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UNIVERSITY FOR
DEVELOPMENT STUDIES

SUSTAINABLE MEAT SAFETY IN THE 21ST CENTURY: Our Responsibility

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Sustainable Meat Safety in the 21st Century: Our Responsibility

By

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Development Studies, Tamale, Ghana**

2022

Inaugural Lecture Delivered under the Chairmanship of

Professor Gabriel Ayum Teye
Vice-Chancellor, UDS

**Venue: Multipurpose Auditorium
Central Administration, Tamale Campus
University for Development Studies**

Friday, August 26, 2022

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Professor of Meat Science and Food Safety

Profile

PROFESSOR FREDERICK ADZITEY (PhD)

Professor Frederick Adzitey is an astute and analytical academician, a prolific writer, an illustrious teacher and researcher, as well as a virtuous, empathic and peaceful leader.

Prof. Frederick Adzitey was born on 23rd August 1980 at Likpe Bakwa, Ghana and obtained his Basic and Junior High School (JHS) education at Maamobi Prisons School, Accra (Primary 1 to JHS 1) and St. Thomas Junior Secondary School, Obuasi (JHS 2 to JHS 3). His Senior Secondary School education was at Awudome Secondary School, Tsito from 1996 to 1999. He was temporarily at Animal Health and Production College, Pong, Tamale in 2000, prior to gaining admission at the University for Development Studies (UDS) in the same year. He graduated from UDS in 2005 with first class in Bachelor of Science (BSc) degree in Agriculture Technology (Animal Science Option). While at UDS, he won the Opopo Transport Award in 2003 for being the best Second Year Agriculture Student. He proceeded to study Master of Science (MSc) in Meat Science and Technology on a Commonwealth Shared Scholarship at the University of Bristol, UK, where he obtained Distinction and won the Maurice Ingram Award for being the best student in the programme. Between 2010 to 2012, he won a Fellowship to study for a PhD in Food Safety at the Universiti of Sains, Malaysia (USM), where he spent 3 years, graduating in 2013. He won the Sanggar Sanjung Awards in 2013 and 2014 for excellent achievements in research paper publication during his PhD studies. Motivated by his love and enthusiasm for teaching and research he enrolled for the postgraduate diploma (PgD) in education at UDS after his PhD, which he completed on time.

Currently, he and a colleague have brought a collaboration between CR72 Ghana Limited (a flagship of rearing for food and job) and the Department of Animal Science to raise layers for distribution to farmers. Prof. Adzitey and his partners also won a grant from the Skills Development Fund (SDF-II-Call 2-0141) to upgrade the skills of employees of Neat Meat Limited (Zebilla) on Meat Handling and Processing, the HACCP (Hazard Analysis and Critical Control Points) Quality Assurance System and Meat Marketing.

Prof. Adzitey has won a number of grants, bursaries and awards including: a Travel Grant from the African Academy of Sciences, an Early Career Researcher Award from The Royal Society of the United Kingdom, The International Committee for Food Microbiology and Hygiene (ICFMH) Studentship Grant, a Bursary from The World Academy of Sciences, a Bursary from the Japanese Society of Science, a grant from the President's Fund of the Society for Applied Microbiology, a Bursary from the EU FP7 Project "Animal Change", and a Bursary from the Wellcome Trust to attend international conferences, training courses or workshops.

He has received funds from the Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, the Global Health Research Unit for Genomic Surveillance and Antimicrobial Resistance - Nigeria, and a Travel Grant Fellowship from the Commonwealth Science Conference to conduct research on whole genome sequencing of *Escherichia coli* and *Salmonella enterica* from meat sources in Ghana.

Prof. Adzitey has risen in this University from the position of Senior Research Assistant (2006 to 2009), Assistant Lecturer (2009 to 2012), Lecturer (2012 to 2015), Senior Lecturer (2015 to 2018), Associate Professor (2018 to 2021) to Professor in Meat Science and Food Safety by hard work and determination. I quote "do not believe in luck, believe in hard work".

He has delivered many courses including (at the postgraduate level): Animal Production, Biochemistry, Meat Microbiology and Hygiene, Livestock and Environment, Refrigeration and Preservation of Meat, and Meat Production and Processing; (at the undergraduate level): Animal Products and Handling, Basic Microbiology, Laboratory Techniques, Animal Biotechnology, Agricultural Zoology, Animal Welfare and Legislation, Introduction to Microbiology 1, Cell Biology, Livestock and Poultry Production, and Cattle, Sheep and Goat Production; (at diploma/certificate level): Cold Chain Management for Food Safety, Meat Processing and Handling, Animal Welfare and Behaviour, and Introduction to Animal Science.

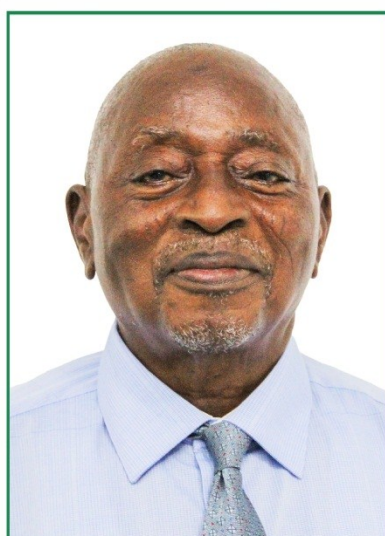
He has supervised 2 PhD students, 12 MPhil Students, 2 MSc students and 55 undergraduate students. Currently, he has 7 PhD Students (2 awaiting graduation), 2 MPhil Students, 1 M.Ed. Education Student, and 10 undergraduate students. He has examined students' theses (PhD or MPhil) from UDS, the University of Fort Hare (South Africa), Stellenbosch University (South Africa), CSIR College of Science and Technology (Ghana), Kwame Nkrumah University of Science and Technology (Ghana), and University of Cape Coast (Ghana). He has also assessed a number of grant applications and promotion documents from both local and international institutions.

Prof. Adzitey has published extensively in reputable peer-reviewed journals and has made presentations at both local and international conferences. He has published over 130 articles, 2 book chapters, 1 book and has over 50 conference proceedings. He has also reviewed over 50 manuscripts for more than 10 different journals.

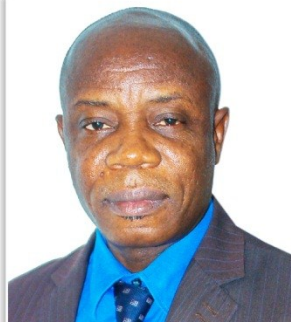
He is an alumnus of the Ghana Young Academy (GhYA) and a Young Affiliate Member of The World Academy of Sciences – a membership achieved by outstanding performance in academia. He is also a member of the Ghana Society for Animal Production (GSAP), the Ghana Animal Science Association (GASA), the International Association for Food Protection (IAFP) and the Society for Applied Microbiology (SfAM). He has served as the Treasurer of GhYA, Vice President of GASA and is currently the Financial Secretary of GSAP. He is currently the Deputy Treasurer of UDS National Alumni. He was the Vice President of UDS Alumni, Northern Chapter, Vice Chair of UDS Judicial Committee, Member of UDS Alumni Constitution Review, and has been Chairman at programmes, Moderator at Conferences, among others.

Professor Adzitey is also involved in Christian activities. He is the patron of the Pentecost Students and Associates, Nyankpala Campus, and Chairman of the Scripture Union Sub-Camp Committee in Northern Region, Ghana. He has given talks on academic excellence, life after school and choosing an appropriate career to students at the University and various Secondary Schools in Tamale.

Currently, Prof. Adzitey is the Head of Department (HoD), at the Department of Animal Science, and has held other positions such as Head - UDS Meats Unit, Exams Officer - Department of Food Science and Technology, Quality Assurance Officer - Department of Veterinary Science, Postgraduate Coordinator - Department of Animal Science, Exams Officer - Department of Animal Science, and Third Trimester Field Practical Training Coordinator - Department of Animal Science. He has been involved in the development and re-accreditation of programmes (MSc, MPhil and PhD Animal Science, BSc Veterinary Science, BSc Agriculture Technology, and Diploma Food Science programmes) at UDS.



Prof. Wayo Seini
CHAIRMAN, UDS COUNCIL



Prof. Gabriel A. Teye
VICE-CHANCELLOR



Prof. Felix K. Abagale
PRO-VICE-CHANCELLOR



Mr. Nurudeen I. Abubakar
REGISTRAR

Programme

Topic	:	Sustainable Meat Safety in the 21st Century: Our Responsibility
Venue	:	Multipurpose Auditorium, Central Administration, Tamale Campus
Chairman	:	Vice-Chancellor, Professor Gabriel A. Teye
2:00 pm	:	Guests Seated
2:15 pm	:	Vice-Chancellor Procession (Audience stand) <ul style="list-style-type: none">- Prayers (Christian and Moslem)- Introduction of Chairman by Mr. Nurudeen Issah Abubakari, Registrar- Welcome Address/Introduction of Speaker by Vice-Chancellor- Lecture on the Topic by Prof. Frederick Adzitey- Chairman Closing Remarks- Vote of Thanks- Announcements- Recession (Audience stand)

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Dedication

*I dedicate my achievement to Almighty God, my mum and
my immediate family. I love you all.*

1.0 Introduction

The Vice-Chancellor

Registrar

Pro-VC and other Principal Officers

Deans and Directors

Heads of Department

Members of the Academic Board

Members of Convocation

Colleague Senior Members

Senior and Junior Staff

Students and Alumni

Distinguished Invited Guests

Family and Friends

The Press

Ladies and Gentlemen

I am humbled and honoured to be given this opportunity by the Vice-Chancellor of our noble University to welcome you all to my inaugural lecture.

I am also very happy to see you all seated here today, including colleagues, family, friends, cherished students and especially all well-wishers who have contributed to this achievement.

I vividly remember when I finished my first degree that Dr. G.A. Teye (now Prof. G.A. Teye and our VC), who had just returned from his PhD studies in the UK requested I do my national service with the Department of Animal Science. By then, I had been posted to a non-governmental organization (NGO) - those were the days that working or doing your service with an NGO was highly prized, so it was a difficult decision for me to do my service with UDS.

As a member of service personnel, I never thought of becoming a Lecturer. Nonetheless, God had His own plans for me, soon He gave me the opportunity to study for my MSc at the University of Bristol from which I returned after a year. After that, it took almost 11 months to be upgraded to the position of an Assistant Lecturer instead of a Lecturer, as I had hoped. That motivated me to

pursue my PhD as soon as possible. To be honest, I told myself I would not come back to UDS after my PhD considering the fact that I was delayed and made an Assistant Lecturer, but the support I received from my then HoD (Dr. H.K. Dei) and Dean (Dr. G.A. Teye) left me no option but to return. I became conscious of being an academician when I returned with my PhD certificate in 2013 coupled with the experience I gained during my time studying in Malaysia.

So, you see, when the actual journey began, it is never too late for you if you are yet to discover what you want to become. I have passed through the ranks of Senior Research Assistant to Professor by God's grace and favour. I must mention that the journey has not been without setbacks and interpersonal animosity, so do not be discouraged when you face such obstacles in life. The key to success is to focus on your goals not obstacles. Do not expect victory without struggling. I also hope people will understand one day that English is a language not a measure of intelligence.

Ladies and gentlemen, I have grown to love research and to write. I work with my students in the laboratory, sometimes finishing at night and even at dawn. I have learnt to take my students' research as my own and have invested my personal resources as well as my time in their work. I have always been mindful of the limited time students have to complete their assignments and have done my best to ensure that they complete on time. I care very much about my students and always want to see them doing well. These are among the motivations that have propelled me this far. I salute all my students.

Ladies and gentlemen (all courtesies observed), this is the 13th inaugural lecture to be delivered by a Professor in UDS, the 3rd from the Department of Animal Science, the 2nd by an Alumnus of UDS and I am the youngest Professor so far in UDS. Work hard in silence, let your success be your noise. My research background is broad in the areas of animal science, meat science and food safety. I have worked on animals, meats, milk, eggs and other foods, in the areas of processing, safety, as well as on the antibiotic resistance and characterization of bacteria isolated from these and their related sources. However, for this inaugural lecture, my focus will be on meat safety – my main

field of specialization. The title of my lecture is “**SUSTAINABLE MEAT SAFETY IN THE 21ST CENTURY: OUR RESPONSIBILITY**”

I am hopeful that after my lecture, we shall all have a take-home message to avoid meat contamination and indirectly food poisoning and illnesses that will result from the consumption of contaminated meat.

The outline of the lecture is as follows:

1. Introduction to research
2. Knowledge and perception of farmers, butchers/meat sellers and consumers in meat safety
3. Contamination of meats by bacteria in Ghana
4. Antibiotic resistance of bacteria isolated from meats in Ghana
5. Antimicrobial residues in meats produced in Ghana
6. Whole genome sequencing of bacteria isolated from meats in Ghana
7. Proposed practical ways to ensuring sustainable meat safety in the 21st Century
8. Conclusions
9. Recommendations
10. Acknowledgements

2.0 Introduction to research

2.1 Meats (nutrient composition, production, consumption and contamination)

Meats are generally the flesh of slaughtered animals that are edible. They are a major component of human diets and serve as an excellent source of protein with high biological value. Nutrients such as fats (omega-3-polyunsaturated fatty acids), minerals (iron, magnesium, potassium, selenium sodium and zinc), and vitamins (vitamin A, vitamin E, B6, B12, niacin, thiamine and riboflavin) can also be found in meats (Ahmad et al., 2018; Smith et al., 2022). Red meats have more myoglobin, the protein responsible for meat colour than white meat. Common red meats in Ghana are beef (cattle), chevon (goat), mutton (sheep) and pork (pig), while white meats are mainly poultry (chicken, guinea fowl, turkey, ducks). In general, red meats have more saturated fat than white meats. Saturated fats can raise your blood cholesterol and increase your

risk of heart disease. If you eat poultry, pork, beef or other meats, choose lean meat, skinless poultry, and unprocessed forms.



Plate 1: A typical white meat



Plate 2: A typical red meat

Globally, an estimated 69 billion chickens, 1.5 billion pigs, 656 million turkeys, 574 million sheep, 479 million goats and 302 million cattle were killed for meat production in 2018 (Ritchie and Poser, 2019). Furthermore, pork is the most consumed meat (average consumption of 16 kg per year), followed by poultry (15 kg), beef/buffalo (9 kg), and mutton and chevon (2 kg each) (Ritchie and Poser, 2019). Meat production (Plate 3) and subsequently consumption, is expected to increase each year. Africa will continue to play a major role in meat production and consumption.

Ghana's meat production increased from 62,493.00 tonnes in 1961 to 290,563.00 tonnes in 2018, a relative change of 365% (Food and Agriculture Organization (FAO), 2020). Nonetheless, Ghana imported 287,930 tonnes of poultry meat and 48,047 tonnes of bovine meat in 2020 (Knoema, 2021). Also, Sasu (2021) reported that about 484 million Ghanaian cedis (approximately 78.4 million dollars) of edible meat offals were imported into Ghana in 2019, and this was the lowest value recorded since 2014. This shows the opportunity and potential for the local animal industry to increase production to fill the gap.

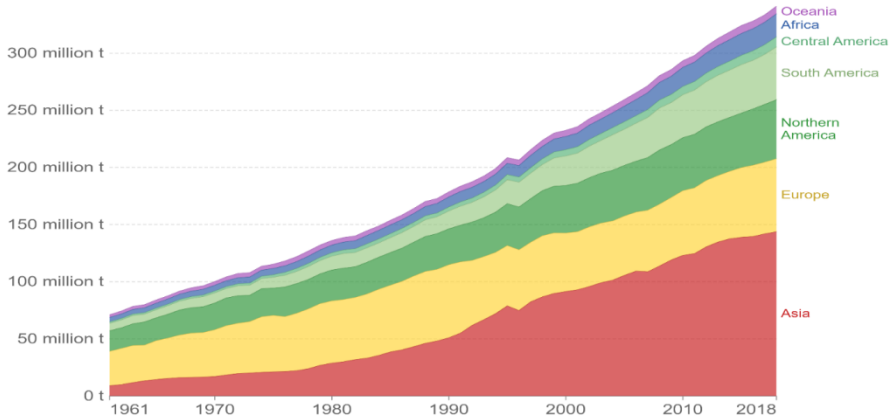


Plate 3: Global meat production from 1961 to 2018.

Source: FAO (2020)

Meat is an essential component of the diet of Ghanaians and local meats are preferred over imported meat (Annan et al., 2018). However, meat and meat products are associated with biological, chemical and physical hazards which pose a threat to food safety and human health. The biological hazards are normally bacteria, viruses, fungal and their associated toxins (United States of America-Food and Drugs Administration (USA-FDA), 2022). Chemical hazards such as animal drug residues, heavy metals, cleaning compounds, allergens and some food additives can be found in meats (Hazaa et al., 2019). Complaints of the presence of physical hazards including bones, plastic, insect, metal, hair, feather, glass, stone, fabric, wood, rubber and foreign objects in meats and meat products have been reported (Cavalheiro et al., 2020).

Of all these hazards much consideration is given to biological meat safety agents and their association with antibiotic resistance (Adzitey et al., 2020a; Adzitey et al., 2020b). This is because meats serve as rich sources of nutrients that promote the growth of pathogenic bacteria (Ahmad et al., 2018). In addition, many animals are colonized with bacteria (and sometimes viruses) which cause illness in both animals and humans – an example is COVID-19 involved in the current pandemic. Also, the presence of pathogenic bacteria which are resistant to many antibiotics (multidrug (antibiotic) resistant) is a global health and development threat (World Health Organization (WHO),

2020). Under the animal food contaminants program of the USA, biological hazards that are routinely monitored include *Salmonella* and *Escherichia coli* (*E. coli*) bacteria (USA-FDA, 2022).

2.2 *Escherichia coli*

Escherichia coli bacteria are Gram-negative rod-shaped, facultative anaerobic and non-spore-forming commonly found in the gastrointestinal tract of humans and animals (Tenaillon et al., 2010). *Escherichia coli* cells are about 2.0µm long and 0.25 to 1.0µm in diameter, with a cell volume of 0.6 to 0.7µm³ (Tenaillon et al., 2010; Yu et al., 2014). They also ferment glucose or lactose and (like the closely related *Salmonella* bacteria) are members of the Enterobacteriaceae family (Tenaillon et al., 2010). *Escherichia coli* grows at 7°C to 50°C, with an optimum temperature of 37 °C (WHO, 2018). They can also grow in low acidic foods with a pH of 4.4 and higher, and in foods with a minimum water activity (a_w) of 0.95 (WHO, 2018). Most *Escherichia coli* strains are harmless and form part of the normal flora of the gastrointestinal tract of animals and humans (Kaper et al., 2014); however, pathogenic strains can cause bloody diarrhoea, anaemia, urinary tract infection, meningitis, peritonitis and even death (Winstead et al., 2020). Plate 4 shows a typical *Escherichia coli* and its parts.

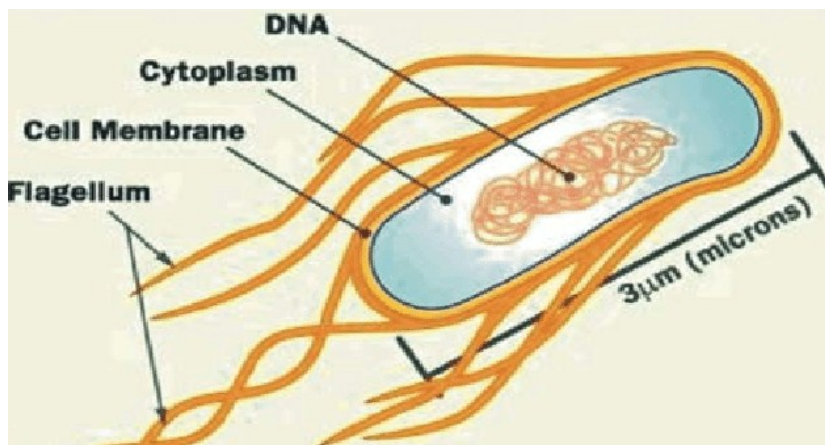


Plate 4: A typical *Escherichia coli* and its parts.

Source: https://www.researchgate.net/figure/Structure-of-E-coli-Source-http-wwwnature-educationorg-waterecolihtml_fig1_337673169

Escherichia coli are classified into two major groups, namely, intestinal or diarrheagenic *Escherichia coli* (DEC) and extraintestinal *Escherichia coli*. The DEC is divided into six pathotypes, namely, Enteropathogenic *E. coli*, Enterohaemorrhagic *E. coli*, Shiga toxin-producing *E. coli*, Enteroinvasive *E. coli*, Enteroaggregative *E. coli*, Enterotoxigenic *E. coli*, Verotoxigenic *E. coli*, and diffusely adherent *E. coli* (Kaper et al., 2014; Winstead et al., 2020). The extraintestinal *E. coli* group includes subtypes causing neonatal meningitis, uropathogenic *E. coli*, and sepsis-associated *E. coli* (Winstead et al., 2020). The various virulence genes present differentiate these pathotypes.

Strains of *Escherichia coli* from meat and meat products that produce dangerous toxins have been implicated in many foodborne outbreaks. Centers for Disease Control and Prevention (CDC) (2019a) showed that an outbreak of *Escherichia coli* from ground beef infected 209 people, out of which twenty-nine (29) were hospitalized, two (2) developed hemolytic uremic syndrome (a type of kidney failure) with no deaths. Also, *Escherichia coli* from ground bison caused thirty-three (33) outbreak cases, eight (8) hospitalizations with no death and hemolytic uremic syndrome (CDC, 2019b). According to the European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC) (2021), Shiga toxin-producing *Escherichia coli* was the fourth most frequent bacterial agent detected in foodborne outbreaks in the EU, with 34 outbreaks, 208 cases, 30 hospitalizations and 1 death recorded. In Ghana, *E. coli* have been isolated from various meat and meat products (Abass et al., 2020; Adzitey, 2015a; Adzitey, 2015b; Adzitey et al., 2018; Adzitey et al., 2019; Adzitey, 2020; Adzitey et al., 2020a; Adzitey et al., 2020b; Adzitey et al., 2021).

2.3 *Salmonella* species

Salmonella species are closely related to *E. coli*, and are also Gram-negative, facultative, rod-shaped, non-spore-forming and predominantly motile bacteria possessing peritrichous flagella (Fàbrega and Vila, 2013; Ryan et al., 2017). They are catalase positive, oxidase negative and are generally small enterobacteria with a diameter ranging from 0.7µm to 1.5µm and a length of 2.0µm to 5.0µm (Fàbrega and Vila, 2013). *Salmonella* species grow optimally at a temperature of 35 to 37°C, pH of 6.5 to 7.5 and water activity of 0.84 to 0.94 (Fàbrega and Vila, 2013; Ryan et al., 2017). The primary source of *Salmonella*

species are the gastrointestinal tracts of humans and animals (WHO, 2018). Plate 5 shows a typical *Salmonella* species.

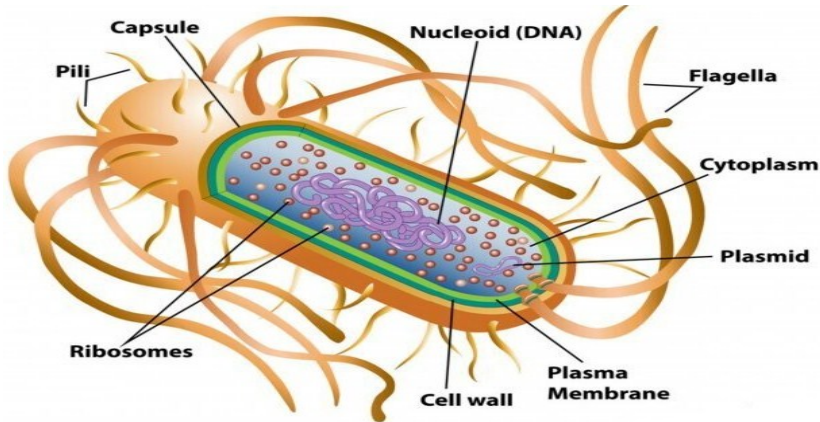


Plate 5: A typical *Salmonella* species and its parts.

Source: <https://biologyease.com/salmonella/>

There are two main species of *Salmonella*, thus, *Salmonella enterica* and *Salmonella bongori* (Ryan et al., 2017). *Salmonella enterica* is further divided into six subspecies that include over 2,600 serotypes (Gal-Mor, et al., 2014). *Salmonella* serotypes can also be divided into two main groups - typhoidal and nontyphoidal (Hendriksen et al., 2011) depending on their ability to develop specific pathologies in humans. Nontyphoidal serotypes are zoonotic and can be transferred from animal-to-human and from human-to-human. Typhoidal serovars (*S. Typhi*, *S. Paratyphi*, *S. sendai*) are specifically human pathogens, and do not infect animals. They can cause foodborne and waterborne infections, typhoid fever, and paratyphoid fever (Gal-Mor et al., 2014). Also people who have recovered from *S. Typhi* infection sometimes continue to excrete *S. Typhi* for years afterwards - and infecting others. Examples of nontyphoidal *Salmonella* serovars are *S. Enteritidis*, *S. Typhimurium*, *S. London*, *S. Dublin*, *S. Gallinarum*, among others.

Salmonella has been studied for very many years (the first scientific papers date back to the 1880s) and causes very many infections/outbreaks in both humans and animals world-wide. According to CDC (2022a), *Salmonella* bacteria caused about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the

United States every year. Food is the source of most of these illnesses. For instance, *Salmonella* outbreak due to backyard poultry was reported by CDC (2022b). This outbreak resulted in 219 illnesses, 27 hospitalizations and one death. In 2021, CDC reported another *Salmonella* outbreak due to backyard poultry, where 1,135 people were taken ill, 273 were hospitalized and 2 deaths were recorded (CDC, 2021). A total of 694 foodborne outbreaks resulting from *Salmonella* were reported to cause 3,686 illnesses, 812 hospitalizations and seven deaths in the EU (EFSA and ECDC, 2021). In addition, *Salmonella* was the second most frequent bacterial agent detected in foodborne outbreaks. It is well-known that numbers of cases of *Salmonella* are underreported world-wide. In Ghana, *Salmonella* species, including the typhoidal strains, can readily be isolated from meat and meat products (Aduah et al., 2021; Adzitey et al., 2014; Adzitey et al., 2015; Adzitey et al., 2020c; Ekli et al., 2020; Tay et al, 2019).

2.4 Antibiotic resistance and residues

Antibiotics are drugs that kill (bactericidal) or slow (bacteriostatic) the growth of bacteria without harming humans or animals. Broad-spectrum antibiotics treat a wide range of infections, while narrow- spectrum antibiotics are effective against only a few types of bacteria (Adzitey, 2015c; Melander et al., 2018). Antibiotics are essential in the treatment of human and animal infections caused by bacteria. Despite the importance of antibiotics, their uses have received much criticism due to the development of resistance by bacteria. The development of antibiotic resistant bacteria normally happens when antibiotics are abused in their usage. Abuse happens when farmers use antibiotics intentionally or unintentionally without the requisite knowledge (Melander et al., 2018).

Similarly, the unnecessary use of antibiotics in human medicine also contributes to the development of antibiotic resistance, which can be passed from harmless bacteria to pathogenic bacteria. Almuzaini et al. (2013) reported that, in some countries antibiotics are available to the public without a prescription and some are substandard and/or counterfeit. Plate 6 shows typical antibiotic tablets.



Plate 6: Typical antibiotic capsules

Antibiotic resistance infections can increase the length and cost of hospitalization, reduce productivity and cause death. For instance, the CDC (2013) estimated the annual impact of resistant infections to be \$20 billion in excess health care costs and 8 million additional hospital days in the United States. In the European Union, resistant infections caused over €1.6 billion and 2.5 million additional hospital days (European Medicine Agency, 2009).

Antibiotic residues are metabolites or compounds present in trace amounts in edible animal tissues or products after the administration of antibiotics (Bacanli and Başaran, 2019). Antibiotic residues occur in animal tissues when animals are treated with antibiotics and withdrawal periods are not observed prior to slaughter. There are maximum permissible levels of antibiotic residues allowed in animal tissues (Codex Alimentarius, 2018). There is evidence of the presence of antibiotic residues in pork, beef, chevon, mutton, chicken, liver, kidney among others of farm animals (Akansale et al., 2019; Ekli et al., 2020; Vishnuraj et al., 2016). Antibiotic residues observed in these meats include amoxycilin, chlorotetracycline, ciprofloxacin, danofloxacin, doxycycline, norfloxacin, oxytetracycline, sulfadiazine and tylosine.

2.5 Whole genome sequencing

Investigating bacteria based on the genetics has been carried out using a number of techniques including enterobacterial repetitive intergenic consensus (ERIC), random amplified polymorphic

deoxyribonucleic acid (RAPD), repetitive extragenic palindromic (REP), pulse-field gel electrophoresis (PFGE), and multi-locus sequence typing (MLST) (Adzitey et al., 2013; Molechan et al., 2017; Pillay et al., 2020). Recent advances have promoted the use of whole genome sequencing (WGS) and metagenomics. Nonetheless, WGS of pathogens has become the reference standard due to its accessibility and affordability, and has revolutionized the field of outbreak investigations (Nutman and Marchaim, 2019). WGS enables the genome of a bacterium to be sequenced and helps in the in silico prediction of various traditional typing methods, which explore short DNA strands with a quick turnaround time comparatively (Lynch et al., 2016). Plate 7 shows the process of whole genome sequencing.

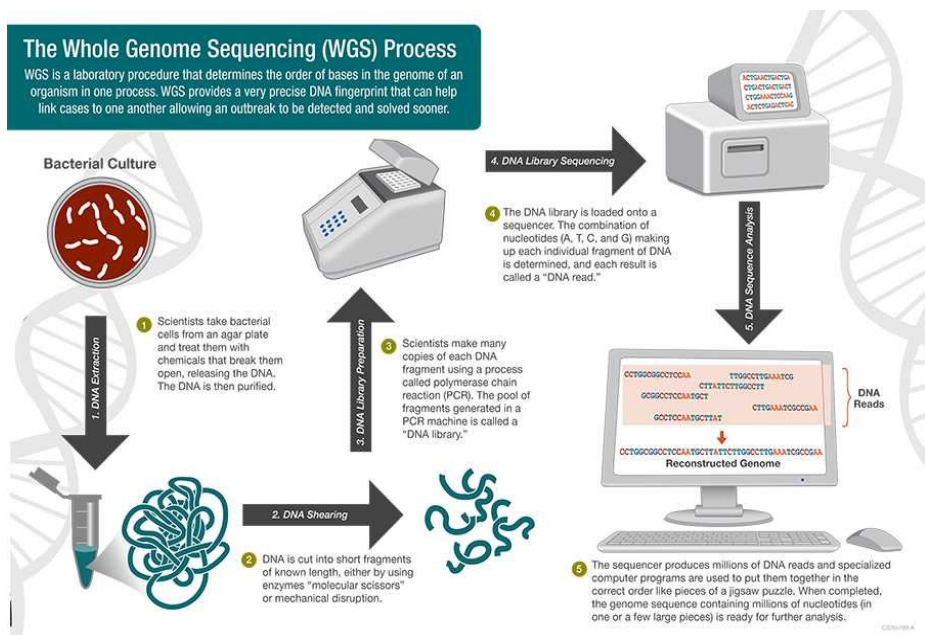


Plate 7: Whole genome sequencing process.

Source: <http://medbox.iab.me/modules/en-cdc/www.cdc.gov/pulsenet/pathogens/protocol-images.html#wgs>

Studies on the knowledge of farmers, butchers/meat sellers and consumers in meat safety in Ghana are limited. Data on contamination rate of *Escherichia coli* and *Salmonella enterica* in meats/meat products, their resistance to antibiotics, as well as antibiotic residues in meats/meat products and the genomic

characterization of *Escherichia coli* and *Salmonella* from meat sources in Ghana are also limited.

3.0 Sample of Relevant Research Studies

3.1 Knowledge and perception of farmers, butchers/meat sellers and consumers in meat safety

Knowledge acquisition and positive perception of farmers, butchers/meat sellers and consumers towards meat safety is very important for the prevention of foodborne disease outbreaks that will result from the consumption of meat. Through this, farmers will engage in sustainable and safe animal production practices including the understanding of animal welfare issues. Butchers/meat sellers will handle meats under hygienic conditions to prevent contaminations by biological, chemical and physical hazards. Consumers can simply demand for animals and meats produced under hygienic conditions using good production practices to propel the agenda of meat safety. Therefore, adherence to meat safety among farmers, butchers/meat sellers and consumers is critical for the prevention of meat-borne diseases to protect public health.

3.1.1 Knowledge and perception of farmers, butchers/meat sellers and consumers in microbiological meat safety

The study showed that majority of the farmers, butchers/meat sellers and consumers (respondents) had heard about microbiological meat safety (Table 1). For farmers, extension officers played a major role in this, while veterinary officers and teachers played a major role in this for butchers/meat sellers and consumers, respectively. The role of media in ensuring that people hear of meat safety cannot be overemphasized. Most of the butchers/meat sellers and consumers also knew poor animal/meat handling can cause the meat to be contaminated with germs/bacteria, but not the farmers. This is worrying since farmers will handle their animals without recourse to best practices. Nonetheless, these farmers knew animals carry bacteria in/on them which can be transmitted onto meats and can cause meat-borne diseases. The majority of butchers/meat sellers and consumers knew that the observance of meat hygiene by meat handlers reduces the risk of meat contamination. Positively, all respondents pointed out that refrigeration was the best method for meat preservation to prevent spoilage. The majority of the respondents had not

heard that specific bacteria such as *Campylobacter* spp., *Salmonella* spp., *E. coli* among others can cause foodborne diseases. However, bacteria, including *Salmonella*, *E. coli*, etc have been implicated in a number of foodborne diseases resulting in illness, hospitalization, recovery or death.

Table 1: Knowledge and perception of farmers, butchers/meat sellers and consumers on microbiological meat safety

Variable	Farmers		Meat sellers/ butchers		Consumers	
	Freq.	Per. (%)	Freq.	Per. (%)	Freq.	Per. (%)
Heard of meat safety						
Yes	93	67	198	71	245	64
No	45	33	80	29	137	36
If, yes from who/where?						
Veterinary officer	2	2	104	53	18	8
Extension officer	62	67	18	9		
Teacher/school					148	62
Media	21	23	76	38	59	25
Colleagues/friends	8	9			12	5
Poor animal/meat handling can cause meat to be contaminated with germs/bacteria						
Yes	41	30	165	62	184	69
No	97	70	103	38	84	31
Observance of meat hygiene by meat handlers reduces the risk of meat contamination						
Yes			174	66	241	73
No			89	34	89	27
Animals carry bacteria in/on them which can be transmitted onto meats and can cause meat-borne diseases						
Yes	87	63				
No	51	37				
Best method to preserve meat to reduce/prevent contamination						
Refrigeration	64	46	167	60	221	61
Salting	43	31	19	7	72	20
Smoking	22	16	28	10	69	19
Drying/frying	9	7	63	23	3	1

Source: Anachinaba et al. (2022a-c)

3.1.2 Knowledge and perception of farmers, butchers/meat sellers and consumers about antibiotic resistant

This study also found that the majority of the respondents had heard about antibiotic resistance (Table 2). Farmers and butchers/meat sellers heard about it mainly from veterinary/health officers, while consumers heard about it mostly from their school teachers. Interestingly, most of the farmers did not know that antibiotic resistance occurs in bacteria/germs, antibiotic resistance occurs when bacteria develop the ability to survive exposure to antibiotics, infections caused by antibiotic resistant bacteria are difficult to treat, humans can consume meat contaminated by antibiotic resistant bacteria and the more antibiotics we use, the higher is the risk that resistance develops and spreads, but most consumers new these. Again, this is disturbing since farmers play a major role in the spread of antibiotic resistance bacteria/germs. The majority of the respondents had ever used antibiotics such as azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin and tetracycline. While, 52% of the farmers disagreed that animals being treated with antibiotics are sometimes slaughtered for sale, 59% of the butchers/meat sellers agreed that animals on antibiotics are sometimes slaughtered for sale. More than half of the farmers (53%) and butchers/meat sellers (67%) disagreed that locally produced meats on the Ghanaian market sometimes contain antibiotic resistant bacteria, but this was not the case for consumers as 74% of them agreed that locally produced meats on the Ghanaian market sometimes carry antibiotic resistant bacteria.

Table 2: Knowledge and perception of farmers, butchers/meat sellers and consumers on antibiotic resistance

Variable	Farmers		Meat sellers/ butchers		Consumers	
	Freq.	Per. (%)	Freq.	Per. (%)	Freq.	Per. (%)
Heard of antibiotic resistance						
Yes	92	69	145	52	213	55
No	42	31	133	48	171	45
If, yes from who/where?						
Veterinary/Health officers	74	80	104	53	24	11
Teacher/School			18	9	119	56
Media	10	11	76	38	58	27

Colleague farmers, friends	8	9	12	6
Antibiotic resistance occurs in bacteria/germs				
Yes	29	27	205	64
No	17	16	94	29
I do not know	62	57	20	6
Antibiotic resistance occurs when bacteria develop the ability to survive exposure to antibiotics				
Yes	12	10	187	51
No	16	13	93	25
I do not know	95	77	87	24
Infections caused by antibiotic resistant bacteria are difficult to treat				
Yes	27	21	187	51
No	16	12	93	25
I do not know	86	67	87	24
Human can consume meat contaminated by antibiotic resistant bacteria				
Yes	18	14	78	21
No	30	23	97	27
I do not know	81	63	189	52
The more antibiotics we use, the higher is the risk that resistance develops and spreads				
Yes	17	13	128	37
No	27	21	103	29
I do not know	84	66	119	34

Source: Anachinaba et al. (2022a-c)

3.1.3 Knowledge and perception of farmers, butchers/meat sellers and consumers in antibiotic residues

This work revealed that most of the butchers/meat sellers and consumers had heard about antibiotic residues, but not the farmers (Table 3). The farmers and butchers/meat sellers who had heard about antibiotic residues heard of it from veterinary officers (45% and 60%, respectively), while consumers heard about it mostly from school or their teachers (58%). Generally, most of the respondents did not know that antibiotic residues are molecules that remain in meat from animals that have been treated with antibiotics, antibiotic residues in meat can be reduced by observing withdrawal periods, antibiotic residues can be transferred from meat to humans via consumption and animal farmers play a significant role in antibiotic resistant residues in meat. The majority of

the consumers had heard about antibiotic residues such as danofloxacin, oxytetracycline, tylosin and chloramphenicol but not the farmers and butchers/meat sellers. The majority of the respondents disagreed that locally produced meats on the Ghanaian market sometimes contain antibiotic residues.

Table 3: Knowledge and perception of farmers, butchers/meat sellers and consumers on antibiotic residues

Variable	Farmers		Meat sellers/ butchers		Consumers	
	Freq.	Per. (%)	Freq.	Per. (%)	Freq.	Per. (%)
Heard of antibiotic residues						
Yes	51	37	126	51	193	53
No	87	63	121	49	168	47
If, yes from who/where?						
Veterinary officer	23	45	75	60	34	18
Teacher/school			16	13	110	58
Colleagues			15	12	12	6
Extension officer	21	41				
Media	7	14	20	16	34	18
Antibiotic residues are molecules that remain in meat from animals that have been treated with antibiotics						
Yes	43	31	74	27	132	35
No	22	16	62	22	79	21
I do not know	73	53	142	51	171	45
Antibiotic residues in meat can be reduced by observing withdrawal periods						
Yes	23	17	65	23	97	26
No	28	20	54	19	121	32
I do not know	87	63	159	57	162	43
Antibiotic residues can be transferred from meat to humans via consumption						
Yes	20	14	62	22	83	22
No	27	20	48	17	137	36
I do not know	91	66	168	60	157	42
Animal farmers play significant role in antibiotic resistant residues in meat						
Yes	22	16	104	37	146	38
No	27	20	46	17	89	23
I do not know	89	64	128	46	146	38

Source: Anachinaba et al. (2022a-c)

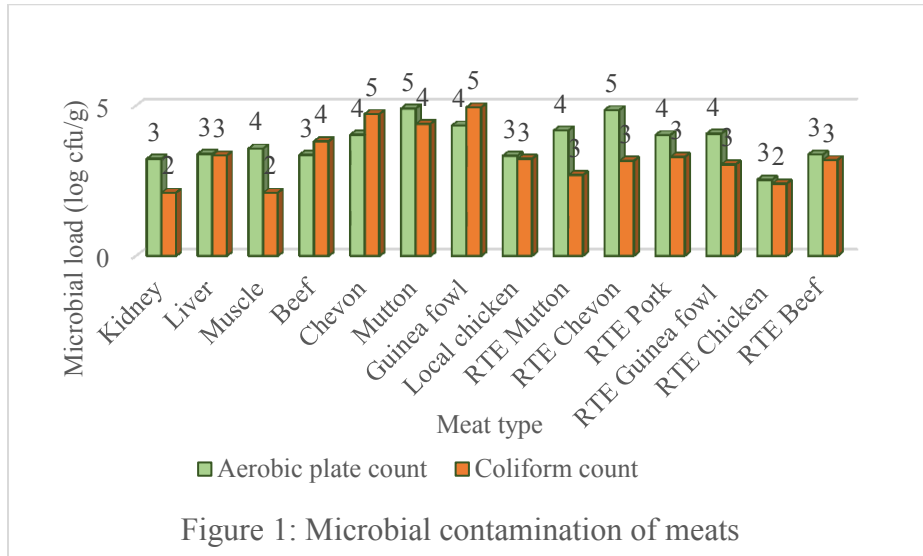
3.2 Contamination of meats by bacteria in Ghana

Meats contaminated by bacteria pose health risks to consumers and threaten public health. Nonetheless, meat consumption will continue to increase as the world gets richer. It has been estimated that 346.14 million tons of meat is consumed globally by humans each year, and in 2030 this number will be 453 million - a 44% increase (The World Counts, 2022). Increase in meat consumption in Ghana is also eminent; as meat serves as part of the daily menu of some Ghanaians (Nkegbe et al., 2013). The muscle of a healthy living animal is essentially sterile. The muscle usually gets contaminated by bacteria after the animal has been slaughtered and the outer covering of the meat removed. These bacteria live in the animal, on the animal and in the environment. Meats normal get contaminated with bacteria through poor animal handling and faulty processing of animals into meat. Also, cross-contamination occurs after meat processing when meats are not handled hygienically. Food-borne diseases and their associated conditions can result from the consumption of contaminated meat.

3.2.1 Contamination of meats using aerobic bacteria count and coliform count

All the meats examined were contaminated when examined by aerobic plate count and coliforms at varying levels (Figure 1). The aerobic plate count is an estimate of bacterial populations in a product. It is commonly employed to indicate the sanitary quality of foods, sensory acceptability, and conformance with good manufacturing practices (Mendonca et al., 2020). Coliform bacteria are also a group of bacteria considered as indicator organisms and their presence signify faecal contamination and the availability of enteric pathogens (Martin et al., 2016). High numbers detected by aerobic plate and coliform count suggest that the meats were processed under relatively unsanitary conditions and/or stored for too long at relatively high temperatures. Adzitey et al. (2018), Sulleyman et al. (2018), Adzitey et al. (2020c) and Adzitey et al. (2020d) confirmed that animals are slaughtered, processed and sold under unhygienic conditions in Ghana. Although most aerobic plate count and coliform bacteria do not necessarily indicate that the bacteria are harmful, some can cause sickness, especially in children and the elderly (Martin et al., 2016; Mendonca et al., 2020). A person that has been exposed to these bacteria may have an upset stomach, vomiting, fever, or diarrhoea. Nonetheless, the aerobic

plate and coliform counts were within acceptable limits ($\leq 5 \log \text{cfu/g}$) set by the Ghana Standard Authority (Ghana Standard Authority, 2020). According to Kim et al. (2018), many countries control meat hygiene by regulating or recommending APC limits in meat. Most countries regulate or recommend the APC level in meat at under $5 \times 10^6 \text{cfu/g}$ or cm^2 ($6.7 \log \text{cfu/g}$ or cm^2).



Source: Abass et al. (2020), Adzitey et al. (2020), Ekli et al. (2020) and Aduah et al. (2021). RTE= ready-to-eat.

3.2.2 Contamination of meats by *Escherichia coli* and *Salmonella enterica*

This study showed that most meat samples were contaminated by *Escherichia coli* and *Salmonella enterica* (Table 4). On average, the contamination rate of the meats by *Escherichia coli* (55%) was higher than *Salmonella enterica* (27%). In addition raw meats were more contaminated than ready-to-eat meats. This is expected since RTE meats in Ghana are prepared using adequate heat, which should destroy all microbes. However, cross-contamination is possible due to improper personal hygiene and handling after preparation. Both *Escherichia coli* and *Salmonella enterica* live in the gastrointestinal tracts (GIT) of farm animals. They contaminate meats when the intestines are ruptured during slaughtering. Cross-contamination can also occur after slaughter as a result of faulty and unhygienic handling of meats. These bacteria can grow and multiply faster on

meats since meat serves as an ideal medium for their growth coupled with the favourable ambient temperature under which meats in Ghana are handled (i.e. normally no refrigeration). Nevertheless, *Escherichia coli* and *Salmonella enterica* should be absent in cooked meats meant for human consumption.

Since most of the meat samples were contaminated with *Escherichia coli* and *Salmonella enterica*, consumers of meat in Ghana are at risk of contracting *Escherichia coli* and *Salmonella* infections from meat consumption. Both *Escherichia coli* and *Salmonella* bacteria can cause diarrhoea, nausea, vomiting, stomach cramps, urinary tract infections, respiratory illness, bloodstream infections, and other illnesses (WHO, 2018; Winstead et al., 2020). *Escherichia coli* and *Salmonella enterica* infections can be lethal, especially in immune-compromised individuals, the young and old (Gal-Mor et al., 2014; CDC, 2022a). The symptoms of *Escherichia coli* and *Salmonella enterica* infections usually begin 12 hours to 6 days after infection and can last for 4 to 7 days (Gal-Mor et al., 2014; WHO, 2018; Winstead et al., 2020; CDC, 2022a). Most people get better within 5 to 7 days, without needing to take antibiotics, but antibiotics are typically used to treat people with severe illnesses or at risk of it.

Table 4: Contamination of meat samples by *Escherichia coli* and *Salmonella enterica*

Source of meat	Area	Contamination rate (%)	
		<i>Escherichia coli</i>	<i>Salmonella enterica</i>
Kidney	Wa	92	10
Liver	Wa	98	32
Muscle	Wa	88	30
Beef	Tamale	87	42
Chevon	Tamale	76	49
Mutton	Tamale	89	73
Local chicken	Tamale	80	29
Guinea fowl	Tamale	89	44
Beef	Tema	67	65
Chicken	Tema	41	13
Pork	Tema	23	8

Beef	Techiman	92	75
RTE Mutton	Bolgatanga	8	2
RTE Chevron	Bolgatanga	20	2
RTE Pork	Bolgatanga	18	2
RTE Guinea fowl	Bolgatanga	6	4
RTE Chicken	Bolgatanga	8	2
RTE Beef	Bolgatanga	16	0
Average		55	27

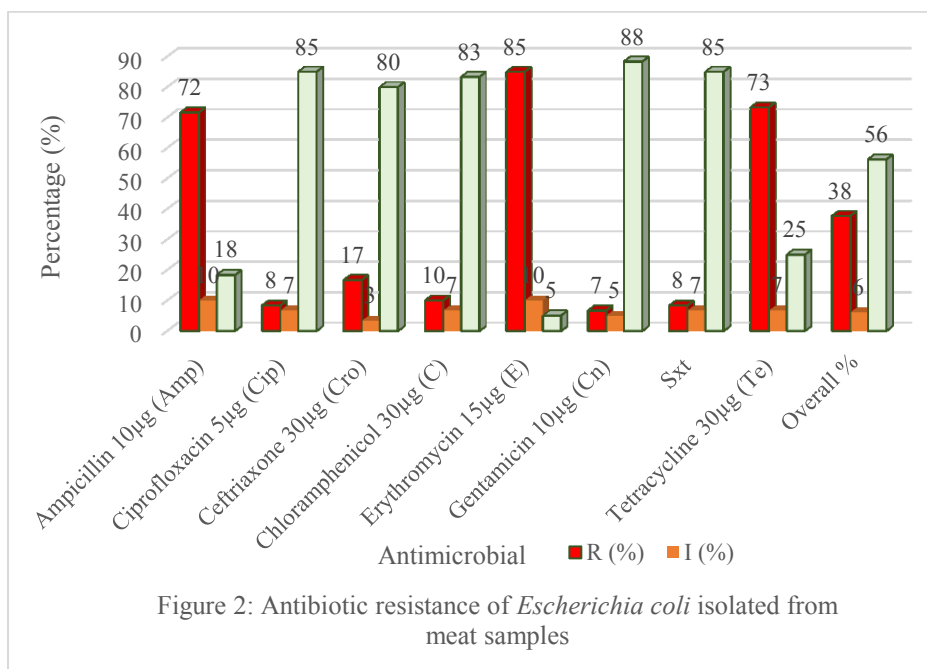
Source: Abass et al. (2020), Adzitey et al. (2020), Ekli et al. (2020) and Aduah et al. (2021). RTE= ready-to-eat.

3.3 Antibiotic resistance of bacteria isolated from meats in Ghana

In Ghana, antibiotics are mainly used by farmers for the treatment of sick animals; only a few farmers use antibiotics for prophylactic and growth promotion purposes (Abass et al., 2020; Akansale et al., 2019; Ekli et al., 2019). Unrecovered or sick animals are sometimes sold to butchers, sold at the market, slaughtered for meat, buried or consumed at home (Abass et al., 2021). It is bad practice for farmers to sell animals that do not recover from their sickness following antibiotic treatments. These animals may end up at the abattoir and be processed into meats, which will enter the food chain. The meats produced from these animals are potential sources of antibiotic resistant bacteria, which can easily be consumed by humans, thus increasing the antibiotic resistance burden. Nonetheless, farmers consult veterinary officers when their animals are sick and rely on both veterinarians and colleague farmers in the administration of drugs to their animals (Abass et al., 2021; Akansale et al., 2019; Ekli et al., 2020). Some farmers in Ghana do not follow withdrawal periods before selling their animals for slaughter (Abass et al., 2021; Ekli et al., 2020). Most farmers were also unaware that misuse of antibiotics can promote pathogen resistance and some do practice self-medication (Akansale et al., 2019). Antibiotics such as tetracycline, gentamicin, penicillin amoxyciline/clavanic and ciprofloxacin were used by farmers in their farming business (Abass et al., 2021; Ekli et al., 2020).

3.3.1 Antibiotic resistance of *Escherichia coli* isolated from meats

Results of this study showed that *Escherichia coli* from raw meat samples were highly resistant ($\geq 72\%$) to ampicillin, erythromycin and tetracycline, but susceptible ($\geq 80\%$) to ciprofloxacin, ceftriaxone, chloramphenicol, gentamicin and suphamethoxazole/trimethoprim (Figure 2). The multiple antibiotic index ranged from 0.11 (resistant to one antibiotic) to 0.56 (resistant to five antibiotics) and 27% of the isolates exhibited multidrug resistance.



S = Susceptible; I = Intermediate; R = Resistant; Sxt = Suphamethoxazole/trimethoprim 25µg Source: Adzitey et al. (2020c)

Escherichia coli isolated from RTE meats also exhibited high resistance ($\geq 71\%$) to amoxicillin/clavulanic acid, azithromycin, teicoplanin and tetracycline but susceptible ($\geq 93\%$) to chloramphenicol (Table 5). The multiple antibiotic index ranged from 0.22 (resistant to one antibiotics) to 0.78 (resistant to seven antibiotics). Multidrug resistance (94%) was high among the *Escherichia coli* isolates.

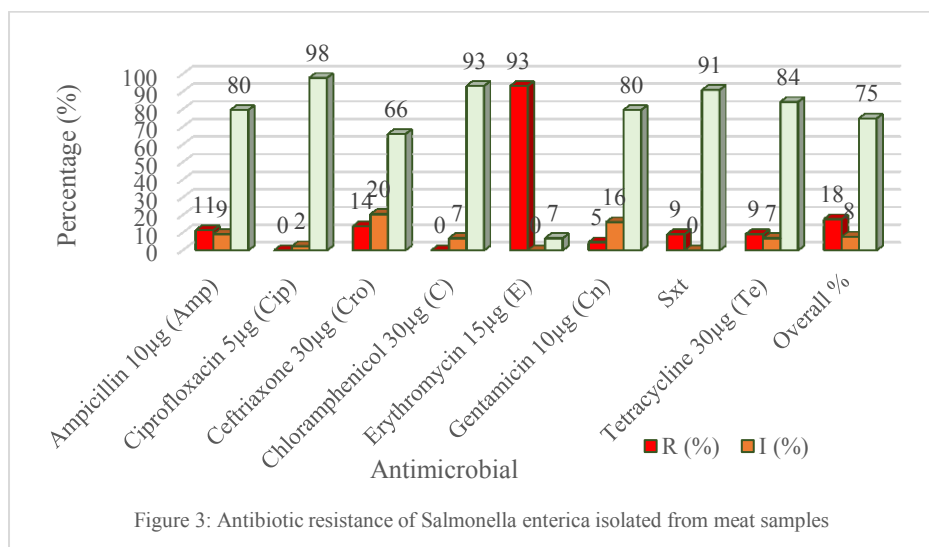
Table 5: Antibiotic resistance of the *Escherichia coli* isolated from ready-to-eat meats

Antimicrobial	R (%)	I (%)	S (%)
Amc	71	0	29
Azithromycin 15µg (Azm)	71	16	13
Ceftriaxone 30µg (Cro)	19	23	58
Chloramphenicol 30µg (C)	6	0	94
Ciprofloxacin 5µg (Cip)	23	16	61
Gentamicin 10µg (CN)	23	39	39
Teicoplanin 30µg (TEC)	97	0	3
Tetracycline 30µg (Te)	94	6	0
Sxt	58	26	16

S = Susceptible; I = Intermediate; R = Resistant; Amc = Amoxycillin/clavulanic acid 30µg; Sxt = Suphamethoxazole/trimethoprim 25µg; Source: Abass et al. (2020)

3.3.2 Antimicrobial resistance of *Salmonella enterica* from meats

The *Salmonella enterica* of meat origin were highly resistant to erythromycin ($\geq 93\%$) but susceptible ($\geq 80\%$) to ampicillin, ciprofloxacin, chloramphenicol, gentamicin, suphamethoxazole/trimethoprim and tetracycline. The multiple antibiotic index ranged from 0.13 (resistant to one antibiotic) to 0.63 (resistant to five antibiotics) and 11% exhibited multidrug resistance.



S = Susceptible; I = Intermediate; R = Resistant; Sxt = Suphamethoxazole/trimethoprim 25µg Source Adzitey et al. (2020b).

Ready-to-eat *Salmonella enterica* isolates showed high resistance ($\geq 83\%$) to azithromycin and teicoplanin, but susceptible ($\geq 83\%$) to chloramphenicol, ciprofloxacin and suphamethoxazole/trimethoprim. The multiple antibiotic index ranged from 0.22 (resistant to two antibiotics) to 0.56 (resistant to five antibiotics) and 67% exhibited multidrug resistance.

Table 6: Antibiotic resistance of the *Salmonella enterica* isolated from ready-to-eat meats

Antimicrobial	R (%)	I (%)	S (%)
Amc	67	0	33
Azithromycin 15µg (Azm)	83	0	17
Ceftriaxone 30µg (Cro)	0	50	50
Chloramphenicol 30µg (C)	0	17	83
Ciprofloxacin 5µg (Cip)	0	17	83
Gentamicin 10µg (Cn)	17	50	33
Teicoplanin 30µg (Tec)	100	0	0
Tetracycline 30µg (Te)	50	50	0
Sxt	0	0	100
Overall %	35	20	44

S = Susceptible; I = Intermediate; R = Resistant; Amc = Amoxycillin/clavulanic acid 30µg; Sxt = Suphamethoxazole/trimethoprim 25µg; Source: Aduah et al. (2021)

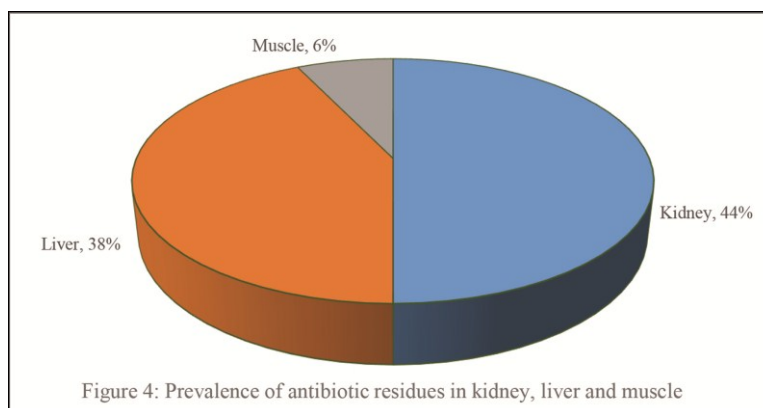
Antibiotic resistance is still a problem in the treatment of bacterial infections, especially where infections are common. Bacteria with a MAR index of greater than 0.2 come from a high-risk source of frequent antibiotic drug usage or feed additives, whereas bacteria with a MAR index of less than 0.2 come from a source of infrequent antibiotic drug usage (Davis and Brown, 2016). The misuse/overuse of antibiotics by livestock farmers in animal farming and by humans as medications have contributed to the rise in antibiotic resistance rates in Ghana. Intermediate resistances were observed against some of the antibiotics. Intermediate resistance isolates are those that are not clearly resistant or susceptible; they suggest potential future resistance and such isolates are difficult to treat when they are involved in infections. Multidrug resistance (resistant to 3 or more different classes of antibiotics) were observed in the *Escherichia coli* and *Salmonella enterica* isolated from the meat sources. Multidrug resistance bacteria species have become a significant public health

problem across the globe in recent years since they restrict therapeutic choices accessible for animals and humans.

3.4 Antimicrobial residues in meats produced in Ghana

The use of antibiotics in Ghana is relatively unregulated. Consequently, animal farmers rarely observe withdrawal periods, contributing to the deposition of antibiotic residues in meats. These residues can cause immunogenic reactions and selectively favour the growth of antibiotic resistant bacteria in meats and consequently in humans when such meats are consumed. Exposure to antibiotic residues can also result in the alteration of microflora, allergy, immunopathological effects, mutagenicity, hepatotoxicity, reproductive disorders, nephropathy (gentamicin), bone marrow toxicity (chloramphenicol), and even carcinogenicity (sulphamethazine, oxytetracycline, furazolidone) (Bacanlı and Başaran, 2019). It has been demonstrated that meats produced in Ghana contain antibiotic residues (Abavelim, 2014; Akansale et al., 2019; Donkor et al., 2011; Ekli et al., 2020).

My studies showed that 44% of kidney, 38% of liver and 6% of muscles from cattle were contaminated by antibiotic residues (Figure 4). In another study, residues of penicillin G, sulfmethoxazole, erythromycin, sulfadizine, sulfamethazine, trimethoprim and sulfaquinoxalin were not detected in the beef, chicken and pork samples (Table 7). However, amoxicillin residues were detected in local beef (0.77 µg/kg) and local chicken (1.31 µg/kg) samples. Also, tetracycline was detected in pork (13.67 µg/kg), beef (5.99 µg/kg) and chicken (3.28 µg/kg) samples.



Source: Ekli et al. (2020)

Table 7: Occurrence of antibiotic residues in beef, chicken and pork

Antibiotic residues ($\mu\text{g}/\text{kg}$)	Beef	Chicken	Pork	Sem	P-value
Penicillin G	ND	ND	ND	ND#	ND#
Amoxicillin	0.77	1.31	ND	0.992	0.063
Tetracycline	5.99 ^b	3.28 ^b	13.67 ^a	3.798	0.001
Sulfmethoxazole	ND	ND	ND	ND#	ND#
Erythromycin	ND	ND	ND	ND#	ND#
Sulfadizine	ND	ND	ND	ND#	ND#
Sulfamethazine	ND	ND	ND	ND#	ND#
Trimethoprim	ND	ND	ND	ND#	ND#
Sulfaquinolaxin	ND	ND	ND	ND#	ND#

ND = not detected; ND#, not done; Means with different superscript along the columns are different at $P < 0.05$ Source: Anachinaba et al. (2022)

Furthermore, antibiotic residues were detected in meats collected from other parts of Ghana (Figure 5). Antibiotic residues such as amoxycillin (18 vs 22 $\mu\text{g}/\text{kg}$), chlorotetracycline (6 vs 7 $\mu\text{g}/\text{kg}$), ciprofloxacin (16 vs 16 $\mu\text{g}/\text{kg}$), danofloxacin (10 vs 10 $\mu\text{g}/\text{kg}$), doxycycline (9 vs 10 $\mu\text{g}/\text{kg}$), norfloxacin (13 vs 14 $\mu\text{g}/\text{kg}$), oxytetracycline (10 vs 9 $\mu\text{g}/\text{kg}$), sulfadiazine (1 vs 1 $\mu\text{g}/\text{kg}$) and tylosin (17 vs 17 $\mu\text{g}/\text{kg}$) were found in the beef and chevon, respectively. Although, antibiotic residues were found in some of the meat samples examined in this study, the concentrations were all below the acceptable maximum residue levels (MRLs) reported by Codex Alimentarius (2018).

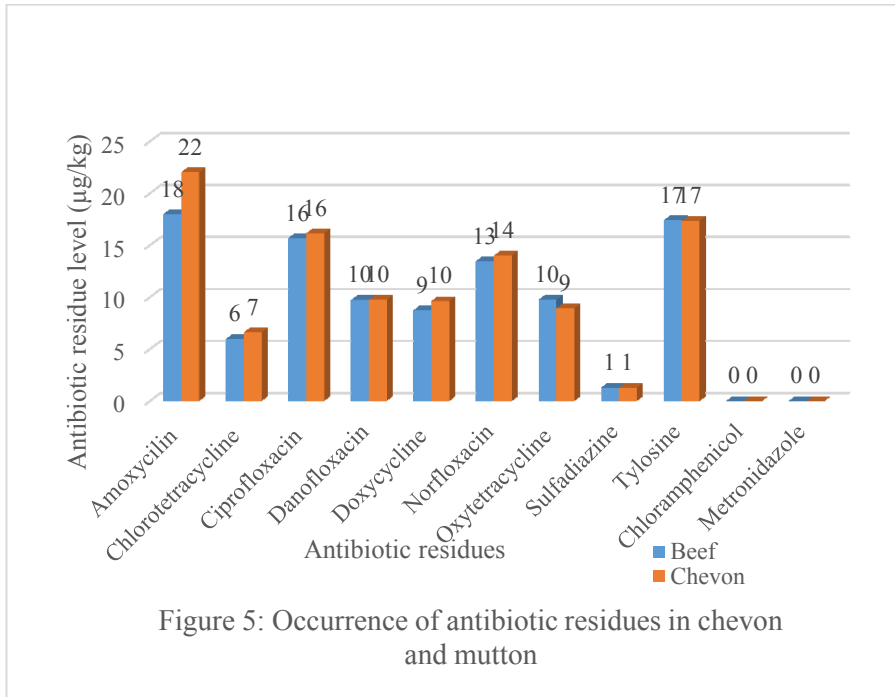


Figure 5: Occurrence of antibiotic residues in chevon and mutton

Source: Akansale et al. (2019)

3.5 Whole genome sequencing of bacteria isolated from meats in Ghana

Whole genome sequencing (WGS) reveals the complete DNA make-up of an organism, enabling us to better understand variations both within and between species. This in turn allows us to differentiate between organisms with a precision that other technologies do not allow. WGS has been widely adapted by the USA Food and Drugs Administration (FDA) for foodborne pathogen identification during foodborne illness outbreaks. FDA (2022) indicated that using WGS for outbreak investigations has helped to reduce foodborne illnesses and deaths over the long term both in the U.S and abroad.

Whole genome sequencing of the *Escherichia coli* strains from Ghana revealed varied MLST types (ST540 to ST1141) as shown in Table 8. The serotypes of the *Escherichia coli* isolates also varied widely (O11 to O113). These variations depict genetic diversity among the *Escherichia coli* strains. All the *Escherichia coli* strains possessed antibiotic resistance genes (ARG's) and 8 (57.1%) had

multiple resistance genes. The most prevalent ARG was *mdf* (A) conferring resistance to macrolide–lincosamide–streptogramin B antibiotics, found in all 14 *Escherichia coli* isolates. Genes that confer resistance to sulfonamides (*sul2* and *sul1*), aminoglycosides (*aph*(3'')-Ib, *aph*(6)-Id and *aadA5*), tetracycline (*tet*(A) and *tet*(B)), β -lactams (*bla*_{TEM-1B} and *bla*_{CTX-M-15}), trimethoprim (*dfrA17* and *dfrA14*), fluroquinolones (*qepA4* and *qnrS1*), fosfomycin (*fosA7*), and phenicols (*catA* and *catA2*) were also predicted to be present in the *Escherichia coli* isolates. Plasmids were present in eleven (11) of the *Escherichia coli* strains, with some strains having multiple plasmids. Plasmids are the extra chromosomal structures in the cells of bacteria which have the ability to self-replicate. Often, the genes carried in plasmids provide bacteria with genetic advantages, such as antibiotic resistance.

Table 8: Whole-genome sequencing characterization of *Escherichia coli* strains isolated from meats in Ghana

No	Isolate	Sample type	MLST ^a	Predicted serotype (s) ^b	Resistance genes ^c	Plasmid replicons ^d
1	AB1_S1	Beef	ST540	O11	<i>mdf</i> (A)	
2	CB1_S2	Beef	ST6646	H39	<i>mdf</i> (A)	IncFIB), IncFIC(FII), IncX1
3	NB12_S13	Beef	ST7483	O8	<i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>mdf</i> (A), <i>sul2</i> , <i>tet</i> (B)	
4	AC1_S3	Chevon	ST44	O162	<i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>bla</i> _{TEM-1B} , <i>qepA4</i> , <i>mdf</i> (A), <i>catA1</i> , <i>catA2</i> , <i>sul2</i> , <i>tet</i> (B)	Col156, IncFIA, IncFIB, IncFII
5	CC6_S5	Chevon	ST469	O8	<i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>bla</i> _{TEM-1B} , <i>mdf</i> (A), <i>sul2</i> , <i>tet</i> (A)	Col156, IncQ1
6	NC3_S4	Chevon	ST1727	O88	<i>fosA7</i> , <i>mdf</i> (A)	IncFIA, IncFIB, IncFII
7	Sg6_S9	Guinea fowl	ST69	O15	<i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>bla</i> _{TEM-1B} , <i>mdf</i> (A), <i>sul2</i> , <i>sul1</i> , <i>tet</i> (A), <i>dfrA17</i>	Col440I, IncFIB, IncFII, IncQ1
8	Tg1_S10	Guinea fowl	ST540	O9	<i>aph</i> (6)-Id, <i>aph</i> (3'')-Ib, <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1B} , <i>qnrS1</i> , <i>mdf</i> (A), <i>sul2</i> , <i>tet</i> (A), <i>dfrA14</i>	IncY
9	Tg5_S14	Guinea fowl	ST7473	O61	<i>mdf</i> (A)	Col156, IncFII, IncI1

10	SLC2_S11	Local Chicken	ST155	H9	<i>aadA5, mdf(A), sul2, tet(A), dfrA17</i>	Col440I, p0111
11	TLC1_S12	Local Chicken	ST297	H9	<i>mdf(A)</i>	IncFII
12	TLC13_S15	Local Chicken	ST155	O132	<i>aadA5, mdf(A), sul2, tet(A), dfrA17</i>	p0111
13	CM4_S8	Mutton	ST155	H40	<i>aadA5, mdf(A), sul2, tet(A), dfrA17</i>	
14	NM11_S7	Mutton	ST1141	O113	<i>qnrS1, mdf(A)</i>	

MLST = Multilocus sequence typing, ^a Using MLSTv.2.0., ^b Using SeroTypeFinderv.2.0, ^c Using ResFinderv.3.0 and ^d Using PlasmidFinderv.1.3. Source: Adzitey et al. (2019).

The tree analyses via the whole genome sequencing single nucleotide polymorphism (WGS SNP) tree differentiated the strains into three clusters (A, B, and C) with each cluster containing sub-clusters (Figure 6). Cluster A had the largest number of isolates (n = 9). The *Escherichia coli* isolates showed significant phylogenetic diversity with the phylogenomics showing greater resolution than the MLST technique. For instance, the sub-cluster C2 contained isolates (CM4, SLC2, and TLC13) from the same clone (ST155), but phylogenetically found on different sub-branches. The tree analyses coupled with metadata showed no patterns among the isolates with respect to their sources and areas.

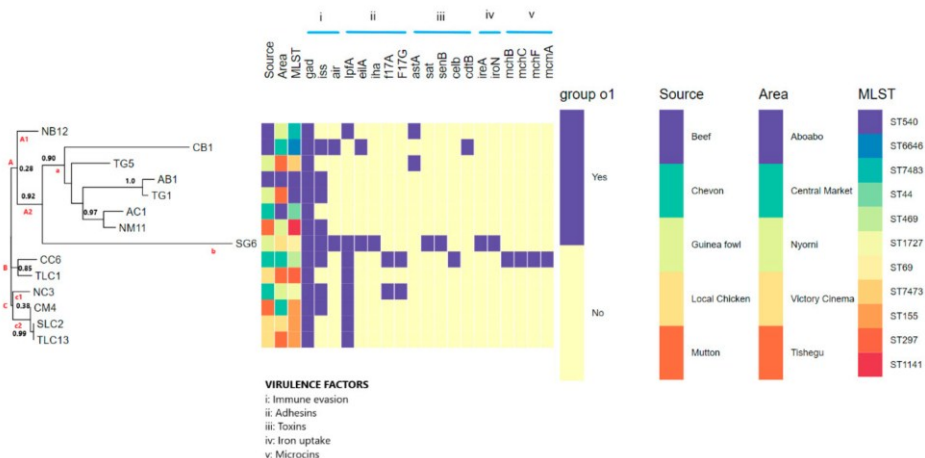


Figure 6: A phylogenomic branch coupled with metadata of *Escherichia coli* isolates using Phandango. The colour codes differentiate the different sources, areas, and STs. The virulence factors are represented by Roman numerals I: immune evasion, II: adhesins, III: toxins, IV: iron uptake, V: microcins. Source: Adzitey et al. (2020a)

The greater resolution power of WGS sequencing over MLST was shown by the clustering of similar STs on the different branches and in sub-clusters, giving credence to the need to shift to genomic investigation for a better understanding of the evolution of pathogens. Furthermore, tree analyses coupled with metadata insights depicted the diversity among the *Escherichia coli* isolates with no correlation with their meat sources and sampling areas. The genomes of the *Escherichia coli* predicted a 0.937 mean probability (P_{score}) of being a human host-pathogen and was found to match pathogenic families ranging between 525 and 1001 (Adzitey et al., 2020a). The whole-virulome analysis predicted differences in the virulence factors possessed by the isolates, that is, nineteen putative virulence genes of the five main classes (adhesins, toxins, immune evasion, iron uptake, and microcin) of *Escherichia coli* (Adzitey et al., 2020a).

Different MLST types were also observed among the *Salmonella enterica* isolates (Table 9). Seven isolates were not typed. This could represent novel *Salmonella enterica* species, although further analysis is required to confirm this observation. Antibiotic resistance gene was observed in one isolate (CM7_S24). Different *Salmonella enterica* serotypes (ST4605 to ST101) were also revealed by the WGS. Differences in MLST and serotypes reveal genetic differences in the *Salmonella enterica* strains. However, certain strains shared the same MLST types and serotypes, such isolates are similar and suggest possible cross-contamination. The *fosA7* gene that confers resistance to fosfomycin was found in one of the *Salmonella enterica* strains. Six of the strains also possessed single plasmid replicons.

Table 9: Whole-genome sequencing characterization of *Salmonella enterica* strains isolated from meats in Ghana

No.	Isolate	Sample type	MLST ^a	Predicted serotype(s) ^b	Resistance genes ^c	Plasmid replicon ^{sd}
1	AB11_S29	Beef	ST4605	Kaapstad		
2	CB5_S22	Beef	ST2469	Lagos		
3	NB10_S20	Beef	ST2609	II 13,22:z:-		
4	AC3_S26	Chevon	Nearest ST2478	Ouakam		
5	CC5_S25	Chevon	ST2469	Lagos		

6	NC6_S16	Chevon	ST603	Infantis		
7	Cg4_S30	Guinea fowl	Nearest ST683	Hato		Incl1
8	Sg14_S27	Guinea fowl	Nearest ST683	Hato		Incl1
9	Tg14_S17	Guinea fowl	Nearest ST683	Hato		Incl1
10	NLC13_S21	Local chicken	Nearest ST683	Hato		Incl1
11	SLC10_S19	Local chicken	ST3899	Hato		
12	TLC7_S23	Local chicken	Nearest ST3899	Hato		Incl1
13	AM10_S28	Mutton	Nearest ST2478	Ouakam		
14	AM9_S6	Mutton	ST4605	Kaapstad		
15	NM14_S18	Mutton	ST4605	Kaapstad		
16	CM7_S24	Mutton	ST101	Africana	fosA7	IncFII(S)

MLST = Multilocus sequence typing, ^a Using MLSTv.2.0., ^b Using SeroTypeFinderv.2.0, ^c Using ResFinderv.3.0 and ^d Using PlasmidFinderv.1.3. Source: Tay et al. (2019)

The phylogenetic relationship and epidemiological distribution of the *Salmonella enterica* isolates from meat samples is depicted in Figure 7. The isolates generally clustered according to their serovars and sequence types (clonal lineages). Of note, there were 7 clades (grouped A–G) and 1 subclade (A1, ST3899) which collaborated with the 8 clonal lineages. This affirms the ability of WGS-based typing methods to accurately differentiate between isolates using appropriately curated databases.

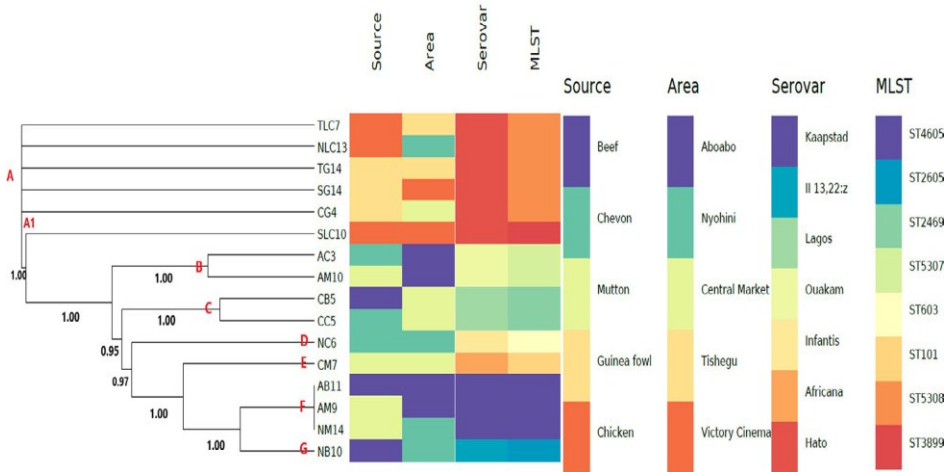


Figure 7: A phylogenomic branch coupled with metadata of *Salmonella enterica* isolates using Phandango. Source: Adzitey et al. (2020c)

The tree analysis coupled with metadata revealed useful insights with regard to the diversity of serovars clones in meat sources and areas of collection (Figure 7). For instance; meat sources; beef, chevon and mutton contained different serovars of *Salmonella enterica* isolates which were clonally distinct. However, all the guinea fowl and chicken samples contained the same serovar; *Salmonella* Hato strain which predominately belonged to the ST5308 clone.

With respect to the area of collection; there was a clonal spread of the *Salmonella* serovars in some areas irrespective of the meat source. For example; the Kaarstad serovar (ST4605) was found in both beef and mutton samples in Aboabo while the Ouakam serovar (ST5307) were also isolated in chevon and mutton in the same area. More so, beef and chevon from the central market area contained the *Salmonella* Lagos (ST2469) strain. A similar trend was also observed in guinea fowl and chicken from both Victory Cinema and Tishegu area. This complex intraspread of multiple *Salmonella enterica* subsp. *enterica* serovars in diverse meat sources in areas in Ghana is very worrying for infection management.

4.0 Proposed practical ways to ensuring sustainable meat safety in the 21st Century

- Observance of good animal husbandry practices such as the provision of proper housing and keeping animals in a clean environment, provision of portable water, provision of nutritious/balanced and safe feed, disease prevention and control practices, avoidance of abuse of veterinary drugs and adherence to animal welfare husbandry practices. Regular vaccination of the animals – e.g. against anthrax.
- Transportation of animals in vehicles/trucks designed for animal transport to the abattoir for slaughter. Overcrowding of animals in vehicles and unnecessarily mixing of animals from different farms and ages should be avoided. Sick animals should not be transported for slaughter. Rest animals on transport every four hours and provide light feeding and water when the journey is more than 8 hours. Loading and offloading of animals should be done gently to avoid stressing them- e.g. ruminant could be made to climb and descend on a gentle slope when boarding vehicles.
- Lairage should be clean and overcrowding should be avoided. Animals that fall sick on transport should be isolated and treated. Animals should be rested between 4 to 8 hours after transportation over long distances. Ante-mortem inspection of live animals should be carried out by experienced and qualified veterinary officers before animals are passed for slaughter. Avoid beating animals and exposing them to any form of stress prior to slaughter to avoid pale soft exudate (PSE) meats.
- Only clean and healthy animals should be slaughtered. Slaughtered animals should not be dressed on the floor but dressed hanging. Ideally the slaughter room temperature should be 8°C to 12°C and dirty offals should be separated from clean carcasses. Post-mortem meat inspection should also be conducted by qualified veterinary officers and only wholesome carcasses should be passed for consumption. Meats should be transported in meat vans and not on motor bikes, motor kings®, in taxis and unapproved vehicles.
- Meat should be sold from netted tables (to avoid flies) and ideally sold from refrigerators at 4°C for fresh meats and -18°C for frozen meats.

Consumers should transport meats in pre-cooled insulated containers and immediately transfer them to their refrigerators when they get home.

- Meat sellers should practice proper self-hygiene, especially good handwashing when selling or handling meats. They should wash their hands thoroughly under running water with soap prior to and after meat handling.
- Consumers should avoid cross-contamination in food preparation areas and in kitchens. Thoroughly wash hands, counters, cutting boards, utensils etc. before and after touching raw meat.
- The golden rule is to **cook meats thoroughly**; cook meat to a minimum internal temperature of 70°C for 15 minutes (until there are no visible red parts in the middle of the meat, and any fluid drip is no longer pink) to destroy all bacteria that may be present. They should then be eaten as soon as possible. If possible, store them in a refrigerator if they are not to be eaten immediately.

5.0 Conclusions

I would like to conclude by saying that, there is a general sense of awareness regarding microbiological meat safety, antibiotic resistance and antibiotic residues among farmers, butchers/meat sellers and consumers in Ghana. Veterinary officers, health officers, teachers, friends and mass media are the sources of creating awareness of meat safety. Unfortunately, over time my research has revealed inadequate knowledge regarding meat contamination through the handling process of animals and meat by our farmers, butchers/meat sellers and consumers. Raw meats in Ghana are contaminated with mostly harmless bacteria and are also within acceptable limits. In addition, raw meats are contaminated with varying levels of *Escherichia coli* and *Salmonella enterica* with numbers of *Salmonella enterica* being absent or low in some ready-to-eat meats. Meats should therefore be properly cooked before consumption. Most of the *Escherichia coli* and *Salmonella enterica* are susceptible to ceftriaxone (cephalosporin), chloramphenicol (chloramphenicol) and ciprofloxacin (quinolone); these antibiotics could be the drug of choice for treating *Escherichia coli* and *Salmonella enterica* infections resulting from the consumption of meats in Ghana. Antibiotic residues are highest or most commonly detected in the liver and kidneys; since the liver metabolizes drugs

into forms that are nontoxic, while the kidneys remove them from the body. Genetic diversity exists among the *Escherichia coli* and *Salmonella enterica* from the meat sources indicating that different strains of these pathogens are present in meats. The presence of antibiotic resistance genes and plasmids in some Ghanaian *Escherichia coli* and *Salmonella enterica* meat isolates highlights their potential pathogenicity. Nevertheless, reported foodborne outbreaks resulting from the consumption of meats in Ghana cannot be found due to lack of surveillance and traceability studies by responsible government authorities. The antibiotic residues in meat are generally below the maximum permissible level. Although, my studies have found low to undetectable traces of antibiotic residues, it is still important to put in a word of caution to our farmers to ensure the right veterinary drug dosing and observance of the withdrawal period.

Finally, a word to young academicians. Gone are the days when professors were seen to be people who were old and had grey/bushy hair on the head/face. Myself and other young professors are testimony, that with determination, courage and hard work, you will get there whilst still relatively young. Young and aspiring academics, the “glass ceiling is broken”, you have an example to follow and you have no reason not to achieve this and better for yourselves. I can only say I am happy when I see a lot of the young ones I have mentored and friends/colleagues achieving and doing better than I have done. I would like to encourage all the young men and women that, it is possible, with determination, commitment and hard work you will get there and even better places as far as you dream.

Remember this: There is no elevator to success, you have to take the stairs.

6.0 Recommendations

- Awareness creation and training on food/meat safety should be provided for farmers, butchers/meat sellers and consumers. This can be done by responsible government authorities (Ghana Standard Authority, Food and Drug Authority etc), non-governmental organizations, scientists and you via social media.

- Personal and environmental hygiene is the key to preventing meat contamination and food poisoning; this should be observed by farmers, butchers/meat sellers and consumers.
- Antibiotic resistance is a major threat to public health and farmers should desist from self-veterinary drug administration without the advice of qualified veterinary personnel. The correct dosage and withdrawal periods should always be adhered to before animals are sold for slaughter.
- Butchers should not slaughter sick animals for food, or animals on antibiotics, and meat sellers should transport and sell meats under hygienic conditions.
- Most healthy people with gastrointestinal symptoms will not need any treatment. If symptoms persist, patients or people who feel unwell should not take antibiotics without prescription from qualified doctors. They should strictly follow dosage instructions when antibiotic therapy is necessary.
- The establishment of state-of-the-art slaughterhouses (preferably one in each region) by the government is necessary to help curb the unhygienic conditions under which animals are slaughtered and meats processed.
- Application of HACCP-based systems backed up by visits to and inspection of abattoirs and periodic surveillance of meats and other food products by the Food and Drugs Authority and Scientists on the Ghanaian market for food hazards are required for early detection, creation of awareness and avoidance of food contaminations and poisonings. There is also the need for monitoring of human diseases followed by identification of sources of disease outbreaks.
- Enforcement of food/meat safety laws by the Ghana Standard Authority, and the Food and Drug Authority is strongly recommended.

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