likely microaerophilic, fastidious, and anaerobic bacteria will not be isolated.

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APPENDIX I

PROCEDURE FOR MALDI TOF OPERATION

1. Pick a bacterial colony and smear it onto a target plate.
2. Add 1-2 µl of a matrix consisting α-Cyano-4-hydroxycinnamic acid (CHCA) dissolved in acetonitrile (50%) and 2.5% trifluoroacetic acid onto it and dry it on the target plate (at room air)
3. Place the target plate into the plating chamber of the mass spectrometer, close it and perform the analysis.

APPENDIX II

PROCEDURE FOR API 20E

1. Confirm the culture is of Enterobacteriaceae. To test this, a quick oxidase test for cytochrome c oxidase may be performed.
2. Pick a single isolated colony (from a pure culture) and make a suspension of it in sterile distilled water.
3. Take the API20E Biochemical Test Strip which contains dehydrated bacterial media/bio-chemical reagents in 20 separate compartments.
4. Using a Pasteur pipette, fill up (up to the brim) the compartments with the bacterial suspension.
5. Add sterile oil into the ADH, LDC, ODC, H2S, and URE compartments.
6. Put some drops of water in the tray and put the API Test strip and close the tray.
7. Mark the tray with the identification number (Patient ID or Organism ID), date, and initials.
8. Incubate the tray at 37^{o}C for 18 to 24 hours.

APPENDIX III

This table shows the diameter cut-off values for the evaluation of resistant and sensitive as per species and antibiotic

ANTIBIOTICS	ENTEROBACTERIACEAE			PSEUDOMONAS, VIBRIO CHOLERAE, AEROMONAS SPECIES			SALMONELLA AND SHIGELLA		
	S	I	R	S	I	R	S	I	R
AMIKACIN 30µg	>=17	15-16	<=14	>=17	15-16	<=14			
AMPICILLIN 10 µg	>=17	14-16	<=13				>=17	14-16	<=13
GENTAMYCIN 10 µg	>=15	13-14	<=12	>=15	13-14	<=12			
CEFEPIME 30µg	>=25	19-24	<=18	>=18	15-17	<=14			
CEFUROXIME 30µg	>=18	15-17	<=14						
CEFTRIAXONE 30µg	>=23	20-22	<=19				>=23	20-22	<=19
PIPERACILLIN TAZOBACTAM 110µg	>=21	18-20	<=17	>=21	18-20	<=17			
COTRIMOXAZOLE 25µg	>=16	11-15	<=10				>=16	11-15	<=10
CIPROFLOXACIN 5µg	>=26	22-25	<=21	>=25	19-24	<=18			
ERTAPENEM 10µg	>=22	19-21	<=18						
IMIPENEM 10µg	>=23	20-22	<=19	>=19	16-18	<=14			
MEROPENEM 10µg	>=23	20-22	<=19	>=19	16-18	<=15			
TIGERCYCLINE 15 µg	>=18		<=18						
AUGMENTIN 30µg	>=18	14-17	<=13						