ANTIMICROBIAL RESISTANCE PATTERNS OF FOODBORNE BACTERIAL ISOLATES FROM HIV/AIDS PATIENTS IN LUSAKA, ZAMBIA

By

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This dissertation is being submitted to the University of Zambia in partial fulfillment of the academic requirements for the Masters of Science degree in Applied Microbiology

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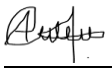
DECLARATION

I, **Aron Rezene Mebrahtu**, declare that this Master's dissertation represents my own work. It has not previously been submitted for a postgraduate degree or any award at the University of Zambia or any other institution. All cited works and materials from other sources have duly been acknowledged and references thereby given.

Signature: 

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This dissertation submitted by Aron Rezene Mebrahtu is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Applied microbiology at the University of Zambia.

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ABSTRACT

Antimicrobial resistance is a major global public health concern and a food safety issue. When pathogens become resistant to antimicrobial agents they can pose a greater human health risk as a result of potential treatment failure, loss of treatment options and increased likelihood of severity of disease. Therefore, this study was designed to determine the antimicrobial drug resistance profile of foodborne bacterial isolates among HIV/AIDS patients. The study was a cross sectional study which was conducted from July to September, 2024 at a microbiology laboratory in the University teaching hospital (UTH). Stool samples were taken from respondents attending UTH hospital with one of the complaints of abdominal pain, vomiting, diarrhea, nausea and fever. Age, sex, residence, and sample origin for each participant were taken and recorded for analysis. Additionally, microbiological identification of bacteria through culturing and antimicrobial susceptibility (AST) pattern test using Kirby-bauer disc diffusion techniques were conducted from the stool samples of the respective participants. The participants came from 32 different areas of Lusaka, Zambia and mainly from Bauleni (12.96%) and Kanyama (7.40%). Most of the study participants (32 participants), were a regular visitor to infectious disease hospital for taking their routine antiretroviral drugs (ARVs) regime while the remaining 22 were from the wards E22, E21 and E12. A total of 77 bacteria were isolated and *Eschercichia coli* was found to be the most prevalent bacteria to be isolated with 27.30% followed by *Protues vulgaris* (15.60%) and *staphylococcus aures* (14.30%). *Eschercichia coli* been found to be highly resistant to ampicillin and sulfamethoxazole/trimethoprim with 95.4% and 80.95% resistance respectively. Foodborne bacteria such as *Staphylococcus aurues* isolates were 100% resistant to azithromycin and 90.90% resistant to methicillin. While *Salmonella paratyphi* isolate was 100% resistant to ampicillin, Sulfamethoxazole/trimethoprim and amoxicillin/clavulanic acid. MDR (multidrug resistance) was seen in 19.40% of the isolates and XDR (extended drug resistance) in 27.80% of the foodborne bacterial isolates. Moreover, MDR foodborne bacteria were significantly associated with sample origin (*p-value*= 0.007). This study has revealed that the issue of drug resistance of foodborne bacteria are at alarming incidence in HIV/AIDS patients. Therefore, proper management in accordance to one health approach should be followed in order to bring a timely solution.

DEDICATION

I dedicate this dissertation to my parents, Mr. Rezene Mebrahtu and Mrs. Hiriyti Yemane who took care of me at all costs in my life and to my brother Mr. Mussie Hailemichael who showed me the way to academic excellence, for his motivation throughout my study years and endless life support.

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LIST OF ABBREVATION

AIDS:	Acquired immunodeficiency syndrome
BFBD:	Bacterial Foodborne Diseases
CD 4:	Cluster of differentiation 4
FAO:	Food and Agriculture Organization
FBB:	Foodborne bacteria
FBD:	Foodborne Diseases
FBI:	Foodborne Infection
HIV:	Human immunodeficiency virus
MDR:	Multidrug Resistance
TH cells:	T helper cells
UTH:	University Teaching Hospital
WHO:	World Health Organization
XDR:	Extended drug resistance

CHAPTER ONE

INTRODUCTION

1.1 Background

Food is defined as any substance which is consumed for a nutritional support by an organism. Despite the fact that it provides the body with essential nutrients, when contaminated by pathogens can result in foodborne diseases (FBD) (Tang *et al.*, 2017). Among the FBD, bacterial foodborne diseases (BFBD) are of specific importance to this research and occur from ingesting bacterial contaminants which are responsible to cause mild to severe health conditions (Abebe, 2020). The main symptoms of BFBD include diarrhea, stomach pain, nausea, vomiting and fever. They can last for a few hours or several days with serious health problems and long term effects including prolonged hospitalization and mortality (Kareem and Al-Ezee, 2020). Each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420,000 deaths with the highest prevalence of 91 million cases of sickness recorded yearly and 137,000 deaths coming from Africa alone (WHO, 2021). Severity of the illness highly depends on immunity of individuals as well as the type of bacteria that contaminated the food. Immunocompromised individuals associated with a greater risk of foodborne infection (FBI) include people with HIV/AIDS, pregnant women, people who have undergone organ transplants, people taking medications that interfere with immune function (e.g., cytotoxic drugs for the treatment of cancer) among others (Hlashwayo *et al.*, 2023).

Antimicrobial resistance is a major global public health concern and a food safety issue. It can pose a greater human health risk as a result of potential treatment failure, loss of treatment options and increased likelihood and severity of disease (Benedict, 2011). Today we are encountering foodborne multi drug resistant microorganisms in clinical and farm settings that are difficult to combat with currently available antibiotics (Benedict, 2011; FAO and WHO, 2023). These multiple resistances have been mainly attributed to the proliferation of resistant genes and to the ease of dissemination of resistant strains between humans and animals especially via food of animal origin or fecal contamination (FAO and WHO, 2023).

Antibiotics used at sub-therapeutic levels to promote rapid growth at the farm-level and to improve feed conversion into meat and milk, can leave residues that then could contribute to the development of resistance to other antimicrobial compounds (Samtiya, 2022). Some studies on the foodborne antibiotic resistance of pathogens isolated from various food products shows that the average frequency of their occurrence in food is greater than eleven percent (Tao *et al.*, 2022). And most of the pathogens show resistance to β-lactam antibiotics, while multidrug resistance (MDR) has been observed among greater than thirty six percent of pathogens (Tao *et al.*, 2022). In recent years, attention has been focused on the emergence of therapeutic-antibiotic resistant strains among the most common foodborne pathogens Sub-Saharan African countries. However, the resources and skills required to perform accurate and reliable microbiology are scarce in low-resource settings (Ombelet *et.al*, 2018). Simply having a laboratory and the latest equipment is not enough. Rather, investing in quality clinical microbiology services that meet a minimum set of standards is of extreme value both for routine clinical care and also for early detection of hospital outbreaks, epidemics and potential pandemics (Ombelet *et.al*, 2018). The emerging resistant phenotypes among foodborne pathogens include *Escherichia coli, Salmonella* spp, *Listeria monocytogenes and Campylobacter* spp (Samtiya, 2022).

The susceptibility to a variety of common and opportunistic foodborne infections in HIV-infected people is generally associated with the progressive decline in the immunological responses as a result of low CD4+ T-lymphocyte cell count (Mwambete, 2014). And pathophysiological complications include the damage of immune system specially the Th 17 cells of lamina propria of the gut by foodborne pathogens such as *salmonella* species to enter to the blood stream (Muller et.al, 2012). Multidrug-resistant *Salmonella enterica serovar Typhi (S. Typhi)* and *nontyphoidal Salmonella* continues to be the leading cause of high mortality and recurrence bacteremia, especially in developing countries, which is typically presented in patients with HIV once the level of CD4+ count falls below 200 cells/μL (Shaklet and Anton, 2010; Muller *et.al*, 2012). Foodborne illnesses by drug resistant pathogens among HIV/AIDS patients are likely to be more serious and last longer. Therefore, it is advised that people living with the virus need to take extra vitamins and minerals to help repair and heal their damaged immune cells (Muller *et.al*, 2012). In addition to that, strict follow up of food safety guidelines proposed by medicals and public health organizations is believed to reduce the risk of foodborne illnesses (Muller *et.al*, 2012). Therefore,

this study was designed to determine the antimicrobial drug resistance profile of foodborne bacterial isolates among HIV/AIDS patients.

1.2 Problem statement

The excessive use and abuse of drug therapies by humans and in animals intended for human consumption are the major reasons for antimicrobial resistance (Wang *et al.*, 2022). Not only that but also poor antimicrobial waste disposition system and lack of food safety practices are increasing the burden of antimicrobial resistant pathogens in recent years (Kim and Ahn, 2022). This phenomenon affects any person regardless of sex, age, origin, or social status and affects mostly immunocompromised patients such as people living with HIV/AIDS (Wang *et al.*, 2022). It is because, the complications from this antimicrobial resistance can result in recurrent enteric infections, septicemia, hemolytic uremic syndrome, anemia, and other opportunistic illnesses among HIV/AIDS patients. Ineffective laboratory diagnosis such as issues concerning the standardization of critical concentrations conducting AST, missing of important antibiotic discs for assay, reproducibility and the reliability of results are affecting AMR results negatively (Olson et.al, 2012). The poor food and water hygienic practices together with high cases of HIV/AIDS in sub Saharan African countries pose a great risk in developing foodborne illnesses and associated complications (Omulo *et al.*, 2015). In 2020, 630 000 [480 000–880 000] people died from HIV-related causes all over the world (WHO, 2021), and 24, 000 (3.8%) were from Zambia. Hence, HIV/AIDS is the primary cause of death in Zambia (CDC Zambia, 2021) and the need to study antimicrobial resistance of bacterial foodborne pathogens in people living with HIV/AIDS becomes important.

1.3 Justification and Significance of the study

Antimicrobial resistance is a major global public health concern and a food safety issue particularly in sub-Saharan African countries like Zambia. Therefore, the study is expected to provide beneficial results on which antibiotics remain effective against foodborne pathogens in HIV/AIDS patients. This allows healthcare providers to make informed decisions about treatment and is essential for improving patient outcomes and reducing unnecessary antibiotic use, which could further exacerbate AMR. Additionally, given the high HIV/AIDS burden in Zambia, understanding the intersection of antimicrobial resistance, foodborne pathogens, and immunocompromised populations can help shape public health interventions. It will also inform policies for better food safety practices, antimicrobial stewardship programs, and infection control measures in healthcare settings. Furthermore, the results of this study will contribute valuable data to regional health authorities in Zambia and neighboring countries, where foodborne infections are a major concern. The findings could help inform broader health initiatives aimed at combatting AMR, improving foodborne disease prevention.

1.4 Scope of the study

The study was limited to microbiological analysis of food poisoned HIV/AIDS patients attending UTH in Lusaka, Zambia which was conducted at a specified period of time.

1.5 Objectives

1.5.1 General objective

To investigate the antimicrobial resistance profile of foodborne bacterial isolates and the socio-demographic factors among HIV/AIDS patients.

1.5.2 Specific Objectives

1.5.2.1 To identify bacterial food pathogens from HIV/AIDS patients at University Teaching Hospital.

1.5.2.3 To evaluate antimicrobial resistance profile of the isolated bacterial food pathogens.

1.5.2.3 To analyze socio-demographic factors of the study participants.

1.6 Research questions

1. What are the common foodborne bacterial species responsible for food poisoning among HIV/AIDS patients in Lusaka, Zambia?
2. What is the antimicrobial resistance profile of the identified bacteria?
3. How are the socio-demographic factors associated with antimicrobial resistance profile of the isolated bacteria?

CHAPTER TWO

LITERATURE REVIEW

2.1 Common Foodborne pathogens

Food can be contaminated by food hazards at any level from harvesting until at a stage where it can be served for nutrition purposes. It can be contaminated by different food hazards, but bacteria are the most common causative food hazard agent (Panwar *et al.*, 2023). Although there are 31 different food-borne bacteria, some of them are particularly important due their consequences in humans and their ability to resist antimicrobials. These includes *Staphylococcus aureus (S. aureus), Salmonella* spp*, Shigella* spp*, Aeromonas species, and Escherichia coli (E. coli)* (Hlashwayo *et al.*, 2023).

A. Staphylococcus aureus

Staphylococcus aureus is one of the most common food-borne pathogens worldwide caused by ingestion of *Staphylococci* enterotoxins (Filipello *et al.*, 2020). It is gram positive coccus, facultative anaerobe, grape like structure under microscopy which can be found as a normal flora in human and animal skin and mucosae (Pal, 2022). *S. aureus*, especially the methicillin-resistant *S. aureus* (MRSA) strain, is highly responsible for various infectious diseases including food poisoning (Aung *et al*., 2017). And researches have mentioned that, the highest carriage of MRSA strains are found in HIV/AIDS patients are seventeen times more likely to contract it when compared to non HIV/AIDS patients (Filipello *et al.*, 2020). *Staphylococcus aureus* is a commensal bacterium and important cause of healthcare-associated infections (Wertheim *et.al*, 2005). Nasal carriage is considered to be the most important site of *S. aureus* colonization and is the best-studied (Wertheim *et.al*, 2005). However, other extra-nasal body sites, including the gastrointestinal tract, are known to harbor *S. aureus* (Rimland and Roberson, 1986). Recent studies have found *S. aureus* in the intestines of healthy humans (Benito *et.al*, 2013; Gurnee *et.al*, 2014) as well as the intestines of hospitalized patients.

B. Salmonellae species

The most common and with highest health consequence among foodborne pathogens is *salmonella* (Kareem and Al-Ezee, 2020). It lives in the gut of most animals which is transmitted by fecal oral route, eating contaminated milk and egg, from person to person via utensils like dishes (Rahman, 2015). *Salmonellae* spp are gram negative facultative anaerobes and are catalase positive, oxidase negative and ferment glucose mannitol and sorbitol to produce acid or acid and gas (Kareem and Al-Ezee, 2020). *Salmonella* is significantly higher in HIV/AIDS patients and is associated with bacteremia a prevalence of 13.7% in contrast to 3.8% non-HIV subjects in a study by udoh and his colleagues (Udoh *et al.*, 2023). *Salmonella* spp. are the leading bacterial causes of food-borne illness in the US (Scallan *et.al,* 2013). The CDC estimates that more than 1 million people in the US contract Salmonellosis each year, with an average of 19,000 hospitalizations and 380 deaths (Scallan *et.al,* 2013). *Salmonella spp.* live in the intestines of most livestock and many wild animals. *Salmonella* spp. infection usually occurs when a person eats food contaminated with the feces of animals or humans carrying the bacteria. Salmonellosis outbreaks are commonly associated with eggs, meat, and poultry, but these bacteria can also contaminate other foods such as fruits and vegetables. More recently, the CDC has reported a total of 258 persons infected with the outbreak strain of *Salmonella bareilly* (247 persons) or *Salmonella nchanga* (11 persons) from 24 states and the District of Columbia (CDC, 2012). Thirty-two ill persons have been hospitalized, and no deaths have been reported. Collaborative investigation efforts of state, local, and federal public health agencies indicate that a frozen raw yellow fin tuna product, known as Nakaochi Scrape, from Moon Marine USA Corporation is the likely source of this outbreak (CDC, 2012).

C. Shigella species

The genus *Shigella* is a member of the family Enterobacteriaceae and possesses four serogroups that have been traditionally treated as species: serogroup A as *Shigella dysenteriae*, serogroup B as *Shigella flexneri*,serogroup C as *Shigella boydii*, and, serogroup D as *Shigella sonnei*. Whereas serogroups A, B, and C consist of 38 serotypes, serogroup D possesses only one (Bacon *et.al*, 2003). Shigella are non-motile, non-spore-forming, facultative anaerobic Gram-negative rods. They can grow at temperatures ranging from 6 to 48 °C, but prefer 37 °C, and *S. sonnei* appears to be able to tolerate lower temperatures better than the other serogroups. Optimum growth occurs

between pH 6.0 and 8.0, although growth has been reported between pH 4.8 and 9.3 (Bacon *et.al*, 2003).

Shigella spp. are closely related to E. coli in their DNA homology and share some biochemical characteristics as well as reactivity to some of the same antibodies, but despite these similarities, their differentiation should be considered clinically significant based, at least in part, on differences in symptoms expressed by infected individuals (Bacon *et.al*, 2003). *Shigella* spp. are found most frequently in environments of compromised sanitation and poor hygiene, and although the primary route of transmission is by person-to-person contact, shigellosis can occur after the ingestion of focally contaminated water or food (Bacon *et.al*, 2003). *Shigella* spp. have not been associated with one specific type of food; foods associated with outbreaks of shigellosis have included milk, salads, chicken, shellfish, and other fresh produce served at a wide range of establishments including restaurants, homes, schools, sorority houses, commercial airlines, cruise ships and military mess halls (Bacon *et.al*, 2003).

D. Aeromonas species

Human infection with *Aeromonas species* is uncommon and most often due to trauma with exposure to contaminated water or soil. *Aeromonas* species are classiefied into two main groups; the psychrophilic non motile *aeromonas,* designated *aeromonas salmonicida* with optimal growth temperatures of 22–25 °C that infects reptiles and fish, and the much larger group of motile mesophilic aeromonads with an optimal growth temperature of 35–37 °C. The motile mesophilic aeromonads are responsible for and are associated with a range of human diseases. The most common species identified are *A. hydrophila*, *A. caviae* and *A. veronii biovar sobria* which cause 85% of *Aeromonas* gastrointestinal infections (David *et.al*, 2010).

E. Escherichia coli

Escherichia coli is also a significant foodborne pathogen characterized as a gram-negative, rod shaped bacterium with five virulence groups, including *enteroaggregative Escherichia coli, enterohemorrhagic E. coli* (also known as Shiga toxin—producing *E. coli* [STEC]), *enteroinvasive E. coli, enteropathogenic E. coli,* and *enterotoxigenic E. coli* (Kabiraz *et al.*, 2023). *E. coli O157:H7* is one of the best known STEC serotypes to contain pathotypes that can cause food-borne infection in HIV/AIDS patients (Ajibola *et al.*, 2023). STEC infection can cause episodes

of mild to severe diarrhea, and 5–10% of infections develop into Hemolytic Uremic Syndrome (HUS)—a severe complication marked by profuse bleeding that can lead to kidney failure and death. STEC strain *O157:H7* is estimated to cause 63,000 illnesses, 2,100 hospitalizations, and 20 deaths each year (Scallan *et.al,* 2011). The principal reservoir for this zoonotic pathogen is the intestinal tract of cattle, but other animals may also serve as reservoirs. *O157:H7* emerged as a significant public health threat in 1982 during two outbreaks of disease that investigators associated with the consumption of undercooked ground meat (Scallan *et.al,* 2011). A wide variety of foods, including fresh produce, have since served as a vehicle for *Escherichia coli O157:H7* outbreaks. Food producers must report the presence of *E. coli O157:H7* to health authorities (Scallan *et.al,* 2011). *Clostridium* spp., *Bacillus* spp., *Vibrio* spp., *Campylobacter* spp., *Pseudomonas* spp., *Cyclospora* spp., *Klebsiella* spp., *and Acinetobacter* spp. can also be associated with BFBD in humans (Kabiraz *et al.*, 2023).

HIV/AIDS patients are highly prone to many foodborne bacterial pathogens where the above mentioned FBB are among the most associated to foodborne illnesses, therefore it is highly recommended to study all the bacteria responsible for food illnesses (Obi *et al.*, 2007). Hence, this study focused on a broad spectrum bacteria isolation from stool samples of HIV/AIDS patients. However, many similar studies in sub Saharan countries are designed to focus on single bacterial isolates which this might lead to data missing on other important foodborne bacterial pathogens associated with complications in HIV/AIDS. For instance a study in Zambia (Momba *et.al,* 2018) focused on specific bacterium isolate *e. coli* and a study conducted in Nigeria focused on campylobacter spp. only (Falodun et.al, 2020).

2.2 Isolation and characterization of foodborne bacterial pathogens from stool samples

Based on properties on growth preferences by foodborne bacterial pathogens, different agar plates are used for isolation purposes from stool samples (Abebe, Gugsa and Ahmed, 2020). MacConkey agar is used for the detection of *E. coli, Salmonella, and Shigella* species based on the principle of fermenting a sugar lactose and Salmonella-Shigella agar (SS) selectively for the latter pathogens. Thiosulfate-citrate-bile salts-sucrose (TCBS) agar used for the detection of *Vibrio species*, Mannitol-Salt Agar (MSA) for *S. aureus* due to its resistance to high salt concentration, Modified Campylobacter Blood-Free Selective Agar Base (mCCDA) for *Campylobacter species*, Listeria

Selective Agar (LSA) for *L. monocytogenes*, CefsulodinIrgasan-Novobiocin (CIN) for *Y. enterocolitica* (Kim *et al.*, 2015). All these culture media have specific incubation temperature and incubation period requirements following the streak method (Obi *et al.*, 2007).

The chance of isolating bacteria increases with immediate isolation process. even though, refrigeration of samples is an option in case of delay in transport which is believed to reduce the chance of isolation of some bacteria. Additionally, suspension of the samples in normal saline and prolonged pre-incubation of the samples in blood culture has shown affirmative increments in chances of isolation (Chang *et al.*, 2019). In this study, the above mentioned points will be incorporated so that to increase the chances of isolation.

There are two ways of characterizing bacteria namely the phenotypic biochemical method and the recent advances of molecular methods (Ryan, 2016 and Gryp *et al.*, 2020). The common biochemical test includes carbohydrate test, Catalase production and coagulase test for gram positive bacteria, indole test, citrate utilization, decarboxylation or deamination of the amino acids, Nitrate reduction, O-Nitrophenyl--D-galactoside (ONPG) breakdown, Oxidase production, Proteinase production, Urease production, and Voges–Proskauer test (Ryan, 2016). But for research purposes, the API 20E identification system designed for identifying members of the Enterobacteriaceae and other nonfastidious Gram-negative rods is the commonly used kit. But others like the API 20NE identification system designed for identifying nonfastidious, nonenteric Gram-negative rods, The API CHB / E identification system intended for the identification of Bacillus and related genera, Microgen GN A and GN B are in action (Awong-Taylor, 2007).

Nowadays the most efficient way of identification is using molecular methods which include two important steps; DNA extraction and identification by polymerase chain reaction (PCR), using gel electrophoresis, or other techniques (Amany et.al, 2014). Different methods of DNA extraction have also been practiced such as boiling method, chemical and enzyme treatment to lyse bacterial cell wall to expose the genetic material (Gryp *et al.*, 2020 and M.I., S.N. and A.E., 2014). The enzymatic extraction of genetic material from a bacterial cell is done by using different kits such as QIAamp DNA Stool Mini kit, PureLink Microbiome DNA Purification kit, QIAamp Power Fecal DNA kit, RNeasy Power Microbiome kit (has been optimized for DNA extraction), Semi-automated NucliSens easy Mag DNA extraction. Based on yield and quality of the DNA, the Power Microbiome kit is found to be the most effective kit when compared with the other kits

(Gryp *et al.*, 2020). The PCR works by amplification of target genes in the bacteria to be identified with steps of denaturation of the template into single strands, annealing of primers to each original strand for new strand synthesis and extension of the new DNA strands (Cheng *et al.*, 2014). The universal used primers in the polymerase chain reaction (PCR) reactions for the amplification of the 16S rRNA gene are the 27F and 1507R (Khabo-Mmekoa and Momba, 2022).

In a study by Taylor. A, all the different methods for biochemical characterization of bacteria were assessed against molecular 16S rRNA. The assessment was based on their ability to identify isolates, corroboration of the data with other methods, cost, ease of use, and web resources. The molecular method was found to be costly and identified only 66% of the isolates tested compared with 74% for API. But using the Microgen identification system appears to be better suited than API or 16s rRNA analysis for identification (Awong-Taylor, 2007).

Performing quality control for every reagents and media is a mandatory process to do because ineffective laboratory diagnosis such as issues concerning the standardization of critical concentrations conducting AST, missing of important antibiotic discs for assay, reproducibility and the reliability of results affects results negatively (Olson et.al, 2012). For instance, in a cross-sectional study conducted in four districts of Lusaka Province, Zambia to determine the antibiotic resistance patterns of *E. coli* isolated from stool samples of broiler poultry farm workers, quality control on culture media and Gram staining were missing in their literature (Mwansa *et.al*, 2023). The turbidity of bacterial suspension for antimicrobial susceptibility testing is compared to 0.5 McFarland standard. A study in Bangladesh, aimed at isolation and identification of *Salmonella* serovars from human stool did not mentioned it (Nesa *et.al*, 2021).

2.3 Food borne disease mechanism

The diseases caused by foodborne pathogens can be classified into three forms: foodborne infection, foodborne intoxication, and foodborne toxico-infection (Tang *et al.*, 2017). The principal route of infection for foodborne pathogens is oral and the primary site of action is the intestine. Most foodborne microorganisms cause localized infection and tissue damage but some spread to deeper tissues to induce systemic infection (Tang *et al.*, 2017).

For successful enteric infection, several factors must work cooperatively in a host (Abebe, 2020). First of all, pathogens must gain access to the host in sufficient numbers to initiate infection. The

primary vehicle of transmission is food and water. However, they can be acquired from direct contact with an animal or a human, such as a food handler, from environments (soil, air) or from an arthropod vector (Tang *et al.*, 2017). Once inside the host, the pathogens must survive in the changing environment, multiply and propagate. Pathogens must find a suitable niche for colonization, which is facilitated by adhesion factors, invasion factors, and chemotaxis (for example, bacterial affinity for iron allows the organism to reach the liver which has a rich source of iron in the form of transferrin) (Abebe, 2020). The microbial cell envelope also helps bacteria to survive in the hostile environment, as the capsule protects the bacteria from being engulfed by phagocytes. In addition, bacterial toxins and enzymes protect cells from elimination by the host immune system (Kareem and Al-Ezee, 2020). Pathogens also damage the host tissues and cells by using exotoxins, endotoxins, or enzymes that cause cell death by apoptosis or necrosis and promote bacterial survival and multiplication (Filipello *et al*., 2020).

Foodborne infection is committed by intact living microorganisms, which must enter the host to cause infection (Tang *et al.*, 2017). Following ingestion with food or water, microorganisms pass through the acidic stomach environment and move to the intestine, where they colonize and cross the intestinal barrier using an active invasion process, or via translocation by phagocytic M cells (Abebe, 2020). Some microorganisms cause local tissue damage and induce inflammation, while others spread to lymph nodes, liver, spleen, brain, or other extra-intestinal sites (Abebe, 2020). Foodborne infection can be acute or chronic. In acute infection, the onset of disease is quick and lasts only for a short duration due to a rapid immunological clearance of the microorganism. In chronic infections, the disease is prolonged and immune clearance is not effective. Often, the prolonged infection is perpetuated by the strong immune response mounted by the host rather than the infective agent itself, such as seen in chronic Shigellosis cases. Patients recovering from a foodborne infection may shed the organism for a while. Some foodborne infections may lead to chronic sequelae such as Reiter's syndrome, arthritis, and Guillain–Barre syndrome (Abebe, 2020).

The infectious dose of pathogens or their toxins varies depending on the immunological status of the host and the natural infectivity of the organism. The infectious dose decreases if consumed with liquid food that traverses stomach rapidly or food (milk, cheese, etc.) that neutralizes the stomach acid. Persons with high gastric pH or those undergoing antibiotic therapy for other ailments are also susceptible to foodborne infections because antibiotics reduce the natural

microflora loads in the intestine, which renders the host more susceptible to foodborne infections (Marya *et.al*, 2022).

2.4 Antimicrobial resistance among foodborne bacteria and the mechanism of resistance

Phenotypically antimicrobial resistance is determined by the Kirby-bauer disc diffusion method (Drew *et al.*, 1972). It is explained by a zone of inhibition of bacterial growth resulting when an antimicrobial concentration in the agar equal to or greater than the minimal inhibitory concentration acts upon a critical population of bacterial organisms which have been inoculated on the agar surface. Based on these results the bacteria can be characterized as resistant, susceptible or intermediate (Amany, 2014). The common antibiotic disc used are Amoxicillin-Clavulanic acid (20/10 µg), Cefoxitin (30 µg), Ceftazidime (10 µg), Cefotaxime (5 µg), Ceftriaxone (30 µg), Cefepime (30 µg); Aztreonam (30 µg), Imipenem (10s µg); Ciprofloxacin (5 µg); Gentamicin (10 µg); Amikacin (30 µg); Chloramphenicol (10 µg); Nitrofurantoin (100 µg); Doxycycline (10 µg); Colistin (30 µg); and Fosfomycin (50 µg) (Falodun *et al.*, 2020). It was observed that some regions of thw world have specific antibiotic resistance patterns. For instance, in Iran, Cephalexin, Penicillin, Nalidixic acid and Azithromycin resistance are of major concern compared to other places (Hassani *et.al*, 2022). In Zambia, Imipenem, ciprofloxacin, Amoxicilli/Sulphamethaxozole, and Tetracyclin resistances are among the top antibiotics being out of favor (Muonga *et.al*, 2021). The primary reason for these cases could be the varying availability of specific antibiotics across different countries or regions (Hassani *et.al*, 2022). In Nigeria, Gentamycin and tetracycline are given to sick pigs and birds in most cases compared to other antibiotics, while in Ghana; Tetracycline and Chloramphenicol are administered for poultry production (Wieters *et.al*, 2024).

Polymerase chain reaction amplification for detection of common antibiotic genes namely CTX-M1, CTX-M2 and mecA is used for molecular antimicrobial resistance analysis (Cheng *et al.*, 2014). In a study done in Saudi Arabia, PCR amplification of CTX-M1 was carried out using the primers: 5`- GGT TAA AAA ATC ACT GCG TC-3` (forward) with an amplicon size of 860 base pairs, CTX-M2: 5`- ATG ATG ACT CAG AGC ATT CG-3` (forward) with an amplicon size of 890 base pairs and for mec A TCCAGATTACAACTTCACCAG-3` with an amplicon size of 162 base pairs. And the result showed Six isolates of *Staphylocuccus aureus* found to be carrying mecA gene, while, CTX-M1 gene was observed in three *E. coli* isolates and CTX-M2 in five *E. coli*

(M.I., S.N. and A.E., 2014). Additionally, marA genes of *Shigella* and *E. coli* isolates are found to be common gene to undergo mutation. Overall mutation rate in marA was 19% and Nucleotide sequencing showed mutations in three positions codon 6 (Deletion of Cytosine), 374 (Addition of Cytosine) (Mehata and Duan, 2011).

The major resistance mechanisms of microbes are decreased drug uptake, efflux pumps, enzymes that inactivate an antimicrobial chemical, target alterations by mutation and formation of biofilms (McEwen and Fedorka-Cray, 2002).

Decreased uptake: This is mainly controlled by porins which are openings in the cytoplasmic membrane through which antimicrobial agents can gain entry, and a reduced number of such porins is one means of antimicrobial resistance (McEwen and Fedorka-Cray, 2002).

Efflux pumps: Some bacteria have a system called an efflux pump. As its name suggests this is a system whereby the bacterium has a pump to expel ingested chemicals. Although some of these drug efflux pumps transport specific substrates, many are transporters of multiple substrates. Antimicrobial efflux pumps are believed to contribute significantly to acquired bacterial resistance because of the very broad variety of substrates they recognize, their expression in important pathogens, and their cooperation with other mechanisms of resistance (Soto *et.al*, 2013).

Enzyme inactivation: Some microorganisms have developed the ability to produce enzymes that are able to inactivate certain antimicrobials. The most notable example is penicillinase that can inactivate penicillin, but there are others. Clavulanic acid can bind penicillinase leaving the antimicrobial amoxicillin to do its work, and also there are the penicillinase resistant penicillins such as methicillin and cloxacillin, but they are still subject to target alterations making them ineffective over time (Soto *et.al*, 2013).

Mutation: When an antimicrobial attacks a specific target, whether it be cell wall peptides, ribosomes or nuclear DNA, it locks on to specific receptors on the target. Bacterial mutation results in the alteration of these receptors so that the antimicrobial can no longer fit and the organism is thus resistant to the effects of the antimicrobial. Examples of clinical strains showing resistance can be found for every class of antimicrobial, regardless of the mechanism of action. Target site changes often result from spontaneous mutation of a bacterial gene on the chromosome and selection in the presence of the antimicrobial. Thus antimicrobials resistant to penicillinase may still be rendered ineffective. This has led to the term methicillin resistant Staphylococcus aureus

(MRSA) the archetypical multi-resistant organism. “The most notable example is penicillinase that can inactivate penicillin, but there are others” (McEwen and Fedorka-Cray, 2002).

Biofilms: Biofilms are complex microbial communities containing bacteria and fungi. The microorganisms synthesise and secrete a protective matrix that attaches the biofilm firmly to a living or non-living surface. At the most basic level a biofilm can be described as bacteria embedded in a thick, slimy barrier of sugars and proteins. The biofilm barrier protects the microorganisms from external threats (Soto *et.al*, 2013).

2.5 HIV infection and Antimicrobial resistance

HIV remains a major global public health issue, having claimed 40.4 million [32.9–51.3 million] lives so far. And 68% in sub Saharan African countries (WHO, 2021). The same is true with Zambia, where a generalized HIV epidemic with most deaths resulting from opportunistic infections was seen the past decade (Avis *et.al*, 2023). The higher prevalence of resistance among HIV/AIDS is of particular concern in low-income settings where alternative treatment options are limited by their availability and cost (Avis *et.al*, 2023). This problem particularly influences the use of different antibiotics so as to increase antimicrobial resistance and made the treatment options to be limited. Moreover, one of the common antibiotics prescribed among HIV/AIDS patients is Co-trimoxazole which is also known as trimethoprim-sulfamethoxazole. It is a broad-spectrum antibiotic, used as a prophylactic agent against opportunistic infections among HIV/AIDS patients (WHO, 2006). World Health Organization has recommended to use co-trimoxazole prophylaxis for immunosuppressed adults and children born to HIV-infected women (WHO, 2006). And the long-term receiving of Co-trimoxazole prophylaxis has led to increased co-trimoxazole resistant bacteria.

The pattern of antibacterial susceptibility of enteric pathogens has been noticed long time and is influenced by the extensive and misuse of antibiotics and changing patient population, especially among immunocompromised patients. β-Lactam antibiotics such as Penicillins, Cephalosporins and Carbapenems have been encountered several times. The production of extended Spectrum β-lactamases (ESBLs) are the predominant drug resistance mechanisms against β-lactam antibiotics among gram-negative bacteria and they are associated with increased morbidity and mortality with immunocompromised individuals (Paterson *et.al*, 2005).

Methicillin resistant *staphylococcus aureus* (MRSA) infection should be considered as a potential etiology for different infections such as pneumonia, given that community outbreaks of MRSA have been seen in men who have sex with men and nasal carriage of MRSA is more common in HIV-infected individuals, particularly at lower CD4 cell counts. Multidrug resistance (MDR) bacteria like ESBL producers and MRSA are a major public health concern worldwide (Paterson *et.al*, 2005). The high prevalence of MRSA colonization in HIV and AIDS patients may be attributed to hospital admissions caused by HIV-related immunosuppression [33]. On the other hand, the association between HIV infection and resistance may be confounded by the presence of high-risk sexual activities reflecting the widespread dissemination of successful MRSA clones 34.

Different studies have shown the resistance of foodborne pathogens in HIV and AIDS patients, among which a study in United states highlighted the increased risk for MDR *Enterobacterales* (MDR-E) infection or colonization, relative to individuals without HIV, due to a greater burden of comorbidities as well as HIV-related intestinal inflammation and microbiota alterations. In their results among 82 participants, MDR-E was present in 20% of participants and non-susceptibility was most common for Penicillins (42/92, 46%), followed by Sulphonamides (38/110, 35%) and first-generation Cephalosporins (33/105, 31%) (Heather *et.al*, 2022).

In south Africa, from 203 HIV and AIDS participants, resistance to trimethoprim Sulphmetaxozole occurred in > 80% of foodborne pathogens. Moreover, carriage of Methicillin Resistant *S. aureus* (MRSA) was significantly associated with being on trimethoprim Sulphmetaxozole at baseline. Minimal inhibitory concentrations (MIC) to Penicillin were determined for 18 isolates of *streptococcus*: 7 (38.9%) were fully sensitive (MIC $\leq$ 0.06 μg/ml), 9 (50%) had intermediate resistance (MIC 0.12 – 1 μg/ml) and 2 (11.1%) had high level resistance (MIC $\geq$2 μg/ml). Fifty percent of *Enterobacteriaceae* produced extended spectrum beta-lactamases (ESBL) (resistant to third generation cephalosporins) and 56% were resistant to Gentamicin. Seventy-seven percent of *S. aureus* were MRSA. Carriage of resistant organisms was not associated with hospitalization (Cotton *et.al*, 2008).

CHAPTER THREE

METHODOLOGY

3.1 Research study design

The study was a cross-sectional type for the determination of antimicrobial resistance patterns of foodborne isolates from HIV/AIDS patients from August 2024 – September 2024.

3.2 Study area

The study was conducted at Adult Infectious Disease Clinic and inpatient wards (E11, E12, E21 and E22), University Teaching Hospital (UTH), Lusaka, Zambia. UTH is a main referral tertiary Hospital in Zambia. The hospital is centrally located in Lusaka which is the capital city and patients are referred from all over the country. The hospital functions as a teaching hospital and is a good center for research.

3.3 Study population and sample size

Fifty-four Stool samples were taken from all HIV/AIDS patients coming to UTH with one of the complaints of abdominal pain, vomiting, diarrhea, nausea and fever associated with foodborne contamination in a stated period of time. Thirty-two of the total participants were from adult infectious disease clinic (AIDC) who visit the center for getting their antiretroviral drugs. While the remaining twenty-two were inpatients from the male and female wards (E11, E12, E21 and E22) who were inpatients getting treatment for several diagnoses. In total fifty-four samples were processed.

3.4 Sampling technique

A purposive sampling method used to select its participants. This was done after consent from participants was granted. Participants were selected on purpose based on their knowledge and understanding of the research as well as the criteria of inclusion.

3.5 Inclusion and Exclusion criteria

3.5.1 Inclusion criteria

All HIV/AIDS patients with one of the complaints of abdominal pain, vomiting, diarrhea, nausea and fever associated to foodborne contamination, who were willing to participate.

3.5.2 Exclusion criteria

All Non HIV/AIDS patients and HIV/AIDS patients who were under an antibiotic therapy (since the desired foodborne pathogens could be lost from the system, making it harder to isolate those bacteria) for two weeks before enrolling in the study (Mwansa *et.al*, 2023).

3.6 Laboratory analysis

3.6.1 Media preparation

Media were procured from the microbiology unit of Department of Biological sciences and Biotechnology, University of Zambia and Microbiology laboratory of University teaching hospital. They were prepared using the powder of the following agar media; [Salmonella Shigella (SS) agar HIMEDIA M108-500G, Xylose lactose dextrose (XLD) agar OXOID CM0469B, Mannitol salt agar (MSA) HG000C26.500, Thiosulfate-Citrate-Bile-Salt Sucrose (TCBS) agar HIMEDIA M189-500G and Muller Hinton agar HIMEDIA M173-500G]. Media were prepared using the manufacturer's instruction but as a general procedure the powder forms of the above agar media were put into a conical flask of distilled water and were left to boil in a heater with a continuous stirring process until it was seen to be thoroughly mixed. The media were sterilized under the temperature of 121 ºC and 15 psi for 15 minutes in an autoclave (SLEFA Medical Instrument Mfg Co) and dispensed into petri dishes. After allowing the media to dry for some time, a proper labelling of date and type of media was done. The prepared agar media were quality controlled for sterility and performance and thereafter kept in a refrigerator for further use (Mwansa, *et al.* 2023).

3.6.2 Sample collection procedure

Fifty-four fresh stool samples for microbiology test were collected from HIV/AIDS patients using rectal swab technique. The swab was inserted beyond the anal sphincter and rotated so that it carries enough stool sample for culturing. The liquid medium consists of an inorganic buffer to stabilize the pH of the medium, and a reducing agent to remove dissolved oxygen from the medium (CLSI, 2022).

3.6.3 Culture and biochemical identification techniques

Stool samples were streaked in to XLD agar, TCBS agar and MSA agar plates upon their arrival to the laboratory and were incubated for 24 hours. Depending on the results of XLD agar based on the lactose and non-lactose fermenters, the non-lactose fermenters colonies which are pink colonies in XLD agar were further streaked in to SS agar for the detection of bacteria of interest such as s*almonella* and *shigella*, (Kim *et al.*, 2015). Biochemical test such as LIA (Lysine Iron Agar), SIM (Sulfide Indole Motility), TSI (Triple Sugar Iron), citrates test and oxidase test were also used for the purpose of biochemical characterization and steps outlined in UTH microbiology scientific operating procedures (SOPs) were also followed. After the incubation period of 24 hours, results were recorded. Moreover, the isolated bacteria were also prepared on slides using normal saline and heat fixing for gram staining. Applying a primary stain (crystal violet) for 1 minute was the first step. After a proper washing, iodine was added to the slides for 1 minute and again after proper washing, rapid decolorization with ethanol for 5 seconds was done. At last, a counterstaining with safranin for 1 min concluded the procedure (Mwansa *et al.* 2023).

3.6.4 Antimicrobial susceptibility testing

Each isolated gram negative and gram positive organism were tested against standard antibiotic discs respectively in mueller hinton agar. The standard antibiotic discs used in the current study were Ampicillin (10 µg), Co-trimoxazole (25 µg), Nitrofurantoin (300 µg), Chloramphenicol (30 µg), Azithromycin (15 µg), Amoxicillin-clavunelic acid (30 µg) and Oxacillin (1 µg). An antibiotic sensitivity test was carried out using the modified Kirby Bauer's agar disc diffusion technique. After the inoculation of test organisms in to the Mueller hinton agar media, the antibiotic discs wereplaced using disc dispenser. The plates with the antibiotic discs were incubated at 37°C for

24 hours to observe the zones of inhibition produced by the standard antibiotics. A quality control of antibiotic discs, and incubation conditions were ensured according the laboratory guidelines (CLSI, 2022; Ngalani *et al.*, 2019).

3.7 Data collection and analysis

Sociodemographic variables such as age, sex, residence, and data for sample origin were taken from medical records of individual participants who were fit for the study. Moreover, microbiological results of the respective participants were incorporated to the existing data of sociodemographic variables. The data were entered in to the Microsoft Excel (2016) and were robustly reviewed and cleaned before further analysis. Finally, they were analyzed using statistical soft wares, IBM statistics SPSS (version 30) and Genstat software *for Windows*® (18th edition). Descriptive summaries and data presentations such as percentages and frequency, stacked bar chart, and tables were used. Multinomial logistic regression was used to analyze the MDR and XDR patterns in comparison to the sociodemographic variables. Odds ratio, *P-values* and confidence intervals were used for checking statistical significance of the data (*P-value* < 0.05 was considered as statistical significant). Model fitting such as goodness of fit and pseudo R-square were used for analysis of multinomial logistic regression. In addition to that, principal component analysis for comparing participant's residence area to the respective foodborne bacterial isolates were done using the software Genstat software *for Windows*® (18th edition).

3.8. Ethical Considerations

Ethical approval to conduct the study was obtained from Natural Science Research and Ethical Committee (NASREC) and University of Zambia Biomedical Research and Ethical Committee (UNZABREC). And approval from University of Zambia School of Natural Sciences, National Health Research Authority (NHRA), and University Teaching Hospital (UTH) were gotten. Further, informed consent statement for adults in English and Nyanja were prepared and given to all study participants. To maintain confidentiality, study participants were allocated study-specific codes, and the study data were strictly kept confidential

CHAPTER FOUR

RESULTS

4.1 Isolation and identification of bacterial isolates

A total of 77 bacteria were isolated with *Eschercichia coli* found to be the most prevalent bacteria at 27.3% followed by *Proteus vulgaris* (15.6%) and *Staphylococcus aures* (14.3%) respectively. On the other hand *Acinetobacter* spp., *Salmonella paratyphi*, *Aeromonas* spp., and *Pseudomonas aeroginosa* were found comprising for only 1.3%. The frequency of the major foodborne pathogens is described in table 1. where *eschercichia coli* became the most prevalent bacteria followed by *Staphylococcus aures* and *Shigella* spp.

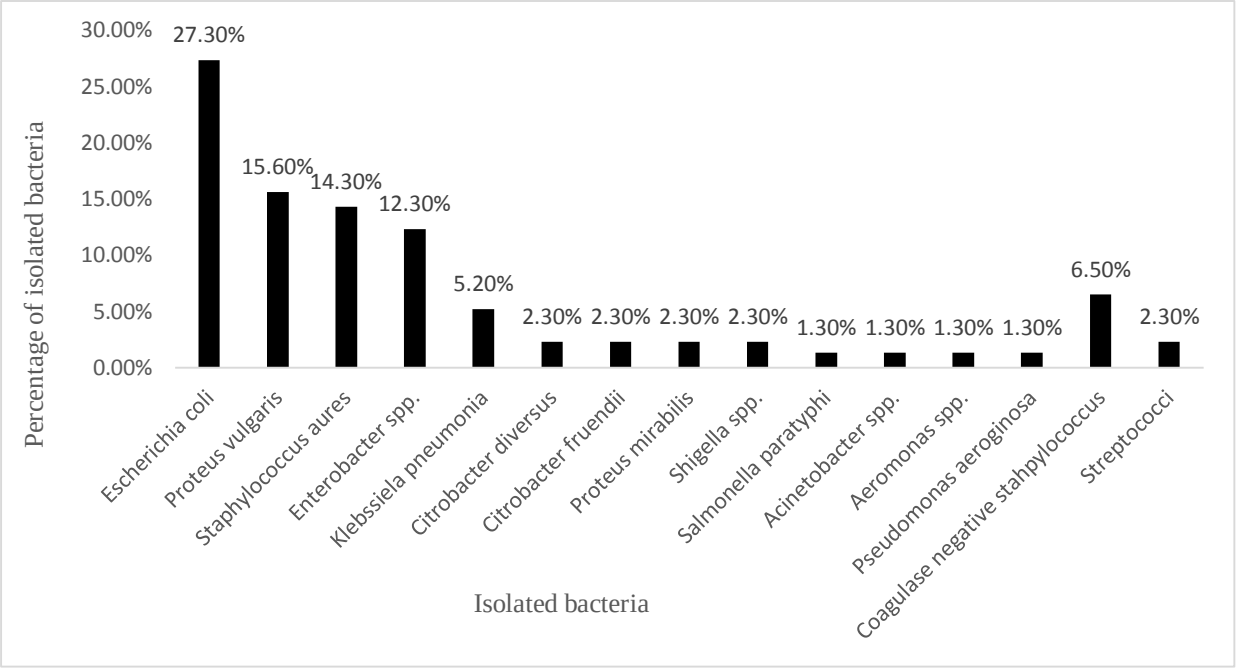

Figure 1. Overall frequency of isolated bacteria

The list of isolates is being categorized according to frequency from higher to lower for gram negative and gram positive differently

Table 1. Frequency of isolated major foodborne bacteria

Food borne bacteria	**Frequency**	**Prevalence**
Escherichia coli	21	27.27%
Staphylococcus aureus	11	14.28%
Shigella spp.	2	2.60%
Salmonella paratyphi	1	1.30%
Aeromonas spp.	1	1.30%
Total FBB	**36**	**46.75%**
Others enteric bacteria	41	53.25%

4.2 Antimicrobial susceptibility test

Escherichia coli was found to be highly resistant to ampicillin (95.24%) and Sulfamethoxazole/trimethoprim (80.95%). It was moderately resistant to Azithromycin (42.85%), Amoxicillin/Clavulanic acid and Nitrofurantoin with 38.04%. In contrast to that, *Escherichia coli* was found to be mainly sensitive to Chloramphenicol with 71.43%. *Salmonella paratyphi* isolate was also highly resistant to ampicillin (100%), Sulfamethoxazole/Trimethoprim (100%) and Amoxicillin/Clavulanic acid (100%) but it was found to be 100% sensitive to Chloramphenicol and Nitrofurantoin. The same was true with *Shigella* spp., which were resistant to Ampicillin, Sulfamethoxazole/Trimethoprim and Nitrofurantoin with (50%). The results are shown in table 2.

Table 2. Antibiotic susceptibility patterns of foodborne bacteria *Escherichia coli, Shigella spp and Salmonella paratyphi* isolated from stool samples.

Antimicrobial categories	Antimicrobials	*Escherichia coli* (21)			*Shigella spp.* (2)			*Salmonella paratyphi* (1)		
		R	I	S	R	I	S	R	I	S
Penicillins	AMP	20 (95.24%)	1 (4.76%)	0(0%)	1(50%)	0(0%)	1(50%)	1(100%)	0(0%)	0(0%)
Folate pathway inhibitor	SXT	17(80.95%)	1(4.77%)	3(14.28%)	1(50%)	0(0%)	1(50%)	1(100%)	0(0%)	0(0%)
Macrolide	AZT	9(42.85%)	7(33.34%)	5(23.81%)	0(0%)	2(100%)	0(0%)	0(0%)	1(100%)	0(0%)
Beta-lactamase inhibitor	AMC	8(38.09%)	9(42.86%)	4(19.05%)	0(0%)	2(100%)	0(0%)	1(100%)	0(0%)	0(0%)
Amphenicol	C	4(19.05%)	2(9.52%)	15(71.43%)	0(0%)	1(50%)	1(50%)	0(0%)	0(0%)	1(100%)
Nitrofuran	F	8(38.09%)	4(19.05)	9(42.86%)	1(50%)	0(0%)	1(50%)	0(0%)	0(0%)	1(100%)

Abbreviations: AMP- Ampicillin; SXT-Sulfamethoxazole/trimethoprim; AZT- Azitromycin; AMC-Amoxicillin/clavulanic acid; C-Chloramphenicol; NIT-Nitrofurantoin. R- resistant, I- Intermidate, S-sensitive

Aeromonas spp. were 100% resistant to Ampicillin, Sulfamethoxazole/Trimethoprim, Chloramphenicol, Azithromycin and Nitrofurantoin. One of the prevalently isolated foodborne bacteria *Staphylococcus aureus* was also among the highly resistant bacteria. It was found to be 100% resistant to Azithromycin and 90.9% resistant to Oxacillin, and it was highly sensitive to Chloramphenicol with 72.72% as shown in table 3.

Table 3. Antibiotic susceptibility patterns of *Aeromonas* spp. and *staphylococcus aureus*

Antimicrobial categories	Antimicrobials	*Aeromonas spp. (1)*			*Staphylococcus aureus (11)*		
		R	I	S	R	I	S
Penicillins	AMP	1(100%)	0(0%)	0(0%)	6(54%)	0(0.0%)	5(46%)
	OX	-	-	-	10(90.9%)	1(9.1%)	0(0.0%)
Folate pathway inhibitor	SXT	1(100%)	0(0%)	0(0%)	-	-	-
Macrolide	AZT	0(0%)	0(0%)	1(100%)	11(100%)	0(0.0%)	0(0.0%)
Beta-lactamase inhibitor	AMC	0(0%)	1(100%)	0(0%)	-	-	-
Amphenicol	C	1(100%)	0(0%)	0(0%)	0(0.0%)	3(27.27)	8(72.72%)
Nitrofuran	F	0(0%)	0(0%)	1(100%)	-	-	-

Abbreviations: AMP- Ampicillin; SXT-Sulfamethoxazole/trimethoprim; AZT- Azithromycin; AMC-Amoxicillin/clavulanic acid; C-Chloramphenicol; NIT-Nitrofurantoin, OX-Oxacillin R- resistant, I- Intermidate, S-sensitive

4.3 Socio-demographic characteristics

4.3.1 Age, Sex and Place of origin of study participants

All of the study participants (a total of 54 participants) were HIV positive with one of the complaints of abdominal pain, vomiting, diarrhea, nausea and fever. Of these 54 participants, 29 (53.7%) are males and 25 (46.3%) are females. The participants came from 32 different areas of Lusaka, Zambia and mainly from Bauleni (12.96%) and Kanyama (7.4%). The youngest participant of the study was 28 years old and oldest being 72 years old. Most of the study participants (32 participants), are a regular visitor to infectious disease hospital and they come to get their routine antiretroviral drugs (ARVs) regime. And the remaining 22 are from the wards E22, E21 and E12. The results are shown in Table 4.

Table 4. Socio-demographic characteristics of the study participants

Variables	Categories	Frequency (Percentage)
Sex	Male	29 (53.70)
	Female	25 (46.30)
Age	25-34	10 (18.52)
	35-44	14 (25.90)
	45-54	16 (29.63)
	55 and above	14 (25.90)
Sample origin	AIDC (Outpatient)	32 (59.25)
	Wards (Inpatient)	22 (40.75)
Address	Bauleni	7 (12.96)
	Kanyama	4 (7.40)
	Kabwata	3 (5.55)
	Kamwala south	3 (5.55)
	Matero	3 (5.55)
	Kalingalinga	3 (5.55)
	Mtendere	2 (3.70)
	Kasupe	2 (3.70)
	Chaisa	2 (3.70)
	Chelston	2 (3.70)
	George compound	2 (3.70)

	Jhon lenge	1 (1.85)
	Lilayi	1 (1.85)
	Kabanana	1 (1.85)
	Kuku	1 (1.85)
	Mandevu	1 (1.85)
	Sos	1 (1.85)
	Chilenje	1 (1.85)
	Obama	1 (1.85)
	Chadleigh	1 (1.85)
	Middle west	1 (1.85)
	6 miles	1 (1.85)
	Kalikiliki	1 (1.85)
	Garden chilulu	1 (1.85)
	15 miles	1 (1.85)
	10 miles	1 (1.85)
	Libala south	1 (1.85)
	Chawama	1 (1.85)
	Chainama	1 (1.85)
	Chalala	1 (1.85)
	Lusaka west	1 (1.85)
	New kasama	1 (1.85)

AIDC refers to Adult infectious disease clinic

4.3.2 Distribution of foodborne bacterial isolates according to participant's residence

Foodborne bacterial isolates were tracked down to know which area are of more prevalence. This was done by plotting the residence of study participants against the respective foodborne isolates. Figure 2 is showing the overall percentage distribution using stacked bar chart and figure 3 is showing the group distribution of foodborne bacteria using principal component analysis respectively. It was noticed that *Escherichia coli*, *Staphylococcus aureus* and *Salmonella paratyphi* are prevalent in patients from Bauleni. *Shigella* spp. are common in John lenge and Matero whereas *Aeromonas* spp. was found to be common in Kasupe.

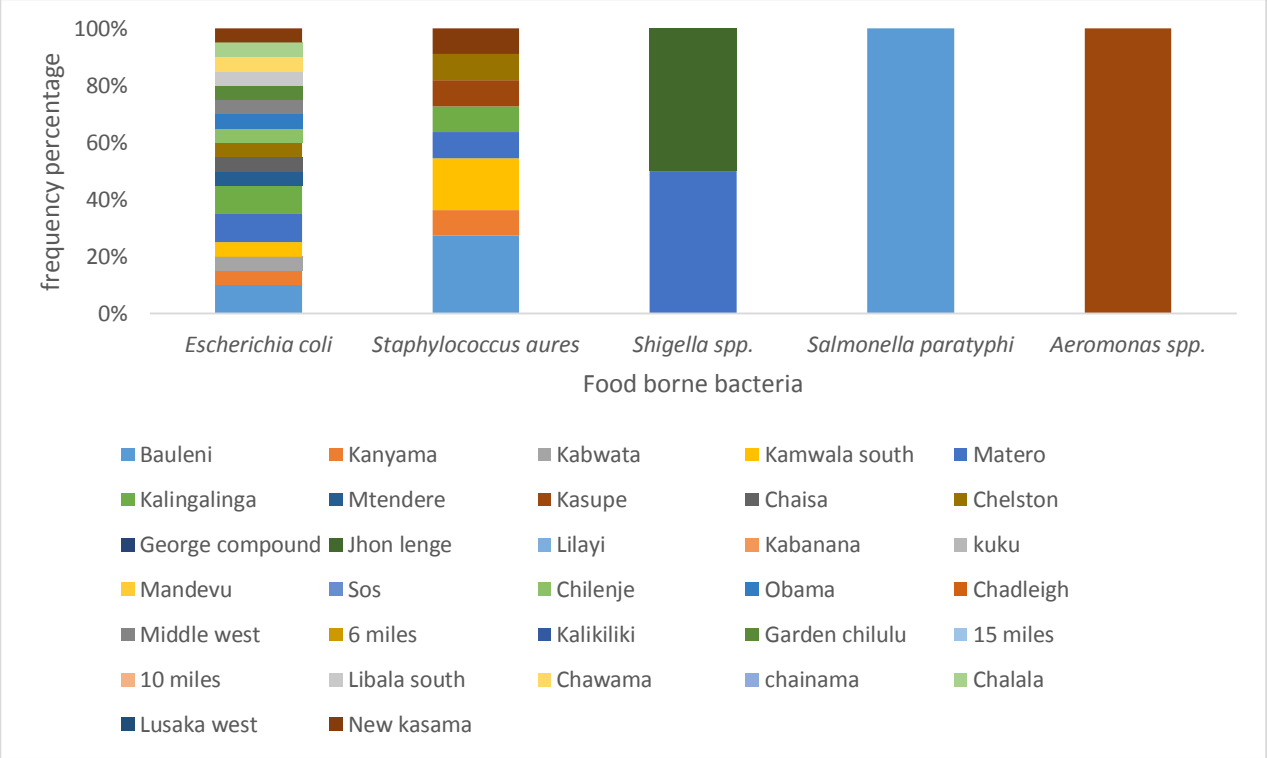

Figure 2. Percentage distribution of foodborne bacteria in patient from different areas of Lusaka province and Central province, Zambia

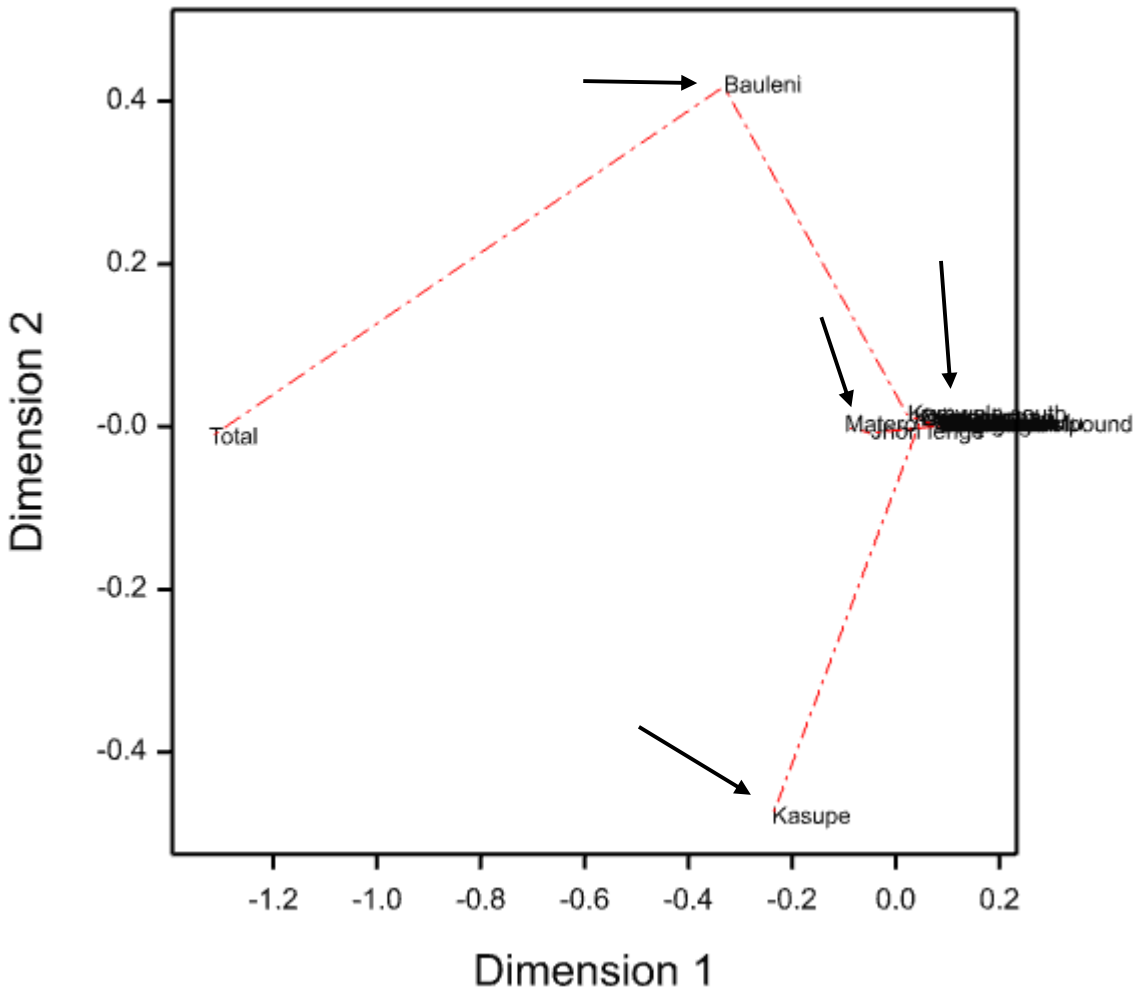

Figure 3. Principal component analysis of distribution of foodborne bacterial isolates in different areas of Lusaka province and Central province, Zambia

N.B: Dimension 1 and 2 are showing the degree of similarity and/or difference among the different groups. Arrows are showing the different groups formed based on similarity matrix

4.3.3 Multinomial logistic regression model

Most of the multidrug resistant foodborne bacteria were isolated from females accounting for 23.53%, from age group 55 and above (42.9%) and samples from wards (35.3%). This study has revealed that sample origin was significantly associated with MDR foodborne bacteria (*p-value*= 0.007). The results are shown in Table 5.

Table 1. Distribution of MDR Foodborne bacterial isolates

Variables	Categories	Total	Negative (19) (52.8%)	MDR (7) (19.4%)	XDR (10) (27.8%)	Odds ratio	CI (95%)	*p-value*
Sex	Male	19	11 (57.9%)	3 (15.80%)	5 (26.3%)	0.637	0.07 - 5.845	0.690
	Female	17	8 (47.06%)	4 (23.53%)	5 (29.41%)			
Age	25-34	7	3(42.9%)	2(28.6%)	2(28.5%)	0.897	0.295 - 2.725	0.848
	35-44	10	6 (60%)	0 (%)	4 (40%)			
	45-54	12	6 (50%)	2 (16.7%)	4 (33.3%)			
	55 and above	7	4 (57.1%)	3 (42.9%)	0 (0%)			
Sample origin	AIDC (Outpatient)	19	16 (84.21%)	1 (5.26%)	2(10.53%)	**0.033**	**0.003 - 0.931**	**0.007**
	Wards (Inpatient)	17	3 (17.65%)	6 (35.3%)	8 (47.05%)			

CHAPTER FIVE

DISCUSSION

The study aimed at characterizing and determining the antimicrobial resistance profile of foodborne bacterial isolates among HIV/AIDS patients attending UTH. Specifically designed to answer research questions implicating on which foodborne bacterial stool species are common among HIV/AIDS patients in Lusaka, Zambia, and how the patterns of antimicrobial resistance profile of the identified foodborne bacteria look and compared. *Escherichia coli* has been the most prevalent enteric bacterial isolated in the study with *staphylococcus aureus* also been ranked next to *E. coli*. And higher sensitivity to Chloramphenicol and higher resistance to Ampicillin and Sulfamethoxazole/trimethoprim are the notable results encountered. The study is expected to provide beneficial results in addressing current status of foodborne antimicrobial resistance patterns among HIV/AIDS patients in Lusaka, Zambia.

5.1 Isolation of foodborne bacteria

Indeed, different foodborne bacteria were isolated from stool samples of participants who are positive for HIV. The isolates include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Shigella* spp. and *Aeromonas* spp. among others isolated enteric bacteria; which are not considered as a major foodborne bacterium but as a minor one such as *Proteus* spp., *Enterobacter* spp., and *Citrobacter* spp. A review study by Bintisis Thomas, has implicated that most enteric bacteria are foodborne pathogens and the main reason for foodborne bacteria to end up as an enteric bacterium in human gut is, that those bacteria which inhabit soil, gut of food animals, different vegetables and fruits get access to human body alongside the food consumed for nutrition (Bintsis, 2017). That's why foods for consumption should pass prevention mechanism before eating (Thobaben, 2010). Washing vegetables with running water, maintaining clean environment for cooking, ensuring enough cooking time and preparing clean utensils for food preparations are basic and essential steps to guarantee food safety (Bintsis, 2017).

But there are other advanced techniques of food preservation methods which are categorized as physical methods, chemical methods and biological methods (Thobaben, 2010). Heat treatment, radiation, light, high pressure processing, pulsed electric fields, and modified atmosphere (vacuum packaging) are considered as physical methods (Lorenzo *et al.*, 2018). These methods work by disrupting the cell membrane and denaturing the nucleic acid, or by creating low oxygen and high carbon dioxide environment for inhibiting aerobic bacteria (Lorenzo *et al.*, 2018). Nowadays, the use of bacteriocins which are ribosomally synthesized antimicrobial peptides, are believed to be more advantageous in that they don't use harmful rays for means of food prevention (Rendueles *et al.*, 2022). This technique is under the biological methods of food preservation, whereas use of chemicals such as sodium benzoate, potassium sorbate, nitrites, and sulfites are among others which are in action in industrial food processing units (Lorenzo *et al.*, 2018).

This study identified about 15 different bacterial species, where the major foodborne bacterial pathogens comprised 46.75% of the total isolates (in total 77 bacterial stool isolates were identified). In addition, foodborne bacteria such as *Escherichia coli* and *Staphylococcus aureus* were found to be the most prevalent bacterial isolates accounting for 27.31% and 14.31% respectively. *Escherichia coli* has been mentioned as the most prevalent enteric bacterial isolate many times in different studies; for instance, a study by Falodun and his colleagues from Nigeria (Falodun *et al.*, 2021) found *Escherichia coli* in 41.6% of all the isolates. Another study by Webale and his colleagues in Kenya showed that 36.4 % of the total isolates were comprised by *Escherichia coli* (Webale *et al.*, 2020). These results are similar to the current study making it as the most commonly encountered bacteria. *Escherichia coli* is considered as a normal flora and has been beneficial in synthesizing vitamins B12 and K but when it invades people with low immunity such as HIV/AIDS patients, it can cause infections such as bacteremia and other adverse conditions (Rajaei *et al.*, 2021).

Studies by Falodun and Webale recorded low percentages of *Staphylococcus aureus* in stool samples unlike the results from the current research which isolated a higher amount (Falodun *et al.*, 2021; Webale *et al.*, 2020).. Generally, *Staphylococcus aureus* is an aerobic bacterium which resides in nasal and skin areas and is not expected to be isolated frequently from stool samples (Filipello *et al.*, 2020). The main reasons for this high amount of isolation from stool sample could be that participants might have eaten foods infested with the bacteria although a deeper investigation is however, needed epidemiologically (Filipello *et al.*, 2020). Secondly, nosocomial

staphylococcus aureus infections which are a common scenario in inpatient departments can contaminate the stool (Alabi *et al.*, 2013). A study done in USA showed that intestinal colonization *by staphylococcus aureus* among hospitalized patients has been associated with increased risk of Staphylococcal infection and could potentially contribute to transmission (Bhalla, Aron and Donskey, 2007). In comparison to nares colonization only, nares and intestinal colonization was associated with increased frequency of positive skin cultures (41% versus 77%; p = 0.001) and trends toward increased environmental contamination (45% versus 62%; p = 0.188) and acquisition on investigator's hands (36% versus 60%; p = 0.057) (Bhalla, Aron and Donskey, 2007). S*taphylococcus aureus* can spread from unwashed hands and contaminate food and after getting in foods, it can multiply and make a toxin that causes food poisoning (Filipello *et al.*, 2020).

Occurrence of *Salmonella paratyphi, Shigella* spp. and *Aeromonas* spp. although minimally seen are worrisome cases from the perspective of foodborne illnesses and infection control. *Shigella* spp. were seen in 2.6% of the isolates and this is found to be lower compared to a study done in Zambia by Hatyoka and her collegues which was 4.8% (Hatyoka *et al.*, 2022). Nevertheless, it is also higher than a study conducted in Dessie, Ethiopia which was 1.8% (Belay *et al.*, 2020). The most common ways people get sick are from eating or drinking contaminated food or water and contact with someone who is sick or has recently been sick after being contaminated with *Shigella spp*. It is also believed to spread during sexual activity with a sick person (Hatyoka *et al.*, 2022).

Salmonella paratyphi was another foodborne bacterium encountered among the HIV/AIDS patients attending UTH. This bacterium is host-restricted pathogen whose reservoir is human. *S. typhi and S. Paratyphi A, B,* and *C* characteristically invade from the gastrointestinal tract into the bloodstream, survive and reproduce within macrophages, and in 1%–4% of cases result in chronic carriage (Hoffman and Luby, 2024). Collectively the typhoidal *salmonellas* are estimated to cause over 135,000 deaths per year (Hoffman and Luby, 2024). In the current study it was seen in 1.3% of the isolates which is lower than a study done in Ethiopia which was 5.2% (Mitiku *et al.*, 2024). Salmonella infection is usually caused by eating raw or undercooked meat, poultry, and eggs or egg products or by drinking unpasteurized milk (Abebe, Gugsa and Ahmed, 2020). The incubation period can be between 6 hours to 6 days (Abebe, Gugsa and Ahmed, 2020).

Another foodborne bacterium to look into are *Aeromonas* spp. *Aeromonas* species are emerging human enteric pathogens which are an environmental opportunistic pathogen (Webale *et al.*, 2020). It is involved in several infectious diseases such as gastroenteritis, septicemia and wound infections (Obi *et al.*, 2007). However, gastroenteritis caused by *Aeromonas* spp. are rare and the clinical relevance of this species in stool specimens is still minimal (Webale *et al.*, 2020).. In the current study it was seen in 1.3% of the isolates. Other minor food bacteria such as *Proteus* spp., *Enterobacter* spp. and *Citrobacter* spp. were also isolated but much emphasis is not sought as they are out of the scope of the study. They can be associated with foodborne illnesses minimally.

5.2 Antimicrobial resistance of foodborne pathogens

Different antibiotics from different antibiotic classes have been used to follow and study the patterns of antimicrobial resistance of the isolated foodborne bacteria. The antibiotics include Timixampicillin, Sulfamethoxazole/Trimethoprim, Azithromycin, Amoxicillin/Clavulanic acid, Chloramphenicol, Nitrofurantoin and Oxacillin.

As in the current study, *Escherichia coli* was found to be highly resistant to ampicillin (with 95.24%) and to Sulfamethoxazole/Trimethoprim (with 80.95%). This bacterium was also found to moderately be resistant to azithromycin (with 42.85%), to Amoxicillin/Clavulanic acid and to Nitrofurantoin (with 38.04%). In contrast to that *Escherichia coli* was found to mainly be sensitive to chloramphenicol (with 71.43%). The resistance patterns of the current study are higher in resistance percentage compared to a study done in Kenya (Webale *et al.*, 2020), in that *Escherichia coli* was resistant to Ampicillin (55.2%) and to Sulfamethoxazole/Trimethoprim (61.4%). On the contrary, 61.2% of the isolates were resistance to Chloramphenicol and this makes a big difference comparing the resistance of chloramphenicol to the current study. Another study in Zambia (Hatyoka *et al.*, 2022), has shown similar results in resistance to Sulfamethoxazole/Trimethoprim which is 90.2% but higher in sensitivity to Amoxicillin/Clavulanic acid with 98.2%. Whereas a study in Cameroon showed similar results in every aspect in that there was 33% resistance to Amoxicillin/Clavulanic acid and 83.3% sensitivity to Chloramphenicol (Ngalani *et al.*, 2019). All in all, higher resistance to Ampicillin and Sulfamethoxazole/trimethoprim and higher sensitivity to Chloramphenicol was noticed in most literatures.

Similarly, *Salmonella paratyphi* isolate was also highly resistant to ampicillin (100%), Sulfamethoxazole/trimethoprim (100%) and Amoxicillin/Clavulanic acid (100%) but it was found to be 100% sensitive to Chloramphenicol and Nitrofurantoin. The study recorded a very high resistance compared to a study in Cameroon which has showed 40% resistance to Amoxicillin/Clavulanic acid (Ngalani *et al.*, 2019). The same study has produced a 20% resistance to Chloramphenicol while it was 100% sensitive to Chloramphenicol in the current study.

The same is true with *Shigella spp.*, which were resistant to ampicillin, Sulfamethoxazole/Trimethoprim and Nitrofurantoin with (50%). Moreover, *Aeromonas spp.* were 100% resistant to ampicillin, Sulfamethoxazole/trimethoprim and chloramphenicol and were found to be 100% sensitive to azithromycin and nitrofurantoin. The use of un-prescribed antibiotics is one of the reasons to result in antimicrobial resistance and is a common problem when it comes to sub-Saharan African countries (Kimera *et al.*, 2020). For that reason, most of the isolates are resistant to ampicillin and Sulfamethoxazole/trimethoprim and moderately sensitive to Chloramphenicol and Nitrofurantoin.

Staphylococcus aureus was also among the highly resistant bacteria. It was found to be 100% resistant to azithromycin and 90.9% resistant to oxacillin, and it was highly sensitive to chloramphenicol with 72.72%. A study by Kates and his colleagues in 2018, has shown that resistance to Oxacillin seen in 43.1% of stool *Staphylococcus aureus* isolates which is lower than resistance to Oxacillin recorded by the current study (90.9%) (Kates *et al.*, 2018). In a study in Vietnam similar results has been recorded as of the current study implicating high resistance to azithromycin up to 82.28% and Oxacillin up to 70% (An *et al.*, 2024). The other angle this study tried to see in accordance to patterns of antimicrobial resistance is the incidence of multi drug resistance (MDR) and extended drug resistance (XDR). Hence high incidence was noticed accounting to 19.4% of MDR cases and 27.8% cases of XDR cases. In other words, those standard antibiotics failed to treat infections from approximately 48.2% of the total isolated foodborne bacteria.

5.3 Socio-demographic variables

The study participants were mainly from Lusaka province but few also came from Central province as well. This suggests that most of the HIV positive patients attending a referral hospital UTH, are

from Lusaka province. It is expected that most patients attending the hospital are from Lusaka; being a capital city and with highest population of approximately 3,079,964 (Lusaka provincial administration, 2025) and a density of 100 per square km. Most of the participants from this study reside in Bauleni (with 12.96%) and Kanyama (with 7.4%). The main reasons for the higher number of participants particularly from this area could be those areas being the most populated places in Lusaka. According to Lusaka city council, Kanyama is the most populated area of Lusaka with around 305,874 people residing and Bauleni with 49,979. Highly populated areas are a common source of bacterial infections throughout the world specially for food and waterborne illnesses (Kim and Ahn, 2022). It is because of the poor sanitation system, poor individual and community based hygienic practices and easy propagation of those bacterial agents from person to person via food and water in accordance to population density (Kim and Ahn, 2022). It was also noticed that most of these foodborne bacteria were sourced from Bauleni. This includes major foodborne bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella paratyphi.* Therefore, accurate measures should be taken in improving sanitation system and individual and community hygienic practices before outbreaks occur.

Multinomial logistic regression models had shown that MDR cases are mainly present in inpatient participants compared to outpatient participants in the current study. In Zambia, as in many other African countries, the healthcare system faces significant challenges related to the control of infections and inappropriate antibiotic orders especially in inpatients (Anne *et al.*, 2020). For instance, a study by (Anne *et al.*, 2020) found that patients who received prolonged antibiotic treatments in hospitals were at a higher risk of developing infections from resistant strains of foodborne pathogens with reasons related to 67% of inappropriately prescribed antibiotics. The prescriptions were inappropriately prescribed to some extent, largely driven by incorrect dose or frequency. Another study in Zambia by (Marjolijn *et.al*, 2017), studied the prevalence of antimicrobial drug resistant bacteria carried by comparing inpatients and outpatients in the resource constraint setting of a secondary care hospital in Zambia. The results were that 90% of inpatients and 48% of outpatients carried one or more *enterobacteriaceae* strains (75% *escherichia coli* and *klebsiella pneumonia*) resistant to gentamicin, ciprofloxacin and/or ceftriaxone ($p < 0.001$). Another study in Kenya by (Jeniffer *et al.* 2022) similarly found that patients in healthcare facilities, especially in ICU settings, were more likely to acquire MDR pathogens reinforcing the notion that being an inpatient increases the risk of MDR bacterial infections. Several global studies

in developed countries have also supported the study findings, for instance a study in the United States by (Jhon *et al.* 2020) showed that patients who were hospitalized for extended periods or who had frequent admissions were more likely to develop infections from MDR foodborne pathogens. In Europe, the European Centre for Disease Prevention and Control (ECDC) regularly reports that healthcare settings are hotspots for the transmission of MDR bacteria (Aleksa *et.al*, 2020). Hence, a large-scale report in Serbia (Aleksa *et.al*, 2020) in 2020 noted that MDR *salmonella* and *staphylococcus aureus* were often linked to hospital-acquired infections, particularly in individuals with weakened immune systems or those receiving antibiotic treatments. Similar study conducted in Iran, also showed that inpatient resistance is higher than outpatient resistance (Akhavizadegan *et al.*, 2021). Therefore, the high cases in MDR and XDR in HIV/AIDS in Zambia is implying that, infections are harder to treat. This often led to longer hospital stays, increased healthcare costs, and, in many cases, higher mortality rates. This is particularly concerning in Zambia, because healthcare infrastructure may struggle to cope with the burden of such resistant infections.

5.4 Limitation of the study

1. Barriers of the principal investigator to speaking and understanding to local languages such as Nyanja and Bemba. Although informed consent in English and Nyanja was prepared, some of the participants were from other tribes who can't understand Nyanja. In such cases study participants were rejected from the study and this can affect the study in that some of the participants representing some tribes were not selected in the study.
2. The use of swabs for sample collection over stool containers. If inaccurate insertion of the tip of the swab in to the anus happens, the tip will end up touching the skin in the vicinity and this might increase the isolation of gram positive bacteria such as staphylococcus aureus bacterial species. Although it was performed with much concentration in performing it, it's better to go for stool containers.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

In conclusion, the results of this study provide valuable insights into the prevalence of foodborne pathogens and the associated antimicrobial resistance patterns among HIV/AIDS patients. The isolation of common foodborne pathogens such as *Escherichia coli, Staphylococcus aureus, Salmonella, Shigella,* and *Aeromonas* from these patients is concerning. HIV/AIDS patients have compromised immune systems, which makes them particularly susceptible to infections, including those caused by foodborne pathogens. These pathogens are often associated with contaminated food and water, which underscores the importance of maintaining strict hygiene practices and food safety measures in this population to prevent infection.

Furthermore, the study reveals that the isolated pathogens exhibit resistance to commonly used antibiotics, such as penicillin and sulfamethoxazole/trimethoprim (*Eschercichia coli* been resistant to ampicillin and sulfamethoxazole/trimethoprim with 95.4% and 80.95% resistance respectively and *Staphylococcus aurues* isolates 100% resistant to azithromycin and 90.90% resistant to methicillin among others). This is particularly alarming as these antibiotics have traditionally been the first line of defense against infections in immunocompromised individuals. The resistance to penicillin is particularly noteworthy, given its widespread use in treating a variety of bacterial infections. Similarly, the resistance to sulfamethoxazole/trimethoprim, a combination frequently used for prophylaxis and treatment in HIV/AIDS patients, suggests a growing challenge in managing infections in this population. However, the results also demonstrate that these pathogens remain sensitive to chloramphenicol, an antibiotic that may still be a viable treatment option. This highlights the importance of susceptibility testing to guide appropriate antibiotic selection, particularly in cases where traditional therapies may no longer be effective.

The study also identifies a concerning trend of high levels of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens (MDR in 19.40% of the isolates and XDR in 27.80% of the isolates), especially in inpatient settings. MDR and XDR organisms are characterized by resistance to multiple classes of antibiotics, limiting the therapeutic options available for treatment.

The high prevalence of these resistant strains in hospitalized patients is particularly troubling, as it suggests that hospital environments may serve as reservoirs for the spread of resistant pathogens. This poses significant challenges not only to patient care but also to infection control efforts in healthcare settings. The presence of MDR and XDR pathogens underscores the critical need for enhanced infection prevention and control measures, along with continuous monitoring and surveillance of antimicrobial resistance trends.

In light of these findings, it is crucial to implement effective antimicrobial stewardship programs, ensure regular screening for antimicrobial resistance, and invest in research for alternative treatment options. The results also highlight the importance of educating healthcare professionals and patients on proper hygiene practices and the risks associated with antibiotic misuse. Ultimately, addressing the rising tide of antimicrobial resistance in HIV/AIDS patients will require a multifaceted approach involving both clinical and public health strategies.

6.2 Recommendations

- Conducting molecular studies of antimicrobial resistance patterns of foodborne bacterial isolates will give a better understanding on resistance mechanism. Therefore, future studies are recommended to focus on this subject matter.

- It is also recommended that future studies should incorporate both clinical samples and food samples for analysis to better understand to what extent are foodborne bacteria propagating in HIV/AIDS. This will give a deeper concept on the ‘One health approach of antimicrobial resistance’.

- Furthermore, those food and water samples for foodborne bacterial analysis should include every participant’s household. These samples alongside the clinical samples from such HIV/AIDS participants will give a complete analysis on foodborne bacteria and will help devise a way to stop transmission of MDR foodborne bacteria.

- The study also recommends that future studies to focus on the attitude, practice and knowledge of AMR among the communities living in highly populated areas of Lusaka. This will help scientists and government bodies to look the issue on a bigger picture and to get a clue of the community on the understanding of AMR.

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APPENDICIES

Informed consent protocol

PROTOCOL SPECIFIC INFORMED CONSENT FORM

ANTIMICROBIAL RESISTANCE PATTERNS OF FOODBORNE BACTERIAL ISOLATES FROM HIV/AIDS PATIENTS IN LUSAKA, ZAMBIA

(In English)

Title of the proposed study: Antimicrobial resistance patterns of foodborne bacterial isolates from HIV/AIDS patients in Lusaka, Zambia

Investigators:

Mr. Aron Rezene Mebrahtu, University of Zambia, School of natural science, Department of biological sciences, Microbiology unit. Tel no +260779075415

Background and rationale for the study:

Antimicrobial resistance is a major global public health concern and a food safety issue. When pathogens become resistant to antimicrobial agents they can pose a greater human health risk as a result of potential treatment failure, loss of treatment options and increased likelihood and severity of disease. The aim of the study is to determine the antimicrobial drug resistance profile of foodborne bacterial isolates among food poisoned HIV/AIDS patients.

Purpose:

The study is expected to provide beneficial results in addressing current status of foodborne antimicrobial resistance patterns among HIV/AIDS patients in Lusaka, Zambia. This will help planners and policy makers in relation to public health and food organizations for the management of drug resistance issues from the approach of one health concept. It's also expected to inform clinicians and researchers on which drugs are highly resistant and which bacterial isolates are showing resistance so that patient management will be enhanced.

Procedures:

In this study, study staff will take stool sample from you and will forward the stool sample to microbiology unit, UTH upon your arrival to the hospital if you have a symptom of abdominal pain, vomiting, diarrhea, nausea and fever associated to foodborne contamination.

Who will participate in the study?

Samples are taken from all HIV/AIDS patients attending UTH with one of the complaints of abdominal pain, vomiting, diarrhea, nausea and fever associated to foodborne contamination in a stated period of time. The participants are only to provide a stool sample once at their time to see a doctor.

Risks/Discomforts:

Participants are only to provide stool sample during their visit to see a doctor. There will be no risk in providing stool samples, however participants may sometimes feel embarrassed or ashamed in providing stool.

Benefits:

There will not be a direct benefit to you. However, researchers may learn some information from this study that may help the community of HIV/AIDS.

Alternatives:

Only participants who want to participate will be part of this study. You can ask questions at any time. You can discuss the study with others before deciding to join. No matter what your decision is, any other care that you get at this clinic will not change.

Questions:

Participants who have study-related questions can reach investigators at any time physically or via the address of the principal investigator.

Questions about participant's rights:

Participants who have questions about their rights as research participants will be given answers and are free to ask UNZABREC about their right anytime. And possible solution for the questions will be addressed.

Statement of voluntariness:

Participation in the proposed study is voluntary and participants may join on their own free will. Participants also have a right to withdraw from the study at any time without penalty.

Confidentiality:

The results of this study will be kept strictly confidential and used only for research purposes. Your identity will be concealed as far as the law allows. Your name will not appear anywhere on the coded forms with the information. Paper and computer records will be kept under lock and key and with password protection respectively.

The interviewer has discussed this information with me and offered to answer my questions. For any further questions, I may contact the Chairperson, UNZABREC.

STATEMENT OF CONSENT/ASSENT

..... has described to me what is going to be done, the risks, the benefits involved and my rights regarding this study. I understand that my decision to participate in this study will not alter my usual medical care. In the use of this information, my identity will be concealed. I am aware that I may withdraw at any time. I understand that by signing this form, I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name:…….Signature of participant ……..Age..……
Date (DD/MM/YY)……

Name of Witness…….. Signature of Witness……
Date (DD/MM/YY)…….
Name……Signature of parent or guardian for minors ……
Date(DD/MM/YY)……

Name……..Signature of Interviewer ……Date (DD/MM/YY)……...

Name……..Signature of Principal Investigator ……
Date (DD/MM/YY)……...

If you have any further questions, please contact the University of Zambia Biomedical Research Ethics Committee

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Federal Assurance No. FWA00000338 IRB00001131 of IORG0000774 NHRAR-REC No 2021-05-0002

FOMU YOVOMEREZA OPHUNZITSIDWA KUSEBENZA NDI INU

KULANGANA PA BAKITERIYA OMWE IVUTA MU NKWALA MU ANTHU ALI NA HIV NDI AIDS OMWE INGENA NDI ZA KUDYA MU LUSAKA, DZIKO LA ZAMBIA

(Nyanja)

Mutu wa kafukufukuyu:

Njira zolimbana ndi tizilombo toyambitsa matenda za mabakiteriya omwe amapezeka m'zakudya omwe ali ndi kachilombo ka HIV/AIDS ku Lusaka, Zambia

Ofufuza:

Bambo Aron Rezene Mebrahtu, University of Zambia, School of Natural Science, Department of Biological sciences, Microbiology unit. Tel no +260779075415

Mbiri ndi zomveka za phunziroli:

Antimicrobial resistance ndi vuto lalikulu laumoyo wa anthu padziko lonse lapansi komanso vuto lachitetezo cha chakudya. Tizilombo toyambitsa matenda tikayamba kugonjetsedwa ndi mankhwala oletsa tizilombo toyambitsa matenda, amatha kukhala pachiwopsezo chachikulu cha munthu chifukwa cha kulephera kwa chithandizo, kutayika kwa njira zochizira komanso kuchuluka kwa mwayi komanso kuopsa kwa matenda. Cholinga cha kafukufukuyu ndi kudziwa mbiri ya antimicrobial kukana mankhwala a mabakiteriya omwe amapezeka m'zakudya pakati pa odwala omwe ali ndi kachilombo ka HIV/AIDS.

Cholinga:

Kafukufukuyu akuyembekezeka kupereka zotsatira zopindulitsa pothana ndi vuto lomwe lilipo pano la antimicrobial resistance pakati pa odwala HIV/AIDS ku Lusaka, Zambia. Izi zidzathandiza okonza mapulani ndi opanga ndondomeko zokhudzana ndi thanzi la anthu ndi mabungwe a chakudya kuti athe kuyang'anira nkhani zotsutsana ndi mankhwala kuchokera ku lingaliro limodzi la thanzi. Izi zikuyembekezekanso kudziwitsa asing'anga ndi ofufuza kuti ndi mankhwala ati omwe samva bwino komanso ndi mabakiteriya ati omwe amadzipatula omwe akuwonetsa kukana kuti chisamaliro cha odwala chiwonjezeke.

Njira:

Mu phunziroli, ogwira ntchito yophunzira adzatenga chitsanzo cha chopondapo kuchokera kwa inu ndipo adzatumiza chitsanzo cha chopondapo ku microbiology unit, UTH mukafika kuchipatala ngati muli ndi chizindikiro cha ululu wa m'mimba, kusanza, kutsegula m'mimba, nseru ndi kutentha thupi komwe kumakhudzana ndi kuipitsidwa ndi chakudya.

Ndani adzachita nawo phunziroli?

Zitsanzo zidzatengedwa kuchokera kwa odwala onse omwe ali ndi kachilombo ka HIV/AIDS omwe amapita ku UTH ndi limodzi mwa madandaulo a ululu wa m'mimba, kusanza, kutsegula m'mimba, nseru ndi kutentha thupi komwe kumakhudzana ndi kuipitsidwa kwa chakudya mu nthawi yodziwika. Kukula kwa omwe atenga nawo mbali kudzakhala 384. Ophunzirawo akuyenera kupereka chitsanzo cha chopondapo kamodzi pa nthawi yawo kuti akawone dokotala.

Zowopsa/Zosasangalatsa:

Ophunzira amangopereka chitsanzo cha chopondapo paulendo wawo wokaonana ndi dokotala. Sipadzakhala chiopsezo chopereka zitsanzo za chimbudzi, komabe otenga nawo mbali nthawi zina amatha kuchita manyazi kapena kuchita manyazi popereka chopondapo.

Ubwino:

Sipadzakhala phindu lachindunji kwa inu. Komabe, ochita kafukufuku angaphunzire zambiri kuchokera mu kafukufukuyu zomwe zingathandize anthu omwe ali ndi kachilombo ka HIV/AIDS.

Njira zina:

Otenga nawo mbali okha omwe akufuna kutenga nawo mbali ndi omwe adzakhale nawo pa kafukufukuyu. Mutha kufunsa mafunso nthawi iliyonse. Mutha kukambirana za phunziroli ndi ena musanaganize zolowa nawo. Ziribe kanthu kuti chisankho chanu ndi chiyani, chisamaliro china chilichonse chomwe mungapeze kuchipatalachi sichidzasintha.

Mafunso:

Otenga nawo mbali omwe ali ndi mafunso okhudzana ndi kafukufuku amatha kufikira ofufuza nthawi iliyonse mwakuthupi kapena kudzera pa adilesi ya wofufuza wamkulu.

Mafunso okhudza ufulu wa otenga nawo mbali:

Ophunzira omwe ali ndi mafunso okhudza ufulu wawo monga ochita nawo kafukufuku adzapatsidwa mayankho ndipo ali ndi ufulu wofunsa UNZABREC za ufulu wawo nthawi iliyonse. Ndipo yankho lotheka la mafunso lidzayankhidwa.

Chidziwitso cha kudzipereka:

Kutenga nawo mbali mu phunziroli ndi kodzifunira ndipo otenga nawo mbali atha kulowa nawo mwakufuna kwawo. Ophunzira amakhalanso ndi ufulu wochoka ku phunziroli nthawi iliyonse popanda chilango.

Chinsinsi:

Zotsatira za kafukufukuyu zidzasungidwa mwachinsinsi komanso kugwiritsidwa ntchito pofufuza. Chidziwitso chanu chidzabisidwa malinga ndi lamulo. Dzina lanu silidzawonekera paliponse pamafomu omwe ali ndi chidziwitso. Zolemba zamapepala ndi zamakompyuta zidzasungidwa pansi pa loko ndi kiyi komanso chitetezo chachinsinsi motsatana.

Wofunsayo wakambirana nane izi ndipo adadzipereka kuti ayankhe mafunso anga. Pamafunso ena aliwonse, nditha kulumikizana ndi Wapampando, UNZABREC.

MAWU OVOMEREZEKA/KUVOMEREZA

..... . Ndikumvetsa kuti kusankha kwanga kutenga nawo mbali mu phunziroli sikungasinthe chithandizo changa chachipatala. Pogwiritsa ntchito chidziwitsochi, chidziwitso changa chidzabisika. Ndikudziwa kuti ndikhoza kuchoka nthawi iliyonse. Ndikumvetsa kuti posayina fomuyi, sindimasiya ufulu wanga uliwonse walamulo koma ndikungosonyeza kuti ndadziwitsidwa za kafukufuku wofufuza momwe ndikuvomera modzifunira kutenga nawo mbali. Kope la fomu iyi lidzaperekedwa kwa ine.

Dzina:……Kusaina kwa otenga nawo mbali……Zaka……..

Tsiku (DD/MM/YY)……

Dzina la Mboni…….. Kusaina kwa Mboni……

Date (DD/MM/YY)…….

Dzina …… Siginecha ya kholo kapena wosamalira ana ……

Tsiku (DD/MM/YY)……

Dzina……..Siginecha ya Interviewer…….

Tsiku (DD/MM/YY)……...

Dzina……..Siginecha ya Principal Investigator ..... Tsiku (DD/MM/YY)……...

Ngati muli ndi mafunso ena chonde lemberani komiti ya University of Zambia Biomedical Research Ethics Committee

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27th June 2024

Your REF. No. 5317-2024

Mr. Aron Mebrahtu,
University of Zambia,
School of Natural Science,
PO Box 32379,
Lusaka.

Dear Mr. Mebrahtu,

RE: ANTIMICROBIAL RESISTANCE PATTERNS OF FOODBORNE BACTERIAL ISOLATES FROM HIV/AIDS PATIENTS IN LUSAKA, ZAMBIA (REF. NO. 5317-2024)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 27th June, 2024. The proposal is **approved**. The approval is based on the following documents that were submitted for review:

a) Study proposal
b) Questionnaires
c) Participant Consent Form

APPROVAL NUMBER : REF. No. 5317-2024.

This number should be used on all correspondence, consent forms and documents as appropriate.

i. **APPROVAL DATE : 27th June 2024** ii. **TYPE OF APPROVAL : Standard** iii. **EXPIRATION DATE OF APPROVAL : 26th June 2025**

iv. After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.

v. **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.

vi. **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).

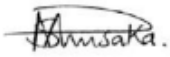
vii. **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.

viii. **NHRA:** You are advised to obtain final study clearance and approval to conduct research in Zambia from the National Health Research Authority (NHRA) before commencing the research project.

ix. **QUESTIONS**: Please contact the UNZABREC on Telephone No. +260977925304 or by e-mail on unzarec@unza.zm.

x. **OTHER**: Please be reminded to send in copies of your research findings/results for our records. You are also required to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study. Use the online portal: unza.rhinno.net for further submissions.

Yours sincerely,



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BMC Infectious Diseases

SYSTEMATIC REVIEW Open Access

A systematic review and meta-analysis of antibiotic resistance of foodborne pathogenic bacteria

Aron Rezene Mebrahtu[1], Likulunga Emmanuel Likulunga[1], Adriace Chauwa[2], Mildred Zulu[3] and Sydney Malama[1*]

Abstract

Antimicrobial drugs are used to treat bacterial pathogens that cause infections in humans and animals. Despite their importance, antimicrobial drugs exhibit inefficiency in treating infections if used irrationally without adherence to standard guidelines. Currently there is a lack of review literatures concerning antimicrobial resistance status in the southern sub Saharan African countries, hence the study is designed for and provides valuable insights into the status and comparison of antimicrobial resistance among foodborne bacteria in Zambia relative to other regions of the world, using systematic literature review and meta-analysis. For meta-analysis of bacterial and AMR prevalence and, generation of forest plots, functions from R packages were used and meta-regression analysis using the random effect model with the R functions "escalc" and "rma" from R "metafor" package was used to determine sample size on bacterial prevalence. A total of 434 articles were identified and downloaded after a systematic research. The study has implicated that the most common foodborne bacteria in the last five years in Zambia are *salmonella spp.*, *E. coli.*, and *L. monocytogens*. Based on the random effect model, the prevalence of bacterial pathogens across all studies in food samples was observed to be 11% and in human samples was 14%. The study found a significant increase in antimicrobial resistance (AMR) burden among foodborne pathogens in Zambia compared to other regions of the world over the past five years. This rise is attributed to the bacteria's ability to develop resistance mechanisms and easily spread between humans, animals, and the environment. Ineffective surveillance, inadequate management by stakeholders, and public unawareness have further exacerbated the problem, requiring effective policy implementations in the health sector.

Keywords Antimicrobial drugs, Antimicrobial resistance, Foodborne bacteria, Meta-analysis