

**PREVALENCE AND ANTIBIOTIC RESISTANCE CHARACTERISTICS OF NON-
TYPHOIDAL *SALMONELLA ENTERICA* SEROTYPES ISOLATED FROM FOOD
AND WATER CONSUMED BY CHILDREN UNDER FIVE YEARS IN KARAMOJA
SUB-REGION**

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**A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND
GRADUATE TRAINING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN FOOD
SECURITY AND COMMUNITY NUTRITION OF GULU UNIVERSITY**

NOVEMBER 2023

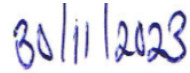
DECLARATION

I, Mpagi Ronald, hereby declare that the content of this dissertation is my original work and has not been previously submitted for any academic award in any university or higher institution of learning.

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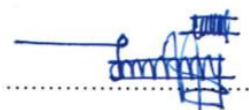
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ACKNOWLEDGEMENTS

I convey my heartfelt thanks to the Lord Jehovah for the strength, wisdom, energy and life during the course of writing this dissertation. His name be praised forever. In addition, I would like to express my thankful gratitude to RUFORUM and Mastercard foundation for funding this work through “Transforming African Agricultural Universities to meaningfully contribute to Africa’s growth and development (TAGDev)” project. Very special thanks to my supervisors Professor Duncan Ongeng and Mr. Lawrence Okidi for their encouragement, guidance and wisdom without which this book would not have been completed. My sincere appreciations also go out to Assoc. professor Richard Echodu and Gulu university multifunctional laboratories for providing me with laboratory space to run my laboratory analysis. Lastly, I thank my parents for the prayers and psychosocial support they rendered during the course of this journey. Thank you very much.

DEDICATION

I dedicate this dissertation to my friends and family. A special feeling of gratitude to my loving family for availing to me the unfailing support and encouragement throughout my years of study and during the process of writing this dissertation. This accomplishment would not have been possible without them. I also dedicate this book to the malnourished community, all policy makers and public health practitioners in the Karamoja sub-region and beyond.

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LIST OF ACRONYMS

AFI	Acute Febrile Illness
AMR	Antimicrobial Resistance
APH	Aminoglycoside Phosphotransferase
ARG	Antimicrobial Resistance Genes
ATCC	America Type Culture Collection
ATP	Adenosine Tri Phosphate
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribo Nucleic Acid
HGT	Horizontal Gene Transfer
HIV/AIDS	Human Immune Virus/Acquired Immune Deficiency Syndrome
IPC	Infection Prevention and Control
IPs	Intestinal Perforations
MDR	Multidrug Resistance
MHA	Muller Hinton Agar
NTS	Non Typhoidal <i>Salmonella</i>
PCR	Polymerase Chain Reaction
UDHS	Uganda Demographic Health Survey
UNAS	Uganda National Academy of Science
UNFPA	United Nations Population Fund
UNHS	Uganda National Household Survey
UNICEF	United Nations International Children Emergency Fund
USAID	United States Agency for International Development
US-CDC	United States Centre for Disease Control
WHO	World Health Organization

ABSTRACT

Food safety is a key factor that influences nutrition outcomes through its impact on the utilization dimension of food security. Karamoja sub-region is an area that has consistently experienced a high prevalence of diarrhoea-associated undernutrition among children under five years over the last decade. *Salmonella* is an important enteric pathogen that occur in foods and water in Karamoja sub-region. However, limited information exists on the serotypes and antimicrobial resistance characteristics of non-typhoidal variants of the pathogen in the area. The overall objective of this study was to determine the prevalence and antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* serotypes that contaminate foods and water consumed by children under five years in Karamoja sub-region. Specifically, the study determined: (i) the prevalence and serotypes; (ii) phenotypic antibiotic resistance characteristics; (iii) genotypic antibiotic resistance characteristics; and (iv) the association between the genotypic and phenotypic antibiotic resistance characteristics of the organisms in question. Two hundred samples of food and water collected from Karamoja sub-region were analysed for *Salmonella enterica* using culture and PCR targeting *invA* gene. The confirmed *Salmonella* isolates were serotyped using multiplex PCR targeting genes conserved in each serotype. The confirmed isolates were further subjected to the disc diffusion test and PCR targeting the corresponding resistance genes to determine the phenotypic and genotypic resistance characteristics, respectively. Results showed the prevalence of non-typhoidal *Salmonella enterica* to be 45%. Four serotypes were identified with *S. typhimurium* (53%) being the most dominant followed by *S. infantis* (16%), *S. enteritidis* (9%) and *S. dublin* (4%). Phenotypically, all the confirmed isolates were resistant to at least one antibiotic recommended for treatment of salmonellosis whereas 37.9% were multidrug resistant. Resistance frequency was highest for azithromycin (91.1%) followed by ciprofloxacin (31.1%), cefixime (20%),

cefotaxime (20%), ceftazidime (15.6%), ceftriaxone (6.7%), cotrimoxazole (6.7%), chloramphenicol (6.7%) and amoxicillin clavulanic acid (2.2%) in decreasing order of magnitude. The most prevalent resistance genes were *mefA* (95.3%), *gyrA* (94.9%), *parC* (92.3%), *bla_{PSE}* (81.3%), *sul1* (66.6%) and *Sul2* (66.6%). Phenotypic resistance to chloramphenicol, beta lactams, cotrimoxazole and ciprofloxacin was strongly correlated with the presence of *floR* (odds ratio (OR)=12.5, 95% confidence interval (CI)0.936-1.000, P=0.031), *bla_{PSE}* (OR=24.7, 95% CI 0.000-0.064, P=0.001) *sul1* (OR=11.9, 95% CI 0.000-0.065, P=0.000)), *parC* (OR=13.3, 95% CI 0.000-0.064, P=0.015) respectively. This study has revealed that food and water consumed by children under five years in Karamoja sub-region are contaminated with antibiotic resistant non-typhoidal *Salmonella enterica* serotypes and are therefore not safe for consumption. Therefore, there is need for concerted effort by concerned authorities to address microbial food safety challenges in Karamoja sub-region as well as develop and priotize prevention measures such as vaccinations and use of antimicrobial peptides as alternatives in managing bacterial infections such as salmonellosis.

Keywords: *Salmonella*, prevalence, serotypes, antimicrobial resistance

CHAPTER ONE: INTRODUCTION

1.1 Background

Malnutrition is a significant risk factor for mortality and poor cognitive development in children under the age of five years. Globally an estimated 149 million (22 %) children under five are stunted and 45 million (6.7 %) are wasted (FAO, IFAD, UNICEF, 2022). The majority of these are concentrated in low-income settings of the world such as Africa (30.7% stunted; 6.0% wasted) (FAO et al., 2022). Regionally, sub-Saharan Africa bears the greatest burden of malnutrition (32.3% stunted; 5.9% wasted) among children under five after middle Africa (FAO et al., 2022). On the other hand in the East African region, Uganda ranks fourth and is characterized by a prevalence of 27.9 % level of stunting and 3.5% of wasting reported among children under five (FAO et al., 2022). Malnourished children under five contribute the greatest burden to the country's prevalence of nutritional challenges with 36% being found in the Karamoja subregion (Uganda Bureau of Statistics (Uganda Bureau of Statistics (UBOS), 2019).

Food safety is a key factor that influences nutritional outcomes through its impact on the utilization dimension of food security (Ahmed et al., 2022). Among the food safety elements (physical, chemical, biological and nutritional), microbial foodborne diseases ostensibly caused by pathogenic protozoa, fungi, viruses and bacteria (Gallo et al., 2020) are a factor that exacerbates malnutrition situation in children under five years mainly through nutrient malabsorption and loss in the form of watery stool. Among diarrheal/invasive agents, *Salmonella* is among the top 31 pathogens with a high capability of causing intestinal or systemic disease in humans (World Health Organization (WHO), 2015). The bacterial genus *Salmonella* is composed of two type species, *bongori* and *enterica*. However, it is *enterica* that

is pathogenic and consists of six sub groups and several serotypes (Xu et al., 2019). In terms of disease, the *enterica* type species can be grouped into those that are typhoidal and those that are non-typhoidal. In this dissertation the word *Salmonella* will refer to type species *enterica* with focus on those that are non-typhoidal.

Salmonella infections are usually self-limiting but in cases of severe gastroenteritis and impaired immunities, the World Health Organization (WHO) recommends the use of antibiotic therapy in management of the disease as well as eliminating the pathogen (WHO, 2015). However, excessive and abusive use of antibiotics by the public has exacerbated the development and propagation of antimicrobial resistance (AMR) among the previously susceptible human bacterial pathogens (Mukami, 2021) such as *Salmonella*. AMR can be defined as a condition where the pathogen has developed the capacity to still grow and multiply in the presence of the antibiotic drug that previously eliminated it (Ikhimiukor et al., 2022). When the same pathogen is able to resist three or more antibiotics that target its elimination, it is termed a multidrug-resistant (MDR) pathogen (Amer et al., 2018). Antibiotic resistance in bacterial pathogens such as *Salmonella* is in most cases associated with genetic changes encoded by chromosomal and plasmid genes (Amer et al., 2018) in the *Salmonella* genomic island. These antimicrobial resistance genes (ARGs) can be inherent or acquired through vertical or horizontal (transduction, transformation and conjugation) mechanisms. However, some form of non-inherited resistance with no genetic alteration among *Salmonella* genus has been reported (Pontes & Groisman, 2019; Rather et al., 2021; Trampari et al., 2021). This form of phenotypic resistance has been associated with specific processes such as the stationary growth phase, persistence and the development of biofilms (Amer et al., 2018).

A study in Uganda revealed that over 40% of hospitalized children diagnosed with diarrhoea had *Salmonella* infections with non-typhoidal salmonellosis being the most predominant

(Appiah et al., 2021). The Karamoja sub-region bears one of the highest diarrhoea prevalence rates (24%) in the country (Uganda Bureau of Statistics (UBOS), 2019). This is partly attributed to the poor sanitary conditions occasioned by low latrine coverage characterized by more than 60% of the population practising open-air defecation (IPC Global Platform, 2021). As a result, the population and most especially those with weak immunity such as children under five are at high risk of exposure to the foodborne pathogens and disease conditions (Nzabakiraho & Gayawan, 2021). A recent study by Okidi et al. (2022) revealed widespread *Salmonella* contamination of foods and water consumed by children under five from all the three agroecological zones that make up Karamoja subregion. The reported massive distribution of the pathogen, which when combined with the emergence and spread of antimicrobial resistance (AMR) creates new challenges with food safety and public health in such a diarrhoea-endemic locality. This is because several strains of multidrug-resistant *Salmonella* have been detected in food and water globally (Rather et al., 2021; Wang et al., 2021; Xu et al., 2020). However, Uganda like many other Sub-Saharan African countries though possess some information on the prevalence of diarrhoea and associated pathogens such as *Salmonella* across its regions (Nantege et al., 2022; Omona et al., 2020), there still exists a significant information gap on the antibiotic resistance properties of such pathogens. As such, it becomes essential to ascertain the AMR situation in *Salmonella* isolated from foods and water consumed by children in the Karamoja sub-region. This provides a better picture of the diarrhoea-associated microbial food safety challenge that children in Karamoja experience and the impact of such a challenge on the endemic child undernutrition situation in Karamoja sub-region.

1.2 Problem Statement

Whereas proper nutrition is known to play a critical role in child development, improving well-being, strengthening immune systems, and reducing the risk of non-communicable diseases (UNICEF & WHO, 2021), Uganda still has 27% (FAO et al., 2022) of children under five malnourished with Karamoja subregion surpassing the country's average and constituting the highest burden (36%) countrywide (UBOS & ICF, 2019). This high burden of malnutrition amongst children under five in Karamoja sub-region is associated with a high prevalence of diarrhea which stands at 24% (UBOS & ICF, 2019). This state of diarrhea has largely been attributed to poor sanitary conditions in the region characterized by low latrine coverage and open air defecation practised by over 60% of the population (IPC Global Platform, 2021). However, limited information exists on the microbial safety of food and water consumed by children and yet food and water are the major vehicles through which enteric pathogens that cause diarrhoea get into the gastro intestinal tract. A recent study by Okidi et al. (2022) reported *Salmonella* contamination of foods and water consumed by children under five from all three agroecological zones that make up the Karamoja subregion. This result highlights the potential diarrhoea- associated food safety challenge that children in Karamoja sub-region is exposed to through food and water but has received limited attention. In addition, majority of the previous studies conducted in Uganda on *Salmonella* focus on typhoidal *Salmonella enterica* serotypes *typhi* and *paratyphi* (Atuyambe, 2020; Ismail et al., 2020; Mirembe et al., 2019) in water sources with less attention given to non-typhoidal *Salmonella enterica* serotypes in foods. The emergence and spread of AMR in enteric foodborne pathogens such as *Salmonella* is a factor that likely exacerbates the condition of diarrhoea to the higher state of undernutrition in children under five in Karamoja sub-region. However, inadequate information exists on the serotypes that circulate in the sub-region and the antibiotic resistance properties of such

diarrhea-causing enteric foodborne pathogens that contaminate food and drinking water consumed by children under five in Karamoja sub-region. The lacuna in information makes it difficult for public health authorities and practitioners to approximate the magnitude of microbial food safety challenge that children under five in Karamoja are exposed to and to prioritize actions to address the challenge. Therefore, addressing the observed information gaps will not only contribute to providing relevant epidemiological information on significant microbial food safety pathogens such as *Salmonella*, it will also guide in the development of effective vaccines geared to prevent bacteria associated foodborne infections such as salmonellosis.

1.3 Objectives of the Study

1.3.1 Overall Objective

The overall objective of this study was to determine the antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* that contaminates food and water consumed by children under five years in Karamoja sub-region.

1.3.2 Specific Objectives

- i. To determine the prevalence and serotypes of non-typhoidal *Salmonella enterica* that contaminate foods and water consumed by children under five in Karamoja sub-region
- ii. To determine the phenotypic antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* that contaminates food and water consumed by children under five in Karamoja sub-region.

- iii. To determine the genotypic antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* that contaminates food and water consumed by children under five in Karamoja sub-region.
- iv. To determine the association between genotypic and phenotypic antibiotic resistance characteristics in non-typhoidal *Salmonella enterica* that contaminates food and water consumed by children under five in Karamoja sub-region

1.4 Research Hypotheses

Ho₁: There is no significant difference in the prevalence and distribution of non-typhoidal

Salmonella enterica serotypes that contaminate food and water in Karamoja sub-region

Ho₂: Non-typhoidal *Salmonella enterica* that contaminates food and water consumed by

children under five in Karamoja sub-region is not resistant to commonly used antibiotics.

Ho₃: Non-typhoidal *Salmonella enterica* that contaminates food and water consumed by

children under five in Karamoja sub-region lacks the markers for antibiotic resistance.

Ho₄: There is no association between genotypic and phenotypic antibiotic resistance in non-

typhoidal *Salmonella enterica* that contaminates food and water consumed by children under five in Karamoja sub-region.

1.5 Significance of the Study

With the present paucity of information on the antibiotic resistance profiles of serious MDR pathogens such as *Salmonella* in countries like Uganda, findings from this study will help in informing effective control policies to reduce on the spread of AMR. The findings of this study will expand on the current clinical antibiogram used to treat *Salmonella* infections. In addition,

findings from this study will help to guide the development of vaccines which are suitable alternatives for antibiotics to prevent bacterial infections such as salmonellosis. This will help to curb the growing pandemic of antibiotic resistance and associated morbidities which greatly affect the immuno suppressed such as children under five and pregnant women. Finally, results from this study can be used as a basis for future research on antibiotic resistance with in Karamoja region and the country at large.

CHAPTER TWO: LITERATURE REVIEW

2.1 *Salmonella* serotypes and associated disease burden

Salmonella is a genus of gram-negative and facultatively anaerobic non-spore forming enteric bacilli (Gallo et al., 2020) consisting of two type species namely; *enterica* (*S. enterica*) and *bongori*. *Bongori* is mostly associated with infections in cold blooded hosts (Liu et al., 2023) and rarely isolated in warm blooded animals. On the other hand, *enterica* sub species which has been associated with occupying a wider ecological niche has been found with ability to colonise animals and humans (Fernández et al., 2018), plants (Burris et al., 2020) and the environment (Taylor & Winter, 2020) . Majority of the animal and human associated *Salmonella* infections are attributed to various serotypes that belong under species *S. enterica* (Lamas et al., 2018). The type species *enterica* is composed of several serotypes. By definition, serotypes are various clusters that make up a particular species of an organism. According to their surface structures, the type species *enterica* can be classified into various serotypes. Scientists base on unique code numbers in O and H antigens to determine these *Salmonella* serotypes.

Over 2500 serotypes of species *S. enterica* have been identified to date (Gossner et al., 2016). Whereas all serotypes can cause human infections, a few of them target specific hosts and are found in particular animal species. Typical examples include serovar *Dublin* and *Choleraesuis* in cattle (Vohra et al., 2019) and pigs (Singh, 2013) respectively. These host specific serotypes have been associated with causing systemic and invasive disease conditions when they affect humans (Singh, 2013). However, majority of *enterica* type species serotypes have been found to occupy a broad range of hosts as well as occupy a wider ecological niche (Seif et al., 2018). These serotypes are often associated with mild gastroenteritis which is usually self-limiting but

among immune compromised individuals such as children under five, they have ability to cause life threatening disease conditions. Typical example of such serotypes includes *typhimurium* and *enteritidis*. However, many other serotypes such as *Agona*, *Uganda*, *Kentucky*, *Infantis*, *London*, *Anatum*, *Newport*, *Montevideo*, *Heidelberg* *Reading*, *Cerro* and many others have been reported to exist (Xu et al., 2019).

Salmonella transmission mainly occurs through the fecal-oral route (LaRock et al., 2015). The organism is mainly harboured in the gut of both domestic and wild animals from where it is shed into the environment and water sources in fecal matter (Gallo et al., 2020). Humans usually get exposed to *Salmonella* infections when they consume food or water contaminated with fecal matter containing the pathogen. Globally, studies have reported the existence of multidrug resistant *Salmonella* in various food products and water sources. For instance Rincón-Gamboa et al. (2021) found *Salmonella* contamination in meat and meat products, Wei et al. (2020) in milk powders, Cho et al. (2020) in water. In Uganda, Ndoboli et al. (2018) found *Salmonella* contamination in fresh vegetables, Onohuean & Igere (2022) in milk and meat products, Kabwama et al. (2017) in water. *Salmonella* infections in Karamoja, may be associated with consumption of contaminated food and water. A study by Okidi et al. (2022) highlighted a widespread contamination of commonly consumed food and water samples in Karamoja sub-region. Despite the findings, there is a notable gap in understanding specific sources. Limited research exists on the dynamics of *Salmonella* contamination in food and local water supply and the factors contributing to persistence. Addressing these gaps is crucial for developing targeted interventions to reduce infections such as salmonellosis and improve the overall health status, especially given the prevailing malnutrition in the region. A comprehensive understanding on the transmission pathways will inform strategies for enhancing food and water safety which in turn will contribute to reducing the burden of

infectious diseases and supporting broader public health efforts, including initiatives to combat malnutrition.

Salmonella infections can be categorized into Typhoidal, Paratyphoidal and Non typhoidal gastroenteritis. Typhoid *Salmonella* infection is caused by serotype *typhi* of type species *enterica* (Hossain et al., 2021). This sickness usually commences 6 to 30 days after being exposed (Hameedullah et al., 2021). Signs and symptoms of typhoidal salmonellosis vary from mild to severe in different individuals and include; high fever, general body weaknesses, abdominal pain, severe diarrhoea in certain individuals, headaches and mild vomiting (Hameedullah et al., 2021). Paratyphoidal salmonellosis is caused by serotype *paratyphi* (A, B and C) of type species *enterica* (CDC, 2021; Crump et al., 2013). Paratyphoidal salmonellosis also presents same signs and symptoms as typhoidal Salmonellosis (CDC, 2021; Crump et al., 2013). Non-typhoidal gastroenteritis can be classified as infections caused by other serotypes of *Salmonella* with an exception of *Typhi* and *Paratyphi*. This infection takes 6 to 72 hours to manifest after exposure. The most common clinical presentation of non-typhoidal salmonellosis includes acute diarrhoea, vomiting, abdominal among others. Recovery from this infection usually takes 3 to 7 days (CDC, 2021). However, some strains of non-typhoidal *Salmonella* (NTS) have been identified to invade the blood stream resulting in bloody diarrhoea in affected victims. These strains are classified as Invasive non-typhoidal *Salmonella* strains (CDC, 2021). The genus *Salmonella* is ranked among the leading 30 bacterial pathogens having potential to cause both intestinal and invasive systemic infections (World Health Organization (WHO), 2015). Among foodborne diseases, *Salmonella* is ranked also as a third leading cause of death particularly in children below five (Rodrigues et al., 2020; WHO, 2015). Non typhoidal *Salmonella* accounts for 534000 (409500-705000) cases and 77,470 (46360-122800) death each year globally with 66,520 deaths occurring in Sub Saharan Africa and particularly

among children below five years. In children, predisposing factors to non-typhoidal Salmonellosis include; weak immunity, malnutrition and malaria parasitemia (GBD, 2020). This disease burden comes with very high economic constraints on governments in attempt to manage such infections. It is important to appreciate that WHO report on *Salmonella* provides estimates only on prevalence of disease condition but limited attention is given to the source of infection such as food and drinking water being investigated in this study. Furthermore, WHO does not report at country level but rather regional prevalence. Thus, there is a paucity of information on Uganda and most especially from most affected localities such as Karamoja sub-region. A recent study by Okidi et al. (2022) provided information on *Salmonella* prevalence of contaminated food and household drinking water consumed by children in Karamoja sub-region. However, no information was provided on serotypes of *Salmonella* involved in food and drinking water contamination in the sub-region. Information on serotypes is important because of differences that have been observed among serotypes in terms of behaviour in food environment as well as severity of the disease condition they elicit (Varga et al., 2020).

2.2 Antibiotic treatment and phenotypic resistance of *Salmonella*

Usually, *Salmonella* infections require no use of antibiotics in their management. However, in situations of severe gastroenteritis and weakened immunities, antimicrobial therapy is recommended in the clinical management of the disease as well as in elimination of the pathogen. Important to note is that inappropriate antibiotic use when managing bacterial infections has resulted in the rise of antibiotic resistance (AR) among previously susceptible bacterial pathogens such as *Salmonella* (Coleman & Smith, 2014). Antibiotic resistance is a condition where bacteria develops capacity to still grow and multiply in presence of the antibiotic drug that previously eliminated it (Ikhimiukor et al., 2022). When the bacterial is

able to resist multiple antibiotics usually greater than three (>3 antibiotics), it is termed a multidrug resistant (MDR) pathogen. Bacterial pathogens achieve this by reducing on penetration of antimicrobials, modifying the antibiotic, altering the drug target site and activation of efflux pumps (Khameneh et al., 2019).

Intrinsic resistance is a condition where the bacterial inherently carries resistance towards a particular antimicrobial (Ibrahim et al., 2021). An example of such inherent properties includes impervious outer membranes in bacteria such as *Salmonella* (gram-negative). This layer hinders penetration of certain classes of antibiotics such as glycopeptides. The absence of this layer in gram-positive bacterial pathogens renders them susceptible to glycopeptides (Ibrahim et al., 2021; Lee, 2019). Some bacteria have an innate ability to express pores on their surfaces through which dangerous substances such as toxins and antibiotics can be ejected. Furthermore the cell membrane remodeling involving various lipo-sugar modifications has been reported to increase the overall positive charge of the cell membrane leading to increased resistance to positively charged antibiotics such polymyxin and colistin in bacterial generations such as *Salmonella* (Olaitan et al., 2014).

Genes that express these inherent resistance characteristics are in most cases encoded in the bacterial genome (Khameneh et al., 2019). On the other hand, developed resistance arises when mobile ARGs are transferred between or within bacterial populations usually from gene carriers to non-gene carriers (susceptible ones). Bacterial pathogens achieve this in three ways: transductions, transformations and conjugations (Frost et al., 2005). Transduction is a process by which viruses (bacteriophage) aid in the transmission of genetic materials between bacteria (Touchon et al., 2017). Conjugation is when transfer of genetic material occurs as a result of cell to cell contact between cells or through a channel usually a sex pilus through which a plasmid is conveyed (Arnold et al., 2022). Unlike conjugations and transductions which rely

on specific MGEs (plasmids and phages), transformation processes in bacteria highly depend on transposons where through genetic recombination fragments of nucleic acid (DNA) from other bacteria fuses with the chromosome of other bacteria. Additionally, mutations in genetic factor may also impose changes alterations in drug target sites in cases where a pathogen is continuously subjected to a particular antibiotic drug.

The rapid emergence of antibiotic-resistant pathogens has been occasioned by abusive use of antibiotics by humans (Shah et al., 2022) that mainly occurs in form of inapt prescriptions, long periods of treatment, self-medication, partial use of medically prescribed drugs and the irregular usage of reserve antibiotic drugs. Earlier studies such as that conducted on the global consumption of antibiotics showed an increase in public consumption rate ever since 2008 (Browne et al., 2021). Regionally, the lowest consumption levels were reported from SSA. However, authors attributed this to scarcity of data from LMICs mostly concentrating on knowledge, attributes and practices the use of antibiotic drugs. The first line antibiotics for *Salmonella* were recommended between 1948 to around the early 1990s (Britto et al., 2018). These antimicrobials included chloramphenicols, co-trimoxazole and ampicillins. Nonetheless, their widespread use resulted to a rise in the development of chloramphenicol resistance (Calderon, 1974; Geddes & Goodall, 1972) and subsequently to ampicillin (Ocharan-Corcuera et al., 1987; Smith et al., 1984) and cotrimoxazole (Ocharan-Corcuera et al., 1987; Williams et al., 1989) leading to MDR *salmonella*. The MDR became established in Asia in the 1990s. Since then, many studies have continued to report and document *Salmonella* resistance to recommended first treatment choice antibiotics worldwide.

Following the emergence of MDR *Salmonella* to first line antibiotics, Fluoroquinolone class of antibiotics became the therapeutic option used to manage salmonellosis in the late 1990s (Britto et al., 2018). These were highly effective in clinical management of salmonellosis until

side effects associated with their use began to emerge among treated children (Britto et al., 2018). Nonetheless, ciprofloxacin and ofloxacin remained the only treatment choices in managing *Salmonella* not until resistance to fluoroquinolones also began to develop. Numerous studies (Chang et al., 2021; Medalla et al., 2021) worldwide have reported *Salmonella* resistance to fluoroquinolone class of antibiotics.

Most recently, azithromycin and third generation cephalosporines have become the preferred option to clinically manage *salmonella* infections in man in presence MDR and Fluoroquinolone susceptibility. However in 1994, resistance among non-typhoidal serotypes in type species *enterica* to 3GCs began to be reported (Tack et al., 2020). The third-generation cephalosporin antibiotic drug tested and resisted was cefotaxime. From then to date , various studies have continued to report and document *Salmonella* resistance to also third-generation cephalosporin with in Africa (Jeon et al., 2019; Pietsch et al., 2021; Wei et al., 2019). Additionally, resistances to azithromycin among serotypes of type species *enterica* have been observed (Chiou et al., 2023; Sajib et al., 2021; Xie et al., 2022). A critical look at the studies that reported antimicrobial resistance in *Salmonella* reveal that data were derived largely from clinical samples from patients. However, limited information exists on the source of infection such as food and water consumed by children in Karamoja sub-region being investigated in this study. The Uganda's Ministry of Health in conjunction with the World Health Organization (WHO) recomend the use of azithromycin (macrolide) chloramphenicol (Phenicol), ciprofloxacin (quinolones), Amoxicillin clauvinic acid (beta lactam), trimethoprim-sulfamethoxazole Cotrimoxazole (sulphonamides) and 3GCs as priority choice in management of *Salmonella* infections. Among the group of 3GCs highlighted, cepheids such as ceftriaxone, cefixime, cefotaxime and ceftazidime are of top priority in the management of the bacterial infection. Thus, it is still not known whether or not *Salmonella* that contaminates food and

water consumed by children in Karamoja are resistant to the antibiotics recommended for disease management.

2.3 Genetic determinants of antibiotic resistance in bacteria

In most cases, phenotypic patterns are influenced by the genotype (Amer et al., 2018). As such, the rise in antimicrobial resistance among bacterial pathogens are usually linked to genetic modifications coded on bacterial chromosomes and MGEs (plasmids, transposons, intergrons and phages) (Bennett, 2008; Blair et al., 2014). For instance numerous reports (Beshiru et al., 2019; Emond-Rheault et al., 2020; Noman et al., 2023) on *Salmonella* have documented a display of antibiotic resistance to relevant antimicrobials in isolates with genetic changes carried on their MGEs. These genetic changes are either through genetic transfers (transductions, transformations and conjugations) or structural modifications through mutations in the elements relevant to antibiotic activity in bacterial pathogen (Amer et al., 2018). In this review, genetic determinants are considered with respect to resistance to aminoglycosides, beta lactams, Phenicols, quinolones and fluoroquinolones, tetracyclines, sulphonamides and trimethoprim, and macrolides.

2.3.1 Resistance to Aminoglycosides

Streptomycin, an aminoglycoside progenitor was discovered in 1943 after isolation from actinomycete *Streptomyces griseus* (Gajdacs, 2019). These drugs are primarily used in combination with other antibiotics and are mostly used in treatment of infections caused by gram-negative bacteria (Urban-chmiel et al., 2022). Most aminoglycosides have a bactericidal effect on bacterial pathogens, with exception of streptomycin, which is bacteriostatic (Alcaine et al., 2007). Aminoglycosides work by inhibiting translation through binding on conserved sequences with in 16S tRNA of 30S ribosomal unit (Urban-chmiel et al., 2022).

Although *Salmonella* seems susceptible to aminoglycosides *in-vitro*, the Clinical and Laboratory Standard Institute (CLSI) has cautioned about their usage in clinical treatment of the infection (CLSI, 2020). *Salmonella* uses its impervious cell layer to reduce aminoglycoside penetration thereby resisting aminoglycoside bactericidal effects. In addition, through mutations the drug target sites are altered and thus interfering with the drug-target site interactions (Alcaine et al., 2007; Shaw et al., 1993; Urban-chmiel et al., 2022). In *Salmonella*, resistance to aminoglycosides is dependent upon the genetic expression of three groups of enzymes which include; acetyltransferases, phosphotransferases, and nucleotidyltransferases (Alcaine et al., 2007).

Aminoglycosides acetyltransferases are enzymes responsible for modification of amino (NH₂) groups in an aminoglycoside molecule. They are classified into four groups; AAC (1), AAC (2), AAC (3), and AAC (6). Their nomenclature is grounded on the position they occupy within adjusted aminoglycoside molecule (Alcaine et al., 2007). *Aac*, a gene mostly located in *Salmonella* genomic islands (Wang et al., 2019), plasmids (Possebon et al., 2021), and intergrons (Zhao et al., 2020). As such, many variants of aminoglycoside acetyltransferases encoding genes (*aac*) have been found various serovars of *Salmonella* (Alcaine et al., 2007).

Aminoglycoside phosphotransferases (APH) catalyze energy-dependent phosphorylation of precise aminoglycoside hydroxyl groups (Alcaine et al., 2007). They are further categorized into sub groups depending on specific sites of phosphorylation. Genetic markers expressing aminoglycoside phosphotransferases enzymes mostly are found on plasmids in *Salmonella* serotypes (Cohen et al., 2020). *Aph* are genes that code expression of these aminoglycoside phosphotransferases. These genes have been reported to exist in various serotypes of *Salmonella* (Alcaine et al., 2007; Cohen et al., 2020). Genes encoding enzyme APH (3') have been found to offer resistance to kanamycin and neomycin (Cohen et al., 2020).

Nucleotidyltransferases target hydroxide molecules and are classified into several sub groups depending on target sites they modify (Alcaine et al., 2007). Genes encoding expression of these enzymes are designated *aad* (for aminoglycoside adenylyltransferases) and *ant* (for aminoglycoside nucleotidyltransferases) (Alcaine et al., 2007). *AadA* are responsible for resistance towards streptomycin in *Salmonella* has been identified in many serotypes. Likewise, *aadB* gene (at times referred to as *ant* (2')), responsible for resistance against gentamycin and tobramycin has also been reported to exist among *Salmonella* serotypes (Ranjbar et al., 2020).

2.3.2 Resistance to Beta Lactams

Beta lactams are made of three categories: Penicillins, cephalosporins and carbapenems (Lima et al., 2020). Penicillins were the first class of beta lactams to be developed (Alcaine et al., 2007; Lima et al., 2020). Structurally, beta lactams consist of penta-member thiazolidine ring bonded with a beta lactam ring (Hutchings et al., 2019). However, their excessive use increased bacterial resistance towards them. As a result, another known as cephalosporins was developed (Alcaine et al., 2007). Cephalosporines comprise a six-member ring fused together with beta lactam ring (Alcaine et al., 2007; Hutchings et al., 2019). This structural modification offers cephalosporines a broader range of action against gram-negative bacteria. In addition, cephalosporines are able to generate greater stability as a result of their structure to beta lactamases (Alcaine et al., 2007). Several classes of cephalosporins are in existence with progressive classes bearing a wider range of action and more effective against gram-negative bacteria than its proceeding class (Alcaine et al., 2007). The bactericidal effect of beta lactams is effected through their potential to affect the Penicillin Binding Proteins (PBP) (Taguchi et al., 2019). PBPs facilitate the manufacture of peptidoglycan required to synthesize microbial cell wall (Taguchi et al., 2019). Beta lactams are considered bactericidal with their activity

varying amongst beta lactams, the organisms and the target Penicillin Binding Protein (Alcaine et al., 2007). In the genus *Salmonella*, the inhibition of these PCBs leads to bactericidal activity of antibiotics. In addition, beta lactams need to be facilitated by OmpC and OmpF porins to be able to traverse bacteria cell membranes so as to reach their PBP targets. Although porin loss (OmpC and OmpF) is a rare mechanism reported in *Salmonella*, cases have been reported where porin loss resulted in increased beta lactam antibiotic (Masi et al., 2022). However, the expression of beta lactamases is the most reported mechanism of beta lactam resistance reported among *Salmonella* serotypes (Lima et al., 2020). Beta lactamases break down beta lactam ring structures yielding beta-amino acids without antimicrobial effect (Lima et al., 2020). Beta lactamase genes are mainly carried on plasmids with few found to exist on bacterial chromosomes (Marcantonio et al., 2022).

According to Amber, beta lactams can be grouped into four classes; Class A, B, C and D. Plasmid borne Class A beta lactamases in *Salmonella* serotypes (Alcaine et al., 2007) have been found to provide resistance against a variety of members in beta lactam class of antibiotics such as penicillins, early generation cephalosporins and carbapenem (Alcaine et al., 2007). There are several families of genes that encode this class of beta lactamase enzymes among which TEM is most common in many serotypes of *Salmonella* (Langata et al., 2019). *Bla_{TEM-1}* has been reported among *Salmonella* serotypes; *typhimurium* (Al-Gallas et al., 2022), *enteritidis* (Cao et al., 2023), *infantis* (Acar et al., 2019) and many others. Other variants such as *bla_{TEM-52}* have also been reported to exist among *Salmonella* serotypes (Alcaine et al., 2007). Other families of this class include *bla_{PSE-1}*/*bla_{CARB-2}* have been observed among *Salmonella* serotypes (Herrera-Sánchez et al., 2020). Recently *bla_{KPC-2}* which confers resistance to carbapenems such as imipenem was also identified in some *Salmonella* serotypes (Patil et al.,

2022). Cefotaximases (*bla_{CTX}*), another family of genes encoding class A beta lactamases has also been found in some *Salmonella* serotypes

(Alcaine et al., 2007). A variant of CTX gene, *bla_{CTX-M}* has also been reported to exist among *Salmonella* serotypes (Alcaine et al., 2007). Another class of beta lactamases mostly reported among serotypes of *Salmonella* is class C. Whereas they are chromosomally encoded in other bacterial species, in *salmonella* C class beta lactamases are harboured on bacterial plasmids. They include *ampC* genes and are associated with cephalosporine resistance. in *Salmonella* serotypes (Souza et al., 2020). *Bla_{CMY-2}* has been reported to confer resistance to ceftriaxone, an extended Spectrum cephalosporine, a drug of choice in managing human salmonellosis (Penha Filho et al., 2019). Various *Salmonella* serotypes have been reported to harbor this gene with several of its variants such as *bla_{CMY4}* and *bla_{CMY-7}* (Alcaine et al., 2007). Class B metallo beta lactamases in *Salmonella* have been known to offer resistance to all beta lactams antibiotics together with carbapenems (Alcaine et al., 2007). They have been found on both plasmids and chromosomes in bacterial pathogens. They include the IMP-1(Ranjbar et al., 2020) and VIM-1 (Rochanski, 2019). Lastly, the class D are rare in *Salmonella* though associated with oxacillin resistance (Alcaine et al., 2007).

2.3.3 Resistance to Phenicols

Phenicols are classified into chloramphenicols and Florfenicol (Alcaine et al., 2007). Chloramphenicols achieve their antimicrobial effect through binding themselves to peptidyl transferase centres within 50s ribosomal unit (Papaneophytou et al., 2019). In *Salmonella*, resistance to chloramphenicols is achieved through two primary ways that is either chloramphenicol-o-acetyltransferase enzymes (CAT) inactivates phenicol antibiotics or efflux systems are expressed to eject drugs from the bacterial cells (Zada et al., 2020). CAT encoding

genes designated as *cat 1* and *cat 2* in *Salmonella* are plasmid (Alcaine et al., 2007). On the hand, the efflux pump in *Salmonella* has been reported to be expressed by two genes that is the *flo R* (Ferreira et al., 2021) and *emlA* gene (Alcaine et al., 2007) with *floR* gene appears to be widely distributed among serotypes of genus *Salmonella* (Alcaine et al., 2007).

2.3.4 Resistance to Quinolones and Fluroquinolones

The first quinolone (nalidixic acid) was manufactured in 1972 (Hong et al., 2021). After subsequent generations of quinolones have emerged with former generations being more effective and possessing a wider range of activity against pathogenic bacteria than the previous one (Hong et al., 2021). Initially, quinolones were designed to target the gyrase component of DNA. However, subsequent groups in addition to the gyrase component (Dighe & Collet, 2020), they also target the topoisomerase IV component in achieving their bactericidal effect (Shaheen et al., 2021). *Salmonella* associated quinolone resistance is achieved in two ways. Firstly, are mutations in the drug determining regions in the gyrase and *par C* sub unit and secondly, is the over expression of the AcrAB-ToIC efflux system that ejects the antibiotics from the bacterial cell (Attia & Elbaradei, 2020).

2.3.5 Resistance to Tetracyclines

Chlortetracycline, a tetracycline progenitor was discovered in 1940 (Alcaine et al., 2007). Tetracyclines achieve their antimicrobial activity through interfering with interaction of tRNA and A sites with in 30s ribosomal unit thereby stopping protein (Tok et al., 2023). *Salmonella* resistance to tetracyclines is associated with expression of an efflux system that eject drugs through porins in bacterial cell walls (Alcaine et al., 2007). Over 25 genes have been found to express both oxytetracycline and tetracycline resistance in bacteria. Of these, *tet(A)* (Khan et al., 2019), *tet(B)* (Khan et al., 2019), *tet(C)* (Mthembu et al., 2019), *tet(D)* (Gambino et al.,

2021), *tet(G)* (Wang et al., 2021), and *tet(R)* (Patel et al., 2020)) have already been identified among *Salmonella* serovars with *tet(A)* reported mostly in *Salmonella* situated in bacterial genomic island, on intergrons and transferrable plasmids (Alcaine et al., 2007). *Tet(B)* has been mostly found in movable plasmids among *Salmonella* serotypes (Alcaine et al., 2007). *Tet(A)* and *tet(B)* are the most prevalent among various *Salmonella* serotypes and are associated with occurrence of MDR in *Salmonella* (Vilela et al., 2019).

2.3.6 Resistance to Sulfonamides and Trimethoprim

Sulphonamides are bacteriostatic antimicrobial drugs (Abutaleb et al., 2022). Their bactericidal effect is mainly through inhibiting dihydropteroate synthetase (DHPS) thereby stopping the formation of tetrahydro folic acid (Patel et al., 2019). On the other hand, the expression of dihydrofolate reductase (DHFR) confers resistance to trimethoprim (Alcaine et al., 2007). *Salmonella* resistance to sulphonamides is associated with expression of *sul* genes (Pavelquesi et al., 2021) which manifests an unresponsive form of dihydropteroate synthetase.

Three categories of *sul* genes have been reported to exist, that is; *sul 1* (Pavelquesi et al., 2021), *sul 2* (Mthembu et al., 2019), and *sul 3* (Zhang et al., 2019). *Sul 1* is most common in serotypes of *Salmonella* and has been identified in serovars *Agona* (Lee et al., 2022), *Albany* (Hong et al., 2021), *Derby* (Pławińska-czarnak et al., 2022), *Enteritidis* (Kanaan et al., 2022), *Hadar* (Gu et al., 2020), *Heidelberg* (Tagg et al., 2019) and *Typhimurium* (Tagg et al., 2019) is often linked with class 1 intergrons possessing other ARGs.

Although found to co-exist with *sul1* in bacteria, *sul2* is mostly plasmid borne (Alcaine et al., 2007). *Salmonella* serotypes *Enteritidis* (Wang, 2020), and *Typhimurium* (Mensah et al., 2019) are also found to possess *sul2*. Recently, another sulphonamides resistant gene, *Sul 3* been found in some *Salmonella* serotype such as *typhimurium* (Oliva et al., 2020), *infantis* (Egorova

et al., 2022), *enteritidis* (Dai et al., 2021) and others. These genes have been associated with plasmids and class I integrons (Alcaine et al., 2007). On the other hand resistance to trimethoprim is associated with DHFR expression alters trimethoprim binding sites (Alcaine et al., 2007). Many *dhfr* and *dfr* variants such as *dhfr1*, *dfrA1*, and *dhfr12* are mostly found to exist in class 1 integrons bearing other sulfonamide determinants such as *sul1* and *sul3*. In addition, they have also been seen to exist on movable plasmids bearing other ARGs (Ambrose & Hall, 2021). Earlier studies revealing the existence of these genes among serotypes of *Salmonella* have been reported (Tok et al., 2023).

2.3.7 Resistance to macrolides

The term macrolides refer to a group of antibiotic drugs consisting of different ring sized macrocyclic lactones attached to a deoxy or amino sugar (Dinos, 2017). Macrolides work through hindering synthesis of bacterial proteins and by binding on their 50S ribosomal subunit (Miklasińska-Majdanik, 2021). From the progenitor erythromycin discovery in 1950 (Chaudhari et al., 2023), various generations of macrolides including azithromycin and clarithromycin have been synthesized possessing better pharmacokinetics and improved stability in acidic conditions (Chaudhari et al., 2023). However, this development came along with serious side effects (Hu, 2020). Resistance to macrolides in bacterial species is mainly achieved through three ways that is; mutations in the ribosomal RNA gene and ribosomal proteins, enzymatic inactivation by esterase/phosphotransferases, through expression of macrolide efflux pumps.

With respect to mutations in ribosomal RNA a nucleotide A2058 of the 23s rRNA has been observed to play a major role in establishing resistance towards macrolide across many bacterial pathogens (Dinos, 2017). Changes in structure of this nucleotide induced by

mutations to A2058G have been viewed to confer resistance to all macrolides including ketolides (Dinos, 2017). Other mutations in position A2059 (Vester & Douthwaite, 2001) and 2057 (Fyfe et al., 2016) though with limited frequency in bacteria are known to confer resistance to macrolides. The mutation in U2609c seen in bacterial pathogens such as *E. coli* was linked to selected resistance against certain ketolide antibiotics such as telithromycin and cethromycin. Addition mutations such as C2610U and C2611U have been also viewed to provide high resistance to macrolides and ketolides across bacterial species (Dinos, 2017). On the other hand, through acquisitions of genes that code for modifications in 23S rRNA, bacterial species have been able to display high resistances to not only macrolides but also other groups of antibiotics such as lincosamides and certain members in streptogramin B family (Dinos, 2017). These genes encode for expression of methyltransferases which catalyze methylation reactions in the A2058 of the 23S rRNA (Dua et al., 1985). This enzyme class is designated Erm for erythromycin resistance methylase and its specific genes, *emra*, *emrb* and many others (Dinos, 2017). These genes have been found in plasmids and within transposons of various *Salmonella* serotypes; *Typhimurium* (Xie et al., 2022), *Enteritidis* (Igbinosa et al., 2021), and *Agona* (Asad et al., 2022). These genes usually co-exist with other genes that express resistance to other antimicrobials.

With regard to structural changes in ribosomes, mutations in genes encoding L4 and L22 ribosomal proteins have been found to confer erythromycin and telithromycin resistance (Pihlajamäki et al., 2002). Changes within highly conserved sequence of *S. pneumoniae* L4 (₆₃KPWRQKGTGRAR₇₄), was observed to result into reduced susceptibility to both macrolides and ketolides (Dinos, 2017). In addition, mutational changes that usually takes place in C-terminal regions of L22 ribosome have been highly associated to express resistance towards macrolides (Farrell et al., 2004).

Through the action of macrolide esterase/phosphotransferases, an ester bond between macrolide aglycons is formed. This bond is known to be a principal substrate to macrolide resistance enzymes (Ere(A) and Ere(B)). The genes that encode for expression of these enzymes are; *ere (A)* and *ere (B)* (Zieliński et al., 2021). The existence of these genetic markers on the MGEs indicates their potential to be propagated and widely spread within and between microbial species (Dinos, 2017) including *Salmonella*. On the other hand phosphotransferases (MPH) are commonly found on the MGEs may be inducible by *mph(A)* or constitutively by *mph(B)* (Rudakova et al., 2020). These enzymes facilitate the transfer of the nucleotide γ -phosphate to desosamine 20-OH group in ring macrolide antibiotics affecting the interaction between a particular macrolide drug and A2058 site (Shakya & Wright, 2010). Responsible genes that express Mph enzymes have been found to exist on MGEs having other antimicrobial resistance genes (Dinos, 2017).

Macrolide efflux pumps are proteins that mediate the replacement macrolide molecule by proton. These proteins are encoded by gene families encoded as *mef* (Kumar et al., 2020; Rédei, 2008). There are two major classes of *mef* genes, that is, *mef(A)* and *mef(E)* gene (Dinos, 2017). These genes encode resistance to 14, 15 but not 16 member macrolides, lincosamides and streptogramins B (Dinos, 2017). The *mef(E)* just like *msr(A)* class of genes are associated with carrying *msr(D)* gene which jointly are responsible to confer more resistance to macrolides (Nunez-Samudio & Chesneau, 2013). These proteins interact in synergy resulting in an increased resistance to macrolides (Dinos, 2017). Recently additional plasmid borne *mef* genes such as *mef(B)*, *mef(O)*, *mef(I)* and *mef(d)* have been identified. All these are reported to confer high levels of resistance to macrolides (Dinos, 2017; Rakitin et al., 2022).

2.3.8 General observations on genetic determinants of antibiotic resistance in *Salmonella*

Arising from literature reviewed under sub-sections 2.3.1-2.3.7 it is apparent that from the time antibiotics began to be used, there has been an observable development and expression of proteins working independently and others synergistically to confer resistance to the various types of antibiotics targeting them (Alcaine et al., 2007). These proteins are encoded by genes which are either on chromosomes or MGEs of the bacterial genomic islands (Lou et al., 2019). As such, these genes have the ability to be spread or distributed within the microbial community either of the same or different species. A summary indicating the class of antibiotics, mode of action and common resistance genes that detected in *Salmonella* are presented in Table 1. Despite the existence of elaborate literature on occurrence of ARGs in *Salmonella*, it is essential to appreciate that the distribution of the resistance genes depend on the serotype, geographical location and pre-exposure history (Varga et al., 2020). Thus, it remains largely unknown the resistance genes that *Salmonella* isolated from food and water consumed by children under five in Karamoja sub-region carry.

Table 1: Genetic markers of *Salmonella* resistance to various class of antibiotics

Class of antibiotic	Mode of action	Common resistance genes in <i>Salmonella</i>
Aminoglycosides	Inhibits protein synthesis	<i>aac</i> , <i>aad(A)</i> , <i>str(A)</i> , <i>str(B)</i> , <i>aph</i> , <i>aad(B)</i>
Beta lactams	Inhibits cell wall formation	<i>bla_{CMY}</i> , <i>bla_{CTX}</i> , <i>bla_{TEM}</i> , <i>bla_{CARB-2}</i> , <i>bla_{OXA}</i>
Chloramphenicol	Inhibits protein synthesis	<i>Cat1</i> , <i>cat 2</i> , <i>floR</i> , <i>emlA</i>
Quinolones	Inhibit topoisomerase	<i>gyrA</i> , <i>gyr B</i> , <i>par C</i>
Tetracyclines	Inhibit protein synthesis	<i>tet (A)</i> , <i>tet(B)</i>
Sulfonamides	Inhibit dihydropteroate formation	<i>sul 1</i> , <i>sul 2</i> , <i>sul 3</i>
Macrolides	Inhibit protein synthesis	<i>mef (A)</i> , <i>erm((B)</i> , <i>erm(A)</i>

2.4 Association between phenotypic and genotypic antibiotic resistance in bacteria

Genetically, the phenotype displayed by an organism is determined or influenced by the genotype (Amer et al., 2018). Some reports have documented an existing association between ARGs (genotype) with displayed resistance (Besharati et al., 2020; Pławińska-czarnak et al., 2022; Serotyping & Manifestations, 2016; Yang et al., 2020; Youssef et al., 2021) thus indicating full expression of genetic resistance. This complete expression of ARGs should be a point of focus to be targeted by various public health agencies since most of them are located on the MGEs with the ability to be distributed between and within bacterial species with in a microbial community. Other studies on *Salmonella* revealed that the pathogen in some situations expressed antibiotic resistance to particular antibiotics even in the absence of the

specific encoding ARGs. For instance a study on systematic evaluation of whole genome sequence- based predictions of *Salmonella* serotype and antimicrobial resistance reported antibiotic susceptibility to streptomycin among the studied isolates despite presence streptomycin resistance genes (Cooper et al., 2020). Similarly, Lapierre et al. (2020) reported colistin resistance among the studied *Salmonella* isolates in absence of the *mrc* genes. This suggests that at times the pathogen may still exhibit antibiotic resistance in the absence of the gene. This could be attributed to other factors that enforce antibiotic resistance but do not involve genetic alteration such as stationary growth phase, biofilm formation and persistency (Amer et al., 2018). Understanding the association between the displayed phenotypes and genotype is paramount in informing policy choices aiming to reduce or prevent further spread of antibiotic resistance in the microbial population. Furthermore, this not only helps to contribute to the existing antibiogram in the management of human salmonellosis but also it guides clinicians to make informed and effective choices in managing *Salmonella* infections. As already indicated above, it is apparent that phenotypic resistance can be displayed in the absence or presence of the genetic markers. On the other hand, previous studies have shown that antibiotic resistance and the association between the phenotype and the genotype is not uniform but varies with the serotypes and strains within a serotype, the substrate from which infection is acquired, and the extent of antibiotic use in a particular geographical locality. In light of these observations, it becomes difficult to predict the association between phenotypic resistance and genotypic resistance of *Salmonella* from food and water in Karamoja sub-region from the results of previous studies. This should be determined empirically.

CHAPTER THREE: METHODOLOGY

3.1 Study Area, Sample collection and transportation sub-region

Samples for the study were collected from Karamoja sub-region. The is located in North East Uganda. It is bordered by Kenya in the East, Lango and Acholi in the West and Teso in the South. The sub-region was selected for the study because it bears the highest prevalence of both child undernutrition and diarrhea in Uganda (UBOS, 2019). It is composed of 9 districts. A random sampling strategy was used to select locations for sample collection. At the district level, Nakapiripirit and Nabilatuk districts were selected followed by two sub counties from each of them using random sampling strategy. Sub counties selected in Nabilatuk district included Lolachat and Lorengedwat while Nakapiripirit TC and Namalu were selected from Nakapiripirit district. As such, 50 samples were collected from each selected sub county. A total sample size of 200 different food and water samples were taken from randomly selected households. This was determined using a sample size equation for a known population attribute (Thrusfield, 2007).

$$n = \frac{z * z * p(1-p)}{e * e} \dots\dots\dots \text{Equation 1}$$

$$n = \frac{1.96 * 1.96 * 0.14(1-0.14)}{0.05 * 0.05}$$

Where **n** is the required sample size, **z** is the confidence level at 95% (Standard value 1.96), **p** is the prevalence of diarrhoea among children under five years admitted in health facilities which stands at 14% (**0.14**) (Appiah et al., 2021) and **e** is the margin of error set at 5% (standard value =0.05). Using equation 1, sample size was found to be 185. However, this was increased to 200 to reduce on the sampling errors. Proportionally samples included cooked cow peas leaves (n=12), cooked meat (n=12) drinking water from households (n=30), water from community water sources (n=26), maize flour (n=12), fresh milk (n=12), fermented milk (n=12), cooked

beans (n=12), sorghum bread (n=12), maize posho (n=12), millet porridge (n=12), millet flour(n=12), cassava flour (n=12) and Sorghum flour (n=12). The sample collection exercise took place in the month of March, 2023. For each food sample, approximately 250 to 400 g were picked using sterile disposable spoons and transferred into sterile disposable bags and placed in already prepared cool box 4°C. These were instantly transported to the laboratory and analysed within 24 hours.

3.2 Isolation of *Salmonella*

In accordance with Food and Drug Authority (FDA) guidelines, *Salmonella* isolation was performed according to the method recommended (FDA, 2018). In brief, 25 mls/grams of the food sample was added to 225 mls of buffered bacteriological peptone (Candalab, Madrid, Spain) and incubated at 37°C for 24 hours for pre-enrichment. Further for selective enrichment, 0.1 ml of the pre-enriched inoculum was transferred to 10 mls of Rapaport Vassiliadis Soy (RVS) broth (Himedia, Mumbai, India) and incubated at 42 °C for 24 hours. Both pre-enrichment and selective enrichment were done under shaking conditions. After enrichment, a loopful of the inoculum were streaked on xylose lysine deoxycholate (XLD) Agar (OXOID, Basingstoke, UK) and incubated at 37°C for 24 hours. The presumptive *Salmonella* colonies appearing pink with dark centres were re-streaked on XLD agar to obtain pure cultures which were screened further for confirmation using Colony Polymerase Chain Reaction (PCR).

3.3 PCR confirmation of *Salmonella* isolates

Colony PCR with a procedure described by Chandra et al. (2013) was performed out to confirm *Salmonella* isolates with primer targeting the *invA* virulence gene (*InvA*- F: 5'-GAGGAAAAAGAAGGGTCG-3'), and (*InvA*-R: 5'-CTCAACTTCAGCAGATACCA-3') (Radhika et al., 2014). The *invA*, the gene that is particularly involved in host recognition and invasion of

the epithelial cells of intestinal mucosa is believed to occur in all *Salmonella* serovars. As such, the *invA* has previously been used in several studies to confirm isolates belonging to the genus *Salmonella* (Alarjani et al., 2021; Tanyag et al., 2021 and Zhanabayeva et al., 2021). The PCR was performed according to procedure optimised by Radhika et al. (2014) with modifications. In brief, the PCR was standardised in a final volume of 25 µL consisting of 12.5 µL Taq 2X master mix, 0.5 µL each of the forward and reverse *invA* primer, 9.5 µL PCR water and 1 to 2 distinct *Salmonella* colonies as the sample DNA. The PCR conditions were as follows: Amplification consisted of 1 cycle of initial denaturation at 94°C for 10 minutes, this was then followed by 30 cycles of denaturation at 94°C for 1 minute, primer annealing at 54°C and extension at 72°C for 1 minute followed by final extension at 72°C for 5 minutes. The PCR product was electrophoresed using 2% agarose gel stained with ethidium bromide and visualised under the Vilber bio print transilluminator set at 250v 50/60 HZ (Vilber smart imaging, France) transilluminator. *Salmonella typhimurium* 14028 and nuclease free water were used as PCR positive and negative controls respectively. The primers and other PCR reagents were obtained from Inqaba Biotechnical industries (Pretoria, South Africa). Following PCR analysis, 45 isolates were confirmed as *Salmonella*.

3.4 PCR Serotyping of *Salmonella* isolates

All the 45 confirmed *Salmonella* isolates were subjected to a multiplex PCR serotyping using genomic DNA. The DNA was extracted using the Quick DNA fungal/bacterial miniprep (Zymo research, California, United States). DNA presence and integrity was then confirmed through 1% (w/v) agarose gel electrophoresis stained with ethidium bromide. The confirmed DNA was stored at freezing conditions of -20°C until used. The PCR was performed in 25 µl volume (Table 3.1). Amplification reactions were performed using the SimpliAmp™ thermal cycler, Singapore programmed as follows: Initial denaturation at 94°C for 10 minutes followed

by 35 amplification cycles of denaturation at 94°C for 30 seconds, annealing for each primer was done at specific temperatures for 1 minute (Table 2), primer extension at 72°C for 30 seconds and final primer extension at 72°C for 4 minutes. Nuclease free water was used as a negative control. The electrophoresis gel was prepared by adding 1% of TBE buffer at a pH of 8.0 and dissolved fully under warm conditions. This was left to cool at room temperature then stained cooled and stained with ethidium bromide (0.5 µg/ml). The prepared gel was then cast for 30 minutes till it completely dried. A volume of 3 µl of PCR product and 2 µl of molecular weight marker with 1000 bp DNA ladder used to determine band sizes. The amplified PCR products were analyzed in a horizontal electrophoresis unit (Consort Electrophoresis model E844, Belgium) for a period of 45 minutes at voltage of 250v. Gel observations were visualized using the Vilber bio print transilluminator projected to a computer screen.

Table 2: List of primers used in the serotyping of the confirmed *Salmonella* isolates

Targeted serotype	Target gene	Primer sequence	Annealing temp °C	Amplicon size (bp)	Reference
<i>Salmonella enteritidis</i>	<i>Sdf 1</i>	F: 5'-TGT GTT TTA TCT GAT GCA AGA GG-3' R: 5' -TGA ACT ACG TTC GTT CTT CTG G-3'	55	304	(Nair et al., 2015)
<i>Salmonella typhimurium</i>	<i>Spy</i>	F: 5- TTG TTC ACT TTT TAC CCC TGA A-3' R: 5' -CCC TGA CAG CCG TTA GAT ATT-3'	58	301	(Nair et al., 2015)
<i>Salmonella Heildberg</i>	1067175	F :5'-ACTTCATGCGCAGACTTGCC-3'	54	105	(Akiba et al., 2011)
	1068152	R: 5'-CAAGAATCGGGAAATTTTGA-3'			
	2541120–	F: 5'-TACCGCCCCATTTGATATCT-3'		199	
	2541716	F: 5'-CCCTTGCGCAACATAAAACT-3'			
	4675756–	F: 5'-GGATCTCAACAAATGAGGT-3'		303	
<i>Salmonella choleraesuis</i>	4676622	F: 5'-ATGCATCACTGCATCTTCGT-3'	54		(Akiba et al., 2011)
	SCH_0971	F: 5'-AGATATGCGGCTTGCTGACT-3' R: 5'-AGCGTGGCTCGAAACAGTAT-3' F: 5'-GGCGAAAGAGCTTAACGTGA-3'		100	
	SCH_2145	R: 5'-TTACCATCGGGACCAAATGT-3' F: 5'-TCGGGATCGTCCTCTATACG-3'		198	
	SCH_4349	R: 5'-GTGGATGTGTTGACCGACTG-3'		305	
<i>Salmonella infantis</i>	3113085–	F: 5'-GGTCATTGTCGGAAACCTG-3'	54	95	(Akiba et al., 2011)
	3114152	F: 5'-ACATTCCCCCTTCCACTGCC-3'			
	3711271–	F: 5'-CGCGAAGAAGTGCATAAACC-3'		198	
	3711921	R: 5'-CGCCACTTTCGTTATCTGAG-3'			
	4621947–	F: 5'-ACCTACTACTATCCCTGATG-3'		304	
<i>Salmonella dublin</i>	4622627	R: 5'- 5'GCGAATTTTGCTACTTGAAG-3'	54		(Akiba et al., 2011)
	SeD_A1226	F: 5'-ATCGGTGCTGGGTAATTTTG-3' R 5'-AGGAACGAGAGAAACTGCTT-3'		105	
	SeD_A1104	F: 5'-ACGCGAAATCTGATGGTCTT-3' R: 5'-GCCCACCAGTTGTGAAAGGC-3'		203	
	SeD_A2276	F: 5'-ATCACCCTCGCAAACCTTGTC-3' R: 5'-TCGGGCAATCAGGTCGCCGA-3'		296	

3.5 Phenotypic antimicrobial susceptibility evaluation of *Salmonella* isolates

The confirmed *Salmonella* isolates were subjected to antibiotic susceptibility test on Mueller Hinton Agar (MHA) (OXOID, Basingstoke, UK) using the Kirby Bauer disc Diffusion method. Prior to the antibiotic disc placement on the MHA plates, colonies of confirmed *Salmonella* isolates were mixed with normal saline and the turbidity of the cell suspension adjusted to 0.5 McFarland standard. This turbidity is estimated to be equivalent to $1-2 \times 10^8$ cfu/ml of *Escherichia coli* (*E. coli*) cells. *E coli* ATCC 25922 was used as a quality control strain. The antibiotics used in susceptibility testing were selected based on the Ministry of Health (MoH) and WHO's recommendation in the management of *Salmonella* illnesses. As such nine different types of antibiotic molecules that fall in different classes of antibiotics were used. They include beta lactams (amoxicillin clauvinic acid or AMC (20/10 µg), Cephems (cefotaxime or CXT (30 µg); ceftriaxone or CRO (30 µg); ceftazidime or CAZ (30 µg); cefixime or CFM (5µg), Sulphonamides (cotrimoxazole or SXT (25 µg), macrolides (azithromycin or AZI (15 µg), Phenicols (chloramphenicol or C (30 µg) and Fluoroquinolone (ciprofloxacin or CIP (5 µg). In conducting the susceptibility tests, the antibiotic discs were put on MHA plates with adequate spacing to avoid overlapping of the zones of inhibition. The MHA plates with discs were incubated at 37°C for 24 hours. The inhibition zone diameters were then measured after the incubation period and the interpretation of the diameters of the zones of inhibition made according to the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2020.). Multiple antibiotic resistance (MAR) index for each isolate was determined by using a formula $MAR = a/b$ where *a* represented the number of antibiotics to which the test isolate depicted resistance and *b* represented the total number of antibiotics to which the test isolate was evaluated for susceptibility.

3.6 Genotypic characterization of antibiotic resistance of *Salmonella* isolates

For all *Salmonella* isolates that showed resistance to one or more of the used antibiotics, the specific ARGs were detected using conventional polymerase chain reaction (PCR). The DNA extracted under subsection 3.5 was used. The PCR was performed in 25 µl volume (Table 3). The PCR amplifications were conducted with a DNA SimpliAmp™ thermal cycler, Singapore programmed as follows: Initial denaturation at 94°C for 10 minutes followed by 35 amplification cycles of denaturation at 94°C for 30 seconds, annealing for primers of the targeted gene (Table 4) for 1 minute, primer extension at 72°C for 30 seconds and final primer extension at 72°C for 4 minutes. Nuclease free water was used as a negative control. The electrophoresis gel was prepared by adding 1% of TBE buffer at a pH of 8.0 and dissolved fully under warm conditions. This was left to cool at room temperature then stained cooled and stained with ethidium bromide (0.5 µg/ml). The prepared gel was then cast for 30 minutes till it completely dried. A volume of 3 µl of PCR product and 2 µl of molecular weight marker with 1000 bp DNA ladder used to determine band sizes. The amplified PCR products were analyzed in a horizontal electrophoresis unit (Consort Electrophoresis model E844, Belgium) for a period of 45 minutes at voltage of 250v. Gel observations were visualized using the Vilber bio print transilluminator projected to a computer screen.

Table 3: The composition of the 25 µl volume of PCR reaction used in the study

Component	25 µl reaction
Taq 2x master mix	12.5 µl
Forward primer	0.5 µl
Reverse primer	0.5 µl
Nuclease free water	9.5 µl
Template DNA	2 µl

Table 4: List of genes and primers with their annealing temperatures used to detect for the genetic resistance of the different antibiotics

Antibiotic	targeted gene	Primer Sequence	Annealing Temperature	Amplicon size	References
Chloramphenicol	<i>cat1</i>	F: 5'-GGCATTTCAGTCAGTTG-3' R: 5'-CATTAAGCATTCTGCCG-3'	50	551	(Kikuvi et al., 2010)
	<i>Flo R</i>	F: 5'-AACCCGCCCTCTGGATCAAGTCAA-3' R: 5'-CAAATCACGGGCCACGCTGTATC-3'	60	548	(Fasaei et al., 2015)
Beta lactams	<i>bla_{TEM}</i>	F: 5'-CCGTGTCGCCCTTATTCCC -3' R: 5'-GCCTGACTCCCCGTCGTGT -3'	71	780	(Kikuvi et al., 2010)
	<i>bla_{PSE}</i>	F: 5'-CGCTTCCCCGTTAACAAGTAC-3' R: 5'-CTGGTTCATTTTCAGATAGCG-3'	58	465	(Kikuvi et al., 2010)
	<i>Bla CTX-M</i>	F: 5'-ATGTGCAGYACCAGTAARGTKATGGC-3' R: 5'-GGGTRAARTARGTSACCAGAAYSAGCGG-3'	58	592	(Mulvey et al., 2003)
Quinolone	<i>par C</i>	F: 5'-CTATGCGATGTCAGAGCTGG-3' R: 5'-TAACAGCAGCTCGGCGTATT-3'	62	270	(Elbadawi , 2017)
	<i>gyrA</i>	F: 5'-AAATCTGCCCCGTGTCGTTGGT-3' R: 5'-GCCATACCTACGGCGATACC-3'	55	343	(Elbadawi , 2017)
Macrolides	<i>erm B</i>	F: 5'-CGTACCTTGGATATTCACCG-3' R: 5'-GTAAACAGTTGACGATATTC-3'	60	224	(Skittrall et al., 2021)
	<i>mef A</i>	F: 5'-CTGTATGGAGCTACCTGTCTGG-3' R: 5'-CCCAGCTTAGGTATACGTAC-3'	60	294	
Sulphonamides	<i>sul 1</i>	F: 5'-GCGCTCAAGGCAGATGGCATT-3' R: 5'-GCGTTTGATACCGGCACCCGT-3'	70	284	(Rahmani et al., 2013)
	<i>Sul 2</i>	F: 5'-GCGCTCAAGGCAGATGGCATT-3' R: 5'-GCGTTTGATACCGGCACCCGT-3'	70	284	(Aarestrup et al., 2003)

3.7 Data analysis

For objective one, descriptive statistics in form of percentages was used to report the prevalence of *Salmonella* and the serotypes observed in the study. For objective two, isolates were scored as either susceptible, intermediate or resistant according to the Clinical and Laboratory Standards Institute (CLSI). Resistance to ≥ 3 or more antimicrobials was classified as Multidrug resistant (MDR). MAR index value >2 indicated high risk source of contamination where antibiotics are often used. Descriptive statistics in form of percentages was used to report the prevalence of AMR phenotypes against each antimicrobial agent. For objective three, descriptive statistics in form of percentages was used to report the prevalence of ARGs. For objective four, a chi-square analysis was performed to determine the association between specific ARGs and AMR phenotypes. Statistically significant associations were shown as odd ratios (ORs) with 95% confidence intervals. An odd ratio of >1 (positive association) indicated increased probability of co-concurrence of the genotype or phenotype being studied with the measured phenotype or genotype. An OR <1 (negative association) indicated decreased probability of co-occurrence of the genotype or phenotype studied with the measured phenotype or genotype. Statistical analysis was performed using SPSS (version 20.0) while a $p < 0.05$ was considered significant.

3.8 Ethical Considerations

Ethical approval to conduct this study was sought from and granted by the Gulu University Research Ethics Committee (Approval number: GUREC-2022-456) (Appendix 1). Consent to collect food samples was sought from household members in the selected sub counties and districts with in Karamoja sub-region (Appendices 3 and 4) for sample of household consent form in English and Karamojong language, respectively.

CHAPTER FOUR: RESULTS

4.1 Prevalence and serotypes of *Salmonella* that occur in food and water consumed by children under five

Before the serotypes could be ascertained, *Salmonella* was detected at the genus level presumptively by culture method and confirmed by PCR. Presumptively out of the 200 food and water samples examined, *Salmonella* was detected in 90 (45%) of them. Recovery of the organism was highest in cooked cowpeas leaves (32,2% of the samples) followed by millet bread (13.3%), cooked meat (8.9%), millet porridge (8.9%), millet bread (7.8%), water from community sources (7.8%), fresh milk (6.7%), sorghum bread (5.6%), fermented milk (4.4%) and drinking water from households (4.4%). No *Salmonella* was detected in dry and processed flours such as maize flour, sorghum flour, cassava flour and millet flour (Table 5).

Table 5: Proportion of food and water samples that tested positive for *Salmonella* presumptively by culture method

Samples	Number of samples	Number of <i>Salmonella</i> positive isolates	Positive rate (%)
Cooked cow peas	12	29	32.2
Cooked meat	12	8	8.8
Water from households	30	4	4.4
Water from community	26	7	7.8
Maize flour	12	0	0
Fresh milk	12	6	6.7
Fermented milk	12	4	4.4
Cooked beans	12	0	0
Sorghum bread	12	5	5.6
Maize posho	12	7	7.8
Millet porridge	12	8	8.9
Millet bread	12	12	13.3
Cassava flour	12	0	0
Sorghum flour	12	0	0
Total	200	90	100

Following the application of PCR targeting the *invA* gene, 45 (50%) isolates were confirmed as *Salmonella*. Of these, 13 (28.8%) isolates were from cooked cow peas leaves, 6 (13.3%)

isolates from millet bread, 5 (11.1%) isolates from cooked meat, 4 (8.8%) isolates were from community water samples, fresh milk and millet porridge, 3 (6.6%) isolates each were obtained from household drinking water, fermented milk and maize posho respectively. Serotyping of the PCR confirmed isolates revealed that 24 (53%) of them were isolates were *S. typhimurium*, 7 (15.56%) were *S. infantis*, 4 (9%) were *S. enteritidis*, and 2 (4%) were *S. dublin*. However, 8 (18%) isolates were unidentified. Serotype *Typhimurium* was the most dominant followed by *infantis*, *enteritidis* and *dublin* in decreasing order of magnitude.

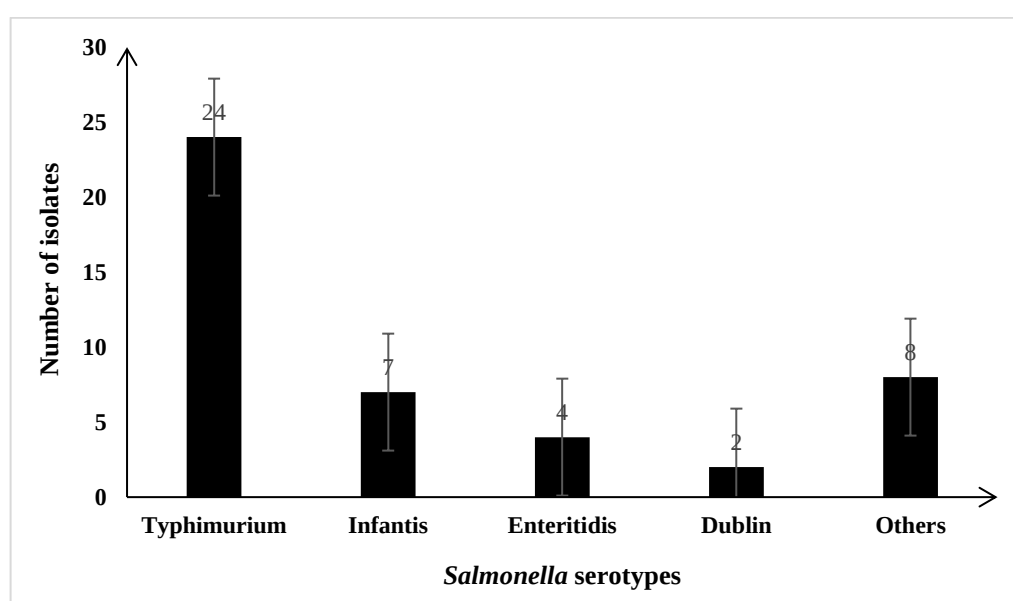


Figure 1: Distribution of *Salmonella* serotypes among the isolates confirmed

4.2 Phenotypic antimicrobial resistance characteristics of *Salmonella* isolates

In general, all the 45 (100%) of the PCR-confirmed *Salmonella* isolates were resistant to at least one antimicrobial agent with 17 (37.9%) isolates displaying MDR and an average MAR index of 0.21 (Table 8). The highest resistance was recorded against azithromycin (91.1%) followed by ciprofloxacin (31.1%), cefixime (20%), cefotaxime (20%), ceftazidime (15.6%), chloramphenicol (6.7%), ceftriaxone (6.7%), cotrimoxazole (6.7%) and amoxicillin clavulanic acid (2.2%) in decreasing order of magnitude (Table 6). Several isolates exhibited resistance

to more than one antibiotic. The resistance pattern is presented in Table 7. A total of 17 resistance patterns were identified among which the dominant ones were Cip-Azi (46.8%) followed by Cip-Azi-CTX (6.7%), Cip-C-20-Azi (6.7%) and Cip-CFM-Azi-CAZ (15.6%)

Table 6: Proportion of *Salmonella* isolates exhibiting various antimicrobial resistance profiles aggregated by antibiotic group

S/N	Antibiotics types	Antibiotic gents	Susceptible	Intermediate	Resistant
01	Beta Lactams	Ceftriaxone (CAX)	41(91.1%)	1(2.2%)	3(6.7%)
		Ceftazidime (CAZ)	37(82.2%)	1(2.2%)	7(15.6%)
		Cefixime (CFM)	36(80.0%)	0	9(20.0%)
		Amoxiclav (AMC)	43(95.6%)	1(2.2%)	1(2.2%)
		Cefotaxime (CTX)	35(77.8%)	1(2.2%)	9(20.0%)
02	Quinolones	Ciprofloxacin (Cip)	6(13.3%)	25(55.6%)	14(31.1%)
03	Macrolides	Azithromycin (Azi)	4(8.9%)	0	41(91.1%)
04	Sulphonamides	Cotrimoxazole (SXT)	42(93.3%)	0	3(6.7%)
05	Phenicol	Chloramphenicol (C-20)	38(84.4%)	4(8.9%)	3(6.7%)

Table 7: Multiple antibiotic resistance patterns exhibited by *Salmonella* isolates

Resistance pattern	No. of Antimicrobial (Agents in the pattern)	No. (%) of <i>Salmonella</i> Isolates with Resistance Patterns (n=45)
Cip-Azi	2	21(46.8)
Cip-CFM	2	1(2.2)
Cip-C-20-CFM-SXT-Azi-CTX-CAZ	7	1(2.2)
Cip-C-20-SXT-Azi-CTX	5	1(2.2)
Cip-CFM-CTX-CAZ-CAX	5	1(2.2)
Cip-Azi-CTX	3	3(6.7)
Cip-CFM-Azi-CAZ	4	2(4.5)
Cip-SXT	2	1(2.2)
AMC-Cip	2	1(2.2)
Cip-C-20-Azi	3	3(6.7)
C-20-Azi-CTX	3	1(2.2)
Cip-CFM-Azi-CAZ-CAX	5	1(2.2)
Cip-CFM-Azi-CTX-CAZ-CAX	6	1(2.2)
AMC-CFM-Azi-CTX-CAX	5	1(2.2)
Azi-CTX	2	1(2.2)
Cip-C-20-CFM-Azi	4	1(2.2)
Cip-Azi-CAZ	3	1(2.2)

Key: CAX-ceftriaxone, Cip-ciprofloxacin-amoxicillin clavulanic acid, CTX-cefotaxime, SXT-cotrimoxazole, CAZ-ceftazidime, CFM-cefixime, C-20- chloramphenicol, Zi-azithromycin

Table 8:: Multiple antibiotic Resistance indices for *Salmonella* isolates (n=45,

Average=0.21)

MAR Index	Number
0.1	25 (55.5%)
0.2	9 (20%)
0.3	3 (6.7%)
0.4	3 (6.7%)
0.5	00
0.6	4 (8.9%)
0.7	1 (2.2%)
0.8	00
0.9	00
1.0	00

4.3 Genotypic antibiotic resistance characteristics of *Salmonella* isolates

Results of PCR analysis conducted to determine genetic markers of antibiotic resistance to the antibiotics investigated are presented in Table 8. Most of the isolates carried multiple resistance genes and were grouped into various clusters. The most common genotypic resistance clusters were: (i) *ParC*; *gyrA*; *mefA* (n=19), *ParC*; *gyrA*; *bla_{PSE}*; *mefA* (n=7), *FloR*; *ParC*; *gyrA*; *mefA* (n=4) and *ParC*; *gyrA* (n=2).

Table 9: Genotypic resistance gene patterns observed in *Salmonella* isolates

Genotypic resistance patterns	No. of Resistance genes	No. (%) of <i>Salmonella</i> Isolates with gene pattern
<i>FloR</i> ; <i>Sul 1</i> ; <i>ParC</i> ; <i>gyrA</i> ; <i>bla_{PSE}</i> ; <i>mefA</i>	6	1(2.2)
<i>FloR</i> ; <i>Sul 1</i> ; <i>Sul 2</i> ; <i>ParC</i> ; <i>gyrA</i> ; <i>bla_{PSE}</i> ; <i>mefA</i>	7	1(2.2)
<i>Flor</i> ; <i>ParC</i> ; <i>gyrA</i> ; <i>mefA</i>	4	4(8.9)
<i>FloR</i> ; <i>bla_{PSE}</i> ; <i>mefA</i>	3	1(2.2)
<i>Sul 2</i>	1	1(2.2)
<i>ParC</i> ; <i>gyrA</i> ; <i>mefA</i>	3	19(42.2)
<i>ParC</i> ; <i>gyrA</i> ; <i>bla_{PSE}</i> ; <i>mefA</i>	4	7(15.7)
<i>ParC</i> ; <i>gyrA</i> ; <i>bla_{PSE}</i> ; <i>mefA</i>	1	1(2.2)
<i>ParC</i>	2	2(4.5)
<i>ParC</i> ; <i>gyrA</i>	2	1(2.2)
<i>GyrA</i> ; <i>mefA</i>	2	1(2.2)
<i>ParC</i> ; <i>mefA</i>	3	1(2.2)
<i>ParC</i> ; <i>gyrA</i> ; <i>bla_{PSE}</i>	1	1(2.2)
<i>bla_{PSE}</i>	2	1(2.2)
<i>bla_{PSE}</i> ; <i>mefA</i>	1	3(6.7)
<i>mefA</i>		

In the context of the five classes of antibiotics investigated, interesting genetic resistance markers were observed with respect to each class (Table 10).

Table 10: Genetic determinants of resistance for *Salmonella* isolates

	<i>bla_{TEM}</i>	<i>bla_{PSE}</i>	<i>bla_{CTX-M}</i>	<i>sul 1</i>	<i>sul 2</i>	<i>gyr A</i>
Positive	0 (0%)	13(81.25%)	0 (0%)	2 (66.6%)	2(66.6%)	37 (94.9%)
Negative	16 (100%)	3 (18.75%)	16 (100%)	1 (33.4%)	1 (33.4%)	2 (5.1%)
Total	16	16	16	3	3	39
	<i>par C</i>	<i>ermB</i>	<i>Mef A</i>	<i>Cat 1</i>	<i>flo R</i>	
Positive	36 (92.3%)	0 (0%)	41 (95.3%)	0 (0%)	7 (100%)	
Negative	3 (7.7%)	43 (100%)	2 (4.7%)	7 (100%)	0 (0%)	
Total	39	43	43	7	7	

Key: *Bla_{TEM}*-resistance gene of beta lactams, *Bla_{PSE}*-resistance gene of beta lactam, *Bla_{CTX-M}*-resistance gene of beta lactams, *Sul 1*- resistance gene of sulphonamides *Sul 2*- resistance gene of sulphonamides, *GyrA*- resistance gene of quinolones, *Par C*- resistance gene of quinolones, *ErmB*- resistance gene of macrolides, *mef A*- resistance gene of macrolides, *Cat 1*- resistance gene of phenicol, *FloR*- resistance gene of Phenicol.

In the case of beta lactams, 16 isolates displayed resistance to the antibiotic class, of which 13 (81.25%) isolates carried one of the genes conferring resistance to beta lactam antibiotics. The most prevalent gene was a penicillinase encoding *bla_{PSE}* (n=13). The extended spectrum beta lactamase (ESBLs) *bla_{TEM}* and the gene for the CTX-M type were not detected in any of the isolates. Three isolates which displayed resistance to beta lactam antibiotics phenotypically did not carry any of the beta lactam ARGs that were investigated. With respect to quinolones, whereas multiple mutations in the quinolone resistance determining regions (QRDR) of the DNA gyrase sub unit gene *gyrA* in combination with mutations in the DNA topoisomerase gene *par C* are expected to result into full resistance to ciprofloxacin, it was apparent that whereas 35 (89.7%) isolates carried both the *gyrA* and the *par C* gene, two isolates carried only *gyrA* while one isolate harboured only the *par C* gene. Worth noting is that whereas one isolate displayed resistance to ciprofloxacin phenotypically, it neither carried the *gyrA* nor the *par C* gene. With regard macrolides, of the 43 isolates that displayed resistance to azithromycin phenotypically, only 41(95.3%) had the macrolide efflux gene *mef A*. The *ermB* gene that

encodes for ribosomal methylase was not detected in any of the isolates that displayed resistance to azithromycin phenotypically. On the other hand, whereas two isolates displayed phenotypic resistance to azithromycin, they did not carry any of the associated ARGs investigated. In the case of phenicols, genes linked to their resistance were present in the 7 isolates that displayed phenotypic resistance to chloramphenicol. The efflux pump gene *FloR* (n=7) was most common among all isolates. However, chloramphenicol acetyltransferase gene *cat A1* was not detected in any of the isolates. For the case of sulphonamides, genes that encode their resistance were detected in all the 3 isolates that displayed phenotypic resistance to cotrimoxazole. Interestingly, only one isolate was found to carry both the *sul 1* and *sul 2* genes while the rest of the cotrimoxazole resistant isolates carried either *sul 1* or *sul 2*.

4.4 Association between genotypic and phenotypic antibiotic resistance characteristics of *Salmonella* isolates

Results of the chi square analysis performed to determine the association between specific ARGs and AMR phenotypes are presented in Table 11.

Significant associations were found between AMR phenotypes and genotypes of the *Salmonella* isolates. Among the 16 isolates that displayed resistance to beta lactams, 13 carried the corresponding ARG. This phenotype was strongly associated with the presence *bla_{PSE}* (OR = 24.7, P = 0.001). However, three isolates did not carry any of the tested ARGs. For the seven isolates that displayed resistance to phenicols, all of them were found to carry the *floR* gene. Nevertheless, *CatA1* was not detected in any of the isolates that displayed phenotypic resistance to Phenicols. Therefore, chloramphenicol resistance was strongly associated with the presence of *floR* (OR = 12.545, P = 0.031).

Table 11: Associations between phenotypes and genotypes of AMR of *Salmonella* isolates

Antibiotic type (Outcome)	Resistance genes (Predictor)	Outcome predictor association		
		OR	95% CI	P-VALUE
β- lactam	<i>bla</i> <i>TEM</i>	-	-	-
	<i>bla</i> <i>PSE</i>	24.7	0.000 - 0.064	0.001
	<i>bla</i> <i>ctx-m</i>	-	-	-
Macrolides	<i>erm</i> <i>B</i>	-	-	-
	<i>mef</i> <i>A</i>	2.3	0.936 - 1.000	0.958
Phenicol	<i>cat</i> <i>A</i> 1	-	-	-
	FloR	12.5	0.000 – 0.065	0.031
Sulphonamides	<i>Sul</i> 1	11.9	0.000 – 0.065	0.000
	<i>sul</i> 2	3.4	0.343 – 0.635	0.583
Fluoroquinolones	<i>Par</i> <i>C</i>	13.3	0.000 – 0.064	0.015
	<i>gyr</i> <i>A</i>	8.4	0.066 – 0.289	0.144

Contrary, all the 41 isolates that displayed resistance to macrolides were found to carry the *mef A* resistant gene. However, none of the macrolide resistant phenotypes carried the *ermB* gene. This phenotype (Azithromycin resistance) was strongly associated with the presence of *mef A* (OR = 2.3, P = 0.958). Among the 39 fluroquinolone resistant isolates detected, all of them were found to carry either the *parC* gene (92.3%, 36/41), the *gyr A* gene (94.9%, 37/39) or both of them. This phenotype was strongly associated with the presence of both *par C* (OR=13.284, p = 0.015) and *gyrA* (OR = 8.418, P = 0.144). Lastly, for the three sulphonamides resistant *Salmonella* isolates, all of them were found to either carry *sul1* or *sul 2* gene. However, 1 out of the 3 isolates was found to carry both *sul 1* and the *sul 2* gene. Resistance to cotrimoxazole was therefore associated with the presence of *sul 1* (OR = 11.9, P = 0.000)

CHAPTER FIVE: DISCUSSION

5.1 Prevalence and serotypes of *Salmonella* associated with foods and water in Karamoja sub-region

At a general scale, the occurrence of *Salmonella* at 45% demonstrates that food and water in Karamoja sub-region is a potential reservoir for pathogens of public health concern such as *Salmonella*. As such, the human population and in particular those that are immune compromised such as children under five years are still at risk of being exposed to infections by *Salmonella*. There is a paucity of information on the prevalence of *Salmonella* in foods and water consumed in Uganda. The *Salmonella* prevalence in foods reported in this study though is higher than 5.8% and 20% reported by Peruzy et al. (2022) and Miao et al. (2022) respectively, it is lower than 60- 70% reported by Hassan et al. (2019) in food products. These variations in *Salmonella* prevalences in food across studies can be attributed to several factors including differences in sample collection, study design, sample size and geographical locations (Sanchez et al., 2007) among others. Nevertheless, the 45% prevalence status of *Salmonella* suggests a higher degree of non-adherence to sanitary food handling behaviours in Karamoja. High levels of poor food sanitary practices in agro pastoral and pastoral communities (Wanjala et al., 2016) such as Karamoja regions have been reported. The observed prevalence of *Salmonella* can also be attributed to the high level of livestock-human interaction that occurs in pastoral regions. The livestock gut is known to be a potential reservoir for zoonotic pathogens such as *Salmonella* which can be shed into the environment through faeces causing environmental contamination (Gallo et al., 2020).

Among food samples investigated, the highest level of *Salmonella* occurrence was found in cowpeas leaves (Table 5). The findings in this study are comparable with those of Okidi et al. (2022) with respect to cowpeas leaves that also reported a high load of *Salmonella* in green

leafy vegetables from Karamoja sub-region. Thus, leafy vegetables such as cowpeas leaves could be some of those food types with high levels of poor food sanitary practices and as such they may be classified as high-risk foods for children under five years. It is interesting to note that no *Salmonella* was detected in cooked beans and dry processed flours such as wheat flour, maize flour, sorghum flour and cassava flour (Table 5). With respect to beans, legumes contain high levels of phytates and lectins. Phytic acid and lectins have been reported to possess significant antimicrobial properties against both gram-negative and positive bacterial pathogens (Zhou et al., 2019). For dried uncooked and milled cereals, the absence of *Salmonella* could be attributed to the low moisture content of flours (Gallo et al., 2020), high shear stress during milling (Chitrakar et al., 2019), and high polyphenolic components (Khoddami et al., 2021) which collaboratively hinder microbial growth. Similar findings indicating less than 2% *Salmonella* prevalence with respect to dry and uncooked cereals have been reported (Danyluk et al., 2007). The observed presence of *Salmonella* in cooked foods and its absence in dry uncooked foods such as flours may suggest contamination to have originated from the water and the cooking equipment used in food preparation or during food handling. This suggestion draws evidence from results presented under sub section 4.1 (Table 5) which shows that 4.4% of the overall *Salmonella* prevalence came from drinking water. Nonetheless, there is need for further investigation to identify other likely source of food contamination. This necessary for comprehensive understanding of *Salmonella* food contamination scenarios in the sub-region

Three major serotypes; *Typhimurium*, *Infantis* and *Enteritidis* were identified to be the most prevalent in this study (Figure 1). Parry-Hanson Kunadu et al. (2020) while investigating the microbial quality of informal raw chicken processing similarly identified *Typhimurium*, *Infantis* and *Enteritidis* as the most prevalent *Salmonella* serovars. Among observed serotypes in this study (Figure 1), *S. typhimurium* was the most dominant (Figure 1). Previous studies by

Tîrziu et al. (2020) and Parry-Hanson Kunadu et al. (2020) also recorded the dominance of the serotype *S. typhimurium* in various food products. In addition, Gutema et al. (2019) further highlighted *S. typhimurium* as the most prevalent and dominant invasive non-typhoidal *Salmonella* in East Africa. The dominance of *S. typhimurium* in food and water samples investigated could be due to cross contamination from the large number of livestock kept within Karamoja sub-region. Livestock is known to be a principal reservoir of *S. typhimurium* (Kijima et al., 2019). This serotype is harboured in the gastro intestinal tract of cattle where it is excreted through fecal matter into the environment (Gallo et al., 2020). On the other hand, *S. typhimurium* is known to possess advanced virulence mechanisms and preadaptation strategies which it can use to tolerate both mild and extreme environmental conditions as well as to adopt to and occupy various niches in the environment including food and water sources (Zarkani & Schikora, 2021). This implies that the *S. typhimurium* isolates detected in food and water samples may also be from other niches. From the results of molecular (PCR) serotyping, 8 isolates were not identified and allocated to any serovar. A concerted effort should be exercised to serotype these isolates. This is because considerable differences have been observed among various serotypes in terms of infectiousness, survival in food, and severity of disease conditions they elicit (Varga et al., 2020).

5.2 Phenotypic antibiotic resistance profiles of *Salmonella* isolates

Despite the advantages of antibiotic drugs in treating infections such as salmonellosis, their extensive, abusive and non-judicious use has resulted into AMR. The uncontrolled use of these drugs exerts selective pressure which in turn results in development of drug resistant *Salmonella* strains in the environment. This coupled together with poor environmental conditions is a potential threat to food safety and public health most especially in such undernutrition-stricken localities like Karamoja. Results in this study revealed that MDR

strains can be obtained from commonly consumed food and water. This suggests potential contamination of the environment including water sources in Karamoja. The average MAR index of 0.21 in this study indicated a high-risk source of contamination in Karamoja sub-region. The resistance patterns this study (Table 6) observed varied between *Salmonella* isolates. The most resisted antimicrobial agent was azithromycin followed by ciprofloxacin. Slightly a higher resistance was recorded by Gomes et al. (2022) in regard to azithromycin (95%). Ciprofloxacin resistance pattern was comparable to findings by Zhang et al. (2019) who recorded 30.26% resistance to ciprofloxacin whereas contrasting those of Ngai et al. (2021) where all the *Salmonella* isolates displayed susceptibility to ciprofloxacin. The difference could be due to differences in strains of the same serovars but circulating in different geographical localities (Varga et al., 2020).

Cefotaxime and cefixime were the most resisted third generation cephalosporins while the least resistance was recorded for ceftriaxone (Table 6). Contrary, to the findings of this study, higher *Salmonella* resistance patterns of more than 50% have been recorded with respect to cefotaxime and cefixime from prior studies (Salman et al., 2022; Vaez et al., 2020; Yasmin et al., 2020). The ceftriaxone resistance pattern in this present study is in agreement with those Awol et al. (2019) who recorded all *Salmonella* isolates as susceptible to ceftriaxone. The observed resistance towards third generation cephalosporins in present study (Table 6) suggests that *Salmonella* may be acquiring resistance to these treatment choice antibiotics. The resistance pattern registered towards chloramphenicol (Table 6) in present was more than the 3.13% resistance to chloramphenicol recorded by Sohail et al. (2021) but lower than the 12.26% and 10.22% recorded by Teng et al. (2022) and Borah et al. (2022) respectively.

The resistance of *Salmonella* to cotrimoxazole (Table 6) was similar with results of a study done in Nepal (4%) (Niroula et al., 2020) but lower than those of (Abebe et al., 2019; Assoumy

et al., 2021; Kamilu & Mukhtar, 2022; Pilau, 2022). The difference may be due to different susceptibility methods breakpoints used (Sharp, 2013). Amoxicillin clavulanic acid showed 2.2% resistance against *Salmonella* isolates which was comparable with findings by Khan et al. (2021) and Rush et al. (2020) who recorded resistance towards amoxicillin clavulanic acid of less than 2%. The low resistance to amoxicillin clavulanic acid could be associated with presence of potassium clavulanate, a beta lactamase inhibitor in the drug combination (Thompson & Ramos, 2017). These resistant isolates observed may work as potential reservoirs for transfer of resistant genes to other highly infectious gram-negative pathogens in the environment. The fact that all the PCR-confirmed isolates displayed antibiotic resistance to recommended antibiotic drugs indicates that food and water consumed by children in Karamoja sub-region is generally unsafe because they expose children to antibiotic resistant *Salmonella*.

5.3 Genotypic antibiotic resistance profiles of *Salmonella* isolates

Among the 45 *Salmonella* isolates resistant to at least 1 antibiotic, 7 resistance genes conferring resistance to 5 classes of antibiotics were identified. The PCR results were consistent with individual resistance phenotypes. Among beta lactamase encoding genes, *bla_{PSE}* a penicillinase encoding gene was the most prevalent (Table 10) in beta lactam resistant isolates. This is similar with those of Feldgarden et al. (2019) who found *bla_{PSE}* to be the most dominant at 95% among *Salmonella* isolates that displayed resistance to beta lactam antibiotics in their study. The widespread of *bla_{PSE}* among *Salmonella* isolates is an important public health concern suggestive of its ability through horizontal transfer to be propagated in either similar or different bacterial species as such leading to proliferation of more antibiotic resistant strains of pathogens in the environment. This could partly explain why resistance against penicillins such as ampicillin and amoxicillin over the years have been reported to be high in many microbial species including *Salmonella*.

Bla_{TEM} and *bla_{CTX-M}* were not detected in any of the 3GCs and cefotaxime resistant isolates in present study. This contrasts with the findings from earlier studies (Langata et al., 2019; Ramatla et al., 2022; Souza et al., 2020) that showed a high prevalence of *bla_{TEM}* and *bla_{CTX-M}* in corresponding resistant *Salmonella* isolates. The Cephalosporine and cefotaxime resistance in present study therefore may be attributed to presence of other resistance genes such as *bla_{SHV}* and *bla_{OXA}* which were not investigated in present study. All cotrimoxazole resistant isolates were found to carry either *sul 1* or *sul 2* resistant genes. This conforms to earlier studies conducted by Pavelquesi et al. (2021) and Seo et al. (2019) that showed presence of *sul 1* and *sul 2* in all cotrimoxazole resistant *Salmonella* isolates. These genes induce the expression of drug insensitive dihydropteroate synthase and dihydrofolate reductase that are responsible for expression of resistance towards sulfonamides and trimethoprim (Li et al., 2021).

Whereas no chloramphenicol acetyltransferase gene *cat A1* was present in any of the chloramphenicol resistant *Salmonella* isolates in present study, all the isolates that displayed resistance to chloramphenicol carried the efflux pump gene *floR* (Table 10). Efflux pump systems in *Salmonella* eject the antibiotic from the bacterial cell thereby maintaining the cellular internal environment (Nishino et al., 2021). The findings of the present study are in agreement with those of Mostafa et al. (2021) and Niu et al. (2020) that revealed a high prevalence of *floR* among chloramphenicol resistant *Salmonella* isolates. Of the 43 azithromycin resistant isolates, 41(95.3%) possessed the macrolide efflux gene *mef A*. The gene *mef A*, is responsible for expressing of the macrolide efflux pumps that eject the antibiotic from the bacterial cell. Similar findings were recorded by Tunsagool et al. (2021) that recorded a higher prevalence of *mef A* genes among azithromycin *Salmonella* resistant isolates.

Thirty-five isolates that displayed resistance to ciprofloxacin in present study were found to carry both the *gyrA* or the *par C* gene (Table 10). Multiple mutations present in quinolone resistance determining regions (QRDR) *gyrA* and topoisomerase gene *par C* are expected to code for full ciprofloxacin resistance. Similar findings have been recorded in previous studies (Shen et al., 2020; Zhan et al., 2019) which showed the occurrence of both the *gyrA* and the *par C* gene among all ciprofloxacin *Salmonella* resistant isolates. The match between the results of the current study and those recorded earlier with respect to ciprofloxacin show the wide spread of *Salmonella* resistance to the antibiotic. On the other hand, this study findings show that most of the *Salmonella* isolated carried the genetic markers for resistance to the antibiotics studied. This further confirms that food and water consumed by children in Karamoja is a vehicle for infection with genetically antibiotic resistant *Salmonella*.

5.4 Association between the genotypic and phenotypic antibiotic resistance profiles among *Salmonella* isolates

Strong associations between the phenotypes and genotypes of AMR were found in this present study (Table 11) such as chloramphenicol with *FloR*, Sulphonamides with *sul 1* and *sul 2*, beta lactams with *bla_{PSE}*, macrolides with *mef A* and quinolones with *parC* and *gyrA*. This indicates that resistance to the antimicrobial agent was caused by the presence of the corresponding ARGs. This finding is similar to results of previous studies (Gow et al., 2008; Rosengren et al., 2009; Van et al., 2020). However, interestingly, this present found some isolates that possessed resistance phenotypes but did not carry corresponding ARGs: for example, 3 isolates that displayed resistance to beta lactams did not carry any associated ARGs investigated. Similar findings were recorded. This could be due to the fact that antibiotic resistance patterns in bacteria are expressed basing on the activation of various diverse genetic factors (Van et al.,

2020) thus *Salmonella* mechanisms of antibiotic resistance recovered from food and water consumed by children under five years in Karamoja require further investigations.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has demonstrated that commonly consumed foods and water in Karamoja sub-region harbour multidrug resistant *Salmonella* pathogens capable of causing life threatening infections in humans especially among the immune compromised individuals such as children under five. *S. typhimurium* was the most dominant serotype. Azithromycin followed by ciprofloxacin were the most resisted antimicrobial agents by *Salmonella* isolates whereas least resistance was recorded against amoxicillin clavulanic acid. The study found a distribution of ARGs among *Salmonella* isolates with strong associations between the AMR phenotypes and genotypes.

6.2 Recommendations

6.2.1 Recommendation for practice

There is need for the community in Karamoja to be sensitized on good food sanitary behaviour and thorough cooking practices to curb the high prevalence of *Salmonella* in food. Amoxicillin-clavulanic acid may be prioritized as a potential drug in treating *Salmonella* infections in Karamoja. Furthermore, amoxicillin clavulanic acid can also be prioritized in developing vaccines targeted to prevent *Salmonella* infections. Lastly, there is need for effort by policy makers to strengthen the implementation of antimicrobial resistance NAP objectives as a way to reduce on the spread of AMR in pathogens.

6.2.2 Recommendations for further Research

Based on the findings of this study, the following recommendations are proposed:

- i. This study found *Salmonella* to be present in majority of the cooked foods but absent in uncooked foods. This may suggest contamination to have arisen from water during food preparation or from food handlers. Therefore, there is need for further studies to track the source of *Salmonella* contamination so as to help inform policy decisions.
- ii. Further studies should look at whole genome sequence of the isolates so as to understand the genomic diversity of the *Salmonella* isolates.

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APPENDICES

APPENDIX 1: ETHICAL CLEARANCE CERTIFICATE

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RESEARCH ETHICS COMMITTEE

To: MPAGI RONALD

20/03/2023

+256783025450

Type: Initial Review

Re: GUREC-2022-456: Antibiotic Resistance profiles of Salmonella isolated from food and water consumed by children under five years in the Karamoja sub region, 2.0, 2023-03-11

I am pleased to inform you that at the 94th convened meeting on 17/11/2022, the Gulu University REC, committee meeting, etc voted to approve the above referenced application.
Approval of the research is for the period of 20/03/2023 to 20/03/2024.

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of 20/03/2024 in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by Gulu University REC:



GULU UNIVERSITY
RESEARCH ETHICS COMMITTEE
APPROVED UNTIL
★ 20 MAR 2024 ★
FACULTY OF MEDICINE
P. O. Box 166, Gulu

APPENDIX 2: COVID-19 AND EBOLA ENGAGEMENT PLAN

Measures for the Prevention and control of the risk of the spread of COVID-19 and Ebola during research undertakings

Study Title: Antibiotic Resistance Profiles of Salmonella Isolates Obtained from Food and Water Consumed by Children Under five years in the Karamoja Sub-region

Principal Investigator (s): Mpagi Ronald

Introduction:

The novel Coronavirus is transmitted from human to human through droplets and direct or close personal contact with an infected individual. Novel Coronavirus signs of infection include respiratory symptoms, fever and cough, shortness of breath and breathing difficulties. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Ebola is transmitted from human to human through direct contact with infected people's blood, secretions, organs or other bodily fluids and surfaces and materials (e.g. bedding, clothing) contaminated with these fluids. Ebola signs of infection include fever, fatigue, muscle, pain, headache, and a sore throat. This is followed by vomiting, diarrhoea, rash, impaired kidney and liver function symptoms, and internal and external bleeding in some cases. Laboratory findings include low white blood cell and platelet counts and elevated liver enzymes.

Implementation of the prevention and control measures

In the implementation of the research activities, the investigators are committed to ensuring the safety of the research team, research participants and communities where the study will be conducted. The study team undertakes to comply with the Standard Operating Procedures issued by the Ministry of Health, and presidential directives to mitigate against the risk of



APPENDIX 3: INFORMED CONSENT FORM (ENGLISH)

Version 2.0

Introduction and consent

Hello, my name is _____, I am conducting a study on the Antibiotic Resistance Profiles of *Salmonella* isolated from food and water consumed by children under five years in the Karamoja sub region. I would much appreciate your participation in this study. I would like to collect some food and water samples commonly feed to children under five years in your household. The samples collected will only be used for laboratory analysis during the study. You will receive a packet of salt and bar of soap for your participation in this study. Your participation is voluntary, but we hope you will participate since your views are important. Do you have any questions?

May I begin to collect the food and water samples?" (If the response is "NO", go to the next Household)

Name of participant..... signature/thumbprint.....

Name of witness.....signature/thumbprint.....date.....

Name of interviewer.....signature.....date.....

General information

District.....subcounty.....Parish.....Village.....

Livelihood Zone.....Household ID.....Date of collection.....

APPENDIX 4: INFORMED CONSENT FORM (LOCAL LANGUAGE: KARAMOJONG)

AKITODIUN KA ESEI

Maata, eraai ekakiro.....Ayai ayong akingitingit nguna etapito epite ngolo itokuruworit ngiko akitiya anakuwan aitungunan koterere iretakini edeke. Akajoikinit ayong kichamu iyong akingisingisia nugu. Tokona ekengisi ayong yong kangisita nguna edolote ngadangikai ngatomoniwini bon. Daadang nguna ilimuni iyong nyekorario lotunga daadang nait isitiyao kotere ayanyun nguna etapit ayakau ka akimuj ka ngipitesio ngulu ititinyikintor iyes alotoma ebuku kus.

Iriamuni iyong achumumbi ke esabuni erai akitalakaret kotere achamakin akingisingiseta.

Emam nyikitingakinio iyong achamakin ngakingisieta nu, nait kejok achamakin Eira anenikon anieraei nguna ilimuni iyong erai nguna itowuikinit ayanyun nguna akire etapito ayakou ka akimuj anege.

Iyakar iyong akingiset adia?

A geikini kona alemuni ngamuja ka ngakipi kon a? (ketem "mam" tolol lokal ngolo chee)

Ekiro aitunganan angini ingisingisio.....Esei.....

Ekiro ke esuada.....esei.....ngirwa ke elap.....

Ekiro ke ekengitingitan.....esei.....ngirwa ke elap.....

APPENDIX 5: DATA RECORD SHEET

Version 2.0

DATA RECORD SHEET

RECORD SHEET			Date	
			Name	
Antimicrobial	Isolate ID		Isolate ID	
	Zone diameter	Interpretation	Zone diameter	Interpretation
Azithromycin (15 µg)				
Amoxicillin-clavulanic acid (20 µg)				
Ceftazidime (30 µg)				
Ciprofloxacin (5 µg)				
Cotrimoxazole (23.75 µg)				
Chloramphenicol (30 µg)				
Cefixime (30 µg)				
Cefotaxime (30 µg)				
Ceftriaxone (30 µg)				



APPENDIX 6: ATTACHMENTS

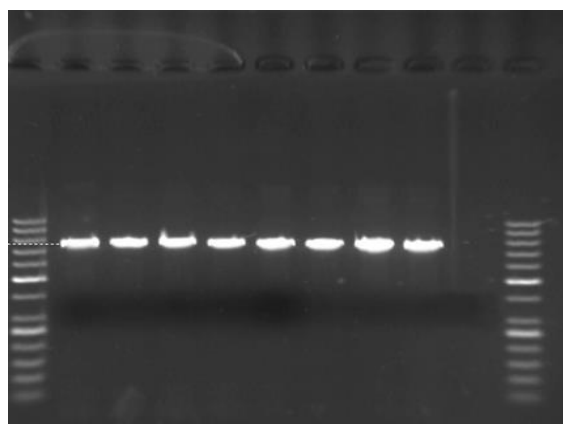


Figure 2: A sample of the diameter zones on inhibition after susceptibility testing

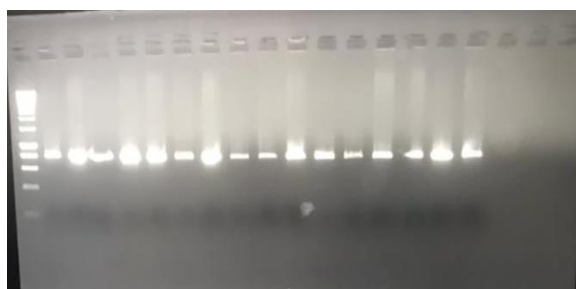


Figure 3: PCR amplicons obtained by primer specific to bla PSE

Lane 1 is a 1000 bp ladder. The other lanes show band of *Salmonella* isolates positive for bla PSE gene

APPENDIX 7: ORIGINALITY REPORT

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PLAGIARISM CHECK

Declaration for Originality Form

This form must be completed and signed for all scholarly work produced.

Name: MR. MPAGI RONALD

Personal File No.(staff) Reg. (student). 21/U/2413/MFN/PS

Faculty/Institute: FACULTY OF AGRICULTURE AND ENVIRONMENT

Department: FOOD SCIENCE AND POST HARVEST TECHNOLOGY

Title and bibliographic description of the work:

PREVALENCE AND ANTIBIOTIC RESISTENCE CHARACTERISTICS OF NON-TYPHOIDAL SALMONELA SEROTYPES ISOLATED FROM FOOD AND WATER CONSUMED BY CHILDREN UNDER FIVE IN KARAMOJA SUB-REGION

Declaration.

1. I understand what plagiarism is and I am aware of the University policy on this regard,
2. I declare that this ... **Dissertation** ... (thesis, dissertation, essay, assignment, paper, report etc.) is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my work has been used, this has been properly acknowledged and referenced in accordance with Gulu University requirements,
3. I have not sought or used the services of any professional agency to produce this work,
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own,
5. I understand that any false claim of this work shall result in disciplinary action in accordance with University Policy on Plagiarism.

Signature

Date

17th / 08 / 2023

Originality Report

The article/thesis/document titled above have been subjected to plagiarism check and the level was found to be.....**16%** (percent) this is classified as:

- ☒ Minor
- ☐ Moderate
- ☐ Serious

Yours sincerely,


Manana. N. Herbert
Ag. University Librarian

