UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

ANTIBIOTIC RESISTANCE AND WHOLE GENOME SEQUENCING OF *E. COLI* ISOLATED FROM READY-TO-EAT (RTE) MEATS IN ONE HEALTH CONTEXT, IN ACCRA METROPOLIS OF GHANA

BONIVENTURE KYIIRU

(UDS/MAN/0003/22)

2024

UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

ANTIBIOTIC RESISTANCE AND WHOLE GENOME SEQUENCING OF *E. COLI* ISOLATED FROM READY-TO-EAT (RTE) MEATS IN ONE HEALTH CONTEXT, IN ACCRA METROPOLIS OF GHANA

BONIVENTURE KYIIRU

(UDS/MAN/0003/22)

A THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, FOOD AND CONSUMAER SCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY (MPHIL) DEGREE IN ANIMAL SCIENCE (MEAT SCIENCE AND TECHNOLOGY OPTION)

MAY, 2024

DECLARATION

Student

I hereby declare that I am the sole author of this thesis and that it has not been submitted for a degree or had any part published anywhere by anyone else. But every other person's work that is mentioned in the text has been properly referenced, and any help that was given to write this thesis has been properly acknowledged.

Candidate's Signature…… Date……

Name: Boniventure Kyiiru

Supervisor

I so certify that the thesis was prepared and presented under proper supervision, in compliance with the thesis supervision guidelines established by the University for Development Studies.

Supervisor's Signature……. Date……

Prof. Frederick Adzitey (PhD)

ACKNOWLEDGMENTS

This project work was put together on the support, talents, encouragement and the advices of numerous individuals than I can acknowledge. Yet, I would like to mention the contributions of some who have aided this. First of all, I am most grateful to Almighty God for the wisdom, knowledge, understanding and guidance inspired me during this project, it would not have been easy for me but through His miracles made this work a success.

I am much grateful to Professor Frederick Adzitey, my supervisor and Head of Department for his sacrificial time, patience and constructive criticism throughout this project work and making it a success. I am honored haven you supervised this project. I equally express my profound gratitude to the entire staffs of the Department of Animal Science for their diverse contributions to this work.

I give much credit to Miss Rejoice Ekli for her support throughout the data collection process, may God reward her greatly. I am grateful to the staffs of Noguchi Memorial Institute for Medical Research, Accra, Ghana for the laboratory work on the whole genome sequencing. I also thank Mr Issah Bagulo for his time spend in assisting me in diverse ways during the work. Also, many thanks to my mates in the persons of Mr. Abass, Madam Nafisha, Mr. Tati, Mr. Mawusi and so on for their support in one way or the other. I am equally grateful to everyone who has contributed to making this work a success.

ABSTRACT

Meat is an essential part of human daily diet due to its nutrient composition and the vital roles it plays in human growth, development and proper functioning. It has equal favorable environment for the growth and multiplication of microorganisms including pathogenic ones. This study seeks to determine the prevalence rate of *Escherichia coli* (*E. coli*) in ready-to-eat (RTE) meats using one health approach. The study also determined the antibiotic resistance and presence of antimicrobial resistance genes, plasmid replicons, virulence genes and multilocus sequence typing (MLST) types of the *E. coli*. In all, 360 ready-to-eat meat and associated samples were randomly collected. The protocol in the USA-FDA Bacteriological Analytical Manual was used for identification of *E. coli*. The disk diffusion method was used for antibiotic resistance tests. Antimicrobial resistance genes, plasmid replicons, virulence genes and MLST types were determined using whole genome sequencing. The findings revealed that 25 samples were contaminated with *E. coli*, representing 7% with RTE beef (15%) being the most contaminated, followed by RTE chicken (10%) and the least was spices (2%) used for preparing RTE meats. The prevalence of *E. coli* in animal (RTE beef, chicken and mutton), human (hands of RTE meat sellers) and environmental (knives for cutting RTE meats and spices for preparing RTE meats) sources was 10%, 3% and 4.2%, respectively. *E. coli* isolates showed resistant mostly to Amoxicillin (100.0%), followed by Suphamethoxazole/trimethoprim (36.0%) and Tetracycline (32.0%), with the lowest resistant being Ceftriaxone (0.0%). The multiple antibiotic resistant (MAR) index ranged from 0.1-0.6. Three of the *E. coli* isolates from Maamobi, Agbogboloshie and Malata beef were resistant to five different antibiotics namely AZM-AML-C-TE-SXT, AML-TE-SXT-AUG-CIP and AML-TE-SXT-AUG-CIP, respectively with MAR index of 0.6. Whole genome sequencing revealed that 52.4% of the *E. coli* isolates harboured antimicrobial resistance genes, and a total of 54 antimicrobial resistance genes made up of 19 different genes were detected. Plasmid replicons were detected in 81.0% of the *E. coli* isolates with a total of 28 plasmid replicons made up of 13 different types. Virulence genes were detected in all the *E. coli* isolates (100%) and in all 156 virulence genes were present made up of 45 different types for multilocus sequence typing, 81.0% were typed, the remaining 19% possessed an unknown MLST types and in all 17 MLST types made up of 12 different types were present. This study reveals the presence of *E. coli* in some of the RTE meats and it associated samples. This *E. coli* exhibited varying resistance to antibiotics and AMR genes, plasmid replicons, virulence genes and known and unknown MLST types were detected.

DEDICATION

I commit this thesis to my spouse, Madam Helen Logh as well as my kids, Bonaventure Y. Kyiiru Junior, Benedicta Y. Kyiiru, Bernice T. Kyiiru and Belinda P. Kyiiru for their encouragement patience and understanding during my studies. Again, to my parents Cletus Kyiiru and Mary Kyiiru for their supplications and assistance throughout my schooling.

TABLE OF CONTENT

LIST OF TABLES

LIST OF ACRONYMS/ABBREVIATIONS

AIEC = Adherent Invasive *E. coli*

AMR = Antimicrobial Resistant

APEC = Avian Pathogenic *E. coli*

CFA = Colonization Factor Antigen

CLSI = Clinical and Laboratory Standards Institute Guidelines

DNA = Deoxyribonucleic Acid

GIT = Gastrointestinal Tracts

EAEC = Enteroaggregative *E. coli*

EFSA = European Food Safety Authority

EHEC = Enterohemorrhagic *E. coli*

EIEC = Enteroinvasive *E. coli*

EPEC = Enteropathogenic *E. coli*

ESBL = Extended Spectrum Beta-Lactamase

ETEC = Enterotoxigenic *E. coli*

EU/EEA = European Union/European Economic Area

FBP = Foodborne Pathogens

FDA-BAM = Food and Drug Administration-Bacteriological Analytical Manual

FSIS = Food Safety and Inspection Service

HC = Hemorrhagic Colitis

HUS = Hemorrhagic Uremic Syndrome

LA = Localized Adherence

NMEC = Neonatal Meningitis *E. coli*

MAR = Multiple Antibiotic Resistant

MLST = Multilocus Sequence Typing

NGS = Next-Generation Sequence

RTE = Ready-to-Eat

STEC = Shiga Toxin-Producing *E. coli*

TPC = Total Plate Count

TVC = Total Viable Count

UPEC = UroPatogenic *Escherichia coli*

USA-FDA = United State of America-Food and Drugs Authority

UTIs = Urinary Tract Infections

WGS = Whole Genome Sequencing

WHO = World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The consumption of meat and meat products is seemingly on the ascendancy in most parts of the world probably due to increase in income levels, population and the high protein levels plus other nutrients such as vitamins, minerals and essential fatty acids present in meats (Smith *et al*., 2022; Ahmad *et al*., 2018; Adzitey *et al*., 2018). Overall world meat production increased by 1.25% in 2017, and by 2027 it is expected to be 8% higher than in the base period in developed countries, whereas in developing regions it is expected to increase by 21% (Marcel *et al*., 2018). According to Rich *et al*. (2022), over the last two decades, meat production on the African continent has almost doubled, from 11.59 million metric tons in 2000 to 19.88 million metric tons in 2020. In Ghana, protein consumption is on the increase such that domestic production does not meet the demand and this in turn has increased the demand for imported meat products (Mingle *et al*., 2021). Meat is a delicate product, susceptible to microbial invasion and subsequently deterioration. Due to the high level of nutrient and water holding capacity of meat, microorganisms including *Escherichia coli* (*E. coli*) can cause visual, texture and organoleptic changes through the production of metabolites (Saucier, 2016; Adzitey *et al*., 2018). Microbes also contaminate meat, causing it to spoil and expose consumers to foodborne diseases.

An earlier study states the essential of the application of DNA sequencing and bioinformatic approaches to study the structure and function of genes present in both humans and pathogens (Kwong *et al*., 2015). Little is known about the genomic characteristics of commensal *E. coli* in produce, their carriage of AMR determinants, virulence traits and potential presence of opportunistic extra intestinal pathogens (Reid

et al., 2020). Due to its ability to provide comprehensive genomic data in a single time, next-generation sequence (NGS) has been increasingly used for genomic characterization of foodborne bacterial pathogens, identification of clonal groups in bacteria of public health importance and molecular characterization of epidemic plasmids harboring AMR or/and virulence genes (Trongjit and Chuanchuen, 2021). Genomic has extensively been used in recent fields of research to study infectious agents. Despite the perceived benefits of sequencing technology to support traditional methods in diagnostic microbiology, there has been limited application in clinical and public health laboratories in other parts of the world (Kwong *et al*., 2015).

Escherichia coli (E. coli) is a Gram-negative bacterium of the enterobacteriaceae family. It lives in the gastrointestinal tracts (GIT) of humans and animals and they commonly end up in the environment due to the close association of animals and humans to their environment (Puvača and Frutos, 2021; Moxley, 2022). *Escherichia coli* is also transmitted to humans primarily through consumption of contaminated foods, including raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts (WHO. 2019). WHO (2019) indicated that, most strains of *E. coli* are harmless but some strains such as Shiga toxin-producing *E. coli* (STEC) can cause severe foodborne diseases. *Escherichia coli* species have been associated with bowel necrosis (tissue death) and hemolytic uremic syndrome (Doyle *et al*., 2006; Adzitey *et al*., 2018). According to Adak *et al*. (2005), foodborne infection can lead to the death of children, affects their growth and cognitive development and heavily affects health care systems. Shiga toxin–producing *Escherichia coli* caused approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States (WHO, 2019; Collins and Greens, 2010).

Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death (Adzitey *et al*., 2021; Tang et al., 2023). Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries and a rise in bacterial resistance to antibiotics complicates treatment of infections (Kibret and Abera, 2011).

1.2 Problem statement

There are some reports on microbial contamination including *Escherichia coli* on ready-to-eat (RTE) meats, meat handlers, equipment and spices in Ghana with the antibiotic resistance of *E. coli* isolated from these RTE meat and its effect on the general public as far as one health concept is concern (Adzitey *et al*., 2021). Generally, the processes in slaughtering animals, processing of the meat and places for sale or marketing are usually unsafe and unhygienic in Ghana and therefore exposing meat to microbial contamination including *Escherichia coli* (Nkegbe *et al*., 2013). These can be transferred to humans through the consumption of undercooked meat and meat products.

1.3 Justification

Despite the fact that there have been some studies of *E. coli* in RTE meats in some parts of the world, such study in Ghana is limited. To the best of my knowledge and via literature search online, such a study in Accra Metropolis was not found especially with regards to whole genome sequencing. This necessitates the need for more studies to determine the level of contamination of RTE meats with microorganisms including *E. coli*, their resistance to antimicrobials, their genetic diversity and to compare the

dynamics of these studies to other parts of the world for epidemiological purposes and public health education/promotion.

1.4 Objectives

The objectives of this study are to determine:

1. the prevalence of *E. coli* in ready-to-eat meats and it associated samples (hands, knives and spices) using one health approach.
2. the antibiotic resistance of selected *E. coli* isolated from ready-to-eat meats and it associated samples.
3. antibiotic resistance genes, plasmid replicons, virulence genes, and MLST types of selected *E. coli* using whole genome sequencing.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Ready-to-eat food (RTE)

Commercially prepared and made for public consumption, ready-to-eat food is available everywhere in the world (Dudeja *et al*., 2017). These are a set of food products that are pre-cleaned, pre-cooked, generally packaged, and ready for consumption without prior preparation or cooking, according to Huang and Hwang (2012). Tshipamba (2018) reported that ready-to-eat meals are produced and sold around the world, particularly in cities and towns to busy employees, students and travellers, and it has become a lucrative business and source of employment. RTE meats are popular in Ghana and are normally sold on the streets, especially at nights, and make a significant contribution to the protein intake of Ghanaians (Abass *et al*., 2020).

2.2 Sources of contamination of ready-to-eat meats

The traditional processing methods that are used in preparation, inappropriate holding temperatures and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended food (Mohammed, 2004). It is easily contaminated by a variety of foodborne pathogens and would be a major source of foodborne diseases. Meat and meat products are considered to be excellent sources of support for the growth of such pathogens (Wang *et al*., 2020). Conditions of food safety include efforts to avoid contamination from biological, chemical agents and other substances that can endanger human health. Microbiological food contamination refers to the presence in food of harmful microorganisms which can cause illness (Bagumire and Karumuna, 2017). The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, processing, storage and distribution at slaughterhouses and retail establishments. Faecal matter is a major source of

contamination and can reach carcasses through direct deposition, as well as by indirect contact through contaminated and clean carcasses, equipment, workers, installations and air (Barros *et al*., 2007). The common pathogenic bacteria found in RTE meats are *Clostridium perfringens*, *Salmonella enterica*, *Staphylococcus aureus* and *E. coli* (Omer *et al*., 2018). Despite ready-to-eat meats being an important source of valuable proteins, they are one of the leading vehicles for microbial pathogens and have been implicated in foodborne disease outbreaks in humans (Shiningeni *et al*., 2019).

2.3 Pathogens in ready-to-eat meats

According to Mohammed (2004), meats products are contaminated with pathogenic Gram- positive and Gram-negative bacteria. This also implies that these meats are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. Improper handling and improper hygiene might lead to the contamination of meats products and this might eventually affect the health of the consumers. The most important bacterial pathogens in meat and meat products that are responsible for food borne infection includes *E. coli*, *Salmonella* and coagulase positive *Streptococcus aureus* (Shaltout *et al*., 2022). The Food safety and inspection service (FSIS) is especially concerned with the presence of *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157: H7, and Staphylococcal enterotoxins in RTE meat and poultry products because of the potential for moderate to severe illness or death, especially among high-risk individuals (Levine *et al*., 2001). Bacterial pathogens associated with RTE food products are *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes*, which result in major disease outbreaks and product recall (Ijabdeniyi *et al*., 2019; Saad *et al*., 2018).

2.4 Foodborne diseases

Foodborne illnesses are infections or irritations of the gastrointestinal (GI) tract caused by food or beverages that contain harmful bacteria, parasites, viruses, or chemicals (Scallan *et al*., 2011). A foodborne illness can affect anyone. However, some people are more at danger than others, including expectant mothers, children, the elderly, and those with weakened immune systems. Every year, there are thought to be 48 million cases of foodborne illnesses (Nazir *et al*., 2023). The most commonly recognized foodborne infections are those caused by the bacteria *Campylobacter, Salmonella*, and *E. coli* O157:H7, and by a group of viruses called calicivirus, also known as the Norwalk and Norwalk-like viruses (Morsy *et al*., 2023).

2.5 Food hygiene issues

Food hygiene is the conditions and measures necessary to ensure the safety of food from production to consumption (Kamboj *et al*., 2020). It is obvious and widely acknowledged that unhygienic food, including contamination of what we consume on a daily basis, has killed millions of people around the world, but especially in Africa where there is a low level of education, poverty, lack of strict enforcement and adherence to public health policies, a lack of qualified staff, and a lack of funding for the health care system, making Africa and Ghana in particular vulnerable to foodborne diseases (Yakubu, 2021). Food hygiene refers to the practices that prevent microbial contamination of food at all points along the chain from farm to table. Food safety refers to the practices that ensure food remains safe at every stage of the food chain from production to harvest, processing, storage, distribution, all the way to preparation and consumption (Kaur *et al*., 2022).

2.6 Impact of foodborne pathogens on public health

The most well-known foodborne pathogens (FBP) include bacteria, viruses, parasites, mycotoxins, and marine biotoxins. Bacteria include *Campylobacter, Salmonella, E. coli, L. monocytogenes, Shigella, Clostridium botulinum, Clostridium perfringens, S. aureus*, and *B. cereus*; viruses include Norovirus, Hepatitis A virus, and Rotavirus; parasites include *Taenia solium* (Hassanain *et al*., 2013). Because of foodborne disease outbreaks linked to unsafe raw food, abused temperature, inadequate cooking, poor personal hygiene, improper handling techniques, and cross-contamination of cooked food with uncooked/raw food, the safety and quality of food produced for human consumption in developing countries has increased over time (Odeyemi, 2016).

2.7 Controlling outbreaks of foodborne diseases

Gargiulo *et al*. (2022) reported that, by concentrating on food preparation, the number of foodborne disease cases in Africa can be significantly decreased. In reality, only this level of intervention can stop foodborne disease cases brought on by viruses, toxins, *Clostridium perfringens, Staphylococci*, and bacteria. Controls in the food production sector may reduce *Campylobacter* and *Salmonella* illnesses, but intervention in the public and private catering and residential sectors may result in the greatest reduction in these infections. As a result, other infections should be the focus of efforts (Newell *et al*., 2017).

2.8 Meat

Meat is any fleshy muscle, such as skeletal muscle, together with any fat or associated connective tissue that is consumed as food (Fish *et al*., 2020). Shook *et al*. (2019) indicated that, after an animal is killed and its flesh is sliced, the process results in the production of meat. According to Jayathilakan *et al*. (2012), the main components of

meat are water and protein, and it is frequently consumed alongside other foods. Although meat can be eaten raw, it is frequently consumed in a variety of ways following cooking and processing. Animal products are often great sources of protein because they include the fundamental amino acids, which are biologically significant and balanced (Day *et al*., 2022). Additionally, meat is the most perishable of all staple foods since it has sufficient nutrients to support bacterial development and production (Luo *et al*., 2022). According to Ahmad *et al*. (2018), animal muscles is roughly made up of 75% water, 20% protein, and 5% of fat, carbohydrates, and other proteins. Meat and meat products are ingested for a variety of reasons, including their protein content, availability of vitamins, minerals, and fats, as well as their savory flavors.

2.9 Types of meat

2.9.1 Chevon and mutton

Meat from goats and sheep are chevon and mutton, respectively. In Ghana, small ruminants are raised (Adams *et al*., 2021). According to Tella and Chineke (2022), their production is crucial for the establishment of jobs, generating income, and providing resources for food (chevon and mutton). Small ruminants are frequently butchered around Christmas, during Islamic holidays, and months before the peasant season (Sime, 2021). As a result, the majority of Ghanaians love chevon and mutton, which are both common meats in Ghana. The majority of these meats are sold to clients in open marketplaces after being chopped into pieces and weighed in pounds (Gewertz and Errington, 2010). While most Ghanaians depend heavily on chevon and mutton for their livelihood, due to poor meat handling practices in some Ghanaian markets, they may also be a substantial source of human pathogenic germs and, consequently, foodborne illnesses (Adams *et al*., 2021).

2.9.2 Beef

Beef is the common name for the bovine carcass or flesh (Schenkel *et al*., 2006). The three Northern regions of Ghana produce the majority of Ghana's cattle, while some are imported from neighboring nations, therefore beef is a convenient source of animal nutrition for many Ghanaians, according to Adzitey *et al*. (2020). The muscle of a healthy living cattle is essentially sterile (Ben *et al*., 2017). Inadequate management of animals before and after slaughter can taint beef (Bonneau and Weiler, 2019).

2.9.3 Chicken

Gallus domesticus, sometimes known as domestic chickens, is the scientific name for the genus Gallus, which is a member of the Phasianidae family (Jarulis *et al*., 2022). As per Alders *et al*. (2018), local chickens possess adaptable traits that enable them to endure and reproduce under unfavorable environmental, dietary, and managerial conditions. According to Moreki *et al*. (2010), there are two types of chicken production in Africa: commercial (high input and high output) and rural (village). According to Haque *et al*. (2020), the commercial sector prioritizes the intense production of meat and eggs. While the village sector is referred to as the traditional, rural, scavenging, family, local, or extensive production system, they use high yield strains that have been grown and supplied by international breeding groups. The meat of local chickens is light, simple to prepare, and low in fat (Danek *et al*., 2021). Additionally, they are more enticing than the rare broiler breeds (Dawkins and Layton, 2012). Local chicken has genetically inferior carcass weight and egg production capacities compared to foreign breeds (Nolte *et al*., 2020). Also, Moges (2010) reported that while 33% of local chicken is sold for profit, 36% of it is consumed by households. Sixteen percent (16%) are used for ceremonies, 13% as presents, and 2% are utilized for other things.

2.9.4 Guinea fowl

Guinea fowls (*Numida meleagris*), raised in Africa, were domesticated for the first time by the ancient Egyptians (Newbold, 1926; Okyere *et al*., 2018). All around the world, their development has accelerated quickly, however, the development of the bird is still in its infancy in Africa (Lawler, 2016). Where it was previously associated with small-scale farmers (Abdul-Rahaman and Adu, 2017). The bird is now bred commercially on a huge scale in France, Belgium, Canada, and Australia (Saina, 2005). Guinea fowls, in contrast to chicken and turkey, are typically resistant to contagious illnesses including Newcastle disease, fowl pox, and gumboro, which have a tendency to obliterate the poultry population. Guinea fowls are expensive in Africa because they have better nutritional value than imported chicken, according to Seneviratna (2013). The meat of guinea fowl, which may weigh up to one kilogram, is incredibly flavorful and almost pheasant-like (Taylor, 2012). Guinea fowl meat is richer in flavor than chicken meat, with the added benefit of having less fat and calories (Soriano-Santos, 2010). The pure white guinea fowl's meat has a lighter color and a little less gamey flavor (Geldenhuys, 2014). According to Feltwell (2011), guinea fowl meat is lean and rich in essential fatty acids, similar to that of a free-range chicken. Compared to turkey meat, the meat has fewer calories and rich in fatty acids. Turkey meat has about 100 kcal per 100 Grams, while guinea hen meat has about 134 kcal per 100 Grams.

2.9.5 Pork

According to Belyaev (2021), a pig is one of the species of the Sus genus, which belongs to the Suidae family of even-toed ungulates. Young pigs are another name for piglets (Mayer and Brisbin, 2008). Pigs include domestic pigs and the well-known Eurasian wild boar (*Sus scrofa*), as well as their offspring and other creatures (Bosse *et al*., 2014). According to Ruvinsky *et al*. (2011), pigs, like all suids, originate from the continents

of Eurasia and Africa, from Europe to the Pacific islands. Asia's pygmy hog is the Babirusa of Indonesia. The African Warthog and another genus of pigs belong to a family of peccaries known as the Suids, which are separate from pigs (Sutherland-Smith, 2015). Pigs are naturally intelligent and gregarious animals (Marino and Colvin, 2015). Pigs are frequently employed in therapeutic experiments involving people because of their genetic similarity to humans (Evangelho *et al*., 2019).

According to Hoan *et al*. (2019), pork is the meat of domestic pigs, it is the most consumed meat in the world, with evidence of pig husbandry dating back to 5000 BC (Harris, 2012). Pork is consumed both raw and preserved. The shelf life of products made from pork is extended by curing (Realini, 2011). According to research by Bergeaud-Blackler (2015), pork is not only one of the most popular meats in the West and Central Europe, but it is also widely consumed in Southeast Asia, India, and Malaysia's eastern and non-muslim regions. Pork is highly prized for its fat content and texture in Asian cuisines, especially in China, according to Höllmann (2013). Pork consumption is prohibited by some cultures and religions, most notably Judaism and Islam (Hamdan *et al*., 2021). According to Wallinga (2009), 36% of the world's meat production comes from pigs, making them the most widely eaten commodity in the world.

2.10 Microbial contamination of meat

If not properly stored, processed and disseminated, meat can become contaminated, ruined, and harmful due to microbial development and activity (Comi, 2017). Das ***et al***. (2019) noted that poultry meat and red meat contain a limited number of bacteria that when exposed to favorable conditions for growth, can multiply to make meat unwholesome. It is known that eggs and deserts are important vectors for the spread of pathogens like *E. coli* (Ajulo *et al*., 2020). Red meat and poultry are more susceptible

to contract pathogens during handling because of their perishable nature (Barberis *et al.*, 2018). Meat is inappropriate for human eating and causes gastrointestinal disorders when harmful germs like *E. coli* and others are present (Ekici and Dümen, 2019). Numerous food safety methods and processes are available in the food industry, notably in the meat industry, to reduce the risk of contamination and disease transmission (Newell *et al.*, 2010). Helminthes, molds, bacteria, and viruses are some of the microorganisms in food hygiene that are of interest (Lorenzo *et al.*, 2018). Bacteria are said to be the most crucial component of these groups, according to EFSA (2010), meat that has undergone post-mortem inspection or that has effective internal parasite control programs or mechanisms in place is unaffected by parasites. The most well-known bacteria linked to meat eating include *Staphylococcus aureus, Campylobacter spp., E. coli., Salmonella spp., Listeria monocytogenes, Clostridium perfringens, Yersinia enterocolitica*, *Bacillus cereus* and *Vibrio parahaemolyticus* (Gourama, 2020). The three most common bacterial pathogens found in fresh meat and poultry are *Campylobacter spp.*, *Salmonella spp.* and *E. coli* (Kegode *et al.*, 2008). Minami *et al.* (2010), stated that foodborne pathogens like *E. coli, Listeria spp, Salmonella enterica* and *Shigella spp*, were isolated from meat samples in supermarkets and meat shops, whereas *Staphylococcus* and *Shigella spp*, were isolated from meat handling equipment. *E. coli* and other bacteria like *Bacillus cereus, Staphylococcus aureus*, and *Clostridium perfringens* were also isolated from meat samples. James and James (2023) argued that in order to prevent bacterial contamination, meat should be frozen or maintained in the coldest region of the freezer.

2.11 Total coliform count and enterobacteriaceae

Coliforms are often not harmful, but their presence in meat samples indicate the potential existence of pathogenic microbes (Cabral, 2010). According to Yohannes

(2021), coliform microorganisms are Gram-negative, non-spore-producing, rod-shaped, motile or non-motile bacteria that can ferment lactose at a temperature of 35 to 37 °C to produce gas and acid. Aerobic coliform may be found in the environment naturally on nearby objects including trees, the ground, and even the faces of people and animals (Van der Loo, 2020). The ubiquity of coliform bacteria in food and water raises the possibility of the presence of other fecal-based harmful microbes, such as bacteria, protozoa, viruses, and various multicellular parasites that cause disease (Faintuch and Faintuch, 2019). According to Shariq *et al*. (2016), coliform bacteria include *E. coli, Citrobacter, Klebsiella*, *Hafnia,* and *Enterobacter*. Total coliforms, fecal coliforms, and thermos-tolerant bacteria are the three types of coliforms. Fecal coliform is a group of bacteria present in feces and includes *Salmonella* and *E. coli*. Total coliform is a term used to describe the enormous variety bacteria that live in the gut. Fecal coliform microbes, which are a subset of the total coliform bacteria found in human or animal intestines and feces, can also be referred to as thermo-tolerant microbes (Yetis and Selek, 2015). Along with *E. coli*, thermotolerant bacteria are suggested as a fecal contamination indicator (Odonkor and Ampofo, 2013). The family of Gram-negative rods known as enterobacteriaceae is large and diverse (Cabral, 2010). Nieder *et al*. (2018), indicated that they can be found in soil, on plants and can contaminate food and lead to gastroenteritis in animals and humans. They are treated as sources of fecal contamination when discovered in foods. According to Cesur *et al*. (2018), enterobacteriaceae are facultative aerobes and anaerobes that may ferment a variety of carbohydrates, have a complicated antigenic structure, produce a number of toxins, as well as other virulence-inducing substances. The genera in this family include *Escherichia coli, Shigella, Salmonella, Yersinia, Enterobacter, Klebsiella, Serratia, Proteus,* and others (Farmer *et al*., 2010). Fully processed meat should have less than 5

log cfu/g of microbial contamination in meat samples gathered from Korea (Hailu, 2023). Kim and Yim (2016) found that the average coliform counts for beef, pig, and chicken samples were 2.90, 3.19, and 3.79log cfu/g, respectively. Khebab samples from Osu had a mean total plate count (TPC) of 5.02, whereas samples from Accra Central had a TPC of 4.08 and samples from Nima had a TPC of 4.80 log 10 cfu/g (Agbodaze et al., 2005). Accra Central samples had the highest mean coliform count of 5.12 log10 cfu/g, with samples from Osu and Nima having 4.41 and 3.70 log10 cfu/g, respectively (Agbodaze *et al*., 2005). Additionally, Accra Central samples had the highest fecal coliforms of 4.4 log10 cfu/g, as opposed to those bought from Osu and Nima, which recorded 3.98 and 3.80 log10 cfu/g, respectively. According to Ampaw (2018), grilled street meat offered at the Banana Inn vending area had a microbiological count of 5.929 ± 1.064 log10cfu/g, while the minimum count at the Tabora vending area was 2.739 ± 0.370 log10cfu/g. In the vending areas, Madina had the highest TVC load of 7. 267 log10 cfu/g, whereas Sowutuom had the lowest TVC of 4.732 log10 cfu/g. The highest total coliform count (TCC) was found in the Banana Inn sales area (6.394 log10 cfu/g), while the lowest level (0.00 log10 cfu/g) was found in the Dome, Legon, North Kaneshie and Tabora sales zones.

2.12 E. coli

Named after the German pediatrician Theodor Escherich, the Escherichia genus is a facultative anaerobic, Gram-negative bacillus belonging to the Enterobacteriaceae family (Torres *et al*., 2009). The majority of facultative anaerobic bacteria identified in the big intestines of humans and other animals are *E. coli*, which is widely distributed in nature (Jang *et al*., 2017). According to Secher *et al*. (2016), there are hundreds of different strains of *E. coli*, some of which are innocuous and live in normal human and animal intestines. According to Pokharel *et al*. (2023), each group of *E. coli* strains has

a unique set of somatic (O) and flagella (1) antigens as well as specific virulence traits that are often plasmid-mediated. *E. coli* is a crucial host organism in biotechnology due to its special traits that are so simple to manipulate, the availability of the complete genome sequence and its capacity to grow in both aerobic and anaerobic settings (Jang *et al*., 2017). Additionally, it is employed in a variety of pharmacological and biological applications. According to Gopal and Kumar (2013), *E. coli* is the microbe most frequently employed in recombinant DNA technology.

2.12.1 Enterohemorrhagic *E. coli* (EHEC)

E. coli O157:H7 and less frequently other *E. coli* (026: HII) serotypes that produce cytotoxins identical to those found in *Shigella dysenteries*, type 1 cause hemorrhagic colitis. According to Bryan *et al*. (2015), these toxins are classified as shiga and include verotoxins and toxins, over the past 20 years, enterohaemorrhagic *E. coli* has been associated with severe and bloody diarrhea, hemorrhagic colitis (HC), and hemorrhagic uremic syndrome (HUS), especially in children (Meng *et al*., 2012). EHEC serotype 0157 H7 is responsible for HUS and bloody diarrhea outbreaks around the world (Joseph *et al*., 2020).

2.12.2 Enteroinvasive *E. coli* (EIEC)

These strains have distinct *E. coli* serotypes that are different from EPEC serotypes (028, 0112, 0115, 0124, 0136, 0143, 0144, 0147, 0152, 0164, and 0167). The EIEC strains may enter the epithelial intestinal cells and are biochemically similar to Shigella (Bumbaugh, 2003). EIEC typically causes a spectrum of diseases ranging from mild, self-limiting diarrhoea to more severe cases resembling bacillary dysentery (shigellosis), symptoms include abdominal cramps, fever, and bloody or inflammatory diarrhoea (Vashisht, 2019). According to Pasqua *et al*. (2017), EIEC strains exhibit

invasive properties, allowing them to enter and multiply within the colonic epithelial cells, the bacteria possess a type III secretion system, which is involved in the injection of effector proteins into host cells, contributing to invasion. EIEC are very invasive. Typically, dysentery brought on by EIEC happens 12 to 72 hours after eating contaminated food. Abdominal pains, diarrhoea, vomiting, fever, chills, and a general malaise are the symptoms of the sickness (Sharif *et al*., 2018).

2.12.3 Enteropathogenic *E. coli* (EPEC)

The serotypes 044, 055, 086, 0111, 0114, 0119, 0125, 0126, 0127, 0128, 0142, and 0158 are those that have historically been associated with the EPEC strains as distinct *E. coli* members (DuPont *et al*., 2009). The enteropathogenic *E. coli* strains cling to the gastrointestinal tract and produce a recognizable gastrointestinal lesion (Lim *et al*., 2010). According to Mare *et al*. (2021) enteropathogenic *E. coli* do not produce enterotoxins and are not invasive. All EPEC are by definition deficient in the genes needed to make shiga toxin (*stx*). Typical EPEC (tEPEC) *E. coli* strains are those that have the phenotypes *eae*, *+bfpA*, and *+stx* (Abe *et al*., 2009). Most of these strains display the localized adherence (LA) phenotype linked to BFP production and are classic O:H serotypes (Trabulsi *et al*., 2002). The *E. coli* common pilus and other recognized adhesins are linked to the LAL pattern in EPEC (Nara *et al*., 2012). Over 200 O-serogroups have been found among EPEC stains; the majority do not fall into the traditional EPEC O-serogroups, and many have been deemed non-typeable (Ochoa and Contreras, 2011).

2.12.4 Enterotoxigenic *E. coli* (ETEC)

According to Steffen *et al*. (2015), ETEC infection is the main cause of traveler's diarrhea and a significant cause of diarrheal illness in low-income countries,

particularly in children. ETEC is spread by contaminated food or water that contains excrement from humans or animals (Smith and Fratamico, 2017). Avoiding or properly preparing meals that could be contaminated with the bacteria, as well as often washing your hands with soap, can help prevent infections (Todd *et al*., 2010).

2.12.5 Enteroaggregative *E. coli*

They can be identified by their distinctive adherence pattern in tissue-culture based assays. On HEP-2 or HeLa cells, all strains exhibit the "stacked brick" adhesion pattern, in contrast to EPEC, which exhibits localized adherence (Carlino *et al*., 2020). More information is needed to understand how *E. coli* diffusely binds to these cell lines (Prorok-Hamon *et al*., 2014). According to Huang *et al*. (2004), EAEC is currently acknowledged as a novel intestinal pathogen. Enterotoxigenic *E. coli* and EAEC are the two most frequent causes of traveler's diarrhea in both adult and pediatric populations (Sanders *et al*., 2019). Over 5000 cases and at least 50 fatalities occurred as a result of a significant epidemic of EAEC in Germany in 2011 (Meng *et al*., 2012). The pathogen was discovered to be an EAEC 0104:H4 strain that was lysogenized by a phage that frequently encodes the adhesin intimin and is generally linked with Shiga toxin-producing *E. coli* (Franz *et al*., 2014). Table 2.1 shows the various pathogenic strains of *E. coli* (Allocati *et al*., 2013).

2.13 Transmission of *E. coli*

E. coli is usually transmitted orally through feces in the digestive tracts of healthy humans and warm-blooded animals (Peek *et al*., 2018). Fegan and Gobius (2012) reported that, nearly all cases of *E. coli* originate in the feces, and they spread through fecal-oral transmission. Furthermore, *E. coli* can spread by contaminated water, cross-contamination during food preparation, or direct human contact (Taulo *et al*., 2009).

Conversely, the main source of exposure is typically contaminated meals, which include raw or undercooked ground beef products, raw milk, and fresh produce (Butt *et al.*, 2021). Sources of *E. coli* infections are through undercooked or raw hamburgers, beef, pork, mutton, chevon, chickens, sprouting's, dry-curated salamis, broccoli, cheese curds, unpasteurized milk or raw milk, contaminated water and ice, and person-to-person transmission (Manning, 2010).

Table 2. 1: Pathogenic *E. coli* strains

Pathotype	Diseases	Symptoms	Virulence factors
EnteroToxigenic *E. coli* (ETEC)	Traveler's diarrhea	Watery diarrhea and vomiting	Heat-labbile and sheat-stable toxins, CFAs, AAFs, cytotoxins
EnteroAggregative *E. coli* (EAEC)	Diarrhea in children	Diarrhea with mucus and vomiting	
EnteroInvasive *E. coli* (AIEC)	Shigellosis-like	Watery diarrhea; dysentery	Cellular invasion, Ipa shiga toxin, hemolysin
Adherent Invasive *E. coli* (AIEC)	Associated with chronic diseases	Persistent intestinal inflammation	Type 1 fimbriae, cellular invasion
Neonatal Meningitis *E. coli* (NMEC)	Neonatal meningitis	Acute meningitis, sepsis	S fimbrie; K1 capsule

Avian Pathogenic *E. coli* (APEC)	Probable source of foodborne disease	Type 1 and P fimbriae; K1 capsule

Bfp: Bundle-forming pili; LEE: Locus for enterocyte effacement, HUS: hemolytic uremic syndrome, CFA: colonization factor antigen, AAF: aggregative adherence fimbria, Daa: diffuse adhesin, AIDA: adhesion involved in diffuse adherence, Ipa Invasion plasmid antigen.

Source: Allocati *et al*. (2013)

2.14 Susceptibility of humans to *E. coli* infection

One particular area of worry is the consumption of harmful foods by those who are more susceptible, such as youngsters and those whose immune systems have been weakened by disease or by the pathogenic response mechanism itself (Gallo *et al.*, 2020). People who work in food preparation facilities, hospitals, nursing homes, slaughterhouses, fisheries, kindergartens, and schools are more susceptible to illness than the general public (Tsai *et al*., 2014).

2.15 Prevention and control of *E. coli*

Access to clean drinking water, efficient methods for managing food contamination, hygiene campaigns, public education, and immunization should all be part of the overall plan for stopping and controlling the spread of *E. coli* (Akinsemolu, 2018). According to a study by Khalil (2021), having access to clean water is the main defense against *E. coli* infections. Boiling and storing food at the right temperatures are also important measures to prevent food contamination (WHO, 2015). Moye *et al.* (2018) stated that food irradiation techniques can be utilized to significantly lower the bacterial burden in high-risk items. Hospital policies that lessen the chance of multi-resistant bacteria spreading include limiting the use of antibiotics and preventing cross-contamination by adhering to fundamental hygienic quality criteria (Saliba *et al.*, 2023). Healthcare workers' hands and pharmaceutical goods are the main areas where *E. coli* infections spread, maintaining proper hand hygiene is essential for preventing cross-contamination (Paul *et al.*, 2011). In order to prevent and treat *E. coli* infections in both humans and animals, antibiotics are essential, nonetheless, it is generally acknowledged that the number of antibiotics taken correlates with antimicrobial resistance (Roth *et al.*, 2019). Lee *et al.* (2013) revealed that, pathogen resistance in both normal human and animal bacterial flora is increased by underdosing and abusing antibiotics,

probiotics may also be used as an alternative to medication therapy for numerous *E. coli* infections. Probiotics are live, stable bacteria that interact with pathogens, primarily Lactobacillus and Bifidobacterium, and can colonize the gastrointestinal system, numerous studies have been done on the possible application of probiotics to prevent or treat gastrointestinal tract infections (Servin, 2004). According to Fuloria *et al*. (2022), probiotic-infused infectious diarrhea chemotherapy has demonstrated beneficial effects by lowering the frequency of diarrhea. Possibly the most effective human defense against the most dangerous strains, like UPEC, NMEC, and ETEC, is immunization. But as of right now, there is not a strong vaccination that can stop these diseases (Naili, 2018).

2.16 Prevalence of *E. coli* in ready-to-eat meats

According to Hassanani *et al*. (2014), the occurrences of *E. coli* were 46.7%, 40%, and 33.3% for hawawshi, kofta, and shawarma ready-to-eat meat samples, respectively that were collected from several fast-food establishments in various areas within the Menofia governorate. Giwa *et al*. (2021) found that out of 85 samples of ready-to-eat meat and meat products, only 5 samples (5.88%) were contaminated with *E. coli*. According to Barua *et al*. (2007), ready-to-eat beef products had the greatest frequency of *E. coli*, 35.21%. Again, Cocker (2022) tested 212 chicken samples, 82(38.7%) were positive for *E. coli*, while from 210 beef samples, 40 (19.0%) tested positive for *E. coli*, 34 (16.3%) tested positive for *E. coli* out of 209 pork samples, and 23 (11.9%) tested positive for *E. coli* out of the 194 turkey samples. Furthermore, the overall prevalence of *E. coli* in food of animal origin in Bangladesh was 37.86%, 49.02% in chicken meat, and 70% in beef (Baidya and Rahman, 2021). *E. coli* was the most common bacteria in meat samples, according to another study conducted in South Africa (Baidya and Rahman, 2021).

2.17 One health

One health is a combined and amalgamated approach that aims to sustainably promote and balance the health of humans, animals and the environment (World Health Organisation (WHO), 2024). It identifies that the health of living organisms (humans, animals, plants) and the broader environment are closely linked and depend on each other (Centers for Disease Control and Prevention (Laith *et al*., 2023). According to WHO (2024), by linking humans, animals and the environment, one health can help to address the full spectrum of disease control – from prevention to detection, preparedness, response and management – and contribute to global health security. Also, healthy relationship and existence among humans, animals, plants and the environment can contribute to addressing emergence of infectious diseases, antimicrobial resistance and food safety, and promote health and sustainable environment. Ludden *et al.* (2019) indicated that, one health genomic surveillance of *E. coli* demonstrated distinct lineages and mobile genetic elements in isolates from humans versus livestock.

One health approach has been used to study the prevalence of bacteria in foods of animal origin. Adzitey *et al*. (2021) reported that *E. coli* (38%) was isolated from raw meats, RTE meats and their related samples using one health context. They found that raw beef (80%), raw chevon (60%), raw chicken (30%), raw guinea fowl (40%), raw mutton (70%), raw pork (60%), RTE beef (50%), RTE chevon (50%), RTE chicken (20%), RTE guinea fowl (20%), RTE mutton (20%), RTE pork (0%) from animal sources. Hand swab from RTE meat sellers (50%) and hand swab of raw meat sellers (10%), and knife swab from raw meat sellers (60%), table swab from raw meat sellers (30%), utensil swab from raw meat sellers (10%), knife swab from RTE meat sellers (30%), table swab from RTE meat sellers (30%) and utensil swab from RTE meat sellers

(30%) from the environment were contaminated with *E. coli*. Ramatla *et al.* (2023) reviewed 126 published articles between 1990 to 2022 using one health approach and reported a prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing *E. coli* to be 33.0%, 33.5% and 56.9% for humans, animals and environment, respectively. They concluded from their study that the use of one health surveillance is very important for tracking and prevention of the spread of antimicrobial resistance. Islam *et al*. (2023), also reported that the prevalence of ESBL producing *E. coli* in humans, animals and environment was 17%, 22% and 39%, respectively and concluded that their results highlight how essential it is to implement comprehensive antimicrobial surveillance using one health approach to mitigate the consequences of antimicrobial resistance.

2.18 Antibiotics

Antibiotics were first defined as any compound that inhibits the growth of microorganisms (Lewis, 2013). However, antimicrobials are chemically synthesized or naturally occurring substances that have the ability to either kill or stop the growth of bacteria (Kamaruzzaman, 2019). Antibacterial medications used to treat bacterial illnesses in humans and animals are referred to as an antibiotic (Hunter *et al*., 2010). They are frequently employed in the monitoring, treatment, and prevention of illnesses and infections (Shaikh *et al*., 2015). Although the understanding of how antibiotics work has grown recently, antibiotics have not been novel for decades (Crofts *et al*., 2017). Centuries ago, Chinese and Central American Indians employed molds to cure infected wounds from the ancient Egyptians, although they were unaware of the mechanism or purpose of these molds (Bottalico *et al*., 2022). In the late 1880s, researchers began to categorize antibiotics that were taken out of molds, which marked a turning point in medical history (Levy, 2013). Penicillin, on the other hand, which is

made from a mold, was not made available until the 1940s. According to Manring *et al*. (2009), its extensive usage as an infection treatment helped save the lives of military soldiers who were injured or crippled during World War II. In the twenty-first century, antibiotics were incorporated by farmers into their organized methods of animal husbandry practices, including their animals' access to clean water, wholesome food, heat and cold protection, immunizations and medical attention when needed (Price and Morris, 2013). Antibiotics are classified as narrow if they exclusively inhibit Gram-positive or Gram-negative bacteria, and as broad spectrum if they also kill Gram-positive and Gram-negative bacteria (Adzitey, 2015).

2.19 Benefits of antibiotics

Due to their pivotal role in the most significant developments in medicine and surgery, antibiotics save lives (WHO, 2017). Antibiotic has also been successful to prevent or treat infections that can affect patients receiving medical care, those with chronic illnesses like diabetes, end-stage renal disease or rheumatoid arthritis, and those who have had complex surgeries like heart surgery, joint replacements or organ transplants (Mercer, 2021). In addition, antibiotics reduce the mortality rate from foodborne and other destitution infections.

2.20 Classes of antibiotics

Antibiotics can be categorized based on their molecular makeup, modes of action (bactericidal and bacteriostatic), and range of activity (wide and limited) (Etebu and Arikekpar, 2016). Oral and injectable administration methods are additional means of identifying antibiotics (Li *et al*., 2019). Stone *et al*. (2020) revealed that, antibiotics usually have comparable time limits, levels of efficacy, and allergic reactions, regardless of their chemical structure or class. Any grouping of antibiotics based on a

chemical or molecular property includes macrolides, quinolones, sulphonamides, beta lactams, aminoglycosides, tetracycline, oxazolidinones, and glycopeptides (Etebu and Arikekpar, 2016). Santos *et al*. (2017) stated that veterinary medications can also be categorized according to the diseases they treat or the kind of sickness the targeted organism causes.

2.21 Common antibiotics used by livestock farmers

2.21.1 Gentamicin

Gentamicin is mostly utilized in veterinary medicine as an oral solution for poultry and as an injection solution for pigs, cattle, and horses (Ibrahim *et al*., 2020). It is widely used in human treatment, usually as an injectable solution for intramuscular delivery and it is formally listed as one of the essential medications for human use by the World Health Organization (González-Vázquez et al., 2017). Gentamicin is effective against a variety of pathogenic bacteria, including P*seudomonas, Proteus, E. coli, Klebsiella pneumoniae, Enterobacter aerogenes, Serratia*, and Gram-positive *Staphylococcus* (Gul *et al*., 2004). It is used to treat infections of these sensitive bacteria in soft tissue, blood, bone, and respiratory tracts as well as urinary tract infections (Gul *et al*., 2004). One antibiotic that contains aminoglycosides is gentamicin, other aminoglycosides include kanamycin, amikacin, tobramycin, neomycin, and streptomycin (Dowling, 2013). Gentamicin is a bactericidal, broad-spectrum antibiotic (except for anaerobic bacteria and *streptococci*), by attaching to 30S ribosomes, it inhibits the synthesis of proteins by bacteria (Safain, 2020). As stated by Timsit *et al*. (2022), gentamicin is used to treat acute, serious infections, such as those brought on by Gram-negative bacteria. Amikacin is more consistently effective against bacterial strains that have developed resistance (Thy *et al*., 2023).

2.21.2 Tetracycline

Access to a variety of semi-synthetic tetracyclines, including minocycline, lymecycline, doxycycline, rolitetracycline and tetracycline, as well as three naturally occurring tetracyclines, namely oxytetracycline, chlortetracycline and demethylchlortetracycline, is possible (Adegboye, 2011). During elimination processes, further classification into three groups is permissible: short-acting (tetracycline, oxytetracycline and chlortetracycline), intermediate-acting (demethylchlortetracycline and metacycline), and long-acting (doxycycline and minocycline) (Abdelrahman *et al*., 2018). Tetracyclines' antibacterial action is indicative of their reversible binding to the bacterial 30 ribosomal subunit (Yao and Moellering, 2011). At a pH of 6 to 6.5, tetracyclines are more potent and effective against bacteria that are growing (Song *et al*., 2017). Tetracyclines are typically the recommended drug for treating mycoplasma and rickettsia. Among the vulnerable species is Wolbachia, an internal rickettsia endosymbiont of worms like *Dirofilaria immitis*. A number of pathogenic *E. coli* isolates, strains of *Pseudomonas aeruginosa, Proteus, Serratia, Klebsiella,* and *Trueperella spp*., are resistant, according to Dingman *et al*. (2010). *Staphylococci* are often resistant to minocycline and doxycycline, but there is general cross-resistance between tetracyclines (Asadi *et al*. 2020). Ali, (2019) recommended against giving tetracyclines to ruminants as therapeutic dosages because of their poor absorption and potential to severely inhibit the microbial activity of the animal.

2.21.3 Suphamethoxazole/trimethoprim

Sulfamethoxazole/trimethoprim, also known by the brand names Co-trimoxazole @, Primsol, Bactrium @, Sulfatrim @, Novo-Trimel @ or Septra @, is a potential antibiotic/antimicrobial sulfonamide that is administered for the treatment of ear infections, urinary tract infections, bronchitis, traveler's diarrhea, and *Pneumocystis*

jiroveci pneumonia (Holmes & Grayson 2017). Trimethoprim inhibits the dihydrofolate reductase enzyme, preventing bacteria from converting folate, this medication causes blood levels of unconjugated bilirubin to rise, according to research by Wróbel *et al*. (2020).

2.21.4 Amoxycillin/clavulanic acid

Amoxicillin/clavulanic acid, commonly known as augmentin, is a combination antibiotic used to treat a variety of bacterial infections (Huttner *et al*., 2020). According to Liu *et al*. (2021), it consists of amoxicillin, a penicillin-like antibiotic, and clavulanic acid, a beta-lactamase inhibitor that helps prevent bacterial resistance. The chemical structure of clavulanic acid, which is produced by *Streptomyces clavuligemus*, is comparable to that of several ß-lactamines, such as penicillin (Pozzi and Ben-David, 2002). Gibson and Veening (2023) reported that amoxicillin inhibits bacterial cell wall synthesis by binding to penicillin-binding proteins, leading to cell death. Prescott (2013) reported that, clavulanic acid inhibits beta-lactamases, enzymes produced by bacteria that can break down and inactivate antibiotics. This enhances the activity of amoxicillin against beta-lactamase-producing bacteria. Amoxicillin/clavulanic acid is commonly used to treat respiratory tract infections, urinary tract infections, skin and soft tissue infections, and other bacterial infections, it is often prescribed empirically for its broad-spectrum coverage (Huttner *et al*., 2020). A study by Fazulbhoy *et al*. (2021) indicated that amoxicillin/clavulanic acid is usually administered orally, and absorption is not significantly affected by food. It has a short half-life, requiring multiple daily doses for optimal efficacy.

2.21.5 Chloramphenicol

According to a study by Singhal *et al*. (2023), chloramphenicol is a broad-spectrum antibiotic that is effective against a variety of bacterial illnesses, including *Rickettsia* and those caused by anaerobic bacteria. Phenicoles block the production of microbial proteins by attaching to the 50S subunits of the 70S ribosome and preventing peptidyl transferase from doing its job (Renata *et al*., 2022). Due to the inhibition of peptide-bond formation, peptides do not elongate (Choi *et al*., 2020). Although chloramphenicol's activity is typically bacteriostatic, at high concentrations it can be bactericidal for some bacteria (Yang *et al*., 2022). Protein synthesis was suppressed in both eucaryotic (mitochondrial) and prokaryotic (bacterial) ribosomes (Miller *et al*., 2009). According to Wilson and O'connor (2021), oral administration of chloramphenicol is another option, and it comes at a reasonable cost. Regrettably, animals and humans who come into contact with this material may also be highly hazardous to them (Lin *et al*., 2022). Ramaiah and Nabity (2011) discovered that in certain animals, chloramphenicol causes suppression of the bone marrow, which is the site of the formation of red and white blood cells. According to Feasey (2013), this is more of a long-term concern, however if the bone marrow is repressed, the issue can usually be resolved by quitting the chloramphenicol therapy. Many nations forbid the use of chloramphenicol in any animal intended for food, while it might not be utilized by many other animals, cats might (De Briyne *et al*., 2014).

2.21.6 Ciprofloxacin

Ciprofloxacin is a broad-spectrum antibiotic that is used to treat infections (Conley *et al*., 2018). Veterinarians may lawfully prescribe this medication as an extra-label treatment even if the Food and Drug Authority has not granted a license for its use in animals (Bedale, 2019). Generally speaking, quinolones, other fluoroquinolones, and

ciprofloxacin are DNA synthesis inhibitors (Millanao *et al*., 2021). According to Khan *et al*. (2018), they mostly bind to the DNA gyrase enzyme, also known as topoisomerase, during bacterial DNA replication. This binding stops the enzyme from carrying out its biological role of cutting, mending, and coiling DNA molecules (Shariati *et al*., 2022). As soon as ciprofloxacin binds to the enzyme topoisomerase, DNA gyrase's function in DNA replication is blocked (Hooper and Jacoby, 2016). The target pathogenic bacteria eventually die as a result of inhibition of DNA synthesis in bacteria, which also hinders other important cellular functions of the organism (Cheng *et al*., 2013). Both Gram-positive and Gram-negative bacterial infections can be treated with ciprofloxacin alone or in conjunction with other antibacterial medications. For anthrax therapy, fluoroquinolones and ciprofloxacin are combined (Shariati *et al*., 2022). Additionally, they are used clinically to prevent chlamydia infections, respiratory tract infections, skin infections, and UTIs (Rafailidis *et al*., 2011). Quinolones, such as nalidixic acid, are mainly used to treat urinary tract infections (UTIs) and to combat gram-negative bacteria, bacterial resistance to quinolone and fluoroquinolone is usually caused by chromosomal alterations that make the organism less sensitive to the medication (Malik *et al*., 2010).

2.21.7 Azithromycin

According to Kirst (2013), erythromycin is a semi-synthetic macrolide that is the source of the antibiotic azithromycin. It is used to treat bacterial infections (Oliver and Hinks, 2021). Since azithromycin has a longer half-life and is used for both dogs and cats more quickly than erythromycin, it is more often used in treatment (Giguère, 2013). Azithromycin, one of the several macrolides, is a bacteriostatic antibiotic that inhibits protein synthesis and is linked to ribosome 50s (Jelić and Antolović, 2016). Although it has some activity against anaerobic bacteria, this treatment has been demonstrated to

be successful against a wide variety of pathogens of both Gram-positive and Gram-negative bacteria (Heidary *et al*., 2022). Azithromycin is effective against bacteria, but have demonstrated that it is also potent against *rickettsia*, *spirochetes*, and *protozoa* (such as *Toxoplasma gondi*, *Giardia spp*. and *Cryptosporidium spp*.) (Sharma *et al*., 2016). Azithromycin is only moderately bioavailable in cats (58%) and humans (37%), whereas it is highly bioavailable in dogs (97%), after per os (PO) injection. A single oral dosage of 10 to 40 mg/kg produced tissue levels in dogs that were proportionate to the dose (Caol *et al*., 2017). Dogs receiving a 20 mg/kg treatment for seven days showed four to seven-fold increases in tissue concentration (Huckle *et al*., 2015).

2.21.8 Ceftriaxone

Ceftriaxone is effective against strains of Haemophilus and Neisseria, just like other third-generation cephalosporins (Hirai, 2011). Contrary to ceftazidime and cefoperazone, ceftriaxone is ineffective against *Pseudomonas aeruginosa* (Chen *et al*., 2021). According to Pana and Zaoutis (2018), ceftriaxone is generally ineffective against Enterobacter species, and its use in treating Enterobacter infections should be avoided. However, because of resistance, the isolate appears susceptible by forming cephalosporinases, which are enzymes that hydrolyze cephalosporins and render them inactive, and some pathogens, like Citrobacter and Providencia, may develop immunity (Tshitshi, 2019). According to Page (2011), ceftriaxone is a broad-spectrum B-lactam (cephalosporin / cephamycin) antibiotic that has been shown in vitro trials with both Gram-positive and Gram-negative aerobic and anaerobic bacteria. Ceftriaxone binds to proteins that bind to penicillin to exert its bactericidal effect, which results from suppression of cell wall production (Dörr, 2021). Ceftriaxone is frequently used in conjunction with aminoglycoside and macrolide antibiotics to treat pneumonia (Lupia

et al. 2020). Additionally, it is the recommended drug for treating bacterial meningitis (Grégoire *et al*., 2019).

2.21.9 Teicoplanin

According to Butler *et al*. (2014), teicoplanin is a glycopeptide antibiotic that has an antibacterial spectrum that is nearly identical to that of vancomycin. The medication is authorized for the management of several Gram-positive infections (Khamesipour *et al*., 2015). According to Cavalcanti *et al*. (2010), teicoplanin has been approved for the treatment of serious infections caused by *Staphylococcus aureus* or *Streptococcal* bacteria that are not adequately responsive to less toxic antibiotics. Accordingly, teicoplanin may be used to treat septicemia, septic arthritis, and osteomyelitis (Peeters *et al*., 2016). Clinicians should be aware that vancomycin may be more effective than teicoplanin in treating certain *Staphylococci*, including *Staphylococcus haemolyticu*s (Falcone *et al*., 2006). Teicoplanin binds to the amino D alanyl D alanine residue, the terminal dipeptide peptidoglycan sequence, and inhibits peptidoglycan contact and cross-linkage, hence preventing cell wall production (Sobral and Tomasz, 2019). Teicoplanin is well absorbed when given intravenously, extensively disseminated as a bound form of plasma protein throughout the body, and eliminated through the urine (Yılmaz and Özcengiz, 2017).

2.22 Importance of farmers' Knowledge in usage of antibiotics

2.22.1 Demographics information

Uddin *et al*. (2014) reported that farmers in Bangladish had varying levels of education, with the majority having completed secondary school. According to another survey, 98.33% of participants were men, 61.94% had completed primary education, 43.33% were 48

years of age or older, and 73.88% had been involved in animal breeding for at least 11 years (Ozturk *et al*., 2019). According to Islam *et al*. (2021), of the 600 respondents, men made up the majority (71%) of the sample, the age range of the group was 21–63, with 80.5% of the participants being older than 30. Less than ten years were spent in the animal husbandry industry by the majority of farmers (57.0%). A significant portion of respondents (31%) lacked literacy, and the majority (70%) raised chickens (Musa, 2018).

2.22.2 Sources where livestock farmers obtained antibiotics

Antibiotics for use by livestock farmers can be obtained from a variety of sources (Manyi-Loh *et al*., 2018). Landfried *et al*. (2018) stated that, majority of farmers were confident that they could obtain antibiotics from a feed shop or online and rely on veterinarians for advice on specific medications to use on their goats, only the medications prescribed by the veterinarians were being used, according to half of the farmers. According to Phu *et al*. (2019), majority of farmers (84.2%) purchased antibiotics over-the-counter without a prescription, 100% did so on a veterinarian's suggestion, 3.9% did so from agro-dealers, and 1.9% did so from other farmers. Farmers obtain antibiotics via veterinary drug stores (12%), animal markets (19%), veterinarians (18%), livestock field officers (9%), and stores that sell agricultural and veterinary implements (29%), neighbors (5%) and spaces used for exhibitions (8%) (Kemp *et al*., 2021). According to a different study, the majority of antibiotic purchases (91.4%) came from veterinarian stores, with the remaining 8.6% coming from local vendors (Shahi, 2022).

2.22.3 Purpose of using antibiotics

Animal antibiotics are frequently used for prevention, growth promotion, and the treatment of sick animals, these applications are crucial for a secure and productive animal business (Scott *et al*., 2019). Therefore, the use of such antibiotics in animals especially food animals may lead to the selection of bacterial strains that are resistant to treatment, which could ultimately result in an infection that affects both humans and animals (Lekshmi *et al*., 2017). Hudson *et al*. (2017) stated that natural foods may be a factor in the spread of resistant bacteria and genes to humans via the food chain, according to research on the molecular identification of antibiotic resistance genes and mobile elements of resistance to antibiotics, this research revealed that bacteria that colonize both humans and animals share similar elements. The usage of fluoroquinolones in feeding animals, for example, has led to the growth of *E. coli* and other ciprofloxacin-resistant bacteria that cause difficult-to-treat infections in humans (Conley *et al*., 2018). According to Rahman *et al*. (2022), the two most common uses of antibiotics by weight were as growth promoters and for the treatment and prevention of illnesses in pigs and poultry. In another study, Van *et al*. (2020) found that the most widely prescribed antibiotic in South Africa was tylosin, one of the four growth promoters, which was followed by tetracyclines, sulphonamides, and penicillin. Abass *et al*. (2020) conducted a survey which revealed that farmers utilized antibiotics for three different purposes: therapeutic (34.16%), prophylactic (14.17%), and combined (40.83%) uses. According to Phares *et al*. (2020), the majority of farmers (80%) indicated they used antibiotics to treat and prevent illnesses, while some did so to promote growth (13.0%) and as prophylaxis (0.7%). Furthermore, a study conducted by Choudhury (2022), found that poultry farmers used antibiotics for growth promoters

(6.9%), preventive purposes (29.3%), and combined prophylactic and therapeutic objectives (32.8%).

2.22.4 Antibiotic drug administration and prescription

Only 85% of antibiotic administration was done by veterinary officials, 65.3% by farm workers, and 23.2% by farm managers (Kahunde *et al*., 2023). In a different survey, half of the chicken farmers indicated that veterinary professionals gave them antibiotics, 43.1% indicated that they self-medicated, and 6.9% of them received antibiotic prescriptions from animal health personnel (Hussain *et al*., 2023).

2.22.5 Antibiotic used by farmers in livestock production

According to a study by Clement *et al*. (2019), tetracyclines (33.6%), fluoroquinolones (26.5%), and beta-lactams/aminoglycosides (20.4%) accounted for the majority of antimicrobials used in animal production in south-western Nigeria. Additionally, frequently used antibiotics were tetracyclines (24.17%), aminoglycosides (17.87%), penicillins (16.51%) and fluoroquinolones (10.55%) (Boamah *et al*., 2016). According to Mutuku *et al*. (2022), the majority of antibiotics are traditionally classified as macrolides or sulfa, with the remaining antibiotics being tetracyclines, aminopenicillin, polymyxin and fluoroquinolones. Tetracycline (61%), sulphadimidine (23%), penicillin-streptomycin (2%) and gentamycin (1%) were found to be among the most widely used and frequently reported antibiotics among Tanzanian livestock keepers by Benta *et al*. (2021). Neoceryl (a commercially produced broad-spectrum antibiotic comprising neomycin, erythromycin, oxytetracycline, streptomycin and colistin) accounted for 36.2% of the commonly used antibiotics, followed by enrofloxacin (27.5%) and furazolidone (20.0%) (Oluwase *et al*., 2014). In a different study, Sekyere (2014) reported that among pig farms in some districts in the Ashanti region of Ghana,

tetracyclines (oxytetracycline, doxycycline, remacycline, and tetracycline) were highly common (64 out of 110 farms). These were followed by streptomycin (48/110), penicillins (48/110), and sulphadimidine (31/1110). The use of aminoglycosides, macrolides, and fluoroquinolones was extremely restricted.

2.23 Antibiotic resistance

Shortly before penicillin was initially used to treat infectious infections in humans the same year, Abraham and Chain (1940) discovered antibiotic resistance in *Bacillus coli* (now known as *E. coli*) (Lalchhandama, 2021). Antibiotic use is thought to be the most important factor in both veterinary and human medicine, contributing to the growth, selection, and spread of antibiotic resistant bacteria (Palma, 2020). According to Sagar *et al*. (2019), bacteria develop resistance to antibiotics when they adapt to their use. An increasing variety of bacterial infections, including pneumonia, TB, gonorrhea, and salmonellosis, are more difficult to treat when the medicines used to treat them lose their effectiveness (Micoli *et al*., 2021). The way that antibiotics are prescribed and used worldwide has to be improved desperately (Carlet *et al*., 2012). Antibiotic resistance will continue to be a significant issue even in the event that new medications are developed if behavior does not change (Lum *et al*., 2017). A change in lifestyle must also involve measures to stop the spread of illness, such as immunization, hand washing, appropriate sexual behavior, and proper food handling (Hawker *et al*., 2019). Many decades after the first patients were treated with antibiotics, bacterial infections are once again a hazard (Mancuso *et al*., 2021). The issue of antibiotic resistance has been connected to both the overuse and misuse of these medications as well as the healthcare industry's inability to produce new antibiotics as a result of stringent regulations and little financial incentives (Muteeb *et al*., 2023). The uncontrolled use of antibiotics in cattle products has led to microbial resistance (Sharma et al., 2018).

Numerous microorganisms have been identified as posing immediate, severe and associated dangers, the majority of them can now significantly impact the healthcare systems, patients, and their families in terms of financial and clinical burden (Ventola, 2015). According to James *et al*. (2011), implementing new ideas, conducting research, and taking crisis management actions all need a large deal of group action: Multidrug resistance has been observed in bacteria that are known to metabolize antimicrobials and use them as a source of nutrients (Zhao *et al*., 2021).

2.24 Multidrug resistant of *E. coli*

Increases in the number of genes on R plasmids, each of which codes for resistance to a specific medication, are the main indicator of multidrug resistance in bacteria (Okoye *et al*., 2022). For instance, integrons integrate many resistance genes in the right spot to offer an effective regulator for their expression, making them incredibly powerful in producing multidrug resistance (Shetty *et al*., 2023). The resistance gene is labeled upon incorporation into an integron, hence facilitating its rapid formation into another integron (Jiao *et al*., 2017). According to Cytryn *et al*. (2017), bacteria that produce antibiotics appear to be the biological source of resistance genes, certain species even arise from their surroundings, particularly soil microorganisms, concerning trend is the selection of mutants with genes for broad resistance recently. According to Samaranayake (2018), R-plasmids are remarkably well-preserved and portable from one cell to another. The resistance nodulation division (RND) super-family pumps are particularly significant in Gram-negative bacteria because they are typically chromosomally coded and readily overexpressed (Henderson *et al*., 2021). Multidrug resistance is a serious medical problem that makes it difficult to cure illnesses and develop new treatments (Tanwar *et al*., 2014). According to a study by Adzitey (2020), the MAR's index of *Escherichia coli* isolated from beef samples varied between 0.11

and 0.56, there were 21 isolates with the most common resistance pattern, Tec (resistant to just teicoplanin), this was accompanied by resistance trends in TecTeSxt (teicoplanin-tetracycline-suphamethoxazole/trimethoprim, n=5 isolates) and AzmTec (azithromycin-teicoplanin, n=6 isolates). According to Rahman *et al*. (2020), every poultry meat had *E. coli* isolates that were resistant to at least three antibiotics, which are frequently used to treat Gram-negative bacteria. Resistance to nearly four drugs was observed in nine (9) *E. coli* isolates, whilst resistance to five antibiotics was found in 15 isolates. Out of the nine antibiotics employed in the study, one strain of *E. coli* had the most resistance to seven distinct antibiotics. ampicillin, tetracycline, sulfamethoxazole trimethoprim, gentamicin, chloramphenicol, nalidixic acid and kanamycin (Alonso *et al*., 2017).

2.25 Whole genome sequencing

A genome is the entire collection of DNAs. The entire DNA sequence of an organism's genome can be found at once using a laboratory technique known as whole genome sequencing (WGS) (Uelze *et al*., 2020). In addition to the DNA present in the mitochondria and in the case of plants, the chloroplasts, it comprises sequencing all chromosomal DNA in an organism (Joshi *et al*., 2023). The fields of clinical microbiology and infectious diseases can benefit greatly from the application of genomics and whole genome sequencing (Kwong *et al*., 2015). Whole genome sequencing can detect an organism's genome's whole DNA sequence all at once, according to Brown *et al*. (2019). In order to compile the massive amount of biological sequence data, it comprises figuring out the base order throughout an organism's entire genome, assisted by computer algorithms and automated DNA sequencing techniques (Yin *et al*., 2019). Whole genome sequencing has evolved into the standard microbial typing technique for epidemic investigations and is being used more frequently for

national monitoring of infectious illnesses in EU/EEA nations and beyond (Harvala *et al.*, 2018).

2.25.1 DNA sequencing and assembly

The process of assembling the pieces is known as sequence assembly, most genome studies currently employ shotgun sequencing, thus genomic DNA is broken up into countless tiny pieces (Ekblom and Wolf, 2014). Pinese *et al*. (2020) stated that genomic DNA for 130 TET-resistant *E. coli* strains was prepared using a modified Nextera library preparation procedure, and the DNA was sequenced using an Illumina HiSeq 2500 device. The assembled genomes were subjected to in silico multilocus sequence typing (MLST) utilizing the WGS data online platform MLST v2.0 (https://cge.cbs.dtu.dk/services/MLST/). An alignment is made to match the reads from these samples to a reference genome after the WGS data has been prepared (Zook *et al.*, 2016). Since the length of the reference genome varies depending on the species, it is computationally and time-consuming to compare several short nucleotide readings to the genome. Hundreds of billions of possible locations could be found in the reference genome (Yin *et al.*, 2019).

2.25.2 Phylogenetic analysis

With its ability to provide high-resolution insights into the evolutionary relationships among microorganisms, whole genome sequencing (WGS) has emerged as a key component of microbial phylogenetics (Kwong *et al.*, 2015). Researchers use WGS data to accurately reconstruct phylogenetic trees, clarifying the genetic relatedness between various strains and species (Ronholm *et al.*, 2016). This methodology is very beneficial for epidemiological research and epidemic analyses. A more accurate understanding of microbial evolution, transmission dynamics, and population structure

is made possible by WGS, which makes it easier to identify genetic variants at the nucleotide level (Oniciuc *et al*., 2018).

2.25.3 Gene screening

According to a study by Kwok *et al*. (2021), whole genome sequencing identifies changes in both coding and non-coding sections of the genome, allowing for thorough gene screening. This method is essential for identifying antibiotic resistance genes, researching genetic diversity among microbial populations, and comprehending the mechanisms underlying pathogenicity. The discovery of new genes and regulatory elements has been transformed by WGS, which has produced invaluable data for the creation of tailored treatments and diagnostics (Satam *et al*., 2023).

2.25.4 Integron analysis

WGS makes it easier to thoroughly analyze integrons, which are genetic components essential to the spread of antibiotic resistance genes in bacteria (Oniciuc *et al*., 2018). According to Hendriksen *et al*. (2019), whole genome sequencing (WGS) offers a comprehensive picture of the genome, which enables researchers to map gene cassettes within integrons, detect integrons, and comprehend their role in antibiotic resistance. This information is vital for surveillance programs and guiding strategies to combat the spread of antibiotic resistance (WHO, 2020).

2.25.5 Virulence-associated genes

Understanding the pathogenicity of microbes requires an understanding of virulence-associated genes, which WGS plays a critical role in finding and characterizing. Researchers can identify genes linked to adhesion mechanisms, secretion systems and virulence factors by analyzing the complete genome (Rahman *et al*., 2023). According

to Klemm and Dougan (2016), this data is essential for researching host-pathogen interactions, creating vaccines, and creating focused treatment approaches.

2.25.6 Next-generation sequencing

Large-scale genomic research has been made possible by next-generation sequencing (NGS) technologies, which are a subset of WGS and allow for high-throughput sequencing of several samples at once (Satam *et al*., 2023). NGS has considerably increased the speed and cost-effectiveness of sequencing. Besser *et al*. (2018) claim that this technical progress has sped up microbial genomes research and increased accessibility for routine applications including surveillance, epidemiology, and diagnostics.

2.25.7 Sequencing options for clinical microbiology: what needs to be considered?

WGS implementation in clinical microbiology involves a number of factors, these include pipelines for bioinformatics, standardization of techniques, data interpretation, and interaction with clinical workflows (Rossen *et al*., 2018). According to Hayeems *et al*. (2020), concerns including data storage, cost-effectiveness, and turnaround time also need to be taken into consideration. It is imperative to optimize these elements in order to successfully integrate WGS into standard clinical practice.

2.25.8 Advantages of whole-genome sequencing

According to a study by Eisfeldt *et al*. (2019), WGS has a number of benefits over conventional sequencing techniques, it offers a thorough perspective of the whole genome, making it possible to identify structural changes, uncommon variants, and intricate genomic rearrangements. Studying population dynamics, the genetics of illness, and the evolution of microbes is made easier by the high resolution of WGS.

Furthermore, WGS makes it possible to analyse several phenotypes at once, which advances our understanding of genetic features in a more comprehensive way (Purushothaman *et al*., 2022).

2.25.9 Key whole-genome sequencing methods

Whole genome sequencing is accomplished by a variety of techniques, each with unique advantages and disadvantages (Gilchrist *et al*., 2015). Some of the techniques, according to Logsdon *et al*. (2020), include long-read technologies like PacBio and Oxford Nanopore sequencing, which can span complicated genomic areas and short-read technologies like Illumina sequencing, which give excellent accuracy and coverage. The goal of hybrid techniques that combine long and short reads is to maximize the benefits of each technology, the particular objectives of the investigation, the size of the genome and the required degree of resolution all influence the sequencing method selection (De Coster *et al*., 2021).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in selected markets (Kaneshie, Malata Nima, Maamobi and Agbogbloshie) in Accra, greater Accra region. Accra is the national capital of Ghana and it shares boundaries to the north with Eastern region, to the east with Volta region, to the west Central region and to the south with the Gulf of Guinea.

3.2 Data collection

A total of 360 samples (animal, human and environment) were randomly collected from street vendors of RTE-meats. The samples collected were from RTE beef (10g), RTE chicken (10g), RTE mutton (10g), spices used for preparing RTE meats (10g), hand swabs of RTE meat sellers ($10cm^2$) and knife swab from knifes use for cutting RTE meats ($10cm^2$) (Table 3.1). These samples (60 each) were randomly collected from 6 different sources from 5 purposely selected markets in Accra. Samples were transported in an ice chest with ice block to the Bruce Hunter Microbiology Laboratory at the University for Development Studies, Nyankpala campus for isolation and analysis of *E. coli*.

Table 3. 1: Number and types of samples analyzed

Samples	Locations	Agbogbloshie	Kaneshie	Malata	Maamobi	Nima	Total
1	Beef	12	12	12	12	12	60
2	Mutton	12	12	12	12	12	60
3	Chicken	12	12	12	12	12	60
4	Knives	12	12	12	12	12	60
5	Spices	12	12	12	12	12	60
6	Hands	12	12	12	12	12	60
	Overall	72	72	72	72	72	360

3.3 Isolation and confirmation of *E. coli*

Isolation and identification of *Escherichia coli* was done using a modified method according to the Food and Drug Administration-Bacteriological Analytical Manual (FDA-BAM) (Adzitey *et al*., 2015). Swabs were moistened with sterile distilled water just before being used for swabbing. The swabs were taken 10cm^2 from knives and hands of RTE- meat handlers and 10g each of the beef, mutton, chicken and spices were taken. Swabs, meat samples and spices were pre-enriched in 10ml Buffered Peptone Water (BPW) and incubated at 37°C for 24 hours, after which aliquots were plated onto Levine's eosin-methylene blue (LEMB) agar and incubated at 37°C for 24 hours.

Presumptive *Escherichia coli* colonies appeared as dark centered and flat, with or without metallic sheen. Such colonies were purified on Nutrient agar and incubated at 37°C for 24 hours. It was identified and confirmed using Gram staining, growth on MacConkey agar, growth in Brilliant green bile broth and *Escherichia coli* latex agglutination test. All media and reagents used were purchased from Oxoid Limited, Basingstoke, UK.

3.4 Phenotypic antimicrobial resistance test

The phenotypic antimicrobial susceptibility test was conducted according to the disk diffusion method (Bauer *et al*., 1966). *E. coli* isolates were subjected to this test using the following nine (9) antimicrobials: Amoxicillin 30µg (AML), moxycillin/clavulanic acid 30µg (AUG), Azithromycin 15µg (AZM), Ceftriaxone 30µg (CRO), Chloramphenicol 30µg (C), Ciprofloxacin 5µg (CIP), Gentamicin 10µg (CN), Tetracycline 30µg (TE), and Suphamethoxazole/trimethoprim SXT 22µg (SXT) purchased from MAST Limited, Liverpool, UK. Purified cultures of *E. coli* were grown in 10 mL trypticase soy broth (Oxoid Limited, Basingstoke, UK) overnight at 37°C and the concentration adjusted to 0.5 McFarland solution. The adjusted solution was spread on Muller Hinton agar (Oxoid Limited, Basingstoke, UK), antimicrobial discs were placed on it and incubated overnight at 37ºC. After incubation, the inhibition zones were measured, and the results interpreted using the Clinical and Laboratory Standards Institute Guidelines (CLSI). The procedure of Paulshus *et al*. (2019), was used to determine the multiple antibiotic resistance index (MAR index) of the *E. coli* isolates. MAR index was defined as a/b where "a" is the number of antibiotics to which a particular isolate was resistant, and "b" is the total number of antibiotics examined. Multidrug resistance was defined as resistant to 3 different classes of antimicrobials (Karam *et al*., 2016).

3.5 Whole genome sequencing of *Escherichia coli* isolates

Sequencing of *Escherichia coli* from ready-to-eat meats and it associated samples was done at the Noguchi Memorial Institute for Medical Research, Accra, Ghana. In all 25 *Escherichia coli* isolates were selected based on their source of isolation and antibiotic resistance pattern, and sent for sequencing. Nonetheless, 21 were sequenced based on the quality of DNA yield after extraction. The 21 *Escherichia coli* isolates sequenced

were from RTE beef (n=9), RTE mutton (n=3), RTE chicken (n=2), knife (n=4), hand (n=2), and spices (n=1).

3.6 Genomic DNA extraction of *Escherichia coli*

QIAamp® DNA mini kit (QIAGEN Inc. GmbH, Holden, Germany) was used to extract DNA from the *Escherichia coli* species by following the manufacturer's instructions. Briefly, 180 µl of ATL buffer was dispersed into DNA free Eppendorf tube and freshly grown colonies of *Escherichia coli* was added. It was mixed for few seconds by vortexing and 20 µl proteinase K was added, mixed and incubated at 56 ºC for 2 hours. After which, 200 µl of buffer AL was added, mixed and incubated at 70 ºC for 10 minutes. It was then centrifuge at 80 rpm for 1 minute and 200 µl ethanol (96-100%) was added, mixed and centrifuge at 80 rpm for additional 1 minute. The mixture was then transferred into QIAamp mini spin column and centrifuged at 8000 rpm for 1 minute. The filtrate was discarded, 500 µl buffer was added and centrifuged again at 14,000 for 3 minutes. Lastly, 50 µl of buffer AE was added, incubated at room temperature for 1 minute and then centrifuged at 8000 rpm for 1 minute. Furthermore, 50 µl of buffer AE was added, incubated at room temperature for 1 minute and centrifuged at 8000 rpm for 1 minute.

3.7 Whole genome sequencing of *Escherichia coli*

This was done using Illumina DNA Prep (M) Tagmentation kit (Illumina Inc. San Diego, CA 92122, United States) by following the manufacture's procedure presented in Appendix I. Fastq files were assessed using Fastqc (v).11.9 and trimmed with Trimmomatic (v.0.39) at quality threshold of ≥ Q20 and above (McCartney *et al.,* 2024; Bolger *et al.,* 2014). Reads ≥ Q20 were subjected to de-novo assembly (minimum contig size of 200 bp, number of contigs < 400 and coverage of 20x) using Unicycler

(v0.4.8). Assembled genomes were analysed using Kmerfinder (v3.2) to determine the identity of the isolates, ResFinder (v4.1) to determine the resistance determinants, VirulenceFindeer (v2.0) to determine the presence of virulence genes, PlasmidFinder (v2.1) to determine the presence of plasmids and MLST option (v2.0.9) to determine the sequence types using the Center for Genomic Epidemiology website available at: https://genomicepidemiology.org/services/index.html (Hasman *et al*., 2014; Bortolaia *et al*., 2020; Joensen *et al*., 2014; Carattoli *et al*., 2014; Larsen *et al*., 2012).

3.8 Statistical analysis

The data obtained was analyzed using binary logistic of IBM Statistical Package for the Social Sciences (SPSS) Version 20. Test for statistical difference was done using Wald Chi- square at 5% significance level and the results were presented in tables.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of *E. coli* in ready-to-eat meats and it associated samples

Prevalence of *Escherichia coli* from the ready-to-eat (RTE) meats and it associated samples is shown in Table 4.1. *Escherichia coli* was isolated from RTE beef (15%), RTE chicken (10%), RTE mutton (5%), hands of RTE meat sellers (3%), knives for cutting RTE meats (7%) and spices for preparing RTE meats (2%). They were significant differences ($P<0.05$) in prevalence among ready-to-eat meats and it associated samples (Appendix II). RTE beef ($P=0.006$) and RTE chicken ($P=0.048$) positive for *E. coli* were significantly higher ($P<0.05$) than those from RTE mutton, hands of RTE meat sellers, knives for cutting RTE meats and spices for preparing RTE meats. Also, 10%, 3% and 4.2% of animal, human and environmental samples, respectively were contaminated with *E. coli*.

Table 4.1: Prevalence of *E. coli* in ready-to-eat meats and it associated samples

Samples	No. Examined	No. positives	% prevalence
RTE Beef	60	9	15
RTE Chicken	60	6	10
RTE Mutton	60	3	5
Hands of RTE meat sellers	60	2	3
Knives for cutting RTE meats	60	4	7
Spices for preparing RTE meats	60	1	2
Overall	360	25	7

Table 4.2: Prevalence of *E. coli* in RTE meats and it associated samples (hands, knives and spices) per location

Samples	Agbogbloshie	Kaneshie	Malata	Maamobi	Nima
Beef	16.7*(2^/12)"	8.3* (1^/12)"	16.7*(2^/12)"	16.7*(2^/12)"	16.7*(2^/12)"
Mutton	8.3* (1^/12)"	8.3*(1^/12)"	0*(0^/12)"	8.3*(1^/12)"	0*(0^/12)"
Chicken	25* (3^/12)"	16.7*(2^/12)"	8.3*(1^/12)"	0*(0^/12)"	0*(0^/12)"
Knives	0* (0^/12)"	0*(0^/12)"	8.3*(1^/12)"	8.3*(1^/12)"	16.7*(2^/12)"
Spices	8.3*(1^/12)"	0*(0^/12)"	0*(0^/12)"	0*(0^/12)"	0*(0^/12)"
Hands	0 *(0^/12)"	8.3*(1^/12)"	0*(0^/12)"	0*(0^/12)"	8.3*(1^/12)"
Overall	9.7*(7^/72)"	6.9*(5^/72)"	5.6*(4^/72)"	5.6*(4^/72)"	6.9*(5^/72)"

N/B*Percentage Prevalence, ^Number of Positive Samples," Total number of samples examined

Table 4.2 shows the prevalence of *E. coli* per samples and location. The results show that samples from Agbogbloshie recorded the highest contamination with overall rate of 9.7% followed by 6.9% for Kaneshie and Nima, the lowest was 5.6% for Maamobi and Malata. It was also revealed that chicken from Agbogbloshie was the most contaminated with 25% contamination rate.

4.2 Phenotypic antimicrobial resistance of *E. coli* isolated from RTE meats and it associated samples

Selected *Escherichia coli* from the ready-to-eat (RTE) meats and it associated samples from the study area were subjected to antimicrobial resistance test against nine (9) antibiotics as in the Table 4.3. The overall results from the antimicrobial resistance test against the *Escherichia coli* isolates were 55.6%, 25.3% and 19.1% for susceptibility, resistance and Intermediate resistance, respectively. The most susceptible antibiotic in this study was Ceftriaxone 30µg with susceptibility of 92% and 0% resistance while Amoxicillin 30µg was most resisted antibiotic with 100% resistance and 0% susceptibility.

Table 4.3: Antibiotic resistance of *E. coli* isolated from RTE meats and it related samples

Antimicrobial	Susceptible (%)	Intermediate resistant (%)	Resistant (%)
Amoxicillin 30µg (AML)	0.0	0.0	100.0
Amoxycillin/clavulanic acid 30µg (AUG)	24.0	68.0	8.0
Azithromycin 15µg (AZM)	84.0	12.0	4.0
Ceftriaxone 30µg (CRO)	92.0	8.0	0.0
Chloramphenicol 30µg (C)	88.0	0.0	12.0
Ciprofloxacin 5µg (CIP)	68.0	12.0	20.0
Gentamicin 10µg (CN)	40.0	44.0	16.0
Tetracycline 30µg (TE)	40.0	28.0	32.0
Suphamethoxazole/trimethoprim SXT 22µg (SXT)	64.0	0.0	36.0
Overall	55.6	19.1	25.3

4.3 Multiple antibiotic resistance index and antibiotic resistance profile of individual *E. coli* isolated from RTE meats and it associated samples

The multiple antibiotic resistant index (MAR) ranged from 0.1(resistant to 1 antibiotic) to 0.6 (resistant to 5 antibiotics). From the 25 isolates, 9 of them were resistant to 1 antibiotic, 6 of the isolates were resistant 2 antibiotics, 7 isolates were also resistant to 3 of the antibiotics whiles the remaining 3 isolates showed the highest antibiotic resistant of 5 each as shown in Table 4.4. Resistant to only Amoxicillin (AML) was the commonest and was exhibited by 9 isolates from different sources and locations. Three isolates were resistant to as many as five different antibiotics. They were *Escherichia coli* isolated from RTE beef in Agbogbloshie (AML-TE-SXT-AUG-CIP), RTE beef in Maamobi (AZM-AML-C-TE-SXT) and RTE beef in Malata (AML-TE-SXT-AUG-CIP).

Table 4.4: Multiple antibiotic resistance index and antibiotic resistance profile of individual *E. coli* isolated from RTE meats and its related samples

Location	Source	Code	No. of antibiotics	Antibiotic resistant profile	MAR index
Kaneshie	Beef	KB9	1	AML	0.1
Agbogbloshie	Spices	AS3	1	AML	0.1
Agbogbloshie	Beef	AB10	1	AML	0.1
Agbogbloshie	Beef	AB2	5	AML-TE-SXT-AUG-CIP	0.6
Kaneshie	Mutton	KM7	1	AML	0.1
Kaneshie	Chicken	KC15	2	AML-TE	0.2
Kaneshie	Hand	KH3	1	AML	0.1
Agbogbloshie	Mutton	AM1	1	AML	0.1
Agbogbloshie	Chicken	AC1	1	AML	0.1
Agbogbloshie	Mutton	AM3	1	AML	0.1
Nima	Beef	NB11	2	AML-SXT	0.2
Agbogbloshie	Chicken	AC6	3	AML-TE-SXT	0.3

Maamobi	Beef	MBB12	5	AZM-AML-C-TE-SXT	0.6
Nima	Knife	NK15	3	AML-TE-CIP	0.3
Nima	Beef	NB14	2	AML-SXT	0.2
Maamobi	Mutton	MBM11	3	CN-AML-CIP	0.3
Maamobi	Knife	MBK11	3	CN-AML-CIP	0.3
Kaneshie	Chicken	KC11	3	AML-TE-SXT	0.3
Malata	Chicken	MAC2	2	AML-SXT	0.2
Malata	Beef	MAB4 2	5	AML-TE-SXT-AUG-CIP	0.6
Maamobi	Beef	MBB11	3	CN-AML-C	0.3
Nima	Hand	NH13	1	AML	0.1
Nima	Knife	NK13	2	CN-AML	0.2
Malata	Beef	MAB14	2	AML-C	0.2
Malata	Knife	MAK11	3	AML-TE-SXT	0.3

Amoxicillin 30µg (AML), Amoxycillin/clavulanic acid 30µg (AUG), Azithromycin 15µg (AZM), Ceftriaxone 30µg (CRO), Chloramphenicol 30µg (C), Ciprofloxacin 5µg (CIP), Gentamicin 10µg (CN), Tetracycline 30µg (TE), and Suphamethoxazole/trimethoprim SXT 22µg (SXT)

4.4 Whole genome sequencing of *Escherichia coli* from RTE meats and it associated samples.

The antimicrobial resistance genes and plasmid replicons of *Escherichia coli* isolated from RTE meats and it associated samples in the Accra Metropolis using whole genome sequencing (WGS) is shown in Table 4.5. Antimicrobial resistance genes were present in 52.4% of the *Escherichia coli* isolates. A total of 54 antimicrobial resistance genes were detected. The 54 antimicrobial resistance genes were made up of 19 different genes. The antimicrobial resistance genes detected were bla_{OXA-1} (3.7%), *qnrS1* (3.7%), *qnrS13* (3.7%), *aadA5* (3.7%), *dfrA17* (5.6%), *aph (3'')*, *Ib* (7.3%), *aph (6)*, *Id* (9.3%), bla_{TEM-1B} (9.3%), *dfrA14* (9.3%), *sul2* (13.0%), and *tet(A)* (14.8%). In addition, one (1) each (1.9%) was detected for *aac(6')*, *aadA1*, $bla_{CTX-M-15}$, bla_{TEM-1C}, *catA1*, *catB3*, *sul1*, and *tet(B)*. Nine (42.9%) *Escherichia coli* isolates possessed three or more antimicrobial resistance genes.

Plasmid replicons were detected in 81.0% of the *Escherichia coli* isolates from the RTE meats and it associated samples. A total of 28 plasmid replicons made up of 13 different types were found. The plasmid replicons found were IncFIB(pB171) (3.6%), IncFIB(pLF82-PhagePlasmid) (3.6%), IncFIC(FII) (3.6%), IncFII(pCoo) (3.6%), IncFII(pSE11) (3.6%), IncI1-I(Alpha) (3.6%), IncI2(Delta) (3.6%), IncX1(3.6%), IncFII (7.1%), IncY (10.7%), IncFIA (14.3), p0111 (17.9%), and IncFIB(AP001918) (21.4%).

Table 4.5: Antimicrobial resistance genes and plasmid replicons of *Escherichia coli* isolated from RTE meats and it related sources in the Accra Metropolis using WGS

No.	Isolate ID	Source	[a]Antimicrobial resistance genes	[b]Plasmid replicons
1	AB2	Beef	*bla$_{TEM-1C}$*	IncY
2	AB10	Beef	-	-
3	AC6	Chicken	-	IncI2(Delta)
4	AM1	Mutton	-	IncI1-I(Alpha)
5	AS3	Spices	*qnrS1, bla$_{TEM-1B}$, sul2, dfrA14, tet(A), aph(6)-Id*	IncFIA, IncFIB(AP001918), IncFII(pSE11)
6	KC11	Chicken	*aadA5, sul2, dfrA17, tet(A)*	p0111
7	KH3	Hand	-	IncFIB(AP001918)
8	KM7	Mutton	*aadA5, aac(6'), bla$_{CTX-M-15}$, bla$_{OXA-1}$, sul1, dfrA17, tet(B), catB3*	IncFIA, IncFIB(AP001918), IncFII
9	MAB14	Beef	-	-
10	MAB42	Beef	*blaOXA-1, tet(B), catA1, aadA1*	IncFIA, IncFIB(AP001918), IncFII(pCoo), IncX1
11	MAC12	Beef	*qnrS13, bla$_{TEM-1B}$, sul2, dfrA14, tet(A), aph(6)-Id, aph(3'')-Ib*	p0111
12	MAK11	Knife	*sul2, dfrA17, tet(A)*	p0111

13	MBB1	Beef	-	IncFIB(pLF82-PhagePlasmid)
14	MBB12	Beef	*aph(6)-Id, aph(3'')-Ib, qnrS1, bla$_{TEM-1B}$, sul2, dfrA14, tet(A)*	IncY
15	MBK11	Knife	-	IncFIB(AP001918), IncFII
16	MBM11	Mutton	-	IncFIA, IncFIB(AP001918), IncFIC(FII)
17	NB11	Beef	*bla$_{TEM-1B}$, sul2, dfrA14, tet(A), aph(6)-Id, aph(3'')-Ib*	IncFIB(pB171), p0111
18	NB14	Beef	*aph(6)-Id, aph(3'')-Ib, qnrS13, bla$_{TEM-1B}$, sul2, dfrA14, tet(A)*	p0111
19	NH13	Hand	-	IncY
20	NK13	Knife	-	-
21	NK15	Knife	*tet(A)*	-

AMR=[a]Antimicrobial resistance; - = Not detected; [a]Using ResFinder (v4.1); [b]Using PlasmidFinder (v2.1)

The virulence genes and multilocus sequencing types of *Escherichia coli* isolated from RTE meats and it associated samples in the Accra Metropolis using whole genome sequencing (WGS) is presented in Table 4.6. Virulence genes were detected in all the *Escherichia coli* isolates. A total of 156 virulence genes made up of 45 different types were present. The predominant (3 or more) virulence genes present were *yehC* (n=3, 1.9%), *yehD* (n=3, 1.9%), *traT* (n=4, 2.6%), *clpK1* (n=5, 3.2%), *ompT* (n=5, 3.2%), *csgA* (n=11, 7.1%), *gad* (n=12, 7.7%), *lpfA* (n= 13, 8.3%), *terC* (n=18, 11.5%), *fimH* (n=21, 13.1%), and *nlpI* (n=21, 13.5%). Two (1.3%) each of *Tia, sitA, iss, hlyE, F17G,* and *F17C*; and one (0.6%) each of *afaA, afaB, afaC, afaD, astA, chuA, cia, cif, cma, cofG cofT, espJ, F17A, F17D, faeA, faeC, faeE, faeF, fyuA, hra, irp2, iucC, iutA, kpsE, kpsMII, nleB, tir,* and *traJ* virulence genes were detected in the *Escherichia coli* isolates. All the 21 *Escherichia coli* isolates possessed 3 or more virulence genes.

For multilocus sequence typing, 81.0% of the *Escherichia coli* were typed, the remaining 19% possessed an Unknown MLST types. In all 17 MLST types made up of 12 different types were present. The MLST types detected were: ST1021, ST1015, ST960, ST169, ST132, ST114, ST108, ST87 and ST2 (n=1 each, 4.8%), ST19 (n=2, 9.8%), and ST999 and ST21 (n=3 each, 14.8%).

Table 4.6: Virulence genes and multilocus sequencing typing of *Escherichia coli* isolated from RTE meats and it related sources in the Accra Metropolis using WGS.

No.	Isolate ID	Source	[a]Virulence genes	[b]MLST types
1	AB2	Beef	*clpK1, fimH, lpfA, nlpI, terC, nleB, nlpI, ompT, terC, tir*	ST21
2	AB10	Beef	*cif, espA, espJ, fimH, gad, lpfA*	N/A
3	AC6	Chicken	*clpK1, csgA, fimH, gad, nlpI, terC, tia*	ST960
4	AM1	Mutton	*cia, clpK1, csgA, fimH, gad, lpfA, nlpI*	N/A
5	AS3	Spices	*csgA, faeA, faeC, faeE, faeF, fimH, gad, lpfA, nlpI, sitA, terC, traT, yehC*	N/A
6	KC11	Chicken	*csgA, fimH, nlpI, terC*	ST999
7	KH3	Hand	*cma, fimH, gad, lpfA, nlpI, terC, tia*	ST108
8	KM7	Mutton	*afaA, afaB, afaC, afaD, fimH, fyuA, hlyE, irp2, iucC, iutA, nlpI, sitA, terC, traT*	ST2
9	MAB14	Beef	*yehD, csgA, fimH, gad, lpfA, nlpI, yehD*	ST1021
10	MAB42	Beef	*F17A, F17C, F17D, F17G, astA, cofG, cofT, fimH, lpfA, nlpI, terC, traT*	ST19
11	MAC12	Beef	*fimH, gad, lpfA, nlpI, terC*	ST21
12	MAK11	Knife	*csgA, fimH, nlpI, terC*	ST999
13	MBB1	Beef	*chuA, fimH, gad*	ST114
14	MBB12	Beef	*clpK1, csgA, fimH, gad, nlpI, terC*	ST169
15	MBK11	Knife	*F17C, F17G, fimH, gad, lpfA, nlpI, terC*	ST87
16	MBM11	Mutton	*fimH, hra, lpfA, nlpI, ompT, terC, traJ, traT*	ST19
17	NB11	Beef	*clpK1, csgA, fimH, gad, hlyE, nlpI, terC*	ST132
18	NB14	Beef	*fimH, lpfA, nlpI, terC*	ST21

19	NH13	Hand	*fimH, gad, iss, lpfA, nlpI, ompT, terC, yehC*	ST1015
20	NK13	Knife	*csgA, fimH, iss, lpfA, nlpI, ompT, terC, yehC, yehD*	N/A
21	NK15	Knife	*csgA, fimH, nlpI, terC*	ST999

[a]Using VirulenceFindeer (v2.0); MLST= [a]Multilocus sequence typing; [a]Using MLST option (v2.0.9)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of *E. coli* from RTE meat

This study found that 25 (7%) RTE meats and it associated samples were positive for *Escherichia coli*. RTE Beef (15%) was the most contaminated, followed by RTE chicken (10%), knives for cutting RTE meats (7%), RTE mutton (5%) and hands of RTE meat sellers (3%). The least contaminated sample was spices (2%) use for preparing RTE meats as indicated in Table 4.1. These contaminations could be as a results of poor hygienic processing procedures including handlers and faecal contaminations. This is therefore a wakeup call for the one health team in Ghana comprising of the animal, human and environmental health workers to join hands to promote public education on hygiene and health on the handling and consumption of RTE foods including meat. Overall, 10% of animal sources (RTE beef, chicken and mutton), 3% of human source (hands of RTE meat sellers) and 4.2% of environmental sources (knives for cutting and spices for preparing RTE meats) were contaminated with *E. coli*. Similarly, to this study, Adzitey *et al*. (2021) found that *E. coli* in raw and RTE meats (animal), on hands of raw and RTE meat sellers (human) and knives, table and utensils of raw and RTE meat sellers (environment) were contaminated with *E. coli*. They reported an overall prevalence of 42%, 30% and 32% for the animal, human and environmental samples, respectively.

The study is in support of that of Abass *et al*. (2020), who also found that RTE beef, chicken and chevon were contaminated with *E. coli*. They reported a prevalence rate of 20%, 16% and 8% for RTE chevon, chicken and beef, respectively. This study was a bit contradictory to an earlier study which recorded zero for *E. coli* in chicken and 7% of *E. coli* in beef obtained from Jordan and Mediterranean country (Osaili *et al*., 2014).

The prevalence of *E. coli* in chicken luncheon as reported by Awadallah *et al.* (2014) was the same as the 10% reported for RTE chicken, while prevalence of *E. coli* in beef luncheon was higher than the present study. Adzitey *et al*. (2024) revealed that *E. coli* contamination was recorded 67%, 41% and 23% for beef, chicken and pork, respectively. The prevalence of *E. coli* in the chicken and beef were higher than the present study. Another study found *E. coli* prevalence rate of 38.9%, 30.5% and 30.5% for chicken, beef and mutton, respectively (Baah *et al*., 2022), these values were higher that the findings of the current study.

5.2 Prevalence of *E. coli* from RTE meat per location

The 25 *E. coli* isolated from the RTE meats and it associated samples in the study area revealed the following overall results, 9.7%, 6.9%, 6.9%, 5.6%, and 5.6% for Agbogboloshie, Kaneshie, Nima, Malata and Maamobi, respectively. This means that RTE meat in Agbogboloshie was the most contaminated followed by Kaneshie and Nima with the least being Malata and Maamobi. This study is tandem to Onyeka *et al*. (2020) who revealed that meat (27%) from East Pretoria was the most contaminated of all the five locations, the most contaminate location in this study was Agbogboloshie. This study is also in agreement with that of Adzitey *et al*. (2018), who reported that beef and it associated samples collected from different locations were contaminated with *E. coli*, who found that samples collected Hansua market (100%), Anyinabrem Market (93.8%), Kenten Market (93.8%), Slaughter House (93.8%), Main Market (87.5%), Nana Abena Market (87.5%), Ahenfie Market (87.5%), Brigade (81.3%), Dwomor Market (81.3%), Site (81.3%), Takofiano Market (75.0%), Zongo-Tamale Station (75.0%), Zongo Market (62.5%), Abanim (50.0%) and Sansama Junction (37.5) were contaminated with *E. coli*. According to Jaja *et al*. (2020) out of the three slaughters points, King William town recorded the most contaminated carcases with *E.*

coli. Another study revealed that butcher shops (66.7%) were the most contaminated compared to restaurants (33.3%) (Beyi *et al*., 2017). The contamination rates of meats with E. coli as reported by Onyeka *et al*. (2020) and Beyi *et al.* (2017) in the various locations were higher than what was found in this study.

5.3 Antimicrobial resistance

Antimicrobials are used in animal production normally for treatment of sick animals, as prophylactics and sometimes as growth promoters (Abass *et al*., 2020; Van *et al*., 2020; Choudhury, 2022). Their used have resulted in the development of resistance by bacteria associated with animals and consequently in meats (Economou and Gousia, 2015; Abass *et al*., 2020; Rahman *et al*., 2022). In Ghana, Ekli *et al*. (2020) evidenced that, farmers in the Wa, municipality used antibiotics such as Ciprofloxacin (32.0%), Sulphamethoxazole/trimethoprim (17.1%), Gentamicin (1.8%), Ceftriaxone (0.9%), Chloramphenicol (0.9%) and Tetracycline (0.9%) as prophylactics or to treat animal diseases and 73.2% of the farmers did not observe withdrawal periods prior to sale and slaughter of animals after antibiotic administration. In this study, Amoxicillin was the most resisted antibiotic (100%) with 0% each for susceptibility and intermediate susceptibility, respectively and this was followed by Suphamethoxazole/trimethoprim with 36% resistant and Tetracycline with 32% resistant. However, the most susceptible antibiotic was Ceftriaxone with 0% resistant, 92% susceptibility and 8% intermediate susceptibility, followed by Chloramphenicol with 88% susceptibility and 12% resistant. This finding suggests that the preferred antibiotic for *E. coli* infection treatment should be Ceftriaxone and Chloramphenicol for effective results. Abass *et al*. (2020) reported that *E. coli* from RTE meat sources were highly susceptible to Chloramphenicol, Ciprofloxacin and Ceftriaxone, but resistant to Amoxycillin/clavulanic acid, which agrees with the findings in this study made to Chloramphenicol and Ceftriaxone.

Adzitey (2020) indicated that, *Escherichia coli* isolated from RTE beef at the Wa municipality were highly resistant to Teicoplanin (97.78%), but were susceptible greater than or equal to eighty percent (≥80%) to Amoxycillin/clavulanic, Ceftriaxone and others. Similarly, this study found susceptibility ≥80% for Ceftriaxone. Another study by Adzitey *et al*. (2021) reported that *E. coli* isolated from meat and its related samples were highly resistant to Amoxicillin (70.9%), Tetracycline (57.0%) and Trimethoprim (55.0%). Again, resistant to Amoxicillin was higher while resistant to Tetracycline was lower in the current study compared to that of Adzitey *et al.* (2021). Rahman *et al.* (2017) found that *E. coli* isolated from chicken meats were resistant to Oxytetracycline (92%), followed by Sulphonamide/trimethoprime (84%), Amoxicillin (76%) and Erythromycin (60%). This study found higher resistance to Amoxicillin (100%) and lower resistance to Sulphonamide/trimethoprim (36%) compared to that of Rahman *et al.* (2017). Ronald *et al.* (2023) reported that *E. coli* isolated from RTE red meats were resistant to Co-trimoxazole (80%), Tetracycline (73%), Streptomycin (67%) and Ampicillin (67%), but susceptible to Ciprofloxacin (100%) and Amikacin (80%). This study reported lower resistance to Tetracycline and lower susceptibility to Ciprofloxacin compared to the study of Ronald *et al*. (2023).

5.4 Multiple antibiotics resistant (MAR) and antibiotic resistance profile of individual *E. coli* isolated from RTE meats and its related samples

Twenty-five (25) *E. coli* isolated from RTE meats and it associated samples were tested against 9 antibiotics for the multiple antibiotics resistant as shown in Table 4.4. The study found that 9, 6, 7 and 3 of the isolates showed resistant to 1, 2, 3 and 5 each of the antibiotics, respectively. This suggests that, in an infection cause by any of these 25 *E. coli* isolates, at least 9 may resist 1 of these 9 antibiotics and at most 3 of them may resist 5 of these antibiotics if used for their treatments. Kathleen *et al*. (2016) indicated

that bacteria with MAR index of greater than or equal to 0.2 comes from source where antibiotics are more commonly used, while those with MAR index of less than 0.2 originate from sources where antibiotics are not frequently used. Therefore, 40% of the *E. coli* isolates were from high-risk sources, while 60% were from low-risk sources. According to Ronald *et al*. (2023), MAR index of *E. coli* isolated from RTE red meats ranged from 0.3 to 0.5, but this study reported MAR index of 0.1 to 0.5. Abass *et al*. (2020) reported that, *E. coli* isolated from RTE meat showed MAR index ranging from 0.2 to 0.9, this presents a relatively higher resistant index as compared to this study. Similarly, to this study, Adzitey (2020) reported MAR index range of 0.1 to 0.6 (resistant to one and five antibiotics) for *E. coli* isolated from beef samples (Adzitey, 2020). Multidrug resistance defines as resistant to three or more different classes of antibiotics was exhibited by 40% of the *E. coli* isolated from RTE meats and it associated samples. The finding of multidrug resistance in RTE meat *E. coli* isolates is consistent with that of Abass *et al.* (2020) and Ronald *et al*. (2023). Some similarities and differences in the resistance patterns were observed in this study. For instance, *E. coli* with codes NB11 and NB14 isolated from RTE beef in Nima exhibited the same resistance pattern of AML-SXT (Amoxicillin-Suphamethoxazole/trimethoprim) and *E. coli* with codes MBM11 and MBK11 isolated from mutton and knife, respectively exhibited the same resistance pattern of CN-AML-CIP (Gentamicin-Amoxicillin-Ciprofloxacin). These reveal possible cross contamination between the RTE meats and it associated samples from the same source. Also, *E. coli* with code AC6 isolated from chicken in Agbogbloshie, *E. coli* isolate with code KC11 isolated from chicken at Kaneshie and *E. coli* with code MAK11 isolated from knife in Malata exhibited the same resistance pattern of AML-TE-SXT (Amoxicillin- Tetracycline -

Suphamethoxazole/trimethoprim). This means that, *E. coli* of the same resistant pattern do circulate among RTE meats from different locations.

5.5 Antimicrobial resistance, plasmid replicons, virulence genes and multilocus sequence typing of *Escherichia coli* isolated from RTE meats and it related sources

Studies on whole genome sequencing of bacteria in Ghana is limited. Ready-to-eat meats require no further cooking prior to eating. Therefore, the presence of diverse antimicrobial resistance genes, plasmid replicons, virulence genes and MLST types in bacteria isolated from RTE meats and it associated samples is worrying and of public health importance. The availability of antimicrobial resistance genes in *Escherichia coli* can increase its' resistance to treatment when they are involved in infections. Antimicrobial resistance genes are often found on plasmids or transposons and can be transferred from one cell to the other via conjugation, transformation or transduction allowing for rapid spread of resistance genes among pathogens (Reygaert, 2018). The findings of this study are comparable to that of others. In the USA, Tadesse *et al.* (2018) examined CTX-M containing *Escherichia coli* isolates from retail meats and cattle, and reported of the presence of $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTXM\text{-}27}$, and $bla_{CTX\text{-}M\text{-}32}$ in the examined samples. This study found $bla_{CTX\text{-}M\text{-}15}$ and mostly $bla_{TEM\text{-}1B}$, but not the other beta-lactamases reported by Tadesse *et al*. (2018). Also, Tadesse *et al*. (2018) identified other antimicrobial resistance genes such *aadA, strA, strB, aac(3)-IId, aac(3)-VIa, aph(3')-Ic,* bla_{TEM}, $bla_{HERA\text{-}3}$, *floR, sul1, sul2, catA1, tetA, tetB, dfrA,* and *qacE*. Similarly, this study found the sulfamethoxazole resistance genes, *Sul1* and *Sul2*, chloramphenicol resistance gene, *CatA1* and tetracycline resistance genes, *tetA* and *tetB*. In Ghana, Adzitey *et al*. (2019) found antimicrobial resistance genes in all *Escherichia coli* isolates of meat origin and reported that 57.1% carried three or more different antibiotic resistance genes. This study found that, 52.4% of the isolates

possessed antimicrobial resistance genes and all positive isolates carried at least three or more resistance genes except one isolate each of RTE beef (with isolate ID AB2) and knife (with isolate ID NK15) origin. Adzitey *et al.* (2019) detected the following antimicrobial resistance genes including *sul2* and *sul1* conferring resistance to sulfonamides, *aph(3")-Ib, aph(6)-Id* and *aadA5*, conferring resistance to aminoglycosides, *tet(A)* and *tet(B)* conferring resistance to tetracycline, $bla_{TEM\text{-}1B}$ and $bla_{CTX\text{-}M\text{-}15}$ conferring resistance to β-lactams, *dfrA17* and *dfrA14* conferring resistance to trimethoprim, *qepA4* and *qnrS1* conferring resistance to fluroquinolones, *catA1* and *catA2* conferring resistance to phenicols, *fosA7* conferring resistance to fosfomycin, and *mdf(A)* conferring resistance to macrolide–lincosamide–streptogra-min B antibiotics. The findings of this study concords with that of Adzitey *et al*. (2019) with the exception of *qepA4, catA2*, *fosA7* and *mdf(A)* which were not found in the current study. Massella *et al*. (2020) reported that, 35.8% of *Escherichia coli* isolated from animals, humans and foods in Italy harbored antimicrobial resistance genes, 71.3% harbored at least three antibiotic resistance genes and 46 different antimicrobial resistance genes were found. In this study, 52.4% of the *Escherichia coli* harbored antimicrobial resistance genes, 42.9% possessed three or more antimicrobial resistance genes, and 19 different antimicrobial resistance genes were observed. Wang *et al*. (2024) found a total of 89 antimicrobial resistance genes in *Escherichia coli* isolated from retail meats in China. Contrarily, this study found a total of 54 antimicrobial resistance genes.

Plasmids, which are extrachromosomal DNA molecules capable of self-replication and have the potential to transfer antimicrobial resistance genes from one *Escherichia coli* to the other. This mechanism will continue to contribute to making bacteria including *Escherichia coli* to be more resistant to antimicrobials thereby impeding treatment. Plasmid prediction using whole genome sequencing has been done by other researchers.

For instance, Adzitey *et al.* (2019) detected plasmid replicons including IncFII, Col440I, IncFIB, IncQ1, IncY, Col156, and IncI1 in *Escherichia coli* of guinea fowl meats origin. This study also detected IncFII and IncY plasmid replicons in the RTE meats and it associated samples. Plasmids similar to IncFIB, that is, IncFIB(pB171) and IncFIB(pLF82-PhagePlasmid), and IncI1, that is, (IncI1-I(Alpha) and IncI2(Delta) were also present in this study. The plasmid replicon, IncFII, was the commonest according Adzitey *et al.* (2019). In this study, the commonest plasmid replicon was IncFIB (AP001918). Massella *et al.* (2020) found 33 different plasmid replicons in *Escherichia coli* isolated from animals, humans and foods, whilst this study found 13 different plasmid replicons in the RTE meats and it associated samples. The commonest replicon was IncF (65.2%), followed by IncI (20.4%) and IncX (16.1%) (Massella *et al.*, 2020). Contrarily, to this study, the predominant plasmid replicons observed were IncFIB (AP001918) (21.4%), p0111 (17.9), IncFIA (14.3%), IncY (10.7%), and IncFII (7.1%). Massella *et al.* (2020) also reported of plasmid replicons across multiple sequence types. In this study, four *Escherichia coli* isolates possessed three or more plasmid replicons, namely KM7 from mutton, MAB42 from beef, MBM11 from mutton and AS3 from spices, belonging to the MLST types ST2, ST19, ST19 and unknown ST, respectively. Päivärinta *et al.* (2020) found various plasmid sequences including IncB/O/K/Z, IncI1, IncFII, IncII, IncFIB, IncFIC, IncX1 and Col-plasmids in broiler flocks, farms and broiler meats. Similarly, IncFII and IncX1 and other strains of IncI1, that is, IncI1-I(Alpha) and IncI2(Delta), IncFIB, that is, IncFIB (AP001918), IncFIB (pB171) and IncFIB(pLF82-PhagePlasmid) and IncFIC, that is, IncFIC (FII) were also found in the present study.

Virulence factor of *Escherichia coli* describes it ability to infect humans or animals and cause a disease. Virulence genes will enable *Escherichia coli* to colonize it hosts and

their identification can lead to the development of rapid screening tests for effective isolation and control measures in samples, sick or diseased individuals and animals. There are some studies reporting on the presence of virulence genes in *Escherichia coli* using whole genome sequencing. A study conducted by Adzitey *et al.* (2020) reported that *Escherichia coli* from guinea fowl meats possessed virulence genes such as *air, astA. eilA, gad, iha, ireA, iroN, iss, IpfA, sat* and *senB.* The current study also found *gad, iss* and *IpfA,* but not, *air, astA. eilA, iha, ireA, iroN, sat* and *senB.* It should be noted that *gad, iss*, and *IpfA* genes are responsible for the production of glutamate decarboxylase, increase serum survival and development of long polar fimbriae, respectively in pathogens. Glutamate decarboxylase catalysis the conversion of glutamate to γ-aminobutyric acid which is the main inhibitory neurotransmitter of the central nervous system (Wang *et al*., 2020). Biran *et al*. (2021) indicated that the *iss* gene is required for the synthesis of capsules, which protects bacteria from bactericidal effect. Torres *et al.* (2009) reported that, the presence of long polar fimbriae in *Escherichia coli* helps it in the adherence, persistence and colonization of intestines. Massella *et al.* (2020) found that *Escherichia coli* from animal, human and food sources possessed different virulence genes such as *fimH, pap, iha, bmaE* and *sfaS* encoding for adhesins, *ibeA* encoding for invasins, *iucD, iutA, fyuA, irp2, iroN, ireA, tsh* and *sitA* encoding for iron acquisition systems, *hlyE, cnf1, cdtB, usp, sat, picU* and *vat* encoding for toxins, and *kpsMT-II, traT, ompT, iss* and *cvaC* encoding for protectins. Similarly, *fimH, iutA, fyuA, irp2, sitA, hlyE, traT* and *ompT* genes were found in this study. Closely associated virulence genes to *kpsMT-I,* and *iucD,* that is, *kpsMII* and *iucC* were also present in the current study.

Multilocus sequence typing directly measures the DNA variations in a set of well conserved housekeeping genes and characterize strains by their unique allelic profiles

(Maiden, 2006). Multilocus sequence typing is considered as gold standard of typing for many species and has also been described as a powerful tool for understanding evolutionary dynamics of pathogens and to gain insight into their genetic diversity (Larsen *et al*., 2012; Nunney *et al*., 2012). The findings of this study are comparable to other studies. Wang *et al*. (2024) reported of the presence of 146 different sequence types which included 20 new STs from retail meats in China. In this study, a total of 17 MLST types made up of 12 different types were identified. Unknown or new STs were four (4) in number. Wang *et al*. (2024) also indicated a high genetic diversity among the *Escherichia coli* from the retail meats which concords to this study. Foster-Nyarko *et al*. (2021) reported STs such as ST155 (n=9), ST9284 (n=1), ST2772 (n=1) in *Escherichia coli* isolated from chickens, and ST155 (n=3), ST540 (n=2), ST212 (n=1), ST2067 (n=1) and ST2614 (n=1) in *Escherichia coli* isolated from guinea fowls, which differs from the STs found in this study. Adzitey *et al*. (2020) found ST69, ST540 and ST7473 in *Escherichia coli* isolated from guinea fowl meats and ST297, ST155 and ST155 in *Escherichia coli* isolated from meat of local chickens. In another study by Adzitey *et al*. (2019), MLST types ST69, ST540 and ST7473 were detected in *Escherichia coli* isolated from guinea fowls. Päivärinta *et al*. (2020) identified MLST types including ST101, ST117, ST212, ST351, ST373, ST1594 and an unknown ST in broiler flocks, farms and broiler meats while, Massella *et al*. (2020) identified ST10, ST23, ST69, ST117 and ST131 in animals and humans. In this study, the MLST types identified were ST1021, ST1015, ST999, ST169, ST132, ST114, ST108, ST87, ST21, STS19 and ST2 which differed from those reported by Adzitey *et al*. (2020), Adzitey *et al*. (2019), Päivärinta *et al*. (2020) and Massella *et al*. (2020).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Escherichia coli was isolated from ready-to-eat (RTE) meats and it associated samples. The overall, prevalence was 7%, and the highest and least contaminated sources were RTE beef and spices for preparing RTE meats, respectively. Also, animal, human and environmental sources were contaminated with *E. coli*. Animal and human sources were the highest and least contaminated sources, respectively. The *E. coli* isolates exhibited highest resistance to Amoxicillin and susceptibility to Ceftriaxone. Overall, the *E. coli* isolates from the RTE

meat and its associated samples exhibited 25.3% resistance, 19.1% intermediate resistance and 55.6% susceptibility. The multiple antibiotic resistant index ranged from 0.1-0.6, that is, resistant to 1 to 5 different antibiotics. Whole genome sequencing of *E. coli* revealed the presence of varying number of antimicrobial resistance genes, virulence genes, plasmid replicons and MLST types in the RTE meat and its associated samples.

6.2 RECOMMENDATIONS

1. The handling and administration/use of antimicrobials should be properly regulated and well enforced by the law enforcement bodies in Ghana.
2. Proper hygienic practices in handling of animals and meats should be observed by farmers, veterinary officers, meat sellers and consumers.
3. More studies on the prevalence, antibiotic resistance and whole genome sequencing of foodborne pathogens in foods should be conducted by researchers and other stake holders to provide data and to create more awareness of foodborne illnesses.

REFERENCES

Abass, A., Adzitey, F., & Huda, N. (2020). *Escherichia coli* of ready-to-eat (RTE) meats origin showed resistance to antibiotics used by farmers. *Antibiotics*, *9*(12), 869.

Abdelrahman, H., Islam, G. M., & Gilbride, K. A. (2018). Effects of tetracycline and ibuprofen on the relative abundance of microbial eukaryotic and bacterial populations in wastewater treatment. *Water Science and Technology*, *2017*(2), 430-440.

Abdul-Rahman, I. I., & Adu, Y. E. (2017). The role of the rural farmer in guinea fowl Numida meleagris value chain, a case study of the Tolon district. *Livestock Research for Rural Development*, *29*(4), 1-13.

Abe, C. M., Trabulsi, L. R., Blanco, J., Blanco, M., Dahbi, G., Blanco, J. E., ... & Elias, W. P. (2009). Virulence features of atypical enteropathogenic *Escherichia coli* identified by the eae+ EAF-negative stx− genetic profile. *Diagnostic Microbiology and Infectious Disease*, *64*(4), 357-365.

Adak, G. K., Meakins, S. M., Yip, H., Lopman, B. A., & O'Brien, S. J. (2005). Disease risks from foods, England and Wales, 1996–2000. *Emerging Infectious Diseases*, *11*(3), 365.

Adams, F., Ohene-Yankyera, K., Aidoo, R., & Wongnaa, C. A. (2021). Economic benefits of livestock management in Ghana. *Agricultural and Food Economics*, *9*, 1-17.

Adegboye, A. O. (2011). Quantitative risk assessment of oxytetracycline and tetracycline residues in slaughtered cattle from three abattoirs in Nigeria (Doctoral dissertation).

Adzitey, F. (2015). Antibiotic classes and antibiotic susceptibility of bacterial isolates from selected poultry; a mini review. *World's Veterinary Journal 5(3): 36-41*,

Adzitey, F. (2020). Incidence and antimicrobial susceptibility of *Escherichia coli* isolated from beef (meat muscle, liver and kidney) samples in Wa Abattoir, Ghana. *Cogent Food & Agriculture*, *6*(1), 1718269.

Adzitey, F., Abu, A., & Teye, G. A. (2020). Impact of COVID-19 on livestock and meat production in northern Ghana. Impact of COVID-19 on Food Production, Nutrition Security and Hospitality in Northern Ghana*: Mitigation Action against the Pandemic. (Editor) Kugbe, JX*, 49-64

Adzitey, F., Anachinaba, I. A., Brown, C. A. Q., Huda, N., & Rambli, M. (2024). Prevalence of resistant *Escherichia coli* isolated from local meats sold in Tema Metropolis, Ghana. *ASEAN Journal on Science and Technology for Development*, *40*(2), 11.

Adzitey, F., Asante, J., Kumalo, H. M., Khan, R. B., Somboro, A. M., & Amoako, D. G. (2020d). Genomic investigation into the virulome, pathogenicity, stress response factors, clonal lineages, and phylogenetic relationship of *Escherichia coli* strains isolated from meat sources in Ghana. *Genes*, 11(12), 1504.

Adzitey, F., Assoah-Peprah, P., & Teye, G. A. (2019). Whole-genome sequencing of *Escherichia coli* isolated from contaminated meat samples collected from the Northern Region of Ghana reveals the presence of multiple antimicrobial resistance genes. *Journal of Global Antimicrobial Resistance*, *18*, 179-182.

Adzitey, F., Ayum, G. T., & Nsoah, J. K. (2018). Contamination of beeves, tables, knives and aprons by *Escherichia coli* in the Techiman Municipality of Ghana-a preliminary study. *Ghanaian Journal of Animal Science*, *9*, 129-134.

Adzitey, F., Huda, N., & Shariff, A. H. M. (2021). Phenotypic antimicrobial susceptibility of *Escherichia coli* from raw meats, ready-to-eat meats, and their related samples in one health context. *Microorganisms*, *9*(2), 326.

Adzitey, F., Sumaila, N., & Saba, C. K. S. (2015). Isolation of *E. coli* from drinking water sources for humans and farm animals in Nyankpala Community of Ghana. *Research Journal of Microbiology*, *10*(3), 126.

Agbodaze, D., Nmai, P. N. A., Robertson, F. C., Yeboah-Manu, D., Owusu-Darko, K., & Addo, K. K. (2005). Microbiological quality of "khebab" consumed in the Accra metropolis. *Ghana Medical Journal*, *39*(2), 46-49.

Ahmad, R. S., Imran, A., & Hussain, M. B. (2018). Nutritional composition of meat. *Meat Science and Nutrition*, *61*(10.5772), 61-75.

Ajulo, H. O., Ajulo, M. O., & Ekereumoh, N. S. E. (2020). Isolation and identification of Salmonella and Escherichia coli from raw goat meat in Uyo Metropolis, Akwaibom State. *Asian Food Science Journal*, *14*(4), 50-60.

Akinsemolu, A. A. (2018). The role of microorganisms in achieving the sustainable development goals. *Journal of Cleaner Production*, *182*, 139-155.

Alders, R. G., Dumas, S. E., Rukambile, E., Magoke, G., Maulaga, W., Jong, J., & Costa, R. (2018). Family poultry: Multiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. *Maternal & Child Nutrition*, *14*, e12668.

Ali, S. F. (2019). Review on rational use of antmicrobials in veterinary practice. *International Journal of Biomedical Engineering and Clinical Science*, *5*(4), 70-77.

Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). *Escherichia coli* in Europe: an overview. *International Journal of Environmental Research and Public Health*, *10*(12), 6235-6254.

Alonso, C. A., Zarazaga, M., Ben Sallem, R., Jouini, A., Ben Slama, K., & Torres, C. (2017). Antibiotic resistance in *Escherichia coli* in husbandry animals: the African perspective. *Letters in Applied Microbiology*, *64*(5), 318-334.

Ampaw, B. A. (2018). Prevalence of *Listeria Monocytogenes* in 'Khebab', a Street-Vended Spicy Grilled Meat (Doctoral dissertation).

Asadi, A., Abdi, M., Kouhsari, E., Panahi, P., Sholeh, M., Sadeghifard, N., ... & Gholami, M. (2020). Minocycline, focus on mechanisms of resistance, antibacterial activity, and clinical effectiveness: Back to the future. *Journal of Global Antimicrobial Resistance*, *22*, 161-174.

Awadallah, M. A., Ahmed, H. A., & Merwad, A. M. (2014). Prevalence of non-O157 shiga toxin-producing *Escherichia coli* and Enterotoxigenic *staphylococci* in ready-to-eat meat products, handlers and consumers in Cairo, Egypt. *Global Veterinaria*, *12*(5), 692-699.

Baah, D. A., Kotey, F. C., Dayie, N. T., Codjoe, F. S., Tetteh-Quarcoo, P. B., & Donkor, E. S. (2022). Multidrug-resistant *Gram-negative* bacteria contaminating raw meat sold in Accra, Ghana. *Pathogens*, *11*(12), 1517.

Bagumire, A., & Karumuna, R. (2017). Bacterial contamination of ready-to-eat meats vended in highway markets in Uganda. *African Journal of Food Science*, *11*(6), 160-170.

Baidya, S., & Rahman, T. (2021). *A* review on the prevalence and detection of Bacterial contamination in common food and associated health risk (Doctoral dissertation, Brac University).

Barberis, S., Quiroga, H. G., Barcia, C., Talia, J. M., & Debattista, N. (2018). Natural food preservatives against microorganisms. *Food Safety and Preservation*, 621-658.

Barros, M. D. A. F., Nero, L. A., Monteiro, A. A., & Beloti, V. (2007). Identification of main contamination points by hygiene indicator microorganisms in beef processing plants. *Food Science and Technology, 27*, 856-862.

Barua, R., Bhilegaonkar, K. N., Agarwal, R. K., Singh, D. K., Malik, S. V. S., Rathore, R. S., ... & Sunil, B. (2007). Prevalence and rapid detection of shiga toxin producing *E. coli (STEC*) in water and ready-to-eat (RTE) foods. *Journal of Veterinary Public Health, 5*(2), 75-83.

Bauer, H., Paronetto, F., Burns, W. A., & Einheber, A. (1966). The enhancing effect of the microbial flora on macrophage function and the immune response: a study in germfree mice. *The Journal of Experimental Medicine, 123*(6), 1013-1024.

Bedale, W. A. (2019). Veterinary drug residues in foods of animal origin. In *Chemical Hazards in Foods of Animal Origin* (pp. 3435-3440). Wageningen Academic Publishers.

Belyaev, R. I., Kuznetsov, A. N., & Prilepskaya, N. E. (2021). How the even-toed ungulate vertebral column works: Comparison of intervertebral mobility in 33 genera. *Journal of Anatomy*, *239*(6), 1370-1399.

Ben Hsouna, A., Ben Halima, N., Smaoui, S., & Hamdi, N. (2017). Citrus lemon essential oil: Chemical composition, antioxidant and antimicrobial activities with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. *Lipids in Health and Disease*, *16*, 1-11.

Benta, M., & Kilonzo, R. (2021). Pastoralists' social networks in access to and use of antibiotics: Implication on drug resistance in Ngorongoro conservation area of Tanzania. *Tanzania Journal of Development Studies*, *19*(2).

Bergeaud-Blackler, F. (2015). The halal certification market in Europe and the world: a first panorama. In *Halal Matters* (pp. 105-126). Routledge.

Besser, J., Carleton, H. A., Gerner-Smidt, P., Lindsey, R. L., & Trees, E. (2018). Next-generation sequencing technologies and their application to the study and control of bacterial infections. *Clinical Microbiology and Infection*, *24*(4), 335-341.

Beyi, A. F., Fite, A.T., Tora, E., Tafese, A., Genu, T., Kaba, T., ... & Cox, E. (2017). Prevalence and antimicrobial susceptibility of *Escherichia coli* O157 in beef at butcher shops and restaurants in central Ethiopia. *BMC Microbiology*, *17*, 1-6.

Biran, D., Sura, T., Otto, A., Yair, Y., Becher, D., & Ron, E. Z. (2021). Surviving serum: the *Escherichia coli* iss gene of extraintestinal pathogenic *E. coli* is required for the synthesis of group 4 capsule. *Infection and Immunity, 89*(10): e0031621.

Boamah, V.E., Agyare, C., Odoi, H., & Dalsgaard, A. (2016). Practices and factors influencing the use of antibiotics in selected poultry farms in Ghana. *Journal Antimicrobial Agents, 2*: 120.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114-2120.

Bonneau, M., & Weiler, U. (2019). Pros and cons of alternatives to piglet castration: Welfare, boar taint, and other meat quality traits. *Animals*, *9*(11), 884.

Bortolaia, V., Kaas, R. S., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., … Aarestrup, F. M. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. *Journal of Antimicrobial Chemotherapy*, 75, 3491–3500.

Bosse, M., Megens, H. J., Madsen, O., Frantz, L. A., Paudel, Y., Crooijmans, R. P., & Groenen, M. A. (2014). Untangling the hybrid nature of modern pig genomes: a mosaic derived from biogeographically distinct and highly divergent Sus scrofa populations. *Molecular ecology*, *23*(16), 4089-4102.

Bottalico, L., Charitos, I. A., Potenza, M. A., Montagnani, M., & Santacroce, L. (2022). The war against bacteria, from the past to present and beyond. *Expert Review of Anti-infective Therapy*, *20*(5), 681-706.

Brown, E., Dessai, U., McGarry, S., & Gerner-Smidt, P. (2019). Use of whole-genome sequencing for food safety and public health in the United States. *Foodborne Pathogens and Disease*, *16*(7), 441-450.

Bryan, A., Youngster, I., & McAdam, A. J. (2015). Shiga toxin producing *Escherichia coli. Clinics in Laboratory Medicine*, *35*(2), 247-272.

Bumbaugh, A. C. (2003). Evolution of invasiveness in *Escherichia coli and Shigella*. Michigan State University. *Michigan State University ProQuest Dissertations Publishing*, 2003. 3115946.

Butler, M. S., Hansford, K. A., Blaskovich, M. A., Halai, R., & Cooper, M. A. (2014). Glycopeptide antibiotics: back to the future. *The Journal of Antibiotics*, *67*(9), 631-644.

Butt, S., Smith-Palmer, A., Shand, A., McDonald, E., Allison, L., Maund, J., ... & Elson, R. (2021). Evidence of on-going transmission of Shiga toxin-producing *Escherichia coli* O157: H7 following a foodborne outbreak. *Epidemiology & Infection*, *149*.

Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health*, 7(10), 3657-3703.

Carattoli, A., Zankari, E., García-Fernández, A., Voldby Larsen, M., Lund, O., Villa, L., Møller Aarestrup, F., & Hasman, H. (2014). In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrobial Agents and Chemotherapy*, 58(7), 3895–3903.

Carlet, J., Jarlier, V., Harbarth, S., Voss, A., Goossens, H., Pittet, D., & Participants of the 3rd world healthcare-associated infections forum. (2012). Ready for a world without antibiotics? The pensières antibiotic resistance call to action. *Antimicrobial Resistance and Infection Control*, *1*, 1-13.

Carlino, M. J., Kralicek, S. E., Santiago, S. A., Sitaraman, L. M., Harrington, A. T., & Hecht, G. A. (2020). Quantitative analysis and virulence phenotypes of atypical enteropathogenic *Escherichia coli* (EPEC) acquired from diarrheal stool samples from a Midwest US hospital. *Gut Microbes*, *12*(1), 1824562.

Cavalcanti, A. B., Goncalves, A. R., Almeida, C. S., Bugano, D. D., & Silva, E. (2010). Teicoplanin versus vancomycin for proven or suspected infection. *Cochrane Database of Systematic Reviews*, (6).

Cesur, M. F., Abdik, E., Güven-Gülhan, Ü., Durmuş, S., & Çakır, T. (2018). Computational systems biology of metabolism in infection. *Metabolic Interaction in Infection*, 235-282.

Chen, C. H., Tu, C. Y., Chen, W. C., Kuo, L. K., Wang, Y. T., Fu, P. K., ... & Lai, C. C. (2021). Clinical efficacy of cefoperazone-sulbactam versus piperacillin-tazobactam in the treatment of hospital-acquired pneumonia and ventilator-associated pneumonia. *Infection and Drug Resistance*, 2251-2258.

Cheng, G., Hao, H., Dai, M., Liu, Z., & Yuan, Z. (2013). Antibacterial action of quinolones: From target to network. *European Journal of Medicinal Chemistry*, *66*, 555-562.

Choi, J., Marks, J., Zhang, J., Chen, D. H., Wang, J., Vázquez-Laslop, N., ... & Puglisi, J. D. (2020). Dynamics of the context-specific translation arrest by chloramphenicol and linezolid. *Nature Chemical Biology*, *16*(3), 310-317.

Choudhury, M. F. (2022). Antibiotic usages and management practices in selected layer farms of Kamarkhanda Upazila of Sirajganj District. *Bangladesh Journal of Public Administration*, *30*(4).

Clement, M., Olabisi, M., David, E., & Issa, M. (2019). Veterinary pharmaceuticals and antimicrobial resistance in developing countries. In *Veterinary Medicine and Pharmaceuticals*. IntechOpen.

Cocker, D. (2022). An investigation into the role of human, animal and environmental factors on transmission of ESBL *E. coli* and ESBL K. pneumoniae in southern Malawian communities (Doctoral dissertation, Liverpool School of Tropical Medicine).

Collins, C., & Green, J. A. (2010). A review of the pathophysiology and treatment of Shiga toxin producing *E. coli* infection. *Pract Gastroenterol*, *34*, 41-50.

Comi, G. (2017). Spoilage of meat and fish. In *The Microbiological Quality of Food* (pp. 179-210). *Woodhead Publishing*.

Conley, Z. C., Bodine, T. J., Chou, A., & Zechiedrich, L. (2018). Wicked: The untold story of ciprofloxacin. *PLoS Pathogens*, *14*(3), e1006805.

Crofts, T. S., Gasparrini, A. J., & Dantas, G. (2017). Next-generation approaches to understand and combat the antibiotic resistome. *Nature Reviews Microbiology*, *15*(7), 422-434.

Cytryn, E., Markiewicz, Z., & Popowska, M. (2017). Antibiotics and antibiotics resistance genes dissemination in soils. Antibiotics and Antibiotics Resistance Genes in Soils: *Monitoring, Toxicity, Risk Assessment and Management*, 151-190.

Danek-Majewska, A., Kwiecień, M., Winiarska-Mieczan, A., Haliniarz, M., & Bielak, A. (2021). Raw chickpea (Cicer arietinum L.) as a substitute of soybean meal in compound feed for broiler chickens: Effects on growth performance, lipid

metabolism, fatty acid profile, antioxidant status, and dietary value of muscles. *Animals*, *11*(12), 3367.

Das, A. K., Nanda, P. K., Das, A., & Biswas, S. (2019). Hazards and safety issues of meat and meat products. In *Food Safety and Human Health* (pp. 145-168). Academic Press.

Dawkins, M. S., & Layton, R. (2012). Breeding for better welfare: genetic goals for broiler chickens and their parents. *Animal Welfare*, *21*(2), 147-155.

Day, L., Cakebread, J. A., & Loveday, S. M. (2022). Food proteins from animals and plants: Differences in the nutritional and functional properties. *Trends in Food Science & Technology*, *119*, 428-442.

De Briyne, N., Atkinson, J., Borriello, S. P., & Pokludová, L. (2014). Antibiotics used most commonly to treat animals in Europe. *Veterinary Record*, *175*(13), 325-325.

De Coster, W., Weissensteiner, M. H., & Sedlazeck, F. J. (2021). Towards population-scale long-read sequencing. *Nature Reviews Genetics*, *22*(9), 572-587.

Demir Yetis, A., & Selek, Z. (2015). Determination of total and fecal coliforms of Akyatan Lagoon in terms of microbiological pollution. *Arabian Journal of Geosciences*, *8*, 1125-1132.

Demir Yetis, A., & Selek, Z. (2015). Determination of total and fecal coliforms of Akyatan Lagoon in terms of microbiological pollution. *Arabian Journal of Geosciences*, *8*, 1125-1132.

Dingman, P., Levy, J. K., Kramer, L. H., Johnson, C. M., Lappin, M. R., Greiner, E. C., ... & Morchon, R. (2010). Association of Wolbachia with heartworm disease in cats and dogs. *Veterinary parasitology*, *170*(1-2), 50-60.

Dörr, T. (2021). Understanding tolerance to cell wall–active antibiotics. *Annals of the New York Academy of Sciences*, *1496*(1), 35-58.

Dowling, P. M. (2013). Aminoglycosides and aminocyclitols. *Antimicrobial Therapy in Veterinary Medicine*, 233-255.

Doyle, M. E., Archer, J., Kaspar, C. W., & Weiss, R. (2006). Human illness caused by *E. coli* O157: H7 from food and non-food sources. *FRI Briefings*, 1-37.

Dudeja, P., Singh, A., Sahni, N., Kaur, S., & Goel, S. (2017). Effectiveness of an intervention package on knowledge, attitude, and practices of food handlers in a tertiary care hospital of north India: A before and after comparison study. *Medical Journal Armed Forces India*, *73*(1), 49-53.

Dupont, C., Armant, D. R., & Brenner, C. A. (2009, September). Epigenetics: definition, mechanisms and clinical perspective. In *Seminars in Reproductive Medicine* (Vol. 27, No. 05, pp. 351-357). © Thieme Medical Publishers.

Economou, V., & Gousia, P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and Drug Resistance*, 8, 49–61.

EFSA Panel on Biological Hazards (BIOHAZ). (2010). Scientific opinion on risk assessment of parasites in fishery products. *EFSA Journal*, *8*(4), 1543.

Eisfeldt, J., Pettersson, M., Vezzi, F., Wincent, J., Käller, M., Gruselius, J., ... & Lindstrand, A. (2019). Comprehensive structural variation genome map of individuals carrying complex chromosomal rearrangements. *PLoS Genetics*, *15*(2), e1007858.

Ekblom, R., & Wolf, J. B. (2014). A field guide to whole-genome sequencing, assembly and annotation. *Evolutionary applications*, *7*(9), 1026-1042.

Ekici, G., & Dümen, E. (2019). *Escherichia coli* and food safety. In *The universe of Escherichia coli*. *IntechOpen*.82375

Ekli, R., Adzitey, F., & Agbolosu A.A. (2020). Farmers' knowledge in antibiotic usage, antibiotic residues, and susceptibility of *Salmonella enterica* in beef samples from

the Wa Municipality, Ghana. *Bulletin of Animal Health and Production in Africa*, 68:89–101

Etebu, E., & Arikekpar, I. (2016). Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. *International Journal Applied Microbiology Biotechnology Res*, *4*(2016), 90-101.

Evangelho, K., Mastronardi, C. A., & De-La-Torre, A. (2019). Experimental models of glaucoma: A powerful translational tool for the future development of new therapies for glaucoma in humans—A review of the literature. *Medicina*, *55*(6), 280.

Faintuch, J., & Faintuch, S. (Eds.). (2019). Microbiome and metabolome in diagnosis, therapy, and other strategic applications. *Academic Press*

Falcone, M., Giannella, M., Raponi, G., Mancini, C., & Venditti, M. (2006). Teicoplanin use and emergence of *Staphylococcus* haemolyticus: is there a link? *Clinical Microbiology and Infection*, *12*(1), 96-97.

Farmer, J. J., Farmer, M. K., & Holmes, B. (2010). The Enterobacteriaceae: general characteristics. *Topley & Wilson's Microbiology and Microbial Infections*, *2*, 1317-59.

Fazulbhoy, R., Khan, A., D'souza, L. A., Dave, N., & Chandu, P. (2021). A comparative analysis of amoxicillin and cefuroxime. *International Journal Science Res Science Technology*, *8*, 96-124.

Feasey, N. A. (2013). Salmonella infections. Manson's Tropical Infectious Diseases*: Expert Consult-Online and Print*, 337.

Fegan, N., & Gobius, K. S. (2012). Pathogenic *Escherichia coli* and one health implications. One Health: The Human-Animal-Environment Interfaces in

Emerging Infectious Diseases: *Food Safety and Security, and International and National Plans for Implementation of One Health Activities, 49-62*.

Feltwell, R. (2011). Small-Scale Poultry Keeping: A Guide to Free-Range Poultry Production. *Faber & Faber.*

Fish, K. D., Rubio, N. R., Stout, A. J., Yuen, J. S., & Kaplan, D. L. (2020). Prospects and challenges for cell-cultured fat as a novel food ingredient. *Trends in Food Science & Technology*, *98*, 53-67.

Foster-Nyarko, E., Alikhan, N. F., Ikumapayi, U. N., Sarwar, G., Okoi, C., Tientcheu, P. E. M., ... & Pallen, M. J. (2021). Genomic diversity of *Escherichia coli* from healthy children in rural Gambia. *PeerJ*, *9*, e10572.

Franz, E., Delaquis, P., Morabito, S., Beutin, L., Gobius, K., Rasko, D. A., ... & Strachan, N. J. (2014). Exploiting the explosion of information associated with whole genome sequencing to tackle Shiga toxin-producing *Escherichia coli* (STEC) in global food production systems. *International Journal of Food Microbiology*, *187*, 57-72.

Fuloria, S., Mehta, J., Talukdar, M. P., Sekar, M., Gan, S. H., Subramaniyan, V., ... & Fuloria, N. K. (2022). Synbiotic Effects of Fermented Rice on Human Health and Wellness: A natural beverage that boosts immunity. *Frontiers in Microbiology*, *13*, 950913.

Gallo, M., Ferrara, L., Calogero, A., Montesano, D., & Naviglio, D. (2020). Relationships between food and diseases: What to know to ensure food safety. *Food Research International*, *137*, 109414.

Gargiulo, A. H., Duarte, S. G., Campos, G. Z., Landgraf, M., Franco, B. D., & Pinto, U. M. (2022). Food safety issues related to eating in and eating out. *Microorganisms*, *10*(11), 2118.

Geldenhuys, G., Hoffman, L. C., & Muller, M. (2014). Sensory profiling of Egyptian goose (*Alopochen aegyptiacus*) meat. *Food Research International*, *64*, 25-33.

Gewertz, D. B., & Errington, F. K. (2010). Cheap meat: flap food nations in the Pacific Islands. *Univ of California Press.*

Gibson, P. S., & Veening, J. W. (2023). Gaps in the wall: understanding cell wall biology to tackle amoxicillin resistance in *Streptococcus* pneumoniae. *Current Opinion in Microbiology*, *72*, 102261.

Gilchrist, C. A., Turner, S. D., Riley, M. F., Petri Jr, W. A., & Hewlett, E. L. (2015). Whole-genome sequencing in outbreak analysis. *Clinical Microbiology Reviews*, *28*(3), 541-563.

Giwa, A. S., Memon, A. G., Ahmed Shaikh, A., Korai, R., Maitlo, G. U., Maitlo, I., ... & Ahmad, J. (2021). Microbiological survey of ready-to-eat foods and associated preparation surfaces in cafeterias of public sector universities. *Environmental Pollutants and Bioavailability*, *33*(1), 11-18.

González-Vázquez, P., Larrañeta, E., McCrudden, M. T., Jarrahian, C., Rein-Weston, A., Quintanar-Solares, M., ... & Donnelly, R. F. (2017). Transdermal delivery of gentamicin using dissolving microneedle arrays for potential treatment of neonatal sepsis. *Journal of Controlled Release*, *265*, 30-40.

Gopal, G. J., & Kumar, A. (2013). Strategies for the production of recombinant protein in *Escherichia coli*. *The Protein Journal*, *32*, 419-425.

Gourama, H. (2020). Foodborne pathogens. *Food safety Engineering*, 25-49.

Grégoire, M., Dailly, E., Le Turnier, P., Garot, D., Guimard, T., Bernard, L., ... & Asseray, N. (2019). High-dose ceftriaxone for bacterial meningitis and optimization of administration scheme based on nomoGram. *Antimicrobial Agents and Chemotherapy*, *63*(9), 10-1128.

Gul, N., Mujahid, T. Y., & Ahmad, S. (2004). Isolation, identification and antibiotic resistance profile of indigenous bacterial isolates from urinary tract infection patients. *Pakistan Journal of Biological Sciences*, *7*(12), 2051-2054.

Hailu, A. (2023). Microbial contaminations of meat and associated risk factors in Harar City, Harari Regional State, Ethiopia (Doctoral Dissertation, Haramaya University).

Hamdan, M. N., Post, M., Ramli, M. A., Kamarudin, M. K., Ariffin, M. F. M., & Huri, N. M. F. Z. (2021). Cultured meat: Islamic and other religious perspectives. *UMRAN-International Journal of Islamic and Civilizational Studies*, *8*(2), 11-19.

Haque, M. H., Sarker, S., Islam, M. S., Islam, M. A., Karim, M. R., Kayesh, M. E. H., ... & Anwer, M. S. (2020). Sustainable antibiotic-free broiler meat production: Current trends, challenges, and possibilities in a developing country perspective. *Biology*, *9*(11), 411.

Harris, M. (2012). The abominable pig. In *Food and Culture* (pp. 73-85). Routledge.

Harvala, H., Broberg, E., Benschop, K., Berginc, N., Ladhani, S., Susi, P., ... & Fischer, T. K. (2018). Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. *Journal of Clinical Virology*, *101*, 11-17.

Hasman, H., Saputra, D., Sicheritz-Ponten, T., Lund, O., Svendsen, C. A., Frimodt-Møller, N., & Aarestrup, F. M. (2014). Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *Journal of Clinical Microbiology*, 52(1), 139–146.

Hassanain, N. A., Hassanain, M. A., Ahmed, W. M., Shaapan, R. M., Barakat, A. M., & El-Fadaly, H. A. (2013). Public health importance of foodborne pathogens. *World Journal of Medical Sciences*, *9*(4), 208-222.

Hassanin, F. S., Reham, A. A., Shawky, N. A., & Gomaa, W. M. (2014). Incidence of *Escherichia coli* and *Salmonella* in Ready to eat Foods. *Benha Veterinary Medical Journal*, *27*(1), 84-91.

Hawker, J., Begg, N., Reintjes, R., Ekdahl, K., Edeghere, O., & Van Steenbergen, J. E. (2019). Communicable disease control and health protection handbook. *John Wiley & Sons.*

Hayeems, R. Z., Dimmock, D., Bick, D., Belmont, J. W., Green, R. C., Lanpher, B., ... & Medical Genome Initiative. (2020). Clinical utility of genomic sequencing: a measurement toolkit. *NPJ Genomic Medicine*, *5*(1), 56.

Henderson, P. J., Maher, C., Elbourne, L. D., Eijkelkamp, B. A., Paulsen, I. T., & Hassan, K. A. (2021). Physiological functions of bacterial “multidrug” efflux pumps. *Chemical Reviews*, *121*(9), 5417-5478.

Hendriksen, R. S., Bortolaia, V., Tate, H., Tyson, G. H., Aarestrup, F. M., & McDermott, P. F. (2019). Using genomics to track global antimicrobial resistance. *Frontiers in Public Health*, *7*, 242.

Hirai, Y. (2011). Treatment Options for Gram-Positive and Gram-Negative Bacteria: Focus on Ceftriaxone. *Clinical Medicine Reviews in Therapeutics*, *3*.

Hoan, N. X., Huy, P. X., Sy, B. T., Meyer, C. G., Son, T. V., Binh, M. T., ... & Velavan, T. P. (2019, September). High hepatitis E virus (HEV) positivity among domestic pigs and risk of HEV infection of individuals occupationally exposed to pigs and pork meat in Hanoi, Vietnam. In *Open Forum Infectious Diseases* (Vol. 6, No. 9, p. ofz306). US: Oxford University Press.

Höllmann, T. O. (2013). *The Land of the Five Flavors: A cultural history of Chinese cuisine*. *Columbia University Press.*

Holmes, N. E., & Grayson, M. L. (2017). Sulfonamides. In *Kucers' The Use of Antibiotics* (pp. 1571-1624). CRC Press.

Hooper, D. C., & Jacoby, G. A. (2016). Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. *Cold Spring Harbor Perspectives in Medicine*, *6*(9).

Huang, D. B., Okhuysen, P. C., Jiang, Z. D., & DuPont, H. L. (2004). Enteroaggregative *Escherichia coli*: an emerging enteric pathogen. *Official journal of the American College of Gastroenterology| ACG*, *99*(2), 383-389.

Huang, L., & Hwang, C. A. (2012). In-package pasteurization of ready-to-eat meat and poultry products. In *Advances in Meat, Poultry and Seafood Packaging* (pp. 437-450). Woodhead Publishing.

Hudson, J. A., Frewer, L. J., Jones, G., Brereton, P. A., Whittingham, M. J., & Stewart, G. (2017). The agri-food chain and antimicrobial resistance: A review. *Trends in Food Science & Technology*, *69*, 131-147.

Hunter, P. A., Dawson, S., French, G. L., Goossens, H., Hawkey, P. M., Kuijper, E. J., ... & Piddock, L. J. (2010). Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. J*ournal of Antimicrobial Chemotherapy*, Volume 65, Issue suppl_1, February 2010, Pages i3–i17,

Hussain, M. A., Mohamed, A. O., Abdelkarim, O. A., Yousef, B. A., Babikir, A. A., Mirghani, M. M., ... & Elhag, R. (2023). Prevalence and Predictors of Antibiotic Self-Medication in Sudan: A Descriptive Cross-Sectional Study. *Antibiotics*, *12*(3), 612.

Huttner, A., Bielicki, J., Clements, M. N., Frimodt-Møller, N., Muller, A. E., Paccaud, J. P., & Mouton, J. W. (2020). Oral amoxicillin and amoxicillin–clavulanic acid:

properties, indications and usage. *Clinical Microbiology and Infection*, *26*(7), 871-879.

Ibrahim, M., Ahmad, F., Yaqub, B., Ramzan, A., Imran, A., Afzaal, M., ... & Ahmed, S. (2020). Current trends of antimicrobials used in food animals and aquaculture. In *Antibiotics and Antimicrobial Resistance Genes in The Environment* (pp. 39-69). Elsevier.

Ijabdeniyi, O. A., Naraindath, K., & Ajayeoba, T. A. (2019). Prevalence of selected foodborne pathogens in the processed meat products from Durban and their growth after treatment with vinegar and lemon juice. *International Food Research Journal*, *26*(6), 1725-1732.

Islam, M. S., Ferdous, M., Islam, U. S., Mosaddek, A. S. M., Potenza, M. N., & Pardhan, S. (2021). Treatment, persistent symptoms, and depression in people infected with COVID-19 in Bangladesh. *International Journal of Environmental Research and Public Health*, *18*(4), 1453.

Islam, M.S., Rahman, A.M.M.T., Hassan, J., and Rahman, M.T. (2023). Extended-spectrum beta-lactamase in *Escherichia coli* isolated from humans, animals, and environments in Bangladesh: A One Health perspective systematic review and meta-analysis. *One Health*, 2023, 16:100526.

Jaja, I. F., Oguttu, J., Jaja, C. J. I., & Green, E. (2020). Prevalence and distribution of antimicrobial resistance determinants of *Escherichia coli* isolates obtained from meat in South Africa. *Plos One*, *15*(5), e0216914.

James, E. H., Wooten, L. P., & Dushek, K. (2011). Crisis management: Informing a new leadership research agenda. *The Academy of Management Annals*, *5*(1), 455-493.

James, S. J., & James, C. (2023). Chilling and freezing. In *Food Safety Management* (pp. 453-474). Academic Press.

Jang, J., Hur, H. G., Sadowsky, M. J., Byappanahalli, M. N., Yan, T., & Ishii, S. (2017). Environmental *Escherichia coli:* ecology and public health implications—a review. *Journal of Applied Microbiology*, *123*(3), 570-581.

Jarulis, J., Nurmeiliasari, N., Haryanto, H., & Vilanda, I. (2022). DNA barcode of red junglefowl Gallus gallus L, 1958 (Aves: Phasianidae) of Sumatra based on mitochondrial COI DNA Gene. *Biosaintifika: Journal of Biology & Biology Education*, *14*(2).

Jayathilakan, K., Sultana, K., Radhakrishna, K., & Bawa, A. S. (2012). Utilization of by products and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology*, *49*, 278-293.

Jiao, Y. N., Chen, H., Gao, R. X., Zhu, Y. G., & Rensing, C. (2017). Organic compounds stimulate horizontal transfer of antibiotic resistance genes in mixed wastewater treatment systems. *Chemosphere*, *184*, 53-61.

Joensen, K. G., Scheutz, F., Lund, O., Hasman, H., Kaas, R. S., Nielsen, E. M., & Aarestrup, F. M. (2014). Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *Journal of Clinical Microbiology, 52(5), 1501–1510.*

Joseph, A., Cointe, A., Mariani Kurkdjian, P., Rafat, C., & Hertig, A. (2020). Shiga toxin-associated hemolytic uremic syndrome: A narrative review. *Toxins*, *12*(2), 67.

Joshi, M., Espeland, M., Huemer, P., deWaard, J., & Mutanen, M. (2023). Species Delimitation Under Allopatry: Genomic Divergences Within and Across Continents in Lepidoptera. *bioRxiv*, 2023-03.

Kahunde, M. A., Odoch, T., Okello Owiny, D., Kankya, C., Kaelin, M., & Hartnack, S. S. (2023). Knowledge, attitudes and practices towards antibiotic use and

resistance in Kyegegwa district, Uganda–a questionnaire study. *medRxiv*, 2023-04.

Kamaruzzaman, N. F., Tan, L. P., Hamdan, R. H., Choong, S. S., Wong, W. K., Gibson, A. J., ... & Pina, M. D. F. (2019). Antimicrobial polymers: the potential replacement of existing antibiotics? *International Journal of Molecular Sciences*, *20*(11), 2747.

Kamboj, S., Gupta, N., Bandral, J. D., Gandotra, G., & Anjum, N. (2020). Food safety and hygiene: A review. *International Journal of Chemical Studies*, *8*(2), 358-368.

Karam, G., Chastre, J., Wilcox, M. H., & Vincent, J. L. (2016). Antibiotic strategies in the era of multidrug resistance. *Critical Care*, *20*(1), 1-9.

Kathleen, M.M., Samuel, L., Felecia, C., Reagan, E.L., Kasing, A., Lesley, M., & Toh, S.C. (2016). Antibiotic resistance of diverse bacteria from aquaculture in Borneo. *International Journal Microbiology*, 2016, 2164761

Kaur, A., Singh, G., Kukreja, V., Sharma, S., Singh, S., & Yoon, B. (2022). Adaptation of IoT with blockchain in Food Supply Chain Management: An analysis-based review in development, benefits and potential applications. *Sensors*, *22*(21), 8174.

Kegode, R. B., Doetkott, D. K., Khaitsa, M. L., & Wesley, I. V. (2008). Occurrence of *Campylobacter species*, *Salmonella species* and generic *Escherichia coli* in meat products from retail outlets in the Fargo metropolitan area. *Journal of Food Safety*, *28*(1), 111-125.

Kemp, S. A., Pinchbeck, G. L., Fèvre, E. M., & Williams, N. J. (2021). A cross-sectional survey of the knowledge, attitudes, and practices of antimicrobial users and providers in an area of high-density livestock-human population in Western Kenya. *Frontiers in Veterinary Science*, *8*, 727365.

Khalil, I., Walker, R., Porter, C. K., Muhib, F., Chilengi, R., Cravioto, A., ... & Bourgeois, A. L. (2021). Enterotoxigenic *Escherichia coli* (ETEC) vaccines: Priority activities to enable product development, licensure, and global access. *Vaccine, 39*(31), 4266-4277.

Khamesipour, F., Hashemian, S. M., Velayati, A. A., & Tabarsi, P. (2015). A review of teicoplanin used in the prevention and treatment of serious infections caused by Gram-positive bacteria and compared its effects with some other antibiotics. *Biomedical and Pharmacology Journal*, *8*(1), 513-521.

Khan, T., Sankhe, K., Suvarna, V., Sherje, A., Patel, K., & Dravyakar, B. (2018). DNA gyrase inhibitors: Progress and synthesis of potent compounds as antibacterial agents. *Biomedicine & Pharmacotherapy*, *103*, 923-938.

Kibret, M., & Abera, B. (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *African Health Sciences*, *11*, 40-45.

Kim, J. H., & Yim, D. G. (2016). Assessment of the microbial level for livestock products in retail meat shops implementing HACCP system. *Korean Journal for Food Science of Animal Resources*, 36(5), 594.

Klemm, E., & Dougan, G. (2016). Advances in understanding bacterial pathogenesis gained from whole-genome sequencing and phylogenetics. *Cell Host & Microbe*, *19*(5), 599-610.

Kwok, A. J., Mentzer, A., & Knight, J. C. (2021). Host genetics and infectious disease: new tools, insights and translational opportunities. *Nature Reviews Genetics*, *22*(3), 137-153.

Kwong, J. C., McCallum, N., Sintchenko, V., & Howden, B. P. (2015). Whole genome sequencing in clinical and public health microbiology. *Pathology*, *47*(3), 199-210.

Laith, A. E., Sendyani, S., & Alnemri, M. (2023). Applications of the One Health concept: Current status in the Middle East. *Journal of Biosafety and Biosecurity*, *5*(1), 21-31.

Lalchhandama, K. (2021). History of penicillin. *WikiJournal of Medicine*, *8*(1), 1-16.

Landfried, L.K., Barnidge, E., Pithua, P., D. Lewis, R., A. Jacoby, J., King, C.C., & Baskin, C.R. (2018). Antibiotic use on goat farms: An investigation of knowledge, attitudes, and behaviors of Missouri goat farmers. *Animals*, *8*(11), 198.

Larsen, M. V., Cosentino, S., Rasmussen, S., Friis, C., Hasman, H., Marvig, R. L., Jelsbak, L., Sicheritz-Pontén, T., Ussery, D. W., Aarestrup, F. M., & Lund, O. (2012). Multilocus sequence typing of total-genome-sequenced bacteria. *Journal of Clinical Microbiology,* 50(4), 1355–1361.

Lawler, A. (2016). Why did the chicken cross the world? the epic saga of the bird that powers civilization. *Simon and Schuster.*

Lee, C. R., Cho, I. H., Jeong, B. C., & Lee, S. H. (2013). Strategies to minimize antibiotic resistance. *International Journal of Environmental Research and Public Health*, *10*(9), 4274-4305.

Lekshmi, M., Ammini, P., Kumar, S., & Varela, M. F. (2017). The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. *Microorganisms*, *5*(1), 11.

Levine, P., Rose, B., Green, S., Ransom, G., & Hill, W. (2001). Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. *Journal of Food Protection*, *64*(8), 1188-1193.

Levy, S. B. (2013). The antibiotic paradox*:* how miracle drugs are destroying the miracle. *Springer.*

Lewis, K. (2013). Platforms for antibiotic discovery. *Nature Reviews and Drug Discovery*, *12*(5), 371-387.

Li, H. K., Rombach, I., Zambellas, R., Walker, A. S., McNally, M. A., Atkins, B. L., ... & Scarborough, M. (2019). Oral versus intravenous antibiotics for bone and joint infection. *New England Journal of Medicine*, *380*(5), 425-436.

Lim, J. Y., Yoon, J. W., & Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157: H7 and its plasmid O157. *Journal of Microbiology and Biotechnology*, *20*(1), 5.

Lin, J., Zhang, K., Jiang, L., Hou, J., Yu, X., Feng, M., & Ye, C. (2022). Removal of chloramphenicol antibiotics in natural and engineered water systems: Review of reaction mechanisms and product toxicity. *Science of The Total Environment*, 158059.

Logsdon, G. A., Vollger, M. R., & Eichler, E. E. (2020). Long-read human genome sequencing and its applications. *Nature Reviews Genetics*, *21*(10), 597-614.

Lorenzo, J. M., Munekata, P. E., Dominguez, R., Pateiro, M., Saraiva, J. A., & Franco, D. (2018). Main groups of microorganisms of relevance for food safety and stability: General aspects and overall description. In *Innovative Technologies for Food Preservation* (pp. 53-107). Academic Press.

Ludden, C., Raven, K.E., Jamrozy, D., Gouliouris, T., Blane, B., Coll, F., de Goffau, M., Naydenova, P., Horner, C., Hernandez-Garcia, J., Wood, P., Hadjirin, N., Radakovic, M., Brown, N.M., Holmes, M., Parkhill, J., & Peacock, S.J. (2019). One health genomic surveillance of *Escherichia coli* demonstrates distinct lineages and mobile genetic elements in isolates from humans versus livestock.

mBio, 10, e02693-18.

Lum, E. P., Page, K., Nissen, L., Doust, J., & Graves, N. (2017). Australian consumer perspectives, attitudes and behaviours on antibiotic use and antibiotic resistance: a qualitative study with implications for public health policy and practice. *BMC Public Health*, *17*, 1-12.

Luo, X., Zaitoon, A., & Lim, L. T. (2022). A review on colorimetric indicators for monitoring product freshness in intelligent food packaging: Indicator dyes, preparation methods, and applications. *Comprehensive Reviews in Food Science and Food Safety*, *21*(3), 2489-2519.

Lupia, T., Corcione, S., Pinna, S. M., & De Rosa, F. G. (2020). New cephalosporins for the treatment of pneumonia in internal medicine wards. *Journal of Thoracic Disease*, *12*(7), 3747.

Maiden MC 2006, Multilocus Sequence Typing of Bacteria. *Annu Rev Microbiol* 60:561-588.

Malik, S., Choudhary, A., Kumar, S., & Avasthi, G. (2010). Quinolones: a therapeutic review. *Journal Pharm Res*, *3*, 1519-1523.

Mancuso, G., Midiri, A., Gerace, E., & Biondo, C. (2021). Bacterial antibiotic resistance: The most critical pathogens. *Pathogens*, *10*(10), 1310.

Manning, S. D. (2010). *Escherichia coli* infections. *Infobase Publishing.*

Manring, M. M., Hawk, A., Calhoun, J. H., & Andersen, R. C. (2009). Treatment of war wounds: a historical review. *Clinical Orthopaedics and Related Research®*, *467*, 2168-2191.

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules*, *23*(4), 795.

Marcel, A., Enciso, S. R. A., Boubaker, B. B., Jonathan, B., Emily, C., Cicerone, G., ... & Wen, Y. (2018). OECD-FAO Agricultural Outlook 2018-2027. In *OECD-FAO Agricultural Outlook 2018-2027* (pp. 1-112).

Mare, A. D., Ciurea, C. N., Man, A., Tudor, B., Moldovan, V., Decean, L., & Toma, F. (2021). Enteropathogenic *Escherichia coli*—A summary of the literature. *Gastroenterology Insights*, *12*(1), 28-40.

Marino, L., & Colvin, C. M. (2015). Thinking pigs: a comparative review of cognition, emotion, and personality in Sus domesticus. *International Journal of Comparative Psychology*, *28*(1).

Massella, E., Reid, C. J., Cummins, M. L., Anantanawat, K.; Zingali, T., Serraino, A., Piva, S., Giacometti, F., & Djordjevic, S. P. (2020). Snapshot study of whole genome sequences of *Escherichia coli* from healthy companion animals, livestock, wildlife, humans and food in Italy. *Antibiotics*, 9, 782.

Mayer, J. J., & Brisbin, I. L. (2008). Wild pigs in the United States: their history, comparative morphology, and current status. *University of Georgia Press.*

McCartney, A. M., Koot, E., Prebble, J. M., Jibran, R., Mitchell, C., Podolyan, A., ... & Chagné, D. (2024). A population genomics analysis of the Aotearoa New Zealand endemic rewarewa tree (Knightia excelsa). *npj Biodiversity*, *3*(1), 7.

Meng, J., LeJeune, J. T., Zhao, T., & Doyle, M. P. (2012). Enterohemorrhagic *Escherichia coli*. Food microbiology*: Fundamentals and Frontiers*, 287-309.

Mercer, A. (2021). Protection against severe infectious disease in the past. *Pathogens and Global Health*, *115*(3), 151-167.

Micoli, F., Bagnoli, F., Rappuoli, R., & Serruto, D. (2021). The role of vaccines in combatting antimicrobial resistance. *Nature Reviews Microbiology*, *19*(5), 287-302.

Millanao, A. R., Mora, A. Y., Villagra, N. A., Bucarey, S. A., & Hidalgo, A. A. (2021). Biological effects of quinolones: A family of broad-spectrum antimicrobial agents. *Molecules*, *26*(23), 7153.

Miller, J. L., Cimen, H., Koc, H., & Koc, E. C. (2009). Phosphorylated proteins of the mammalian mitochondrial ribosome: implications in protein synthesis. *Journal of Proteome Research*, *8*(10), 4789-4798.

Minami, A., Chaicumpa, W., Chongsa-Nguan, M., Samosornsuk, S., Monden, S., Takeshi, K., ... & Kawamoto, K. (2010). Prevalence of foodborne pathogens in open markets and supermarkets in Thailand. *Food Control*, *21*(3), 221-226.

Mingle, C. L., Darko, G., Asare-Donkor, N. K., Borquaye, L. S., & Woode, E. (2021). Patterns in protein consumption in Ghanaian cities. *Scientific African*, *11*, e00684.

Moges, F. (2010). Indigenous chicken production and marketing systems in Ethiopia: *Characteristics and opportunities for market-oriented development* (Vol. 24). ILRI (aka ILCA and ILRAD).

Mohammed, A. M. (2004). Contamination of ready to eat vended food of meat origin with aerobic bacteria in Khartoum State. *University of Khartoum*.

Moreki, J. C., Dikeme, R., & Poroga, B. (2010). The role of village poultry in food security and HIV/AIDS mitigation in Chobe District of Botswana. *Livestock Research for Rural Development*, *22*(3), 1-7.

Morsy, T. A., Abou-Elmagd, A. I., Abdallah, A. M., & El-Shahat, S. A. (2023). Assessment on foodborne diseases (gastroenteritis) with increased climatic conditions: A review artcile. *Journal Of the Egyptian Society of Parasitology*, *53*(1), 165-184.

Moxley, R. A. (2022). Enterobacteriaceae: Escherichia. *Veterinary Microbiology*, 56-74.

Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage applications for food production and processing. *Viruses*, *10*(4), 205.

Musa, E. O. M. (2018). Adoption of draught animals by farmers in (West and South Kordofan State (Doctoral dissertation, Sudan University of Science and Technology).

Muteeb, G., Rehman, M. T., Shahwan, M., & Aatif, M. (2023). Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals*, *16*(11), 1615.

Mutuku, C., Gazdag, Z., & Melegh, S. (2022). Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches. *World Journal of Microbiology and Biotechnology*, *38*(9), 152.

Naili, I. (2018). Prevention of pathogenic *Escherichia coli* diseases: immune response to a broad-spectrum *E. coli* antigen, and role of nitric oxide in enterohemorrhagic *E. coli* virulence. *Available at:* https://hal.science/tel-03903127 accessed on 15/09/2024.

Nara, J. M., Pimenta, D. C., Abe, C. M., Abreu, P. A., Moraes, C. T., Freitas, N. C., ... & Piazza, R. M. (2012). Low-molecular mass comparative proteome of four atypical enteropathogenic *Escherichia coli* isolates showing different adherence patterns. *Comparative Immunology, Microbiology and Infectious Diseases*, *35*(6), 539-549.

Nazir, A., Ochani, S., Nazir, A., Fatima, B., Ochani, K., Al Hasibuzzaman, M., & Ullah, K. (2023). Rising trends of foodborne illnesses in the US. *Annals of Medicine and Surgery*, *85*(5), 2280.

Newbold, D. (1926). A historical note on the guinea-fowl. *Sudan Notes and Records*, *9*(1), 125-129.

Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., ... & Kruse, H. (2010). Food-borne diseases—the challenges of 20 years ago still persist while new ones continue to emerge. *International Fournal of Food Microbiology*, *139*, S3-S15.

Newell, D. G., Mughini-Gras, L., Kalupahana, R. S., & Wagenaar, J. A. (2017). *Campylobacter* epidemiology—Sources and routes of transmission for human infection. In *Campylobacter* (pp. 85-110). Academic Press.

Nieder, R., Benbi, D. K., Reichl, F. X., Nieder, R., Benbi, D. K., & Reichl, F. X. (2018). Soil as a transmitter of human pathogens. *Soil Components and Human Health*, 723-827.

Nkegbe, E. K., Aikins-Wilson, S., Assuming-Bediako, N., & Munkaila, L. (2013). Consumers Perception on Meat Handling in Ghana. *Asian Journal of Agriculture and Food Sciences*, *1*(4).

Nolte, T., Jansen, S., Weigend, S., Moerlein, D., Halle, I., Link, W., ... & Sharifi, A. R. (2020). Growth performance of local chicken breeds, a high-performance genotype and their crosses fed with regional faba beans to replace soy. *Animals*, *10*(4), 702.

Nunney L, Elfekih S, Stouthamer R. The importance of multilocus sequence typing: cautionary tales from the bacterium Xylella fastidiosa. *Phytopathology.* 2012 May;102(5):456-60. doi: 10.1094/PHYTO-10-11-0298. PMID: 22236051

Ochoa, T. J., & Contreras, C. A. (2011). Enteropathogenic *E. coli* (EPEC) infection in children. *Current Opinion in Infectious Diseases*, *24*(5), 478.

Odeyemi, O. A. (2016). Public health implications of microbial food safety and foodborne diseases in developing countries. *Food & Nutrition Research*, *60*(1), 29819.

Odonkor, S. T., & Ampofo, J. K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research*, *4*(1), e2.

Okoye, E. L., Kemakolam, C., Ugwuoji, E. T., & Ogbonna, I. (2022). Multidrug resistance tracing by plasmid profile analysis and the curing of bacteria from different clinical specimens. *Advanced Gut & Microbiome Research*, *2022*.

Okyere, K., Kwame, K. A. J., Yaw, A. S., Akwasi, A. A., & Clement, K. G. (2018). Haematological, biochemical and hormonal profile of guinea fowl (numida meleagris) layers fed different crude protein diet in the seasons in Ghana. *Journal of Animal Research*, *8*(6), 945-954.

Oluwasile, B. B., Agbaje, M., Ojo, O. E., & Dipeolu, M. A. (2014). Antibiotic usage pattern in selected poultry farms in Ogun state. *Sokoto Journal of Veterinary Sciences*, *12*(1), 45-50.

Omer, M. K., Álvarez-Ordoñez, A., Prieto, M., Skjerve, E., Asehun, T., & Alvseike, O. A. (2018). A systematic review of bacterial foodborne outbreaks related to red meat and meat products. *Foodborne Pathogens and Disease*, *15*(10), 598-611.

Oniciuc, E. A., Likotrafiti, E., Alvarez-Molina, A., Prieto, M., Santos, J. A., & Alvarez-Ordóñez, A. (2018). The present and future of whole genome sequencing (WGS) and whole metagenome sequencing (WMS) for surveillance of antimicrobial resistant microorganisms and antimicrobial resistance genes across the food chain. *Genes*, *9*(5), 268.

Onyeka, L. O., Adesiyun, A. A., Keddy, K. H., Madoroba, E., Manqele, A., & Thompson, P. N. (2020). Shiga toxin–producing *Escherichia coli* contamination of raw beef and beef-based ready-to-eat products at retail outlets in Pretoria, South Africa. *Journal of Food Protection*, *83*(3), 476-484.

Osaili, T. M., Al-Nabulsi, A. A., Shaker, R. R., Jaradat, Z. W., Taha, M., Mohammed, A. K., ... & Holley, R. (2014). Prevalence of *Salmonella serovars*, *Listeria monocytogenes*, and *Escherichia coli* O157: H7 in Mediterranean ready-to-eat meat products in Jordan. *Journal of Food Protection*, *77*(1), 106-111.

Ozturk, Y., Celik, S., Sahin, E., Acik, M. N., & Cetinkaya, B. (2019). Assessment of farmers' knowledge, attitudes and practices on antibiotics and antimicrobial resistance. *Animals*, *9*(9), 653.

Page, M. G. (2011). Beta-lactam antibiotics. In *Antibiotic Discovery and Development* (pp. 79-117). Boston, MA: Springer US.

Päivärinta, M., Latvio, S., Fredriksson-Ahomaa, M., & Heikinheimo, A. (2020). Whole genome sequence analysis of antimicrobial resistance genes, multilocus sequence types and plasmid sequences in ESBL/*AmpC Escherichia coli* isolated from broiler caecum and meat. *International Journal of Food Microbiology*, 315, 108361,

Palma, E., Tilocca, B., & Roncada, P. (2020). Antimicrobial resistance in veterinary medicine: An overview. *International Journal of Molecular Sciences*, *21*(6), 1914.

Pana, Z. D., & Zaoutis, T. (2018). Treatment of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBLs) infections: what have we learned until now? *F1000Research*, *7*.

Pasqua, M., Michelacci, V., Di Martino, M. L., Tozzoli, R., Grossi, M., Colonna, B., ... & Prosseda, G. (2017). The intriguing evolutionary journey of enteroinvasive *E. coli* (EIEC) toward pathogenicity. *Frontiers in Microbiology*, *8*, 2390.

Paul, R., Das, N. K., Dutta, R., Bandyopadhyay, R., & Banerjee, A. K. (2011). Bacterial contamination of the hands of doctors: a study in the medicine and dermatology wards. *Indian Journal of Dermatology, Venereology and Leprology*, *77*, 307.

Paulshus, E., Kühn, I., Möllby, R., Colque, P., O'Sullivan, K., Midtvedt, T., ... & Sørum, H. (2019). Diversity and antibiotic resistance among *Escherichia coli* populations in hospital and community wastewater compared to wastewater at the receiving urban treatment plant. *Water Research*, *161*, 232-241.

Peek, S. F., Mcguirk, S. M., Sweeney, R. W., & Cummings, K. J. (2018). Infectious diseases of the gastrointestinal tract. *Rebhun's Diseases of Dairy Cattle*, 249.

Peeters, O., Ferry, T., Ader, F., Boibieux, A., Braun, E., Bouaziz, A., ... & Valour, F. (2016). Teicoplanin-based antimicrobial therapy in *Staphylococcus aureus* bone and joint infection: tolerance, efficacy and experience with subcutaneous administration. *BMC Infectious Diseases*, *16*, 1-9.

Phares, C. A., Danquah, A., Atiah, K., Agyei, F. K., & Michael, O. T. (2020). Antibiotics utilization and farmers' knowledge of its effects on soil ecosystem in the coastal drylands of Ghana. *PloS one*, *15*(2), e0228777.

Phu, D. H., Giao, V. T. Q., Truong, D. B., Cuong, N. V., Kiet, B. T., Hien, V. B., ... & Carrique-Mas, J. (2019). Veterinary drug shops as main sources of supply and advice on antimicrobials for animal use in the Mekong Delta of Vietnam. *Antibiotics*, *8*(4), 195.

Pinese, M., Lacaze, P., Rath, E. M., Stone, A., Brion, M. J., Ameur, A., ... & Thomas, D. M. (2020). The Medical Genome Reference Bank contains whole genome and phenotype data of 2570 healthy elderly. *Nature Communications*, *11*(1), 435.

Pokharel, P., Dhakal, S., & Dozois, C. M. (2023). The diversity of *Escherichia coli* pathotypes and vaccination strategies against this versatile bacterial pathogen. *Microorganisms*, *11*(2), 344.

Pozzi, S. P., & Ben-David, Z. (2002). Use of amoxycillin+ clavulanic acid combination in veterinary medicine and possible antibiotic resistance of human pathogens. *Israel Journal of Veterinary Medicine*, *57*(3), 95-100

Prescott, J. F. (2013). Other beta-lactam antibiotics: beta-lactamase inhibitors, carbapenems, and monobactams. *Antimicrobial Therapy in Veterinary Medicine*, 175-187.

Price, C. S., & Morris Jr, J. A. (2013). Marine cage culture and the environment: Twenty-first century science informing a sustainable industry. *Informationen für die Fischwirtschaft Volume 11*

Prorok-Hamon, M., Friswell, M. K., Alswied, A., Roberts, C. L., Song, F., Flanagan, P. K., ... & Campbell, B. J. (2014). Colonic mucosa-associated diffusely adherent afaC+ *Escherichia coli* expressing lpfA and pks are increased in inflammatory bowel disease and colon cancer. *Gut*, *63*(5), 761-770.

Purushothaman, S., Meola, M., & Egli, A. (2022). Combination of whole genome sequencing and metagenomics for microbiological diagnostics. *International Journal of Molecular Sciences*, *23*(17), 9834.

Puvača, N., & de Llanos Frutos, R. (2021). Antimicrobial resistance in *Escherichia coli* strains isolated from humans and pet animals. *Antibiotics*, *10*(1), 69.

Rafailidis, P. I., Polyzos, K. A., Sgouros, K., & Falagas, M. E. (2011). Prulifloxacin: a review focusing on its use beyond respiratory and urinary tract infections. *International Journal of Antimicrobial Agents*, *37*(4), 283-290.

Rahman, M. A., Rahman, A. K. M. A., Islam, M. A., & Alam, M. M. (2017). Antimicrobial resistance of *Escherichia coli* isolated from milk, beef and chicken meat in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, *15*(2).

Rahman, M. M., Husna, A., Elshabrawy, H. A., Alam, J., Runa, N. Y., Badruzzaman, A. T. M., ... & Ashour, H. M. (2020). Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. *Scientific Reports*, *10*(1), 21999.

Rahman, M. M., Sadekuzzaman, M., Rahman, A., Siddique, M. P., Uddin, M. A., Haque, E., ... & Islam, A. (2023). Complete genome sequence analysis of the multidrug resistant Aeromonas veronii isolated for the first time from stinging catfish (Shing fish) in Bangladesh. *Journal of Advanced Veterinary and Animal Research*, *10*(3), 570-578.

Rahman, M. R. T., Fliss, I., & Biron, E. (2022). Insights in the development and uses of alternatives to antibiotic growth promoters in poultry and swine production. *Antibiotics*, *11*(6), 766.

Ramaiah, S. K., & Nabity, M. B. (2011). Blood and Bone marrow toxicity. *Veterinary Toxicology: Basic Clinical Principles*, *277*.

Ramatla, T., Mafokwane, T., Lekota, K., Monyama, M., Khasapane, G., Serage, N., Nkhebenyane, J., Bezuidenhout, C., & Thekisoe, O. (2023). “One Health” perspective on prevalence of co-existing extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* pneumoniae: a

comprehensive systematic review and meta-analysis. *Annals of Clinical Microbiology and Antimicrobials*, 22, 88.

Realini, C. E., Guàrdia, M. D., Garriga, M., Pérez-Juan, M., & Arnau, J. (2011). High pressure and freezing temperature effect on quality and microbial inactivation of cured pork carpaccio. *Meat Science*, *88*(3), 542-547.

Reid, C. J., Blau, K., Jechalke, S., Smalla, K., & Djordjevic, S. P. (2020). Whole genome sequencing of *Escherichia coli* from store-bought produce. *Frontiers in microbiology*, *10*, 3050.

Renata, U. C., Agnieszka, M., Dagmara, S. P., Kinga, W., Marta, D., Anna, N., & Jacek, O. (2022). Antibiotic Resistance in Bacteria—A Review.

Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol.* 2018 Jun 26;4(3):482-501

Rich, K., Schaefer, K., Thapa, B., Hagerman, A., Shear, H. (2022). An overview of meat processing in Africa. pp. 33-43. https://doi.org/10.54067/9781737916444

Ronald, C., Matofari, J. W., & Nduko, J. M. (2023). Antimicrobial resistance of *E. coli* strains in ready-to-eat red meat products in Nakuru County, Kenya. *The Microbe*, *1*, 100022.

Ronholm, J., Nasheri, N., Petronella, N., & Pagotto, F. (2016). Navigating microbiological food safety in the era of whole-genome sequencing. *Clinical Microbiology Reviews*, *29*(4), 837-857.

Rossen, J. W., Friedrich, A. W., & Moran-Gilad, J. (2018). Practical issues in implementing whole-genome-sequencing in routine diagnostic microbiology. *Clinical Microbiology and Infection*, *24*(4), 355-360.

Roth, N., Käsbohrer, A., Mayrhofer, S., Zitz, U., Hofacre, C., & Domig, K. J. (2019). The application of antibiotics in broiler production and the resulting antibiotic

resistance in Escherichia coli: A global overview. *Poultry science*, *98*(4), 1791-1804.

Ruvinsky, A., Rothschild, M. F., Larson, G., & Gongora, J. (2011). Systematics and evolution of the pig. In *The genetics of the pig* (pp. 1-13). Wallingford UK: CABI.

Saad, S. M., F Khater, D., & Zaki, S. M. (2018). Food poisoning bacteria in ready-to-eat meat and chicken meat products. *Benha Veterinary Medical Journal*, *35*(1), 301-310.

Safain, K. S. (2020). Detection of antibiotic resistance spectrum and resistance genes for aminoglycoside, macrolide, and β-lactam antibiotics using wound swab samples (Doctoral dissertation, Brac University).

Sagar, S., Kaistha, S., Das, A. J., & Kumar, R. (2019). Antibiotic Resistant Bacteria*: A* Challenge to Modern Medicine. *Springer Singapore*.

Saina, H. (2005). Guinea fowl (Numidia meleagris) production under smallholder farmer management in Guruve District, Zimbabwe. *Master of Philosophy Thesis*.

Saliba, R., Zahar, J. R., Dabar, G., Riachy, M., Karam-Sarkis, D., & Husni, R. (2023). Limiting the spread of multidrug-resistant bacteria in low-to-middle-income countries: one size does not fit all. *Pathogens*, *12*(1), 144.

Samaranayake, L. (2018). Essential microbiology for dentistry-E-Book. *Elsevier Health Sciences.*

Sanders, J. W., Riddle, M. S., Taylor, D. N., & DuPont, H. L. (2019). Epidemiology of travelers' diarrhea. In *Travel Medicine* (pp. 187-198). Elsevier.

Santos, R., Ursu, O., Gaulton, A., Bento, A. P., Donadi, R. S., Bologa, C. G., ... & Overington, J. P. (2017). A comprehensive map of molecular drug targets. *Nature Reviews Drug Discovery*, *16*(1), 19-34.

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., ... & Malonia, S. K. (2023). Next-generation sequencing technology: current trends and advancements. *Biology*, *12*(7), 997.

Saucier, L. (2016). Microbial spoilage, quality and safety within the context of meat sustainability. *Meat Science*, *120*, 78-84.

Scallan, E., Griffin, P. M., Angulo, F. J., Tauxe, R. V., & Hoekstra, R. M. (2011). Foodborne illness acquired in the United States—unspecified agents. *Emerging Infectious Diseases*, *17*(1), 16.

Schenkel, F. S., Miller, S. P., Jiang, Z., Mandell, I. B., Ye, X., Li, H., & Wilton, J. W. (2006). Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, *84*(2), 291-299.

Scott, H. M., Acuff, G., Bergeron, G., Bourassa, M. W., Gill, J., Graham, D. W., ... & Wittum, T. E. (2019). Critically important antibiotics: criteria and approaches for measuring and reducing their use in food animal agriculture. *Annals of The New York Academy of Sciences*, *1441*(1), 8-16.

Secher, T., Brehin, C., & Oswald, E. (2016). Early settlers: which *E. coli* strains do you not want at birth? *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *311*(1), G123-G129.

Sekyere, J. O. (2014). Antibiotic types and handling practices in disease management among pig farms in Ashanti Region, Ghana. *Journal of Veterinary Medicine*, *2014*.

Seneviratna, P. (2013). Diseases of Poultry:(Including Cage Birds). Elsevier.

Servin, A. L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS microbiology reviews*, *28*(4), 405-440.

Shahi, M. K. (2022). Situation analysis and kap on antimicrobial use and resistance among veterinarians and broiler poultry farmers of Nepal. *Chulalongkorn University Theses and Dissertations (Chula ETD)*. 6080. Available at: *https://digital.car.chula.ac.th/chulaetd*/6080 accessed on 16/09/2024.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D., & Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi Journal of Biological Sciences*, *22*(1), 90-101.

Shaltout, F. A. E., Barr, A. A. H., & Abdelaziz, M. E. (2022). Pathogenic Microorganisms in Meat Products. *Biomedical Journal of Scientific & Technical Research*, *41*(4), 32836-32843.

Shariati, A., Arshadi, M., Khosrojerdi, M. A., Abedinzadeh, M., Ganjalishahi, M., Maleki, A., ... & Khoshnood, S. (2022). The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic. *Frontiers in Public Health*, *10*, 1025633.

Sharif, M. K., Javed, K., & Nasir, A. (2018). Foodborne illness: threats and control. In *Foodborne Diseases* (pp. 501-523). Academic Press.

Shariq, M., Singh, S., Farooq, U., Dhariyal, K. K., Singh, K., & Kaur, N. (2016). Presumptive coliform count in water sample collected from different sites of a university, Moradabad, Uttar Pradesh, India. *International Journal of Scientific Study*, *3*(12), 91-96.

Sharma, C., Rokana, N., Chandra, M., Singh, B. P., Gulhane, R. D., Gill, J. P. S., ... & Panwar, H. (2018). Antimicrobial resistance: its surveillance, impact, and alternative management strategies in dairy animals. *Frontiers in Veterinary Science*, *4*, 237.

Sharma, N., Aron, N., Agarwal, T., Sharma, C., Aron, N., Agarwal, T., & Sharma, N. (2016). Antimicrobial agents for ocular use: bacterial, fungal, viral, and protozoal infections. In *Pharmacology of Ocular Therapeutics* (pp. 285-332). Cham: Springer International Publishing.

Shetty, V. P., Akshay, S. D., Rai, P., & Deekshit, V. K. (2023). Integrons as the potential targets for combating multidrug resistance in Enterobacteriaceae using CRISPR-Cas9 technique. *Journal of Applied Microbiology*, *134*(7), lxad137.

Shiningeni, D., Chimwamurombe, P., Shilangale, R., & Misihairabgwi, J. (2019). Prevalence of pathogenic bacteria in street vended ready-to-eat meats in Windhoek, Namibia. *Meat Science*, *148*, 223-228.

Shook, B., Nelson, K., Aguilera, K., & Braff, L. (2019). Early Members of the Genus Homo. *Explorations*.

Sime, A. G. (2021). Production Performance, Consequence and Constraints of Small Ruminants in Ethiopia: A Review. *world (Kassahun, 2004)*, *90*.

Singhal, M., Agrawal, M., Bhavna, K., Sethiya, N. K., Bhargava, S., Gondkar, K. S., ... & Arora, M. K. (2023). Chloramphenicol and tetracycline (broad spectrum antibiotics). In *Antibiotics-Therapeutic Spectrum and Limitations* (pp. 155-165). Academic Press.

Smith, J. L., & Fratamico, P. M. (2017). *Escherichia coli* as a Pathogen. In *Foodborne Diseases* (pp. 189-208). Academic Press.

Smith, N. W., Fletcher, A. J., Hill, J. P., & McNabb, W. C. (2022). Modeling the contribution of meat to global nutrient availability. *Frontiers in Nutrition*, *9*, 73.

Sobral, R., & Tomasz, A. (2019). The *staphylococcal* cell wall. *Microbiology Spectrum*, *7*(4), 10-1128.

Song, J., Rensing, C., Holm, P. E., Virta, M., & Brandt, K. K. (2017). Comparison of metals and tetracycline as selective agents for development of tetracycline resistant bacterial communities in agricultural soil. *Environmental Science & Technology*, *51*(5), 3040-3047.

Soriano-Santos, J. (2010). Chemical composition and nutritional content of raw poultry meat. *Handbook of Poultry Science and Technology, 1*, 467-489.

Steffen, R., Hill, D. R., & DuPont, H. L. (2015). Traveler's diarrhea: a clinical review. *Jama*, *313*(1), 71-80.

Stone Jr, C. A., Trubiano, J., Coleman, D. T., Rukasin, C. R., & Phillips, E. J. (2020). The challenge of de-labeling penicillin allergy. *Allergy*, *75*(2), 273-288.

Sutherland-Smith, M. (2015). Suidae and Tayassuidae (Wild pigs, peccaries). *Fowler's Zoo and Wild Animal Medicine, Volume 8*, 568.

Tadesse, D. A., Li, C., Mukherjee, S., Hsu, C. H., Bodeis Jones, S., Gaines, S. A., ... & Zhao, S. (2018). Whole-genome sequence analysis of CTX-M containing *Escherichia coli* isolates from retail meats and cattle in the United States. *Microbial Drug Resistance*, *24*(7), 939-948.

Tang, K. W. K., Millar, B. C., & Moore, J. E. (2023). Antimicrobial resistance (AMR). *British Journal of Biomedical Science*, *80*, 11387.

Tanwar, J., Das, S., Fatima, Z., & Hameed, S. (2014). Multidrug resistance: an emerging crisis. *Interdisciplinary Perspectives on Infectious Diseases*, *2014*.

Taulo, S., Wetlesen, A., Abrahamsen, R. K., Narvhus, J. A., & Mkakosya, R. (2009). Quantification and variability of *Escherichia coli* and *Staphylococcus aureus* cross-contamination during serving and consumption of cooked thick porridge in Lungwena rural households, Malawi. *Food Control*, *20*(12), 1158-1166.

Taylor, S. (2012). John Gould's extinct and endangered birds of Australia. *National Library Australia.*

Tella, A., & Chineke, C. A. (2022). Improving global foods system, human health, and alleviating poverty through small ruminant production: the nigerian gains. *Global Journal of Agricultural Sciences*, *21*(1), 13-25.

Thy, M., Timsit, J. F., & de Montmollin, E. (2023). Aminoglycosides for the treatment of severe infection due to resistant Gram-Negative pathogens. *Antibiotics*, *12*(5), 860.

Timsit, J. F., Wicky, P. H., & de Montmollin, E. (2022). Treatment of severe infections due to metallo-betalactamases enterobacterales in critically ill patients. *Antibiotics*, *11*(2), 144.

Todd, E. C., Michaels, B. S., Smith, D., Greig, J. D., & Bartleson, C. A. (2010). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 9. Washing and drying of hands to reduce microbial contamination. *Journal of Food Protection*, *73*(10), 1937-1955.

Torres AG, Blanco M, Valenzuela P, Slater TM, Patel SD, Dahbi G, López C, Barriga XF, Blanco JE, Gomes TA, Vidal R, Blanco J. Genes related to long polar fimbriae of pathogenic *Escherichia coli* strains as reliable markers to identify virulent isolates. *Journal Clinical Microbiology.* 2009 Aug;47(8):2442-51.

Trabulsi, L. R., Keller, R., & Gomes, T. A. T. (2002). 10.321/eid0805. Typical and Atypical Enteropathogenic *Escherichia coli*. *Emerging Infectious Diseases*, *8*(5), 508.

Trongjit, S., & Chuanchuen, R. (2021). Whole genome sequencing and characteristics of *Escherichia coli* with co-existence of ESBL and mcr genes from pigs. *Plos one*, *16*(11), e0260011.

Tsai, R. J., Luckhaupt, S. E., Schumacher, P., Cress, R. D., Deapen, D. M., & Calvert, G. M. (2014). Acute myeloid leukemia risk by industry and occupation. *Leukemia & Lymphoma*, *55*(11), 2584-2591.

Tshipamba, M. E. (2018). Foodborne pathogens and their antibiotic resistance profiles in ready-to-eat meat sold around Johannesburg Central Business District, Gauteng Province (Doctoral dissertation, North-West University (South Africa).

Tshitshi, L. (2019). Molecular characterization of carbapenem resistant extended spectrum Beta-Lactamase (ESBL) producing enterobacteriaceae (Doctoral dissertation, North-West University (South Africa).

Uddin, M. N., Bokelmann, W., & Entsminger, J. S. (2014). Factors affecting farmers' adaptation strategies to environmental degradation and climate change effects: A farm level study in Bangladesh. *Climate*, *2*(4), 223-241.

Uelze, L., Grützke, J., Borowiak, M., Hammerl, J. A., Juraschek, K., Deneke, C., ... & Malorny, B. (2020). Typing methods based on whole genome sequencing data. *One Health Outlook*, *2*, 1-19.

Van der Loo, C. (2020). Analysis of borehole water with specific emphasis on the detection of free-living amoebae and amoeba-resistant bacteria. *University of Johannesburg (South Africa).*

Van, T. T. H., Yidana, Z., Smooker, P. M., & Coloe, P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. *Journal of Global Antimicrobial Resistance*, *20*, 170-177.

Vashisht, J. (2019). Identification and characterization of major causative bacterial agents of diarrhea from regions of himachal Pradesh (Doctoral dissertation, *Jaypee University of Information Technology, Solan, HP).*

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics*, *40*(4), 277.

Wallinga, D. (2009). Today's food system: how healthy is it? *Journal of Hunger & Environmental Nutrition*, *4*(3-4), 251-281.

Wang D, Huang K, Lin Z, Zhang Y, Liu G, Wu Y, Wang S. Glutamic acid decarboxylase antibodies in neurocritical patients: a culprit or a bystander? *Neurol Science.* 2020 Dec;41(12):3691-3696

Wang, Q., Han, Y. Y., Zhang, T. J., Chen, X., Lin, H., Wang, H. N., & Lei, C. W. (2024). Whole-genome sequencing of *Escherichia coli* from retail meat in China reveals the dissemination of clinically important antimicrobial resistance genes. *International Journal of Food Microbiology*, *415*, 110634.

Wang, S. K., Fu, L. M., Chen, G. W., Xiao, H. M., Pan, D., Shi, R. F., ... & Sun, G. J. (2020). Multisite survey of bacterial contamination in ready-to-eat meat products throughout the cooking and selling processes in urban supermarket, Nanjing, China. *Food Science & Nutrition*, *8*(5), 2427-2435.

Wilson, R. D., & O'Connor, D. L. (2021). Maternal folic acid and multivitamin supplementation: International clinical evidence with considerations for the prevention of folate-sensitive birth defects. *Preventive Medicine Reports*, *24*, 101617.

World Health Organisation (WHO) (2024). One health. Available at: https://www.who.int/health-topics/one-health#tab=tab_1 accessed on 3/7/2023.

World Health Organization. (2015). Food Safety*: What you should know* (No. SEA-NUT-196). World Health Organization

World Health Organization. (2017). Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline, including tuberculosis (No. WHO/EMP/IAU/2017.11). World Health Organization.

World Health Organization. (2019). Shiga Toxin-producing Escherichia Coli (STEC) and Food: *Attribution, Characterization and Monitoring* (Vol. 19). World Health Organization.

World Health Organization. (2020). GLASS whole-genome sequencing for surveillance of antimicrobial resistance.; *https://creativecommons.org*

Wróbel, A., Arciszewska, K., Maliszewski, D., & Drozdowska, D. (2020). Trimethoprim and other nonclassical antifolates an excellent template for searching modifications of dihydrofolate reductase enzyme inhibitors. *The Journal of Antibiotics*, *73*(1), 5-27.

Yakubu, A. L. H. A. S. S. A. N. (2021). Food Hygiene Practices Among Street Food Vendors in The Sagnarigu Municipality of The Northern Region of Ghana (Doctoral Dissertation).

Yang, J., Barra, J. T., Fung, D. K., & Wang, J. D. (2022). Bacillus subtilis produces (p) ppGpp in response to the bacteriostatic antibiotic chloramphenicol to prevent its potential bactericidal effect. *Mlife*, *1*(2), 101-113.

Yao, J. D., & Moellering Jr, R. C. (2011). Antibacterial agents. *Manual of Clinical Microbiology*, 1041-1081.

Yılmaz, Ç., & Özcengiz, G. (2017). Antibiotics: Pharmacokinetics, toxicity, resistance and multidrug efflux pumps. *Biochemical Pharmacology*, *133*, 43-62.

Yin, Y., Jiang, Y., Lam, K. W. G., Berletch, J. B., Disteche, C. M., Noble, W. S., ... & Shendure, J. (2019). High-throughput single-cell sequencing with linear amplification. *Molecular Cell*, *76*(4), 676-690.

Yohannes, L. (2021). Microbiological Quality and Level of Heavy Metals in Edible Vegetable Oils Produced and Marketed in Gondar City (Doctoral Dissertation, Uog).

Zhao, X. L., Chen, Z. G., Yang, T. C., Jiang, M., Wang, J., Cheng, Z. X., ... & Peng, X. X. (2021). Glutamine promotes antibiotic uptake to kill multidrug-resistant uropathogenic bacteria. *Science Translational Medicine*, *13*(625), eabj0716.

Zook, J. M., Catoe, D., McDaniel, J., Vang, L., Spies, N., Sidow, A., ... & Salit, M. (2016). Extensive sequencing of seven human genomes to characterize benchmark reference materials. *Scientific Data*, *3*(1), 1-26.

APENDIX I: Protocol for whole genome sequencing

Before start checklist

Materials	Consumables	Equipment
☐ 50 ng high molecular weight genomic DNA per sample	☐ 1.5 ml Eppendorf DNA LoBind tubes	☐ Ice bucket with ice
☐ For R10.3 flow cells, 50-200 ng high molecular weight genomic DNA per sample	☐ 2 ml Eppendorf DNA LoBind tubes	☐ Microplate centrifuge, e.g. Fisherbrand™ Mini Plate Spinner Centrifuge (Fisher Scientific, #11766427)
☐ Rapid Barcoding Kit 96 (SQK-RBK110.96)	☐ 0.2 ml thin-walled PCR tubes	☐ Timer
	☐ Eppendorf twin.tec® PCR plate 96 LoBind, semi-skirted (Cat # 0030129504) with heat seals	☐ Thermal cycler or heat block at 30°C and 80°C
	☐ Nuclease-free water (e.g. ThermoFisher, cat # AM9937)	☐ Magnetic rack
	☐ Freshly prepared 80% ethanol in nuclease-free water	☐ Hula mixer (gentle rotator mixer)
		☐ Pipettes and pipette tips P2, P20, P100, P200, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
Library preparation	
☐ Program the thermal cycler: 30°C for 2 minutes, then 80°C for 2 minutes. Thaw kit components at RT, spin down briefly using a microfuge and mix by pipetting as indicated by the table below: ☐ Rapid Barcode plate (RB96): not frozen, briefly spin down, mix well by pipetting ☐ Rapid Adapter F (RAP-F): not frozen, briefly spin down, mix well by pipetting ☐ SPRI beads (SPRI): thaw at RT, briefly spin down, mix by pipetting or vortexing immediately before use ☐ Sequencing Buffer II (SBII): thaw at RT, briefly spin down, mix well by pipetting* ☐ Loading Beads II (LBII): thaw at RT, briefly spin down, mix by pipetting or vortexing immediately before use ☐ Elution Buffer (EB): thaw at RT, briefly spin down, mix well by pipetting ☐ Flush Buffer (FB): thaw at RT, briefly spin down, mix by vortexing ☐ Flush Tether (FLT): thaw at RT, briefly spin down, mix well by pipetting Prepare the DNA in Nuclease-free water. ☐ Transfer 50 ng genomic DNA per sample (if using R9.4.1 flow cells) or 50-200 ng (if using R10.3 flow cells) into a 1.5 ml Eppendorf DNA LoBind tube ☐ Adjust the volume to 9 µl with Nuclease-free water ☐ Mix by pipetting up and down ☐ Spin down briefly in a microfuge	

MASSFLOWINSTRUCTIONS	NOTES/OBSERVATIONS
In 0.2 ml thin-walled PCR tubes or an Eppendorf twin.tec® PCR plate 96 LoBind, mix the following. The Rapid Barcodes can be transferred using a multichannel pipette: ☐ 9 µl 50 ng template DNA ☐ 1 µl Rapid Barcodes (RB01-96, one for each sample) ☐ Ensure the components are thoroughly mixed by pipetting. Seal the plate and spin down in a centrifuge.	

☐ Incubate the tubes or plate at 30°C for 2 minutes and then at 80°C for 2 minutes. Briefly put the tubes or plate on ice to cool. ☐ Pool all barcoded samples in your desired ratio, noting the total volume. ☐ Resuspend the SPRI beads by vortexing. ☐ To the entire pooled barcoded sample from Step 7, add an equal volume of resuspended SPRI beads and mix by flicking the tube. ☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. ☐ Prepare at least 3 ml of fresh 80% ethanol in Nuclease-free water. ☐ Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant. ☐ Keep the tube on the magnet and wash the beads with 1.5 ml of freshly-prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard. ☐ Repeat the previous step. ☐ Briefly spin down and place the tube back on the magnet. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellet to the point of cracking. ☐ Remove the tube from the magnetic rack and resuspend the pellet in 15 µl Elution Buffer (EB). Incubate for 10 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute. Remove and retain 15 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Remove and retain the eluate which contains the DNA ☐ library in a clean 1.5 ml Eppendorf DNA LoBind tube Dispose of the pelleted beads	

Quantify 1 µl of eluted sample using a Qubit fluorometer.	
☐ Transfer 11 µl of the sample into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Add 1 µl of Rapid Adapter F (RAP F) to 11 µl of barcoded DNA. ☐ Mix gently by flicking the tube, and spin down. ☐ Incubate the reaction for 5 minutes at RT.	
The prepared library is used for loading into the MinION flow cell. Store the library on ice until ready to load.	

INSTRUCTIONS	NOTES/OBSERVATIONS
Priming and loading the SpotON Flow Cell	
Using the Loading Solution ☐ Thaw the Sequencing Buffer II (SBII), Loading Beads II (LBII) or Loading Solution (LS, if using), Flush Tether (FLT) and Flush Buffer (FB) at RT before mixing the reagents by vortexing, and spin down the SBII and FLT at RT. ☐ Mix the Sequencing Buffer II (SBII), Flush Buffer (FB), Flush Tether (FLT) and Loading Solution (LS, if using) tubes by vortexing. Spin down the SBII and FLT at RT. ☐ To prepare the flow cell priming mix, add 30 µl of thawed and mixed Flush Tether (FLT) directly to the tube of thawed and mixed Flush Buffer (FB), and mix by vortexing at RT. ☐ Open the MinION Mk1B lid and slide the flow cell under the clip. ☐ Slide the priming port cover clockwise to open the priming port.	
IMPORTANT ☐ Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.	
After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles (a few µl): ☐ Set a P1000 pipette to 200 µl ☐ Insert the tip into the priming port ☐ Turn the wheel until the dial shows 220-230 ul, to draw back 20-30 ul, or until you can see a small volume of buffer entering the pipette tip Note: Visually check that there is continuous buffer from the priming port across the sensor array. ☐ Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes. During this time, prepare the library for loading by following the steps below. ☐ Thoroughly mix the contents of the Loading Beads II (LBII) by pipetting.	
IMPORTANT ☐ The Loading Beads II (LBII) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.	
In a new tube, prepare the library for loading as follows: ☐ 37.5µl Sequencing Buffer II (SBII ☐ 25.5µl Loading Beads II (LBII) mixed immediately before use, or Loading Solution (LS), if using ☐ 12 µl DNA library Complete the flow cell priming: ☐ Gently lift the SpotON sample port cover to make the SpotON sample port accessible. ☐ Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles.	

APENDIX II: Data Analysis Output (Prevalence of *E.* coli from RTE meats and it associated samples)

```
GET DATA /TYPE=XLSX
/SHEET=name 'Sheet1'
 /CELLRANGE=full
 /READNAMES=on
 /ASSUMEDSTRWIDTH=32767.
EXECUTE.
DATASET NAME DataSet1 WINDOW=FRONT.
* Generalized Linear Models.
GENLIN Result (REFERENCE=LAST) BY Sample (ORDER=ASCENDING)
 /MODEL Sample INTERCEPT=YES
 DISTRIBUTION=BINOMIAL LINK=LOGIT
 /CRITERIA METHOD=FISHER (1) SCALE=1 COVB=MODEL
MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-
006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95
CITYPE=WALD LIKELIHOOD=FULL
 /EMMEANS TABLES=Sample SCALE=ORIGINAL COMPARE=Sample
CONTRAST=PAIRWISE PADJUST=LSD
 /MISSING CLASSMISSING=EXCLUDE
 /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
```

Generalized Linear Models

Notes

Output Created		01-FEB-2024 17:47:22
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	360
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable

Notes

Syntax		GENLIN Result (REFERENCE=LAST) BY Sample (ORDER=ASCENDING) /MODEL Sample INTERCEPT=YES DISTRIBUTION=BINOMIAL LINK=LOGIT /CRITERIA METHOD=FISHER(1) SCALE=1 COVB=MODEL MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 CITYPE=WALD LIKELIHOOD=FULL /EMMEANS TABLES=Sample SCALE=ORIGINAL COMPARE=Sample CONTRAST=PAIRWISE PADJUST=LSD /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.14
	Elapsed Time	00:00:00.16

[DataSet1]

Model Information

Dependent Variable	Result[a]
Probability Distribution	Binomial
Link Function	Logit

a. The procedure models 0 as the response, treating 1 as the reference category.

Case Processing Summary

	N	Percent
Included	360	100.0%
Excluded	0	0.0%
Total	360	100.0%

Categorical Variable Information

			N	Percent
Dependent Variable	Result	0	335	93.1%
		1	25	6.9%
		Total	360	100.0%
Factor	Sample	Hands of RTE meat sellers	60	16.7%
		Knives for cutting RTE meats	60	16.7%
		RTE Beef	60	16.7%
		RTE Chicken	60	16.7%
		RTE Mutton	60	16.7%
		Spices for preparing RTE meats	60	16.7%
		Total	360	100.0%

Goodness of Fit[a]

	Value	df	Value/df
Deviance	.000	0	.
Scaled Deviance	.000	0	
Pearson Chi-Square	.000	0	.
Scaled Pearson Chi-Square	.000	0	
Log Likelihood[b]	-9.072		
Akaike's Information Criterion (AIC)	30.145		
Finite Sample Corrected AIC (AICC)	30.383		
Bayesian Information Criterion (BIC)	53.461		
Consistent AIC (CAIC)	59.461		

Dependent Variable: Result

Model: (Intercept), Sample[a]

a. Information criteria are in small-is-better form.

b. The full log likelihood function is displayed and used in computing information criteria.

Omnibus Test[a]

Likelihood Ratio Chi-Square	df	Sig.
10.926	5	.053

Dependent Variable: Result

Model: (Intercept), Sample[a]

a. Compares the fitted model against the intercept-only model.

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	Df	Sig.
(Intercept)	116.511	1	.000
Sample	9.241	5	.100

Dependent Variable: Result

Model: (Intercept), Sample

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test
			Lower	Upper	Wald Chi-Square
(Intercept)	4.078	1.0084	2.101	6.054	16.349
[Sample=Hands of RTE meat sellers]	-.710	1.2386	-3.138	1.717	.329
[Sample=Knives for cutting RTE meats]	-1.438	1.1335	-3.660	.783	1.611
[Sample=RTE Beef]	-2.343	1.0713	-4.443	-.243	4.783
[Sample=RTE Chicken]	-1.880	1.0964	-4.029	.269	2.941
[Sample=RTE Mutton]	-1.133	1.1695	-3.425	1.159	.939
[Sample=Spices for preparing RTE meats]	0[a]	.	.	.	.
(Scale)	1[b]				

Parameter Estimates

Parameter	Hypothesis Test	
	df	Sig.
(Intercept)	1	.000
[Sample=Hands of RTE meat sellers]	1	.566
[Sample=Knives for cutting RTE meats]	1	.204
[Sample=RTE Beef]	1	.029
[Sample=RTE Chicken]	1	.086
[Sample=RTE Mutton]	1	.333
[Sample=Spices for preparing RTE meats]	.[a]	.
(Scale)		

Dependent Variable: Result

Model: (Intercept), Sample

a. Set to zero because this parameter is redundant.

b. Fixed at the displayed value.

Estimated Marginal Means: Sample

Estimates

Sample	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
Hands of RTE meat sellers	.97	.023	.88	.99
Knives for cutting RTE meats	.93	.032	.84	.97
RTE Beef	.85	.046	.74	.92
RTE Chicken	.90	.039	.79	.95
RTE Mutton	.95	.028	.86	.98
Spices for preparing RTE meats	.98	.017	.89	1.00

Pairwise Comparisons

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	df	Sig.
Hands of RTE meat sellers	Knives for cutting RTE meats	.03	.040	1	.401
	RTE Beef	.12[a]	.052	1	.024
	RTE Chicken	.07	.045	1	.140
	RTE Mutton	.02	.036	1	.648
	Spices for preparing RTE meats	-.02	.028	1	.558
Knives for cutting RTE meats	Hands of RTE meat sellers	-.03	.040	1	.401
	RTE Beef	.08	.056	1	.138
	RTE Chicken	.03	.050	1	.508
	RTE Mutton	-.02	.043	1	.697
	Spices for preparing RTE meats	-.05	.036	1	.167
RTE Beef	Hands of RTE meat sellers	-.12[a]	.052	1	.024
	Knives for cutting RTE meats	-.08	.056	1	.138
	RTE Chicken	-.05	.060	1	.406
	RTE Mutton	-.10	.054	1	.064
	Spices for preparing RTE meats	-.13[a]	.049	1	.006
RTE Chicken	Hands of RTE meat sellers	-.07	.045	1	.140
	Knives for cutting RTE meats	-.03	.050	1	.508
	RTE Beef	.05	.060	1	.406

RTE Mutton	RTE Mutton	-.05	.048	1	.296
	Spices for preparing RTE meats	-.08[a]	.042	1	.048
	Hands of RTE meat sellers	-.02	.036	1	.648
	Knives for cutting RTE meats	.02	.043	1	.697

Pairwise Comparisons

(I) Sample	(J) Sample	95% Wald Confidence Interval for Difference	
		Lower	Upper
Hands of RTE meat sellers	Knives for cutting RTE meats	-.04	.11
	RTE Beef	.02[a]	.22
	RTE Chicken	-.02	.16
	RTE Mutton	-.05	.09
	Spices for preparing RTE meats	-.07	.04
Knives for cutting RTE meats	Hands of RTE meat sellers	-.11	.04
	RTE Beef	-.03	.19
	RTE Chicken	-.07	.13
	RTE Mutton	-.10	.07
	Spices for preparing RTE meats	-.12	.02
RTE Beef	Hands of RTE meat sellers	-.22[a]	-.02
	Knives for cutting RTE meats	-.19	.03
	RTE Chicken	-.17	.07
	RTE Mutton	-.21	.01
	Spices for preparing RTE meats	-.23[a]	-.04
RTE Chicken	Hands of RTE meat sellers	-.16	.02
	Knives for cutting RTE meats	-.13	.07

RTE Mutton	RTE Beef	-.07	.17
	RTE Mutton	-.14	.04
	Spices for preparing RTE meats	-.17[a]	.00
	Hands of RTE meat sellers	-.09	.05
	Knives for cutting RTE meats	-.07	.10

Pairwise Comparisons

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	df	Sig.
RTE Mutton	RTE Beef	.10	.054	1	.064
	RTE Chicken	.05[a]	.048	1	.296
	Spices for preparing RTE meats	-.03	.033	1	.307
	Hands of RTE meat sellers	.02	.028	1	.558
	Knives for cutting RTE meats	.05	.036	1	.167
Spices for preparing RTE meats	RTE Beef	.13	.049	1	.006
	RTE Chicken	.08	.042	1	.048
	RTE Mutton	.03	.033	1	.307

Pairwise Comparisons

(I) Sample	(J) Sample	95% Wald Confidence Interval for Difference	
		Lower	Upper
RTE Mutton	RTE Beef	-.01	.21
	RTE Chicken	-.04[a]	.14
	Spices for preparing RTE meats	-.10	.03
	Hands of RTE meat sellers	-.04	.07
	Knives for cutting RTE meats	-.02	.12
Spices for preparing RTE meats	RTE Beef	.04	.23
	RTE Chicken	.00	.17
	RTE Mutton	-.03	.10

Pairwise comparisons of estimated marginal means based on the original scale of dependent variable Result

a. The mean difference is significant at the .05 level.

Overall Test Results

Wald Chi-Square	df	Sig.
10.865	5	.054

The Wald chi-square tests the effect of Sample. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.