Sokoine University of Agriculture

MSc Dissertation

Prevalence and Antimicrobial Resistance Profile of Foodborne *Salmonella Enterica* and *Escherichia Coli* from Rodents and Shrews in Morogoro Municipality, Tanzania

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PREVALENCE AND ANTIMICROBIAL RESISTANCE PROFILE OF FOODBORNE *SALMONELLA ENTERICA* AND *ESCHERICHIA COLI* FROM RODENTS AND SHREWS IN MOROGORO MUNICIPALITY, TANZANIA

Dissertation Submitted to Sokoine University of Agriculture in Partial Fulfillment of the Requirements for the Degree of Master of Science in Public Health Pest Management

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EXTENDED ABSTRACT

Rodents and shrews were identified as transmitters and carriers of *Salmonella enterica*, *Escherichia coli* (*E. coli)*, and their antimicrobial resistant strains; however, few researchers have examined rodents feces infections. Antimicrobial resistance (AMR) is a pressing issue in the field of global public health, impacting both human and animal health. Small mammals such as rodents and shrews excrements constitute a significant reservoir of zoonotic pathogens, including bacteria resistant to antimicrobials. To date, there is a need for more documented research specifically addressing *Escherichia coli* (*E. coli*) and *Salmonella enterica* strains derived from small mammal feces samples collected in Morogoro Municipality. This study aimed to determine the prevalence and antimicrobial resistance profile of *Salmonella enterica* and *E. coli* associated with rodents and shrews in five wards selected in Morogoro Municipality, Tanzania, from March to November 2023.

Questionnaires were used to assess risk factors of *Salmonella enterica* and *E. coli* associated with house rodents and shrews. This study isolated bacteria extracted from fecal samples of rodents and shrews by using a culture test and identified them with biochemical tests. Molecular tests were used to screen out bacteria-targeted. The isolates bacteria were analyzed for AMR using the disc diffusion method for susceptibilities test to the selected antibiotics. Molecular analysis was used to identify the species of bacteria using *16S rRNA* and *InvA*. Polymerase chain reaction (PCR) was used to find resistance genes in each isolate, including *Bla TEM, Bla SHV*, *Bla CTXM*, *Sul 1*, and *Sul 2*.

A total of 148 small mammals were captured, 145 (98%) were rodents and 3(2%) were shrews. These small mammals were captured from domestic, peri-domestic, and marketplaces. *Salmonella enterica* was detected in 3/148 (2%), and *E. coli* was found in 54/148 (36.5%) samples. Most rodents infected by *E. coli* and *Salmonella enterica* were *Mus* species, with 16.2% and 1.3% respectively. Regarding habitat, the high prevalence of *E. coli* was

observed in open markets, at 16.9%, while *Salmonella enterica* was high inside households, with 1.3%. Regarding the risk factors associated with house rodents and shrews, 83.7% of respondents found rodents feces in uncooked or cooked food, 30.4% found rodents feces in the water storage, 93.2% found food eaten by rodents, whereby 66.9 % of households used food contaminated with feces or eaten by rodents. Twenty-seven percent (27%) of the respondents were diagnosed with Diarrhea, which may be linked with pathogens from rodents and shrews.

The AMR on Amoxicillin was observed in *shrews* at 100%, in *Rattus rattus* was 89.5%, and *Mus* spp was 87.5%, while AMR to Ampicillin on *Rattus rattus* was 74% and in *Mus* spp was 62.5%. *Salmonella Enterica* was more resistant than *E. coli* for Sulphamethoxazole/Trimethoprim, Ciprofloxacin, and ampicillin. *E. coli* was more resistant to Amoxicillin than *Salmonella Enterica.* Concerning the genes linked to *E. coli* and *Salmonella enterica,* the results showed that *Salmonella Enterica* harbored more resistance genes (20%) than *E. coli* (12%). Two (2) isolates, out of 13 contained Sulphonamide-resistant genes as follows: *Sul 1* (n=1) of *Salmonella enterica* and *Sul 2* (n=1) of *E. coli*, both representing 15.4% of the total resistant gene analyzed in this study. *β-lactamases* (*Bla TEM* , *Bla SHV*, *Bla CTXM*) were found in 7 isolates (53.8%), with *Salmonella enterica* harboring more resistance genes than *E. coli*. The results of this study indicated that the public health significance of pathogens in rodents and shrews from the study area requires further investigation because these animals live close to humans and are also able to move from one place to another, which can increase the transmission of pathogens harbored by them to humans or the environment. Also, this research reveals the presence of resistant *Escherichia coli* and *Salmonella enterica* in small mammals, which indicate the potential role of rodents and shrews as a reservoir for AMR *E. coli* and *Salmonella enterica* that can be transferred to humans.

IKISIRI KUU

Panya na virukanjia viligunduliwa kama wabebaji bacteria wa *Salmonella enterica*, *Escherichia coli* (*E. coli*), na aina zao zenye upinzani wa antibiotiki hata hivyo, watafiti wachache wamechunguza maambukizi ya kinyesi cha panya. Upinzani wa antibiotiki (AMR) ni suala linalotia shinikizo katika uga wa afya ya umma duniani, likiathiri afya ya binadamu na wanyama. Wanyama wadogo kama panya na virukanjia wanawakilisha hazina kubwa ya vimelea vya zoonotiki, ikiwa ni pamoja na bakteria wenye upinzani wa antimicrobial. Hadi sasa, kuna haja ya utafiti zaidi uliodhibitishwa unaohusu aina ya *E. coli* na *Salmonella enterica* zinazotokana na sampuli za kinyesi za wanyama wadogo zilizokusanywa katika Manispaa ya Morogoro. Utafiti huu ulilenga kutambua kiwango na maelezo ya upinzani wa antimicrobial wa *Salmonella enterica* na *E. coli* zinazohusiana na panya na virukanjia katika mabanda matano yaliyochaguliwa katika Manispaa ya Morogoro, Tanzania, kuanzia Machi hadi Novemba 2023.

Maswali yalitumika kutathmini mambo ya hatari ya *Salmonella enterica* na *E. coli* yanayohusiana na panya na virukanjia wa nyumbani. Utafiti huu uliainisha bakteria zilizotengwa kutoka kwa sampuli za kinyesi za panya na virukanjia kwa kutumia jaribio la utamaduni na kuzitambua kwa vipimo vya biochemical. Vipimo vya molekuli vilivyotumika kufanya uchunguzi wa bakteria walengwa. Bakteria zilizotengwa zilichambuliwa kwa AMR kwa kutumia mbinu ya upimaji wa unyevu wa diski kwa ajili ya vipimo vya kupima viungo vya antibiotiki zilizochaguliwa. Uchambuzi wa molekuli ulitumika kutambua spishi za bakteria kwa kutumia *16S rRNA* na *InvA*. Mbinu ya mnyororo wa polima (PCR) ilitumika kutambua jeni za upinzani katika kila kiumbe kilichotengwa, ikiwa ni pamoja na *Bla TEM, Bla SHV, Bla CTXM, Sul 1,* na *Sul 2.*

Jumla ya wanyama wadogo 148 walinaswa, 145 (98%) walikuwa ni panya na 3(2%) walikuwa ni virukanjia. Wanyama hawa walinaswa kutoka kwenye makazi ya ndani, kando ya nyumba, na masoko. *Salmonella enterica* iligunduliwa katika 3/148 (2%), na *E. coli* ilipatikana katika sampuli za 54/148 (36.5%). Panya wengi

walioambukizwa na *E. coli* na *Salmonella enterica* walikuwa ni wa spishi ya *Mus*, kwa mtiririko huo 16.2% na 1.3%. Kuhusu makazi, ueneaji mkubwa wa *E. coli* uliwekwa katika masoko ya wazi, kwa 16.9%, wakati *Salmonella enterica* ilikuwa juu ndani ya nyumba, kwa 1.3%. Kuhusu mambo ya hatari yanayohusiana na panya wa nyumbani na virukanjia, 83.7% ya washiriki waligundua kinyesi cha panya katika chakula kisichopikwa au kilichopikwa, 30.4% waligundua kinyesi cha panya katika uhifadhi wa maji, 93.2% waligundua chakula kilicholiwa na panya, huku 66.9% ya nyumba zikitumia chakula kilichochafuliwa na kinyesi au kilicholiwa na panya. Asilimia ishirini na saba (27%) ya washiriki waligunduliwa na kuhara, ambayo inaweza kuwa inahusiana na vimelea kutoka kwa panya na virukanjia. Upinzani wa AMR kwa Amoxicillin ulionekana kwa vituvi kwa 100%, kwa *Rattus rattus* ilikuwa 89.5%, na *Mus* spp ilikuwa 87.5%, wakati upinzani kwa Ampicillin kwa *Rattus rattus* ilikuwa 74% na *Mus* spp ilikuwa 62.5%. *Salmonella enterica* ilikuwa na upinzani zaidi kuliko *E. coli* kwa Sulphamethoxazole/Trimethoprim, Ciprofloxacin, na ampicillin. *E. coli* ilikuwa na upinzani zaidi kwa Amoxicillin kuliko *Salmonella enterica*. Kuhusu jeni zinazohusiana na *E. coli* na *Salmonella enterica*, matokeo yalionyesha kwamba *Salmonella enterica* ilikuwa na jeni nyingi za upinzani (20%) kuliko *E. coli* (12%). Isolati mbili (2), kati ya 13, zilikuwa na jeni zenye upinzani wa Sulphonamide kama ifuatavyo: Sul 1 (n=1) ya *Salmonella enterica* na Sul 2 (n=1) ya *E. coli*, zote zikiwakilisha 15.4% ya jumla ya jeni zenye upinzani zilizochambuliwa katika utafiti huu. *β-lactamases* (*Bla TEM, Bla SHV, Bla CTXM*) ziligunduliwa kwa isolati 7 (53.8%), na *Salmonella enterica* ikishikilia jeni nyingi za upinzani kuliko *E. coli*. Matokeo ya utafiti huu yalionyesha umuhimu wa afya ya umma wa vimelea katika panya na virukanjia kutoka eneo la utafiti unahitaji uchunguzi zaidi kwa sababu wanyama hawa huishi karibu na binadamu na pia wanaweza kutoka se.

DECLARATION

I, Marie Chantal Uwanyirigira, declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work done within the registration period and that it has neither been submitted nor concurrently submitted in any other institution.

_____ _____

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The declaration is hereby confirmed by;

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Prof. Abdul A.S. Katakweba Date

(Supervisor)

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Prof. Sharadhuli KIMERA Date

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DEDICATION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

AML	Amoxicillin
AMP	Ampicillin
AMR	Antimicrobial resistance
BLAST	Basic Local Alignment Search Tool
Bp	base pairs
BPW	Buffered Peptone Water
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone,
CTX	Cefotaxime
DNA	Deoxyribonucleic acid
E. coli	*Escherichia Coli*
ESBL	Extended Spectrum Beta Lactamases
FBD	Food Borne Disease
FIG	Figure
GIS	Geographic information system
I	Intermediate
IMViC	Indole, Methyl red, Voges-Proskauer and Citrate
MDR	Multi drug resistance
ml	Microliter
PCR	Polymerase chain reaction
R	Resistance
rRNA	Ribosomal ribonucleic acid
RVS	Rappaport-Vassiliadis Soya
S	susceptible
SIM	Sulfur, Indole, Motility
SPP	Species
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
SXT	Sulphamethoxazole/Trimethoprim
TSI	Triple Sugar Iron.
UV	Ultraviolet
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Rodents and shrews have been linked to the spread of foodborne diseases found in humans, Numerous significant zoonotic infections affecting people and animals are mainly carried and hosted by rodents and shrews (Ramatla *et al.,* 2022). It is widely acknowledged that they contribute to the spreading and transmitting of human diseases. According to (Jemilehin *et al.,* 2016), rodents feed on stored food and contaminate it with urine and feces that may be harmful to human health.

Rodents and shrews are highly significant to public health because they are reservoirs for pathogens, such as *Salmonella* spp and (*E. coli).* These small mammals were identified as transmitters and carriers of resistant *Salmonella* and *E. coli* strains (Nkogwe et al., 2011; Greig et al., 2015; Pimentel Sobrinho *et al.,* 2020). The World Health Organization (WHO) has categorized *Enterobacteriaceae*, including *E. coli* and *Salmonella* spp., as pathogens of critical priority for AMR investigation (Mogasale *et al.,* 2021). Many bacteria extracted from rodents and shrews have been reported to be resistant, particularly *Salmonella* spp. and *E. coli* (Nkogwe et al., 2011). Previous studies have demonstrated the presence of AMR *E. coli* and *Salmonella* spp in rodents and shrews inhabiting both urban and rural environments (Katakweba, 2014; Ndakidemi et al., 2022; Kimwaga et al., 2022). Most prior investigations have examined the resistance phenotype of AMR *E. coli* and *Salmonella* spp strains obtained from rodents (Ball *et al.,* 2019). Therefore, rodents and shrews residing in proximity to human settlements have the potential to participate in the dissemination of AMR genes (Benavides et al., 2021). The Antimicrobial resistance found in *E. coli* and *Salmonella* spp. can be horizontally transferred to diverse bacterial populations(Ripanda *et al.,* 2023). This phenomenon poses a possible risk of dissemination to both humans and the environment, exacerbating AMR(Nelson *et al.,* 2008).

The bacteria, which are excreted in feces, are known to cause significant illness in humans with gastroenteritis and foodborne diseases (Himsworth *et al.,* 2015; Zhang *et al.,* 2015; Pimentel *et al.,* 2020). All of these diseases are primarily spread to people by eating contaminated food that has come into contact with rodent excrement and urine, pests (including shrews), human hands, or rodent-infested water and soil. Jawad *et al.* (2013) and Torgerson et al. (2015) report that rodent infestation occurs along the entire "food chain," irrespective of the forms of transportation. Foodborne diseases (FBD) are illnesses resulting from the consumption of contaminated or naturally harmful food or drink with pathogenic bacteria and or their toxins (Rakshna *et al.,* 2020). Many FBDs are zoonotic since they can spread from animals to people (Chlebicz, 2018). Some of these are also novel diseases or illnesses that are changing in their hosts, locations, or effects (Cissé, 2019).

The primary means of preventing the spread of FBDs is maintaining hygiene throughout the food supply chain during storing, preparing, distributing, and selling food, minimizing contact between rodent infestations and human food (Greig *et al.,* 2015; Cissé, 2019). Trematerra (2013) recommends incorporating a highly effective pest control strategy to avoid food contamination throughout the supply chain. Many countries in Sub-Saharan Africa, including Tanzania, pay less attention to rodents and shrews management but instead focus on prevention of diseases with other methods. Nevertheless, constant improvement in knowledge, competence, and practice in the management of rodent pests in food handling at both commercial and domestic levels to reduce the currently heavy burden placed by foodborne diseases on overstretched public health and welfare is required (Mangombi *et al.,* 2021; Samiji, 2022).

1.1 Rodents and shrews as reservoirs of Bacteria

Rodents and shrews are widely dispersed worldwide, with more concentration in urban areas where people live and work (Greig *et al.,* 2015; Cissé, 2019). Despite the likelihood that rodents serve as

zoonotic disease reservoirs, little is known about the diversity of microbes found in the urban rodents and shrews population. These small mammals are infected with bacterial pathogens known to cause acute or mild gastroenteritis in people, including atypical entero-pathogenic *E. coli*, *Clostridium difficile*, and *Salmonella enterica,* as well as infectious agents that have been associated with undifferentiated febrile sicknesses, including *Bartonella* spp, *Streptobacillus moniliformis*, *Leptospira interrogans*, and *Seoul Hantavirus* (Greig *et al.,* 2015; Cissé, 2019). It has been concluded that rodents serve as reservoirs for an enormous variety of microorganisms that may have an impact on human health and that more surveillance and understanding of the disease risks associated with urban rodent infestation are required (Firth *et al.,* 2014).

The study conducted by Katakweba (2018), *Rattus rattus* and *Mastomys natalensis* captured in pre-domestic houses and domestic sites of Morogoro municipality. Therefore, Small mammals, especially rodents and shrews, are known to frequently come into touch with humans, which may increase disease transmission through contaminated food with rodent's feces and urine (Katakweba, 2018; Kimwaga et al., 2022).

1.2. *Salmonella* spp and *E. coli* associated with Rodents and Shrews

Rodents and shrews can be carriers for pathogens that are transmitted to humans through the consumption of contact-contaminated food; hence rodents constitute a significant threat to zoonotic diseases that bacterial pathogens could cause (Nkogwe *et al*., 2011). Salmonellosis and infection related to *E. coli* are the most frequent and significant part of foodborne infections worldwide (Akbar *et al.,* 2011). In the study conducted by Nkogwe *et al.* (2011) on the rodents on the Caribbean Islands, *E. coli*, *Salmonella* spp., and *Campylobacter* spp were isolated, the majority of the rodents trapped were in areas close to human habitation and market areas, infesting human foods and the environment. With the unique

condition of salmonellosis and other infections, failure to manage rodent populations in some geographic areas has continued to pose health risks to humans.

1.3 Prevalence of *Salmonella* and *E. coli* in Rodents and Shrews

The different pathogens of public health significance for both human and animal diseases were found in a study carried out in North West Province, South Africa, by Ramatla *et al.* (2022). *Shigella* spp, *Salmonella* spp, and *E. coli* were found to be present in 3.3%, 29.9%, and 20.7%, respectively, of the 154 samples of captured rodents that were tested for zoonotic bacterial pathogens in the study on specific pathogens from rodents that live in poultry houses. The study conducted by Mehmood *et al.* (2012) in the poultry farms at Rawalpindi, Pakistan, revealed that *shrews* trapped in the poultry farms are reservoirs of different bacteria, including *Escherichia Coli*, *Proteus* spp. and *Salmonella* spp which are harmful to human and animals' health. In the same study, the high prevalence report in *Salmonella* spp was 69.2% in fecal and urine samples, and *Escherichia Coli* and *Proteus* spp were 61.5% and 15.38%, respectively, in fecal samples; this indicates a high risk of the passage of the infection from one host individual to another. The presence of *Salmonella* spp. and *E. coli* bacteria linked to rodents and shrews, which are responsible for diseases, is still unknown in Morogoro Urban (Municipality), Tanzania.

1.4 The Factors that influence foodborne diseases transmitted by bacteria associated with rodents and shrews

Local habitat features such as sanitation and food waste may be strong determinants of spatial heterogeneity in rodent and shrew infection because of their role in pathogen and ecology. For example, the presence of food waste in the garbage can promote rodents infestations (Murray *et al.,* 2018) and potentially the transmission of fecal-oral pathogens, such as *E. coli* and *Salmonella* spp*,* due to fecal contamination and contact with food (Nelson et al.,

2008). Rodents and shrews individual characteristics may also contribute to variation in infection risk since older and more injured rodents may be more likely to harbor certain infections (Himsworth *et al.,* 2014). Seasonal peaks in population density, the persistence of microorganisms in the environment, and seasonal weather variations may also have an impact on pathogen transmission (Van *et al.,* 2011).

1.5 Antimicrobial resistance of *Salmonella and E. coli*

Antimicrobial resistance, according to the (WHO, 2015), happens when bacteria, viruses, fungi, and parasites alter over time and cease to respond to medications, making illnesses more difficult to treat and raising the risk of disease spread, serious illness, and death. AMR is an emerging issue problem among common diseases and foodborne pathogens in particular. A lot of recent diseases take pride in their resistance to medications or antimicrobial typically used to treat harmful microorganisms. It is believed that the extensive use of antibiotics in animal feed is the cause of the rise in AMR (Akbar *et al.,* 2011). Inappropriate antibiotic prescription and sales, the use of antibiotics outside of the healthcare industry, and genetic features inherent to bacteria are only a few of the many causes that have contributed to antimicrobial resistance(Katakweba, 2014; Hilary *et al.,* 2016). Previous studies reported AMR of *E. coli* and *Salmonella* spp*.* on different antimicrobials in humans, livestock, and wild animals (Sonola *et al.,* 2021).

1.6 Food and water control from rodents and shrews

Foodborne pathogens from rodents and shrews can cause serious health problems in humans (Jahan, 2021). Therefore, the elimination of these pathogens in the first part of the food chain should have priority, especially in the context of food and water handling, where there is a possibility to contaminate food and water by leaving behind their excrement with harmful pathogens(Himsworth et al., 2015).In order to control the transmission of FBDs, we need to prepare and store our food very carefully. Hence, the main reasons

for control programs are to eliminate or reduce contamination of products, damage to food stocks and properties, and the spreading of disease(Torgerson *et al.,* 2015). Monitoring the safety of the food should be considered from the crop stage through to the storage of food in both commercial and domestic premises (García *et al.,* 2008). A rodent control program that includes sanitation, rodent-proofing, population reduction, and evaluating and monitoring the rodent situation is a critical part of every management program (Fernández *et al.,* 2007). Pests require food, water, and shelter to survive; hence, food operators need to eliminate these conditions to prevent rodent infestations (Trematerra, 2013). Moreover, they should reduce the risk of foodborne diseases transmitted by rodents and shrew through considering Integrated Pest Management (IPM) approach.

1.7 Antimicrobial resistance bacteria shedding control

Antimicrobial resistance has emerged in zoonotic enteropathogens like *Salmonella* spp., *Campylobacter* spp., and *E. coli*, but the prevalence of resistance varies(Bhat, 2021). Antimicrobial resistance emerged from the use of antibiotics in animals and the subsequent transfer of resistance genes and bacteria among animals, animal products, and the environment, then later to humans (Singer, 2003). Shedding of antimicrobial resistance bacteria through the feces of rodents and shrews can emerge resistant in the environment; for instance, *S. Enterica* subsp. *Enterica* serovar *Choleraesuis* persists in dry feces 13 months post-shedding and after the disinfection process and survives in soil between 25 and 200 days (Blaha, 2009; Gwenzi et al., 2021). Reduction or elimination of these small mammals is an essential part of the prevention strategies in the control of antimicrobial resistance bacteria shedding.An effective control program should keep rodent numbers at the lowest possible level by using the approach of integrated pest management (IPM) (Lamichhane *et al.,* 2024).

1.8. Problem Statement and Justification

Rodents and shrews are found in houses, peri-domestic areas, shops, and food market areas and share the environment with humans (Taylor *et al.,* 2012). The movement of these rodents and shrews increases the shedding of pathogens in the environment, water, and food.(Jahan *et al.,* 2021).Given the wide variety of about 1700 species of small mammals in the world, particularly rodents and shrews that live in close proximity to humans and are known to be reservoirs or vectors of various FBDs, thus interaction may have a significant negative impact on human health (Saad *et al.,* 2015; Rabiee *et al.,* 2018). Many infectious illnesses are known to be transmitted to humans by rodents and shrews (Kijlstra *et al*., 2008; Tijjani et al., 2020). Some of these are viral (like Lassa fever, Hantavirus disease, tick-borne encephalitis, Argentine and Bolivian hemorrhagic fever), bacterial (such as Typhoid fever, plague, leptospirosis, Lyme disease, relapsing fevers, salmonellosis), and protozoans (*e.g., leishmaniasis, toxoplasmosis, cryptosporidium, Entamoeba histolytica, Giardia)* or helminths (*e.g., trichinellosis, echinococcosis*)(Chauhan *et al.,* 2020; Mustapha et al., 2019; Tijjani *et al.,* 2020). There are two basic types of rodent-borne illnesses: diseases that are directly or indirectly transferred. In the first, infections are spread through bites or inhaling rat excrement-borne germs, but in the latter, humans become ill after consuming food or water tainted with rodent urine or feces (Temmam *et al.,* 2014; Kumar Praveen *et al*., 2016). The strains of *Salmonella* spp*.* and *E. coli* that can be carried and transmitted by rodents are an emerging public health concern due to antimicrobial resistant (AMR) (Greig et *al.,* 2015). AMR associated with rodent-borne salmonellosis and *E. coli* accounts for economic and public health threats (therapeutic implications) and should not be ignored (Rabiee *et al.,* 2018). Currently, there is limited information on foodborne pathogens and their resistant that are harbored by rodents and shrews regardless of their proximity to human and animal localities in Tanzania. Hence this paucity of research information necessitates a study to estimate the burden and role played by rodents and shrews in the

transmission of *Salmonella* spp and *E. coli* with their resistant to antimicrobials, this study aims to determine the prevalence and antimicrobial resistance profile of *Salmonella enterica* and *E. coli* associated with rodents and shrews as a public health concern in the Morogoro Municipality district of Tanzania.

Isolation, identifying, characterizing, and investigating the antimicrobial susceptibility profile of *Salmonella enterica* and *E. coli* isolated from rodents and shrews in the study area will provide information on the burden of foodborne pathogens and their resistant isolates in rodents and shrews with conceivable causes of multidrug resistance in humans and domestic animals implicating significant public health threat. Different studies have been conducted on rodent-borne diseases, but little is known about pathogens, specifically *Salmonella enterica* and *E. coli* from rodents and shrews. The information from this study will help in setting of prevention and control of rodents and shrews. This study will also help the decision-makers in the public health sector to improve control strategies for foodborne diseases. Additionally, it will aid in enhancing treatment protocols for both humans and veterinary animals afflicted with illnesses by identifying resistance patterns of routinely employed antimicrobial medications.

1.9 Objectives

1.9.1 Overall objective

To determine the prevalence and antimicrobial resistance profile of *Salmonella enterica, E. coli,* and their risk factors associated with rodents and shrews in Morogoro Municipality.

1.9.2 Specific objectives

1. To determine the prevalence of *Salmonella enterica* and *E. coli* bacteria in rodents and shrews in Morogoro municipality, Tanzania.

2. To determine the antimicrobial resistance profile of *Salmonella enterica and E. coli* in rodents and shrews in Morogoro municipality, Tanzania.
3. To evaluate risk factors of *Salmonella enterica* and *E. coli* infections associated with rodents and shrews in Morogoro Municipality, Tanzania.

1.10 Hypotheses

1. Rodents and shrews have a significant prevalence of *Salmonella enterica* and *E. coli* pathogens.
2. *Salmonella enterica* and *E. coli* pathogens have resistance to certain antimicrobials in Morogoro municipality.
3. Risk factors of *Salmonella enterica and E. coli associated* with rodents and shrews have significant to human health.

CHAPTER TWO

Manuscript One

PREVALENCE OF *SALMONELLA ENTERICA, ESCHERICHIA COLI,* AND THEIR RISK FACTORS ASSOCIATED WITH RODENTS AND SHREWS IN MOROGORO MUNICIPALITY, TANZANIA

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Abstract

Rodents and shrews are known to be the source of foodborne diseases; however, few researchers have examined rodents' feces infestations. This study aimed to determine the prevalence of *Salmonella enterica and Escherichia coli* (*E. coli)* bacteria and their

risk factors associated with rodents and shrews in five wards selected in Morogoro Municipality, Tanzania from March to November 2023. Rodents and shrews were captured from domestic, peri-domestic, and marketplaces using live traps baited with peanut butter and maize bran, Tomatoes, Sweet potatoes, Avocado, Green maize, and ripened Bananas. Questionnaires were used to assess risk factors of *Salmonella enterica* and *E. coli* associated with house rodents and shrews. This study isolated bacteria from fecal samples extracted from rodents and shrews using a culture test and identified them with biochemical tests. Molecular tests were used to screen out bacteria targeted . A total of 148 mammals were captured. Among them, rodents were 145(98%), and Shrews were 3(2%). Among rodents, *Rattus rattus* were 76(51.4%), *Mus* 61(41.2%) and *Cricetomy* 8(5.4%). *Salmonella enterica* was detected in 3/148 (2%), and *E. coli* was found in 54/148 (36.5%) samples. Most rodents infected by *E. coli* and *Salmonella enterica were Mus* species, with 16.2% and 1.3% respectively.

Regarding habitat, the high prevalence of *E. coli* was observed in open markets, at 16.9%, while *Salmonella enterica* was high inside households, with 1.3%. Regarding the risk factors associated with house rodents and shrews, 83.7% of respondents found rodents feces in uncooked or cooked food, 30.4% found rodents feces in the water storage, 93.2% found food eaten by rodents, whereby 66.9 % of households used food contaminated with feces or eaten by rodents. Twenty-seven percent (27%) of the respondents were diagnosed with Diarrhea, which may be linked with pathogens from rodents and shrews. The results of this study indicated that the public health significance of pathogens in rodents and shrews from the study area requires further investigation.

Keywords: Rodents, Shrews, *Salmonella enterica, E. coli*, Prevalence, Risk factors, Morogoro, Tanzania

INTRODUCTION

Rodents comprise more species than any other mammal order in the world (K. Zhang *et al.,* 2022). Most rodents are considered keystone species in their ecological communities; hence, the survival of many different species in the ecosystem depends on them (Delibes-Mateos *et al.,* 2011). For public health points, this is particularly important for rodent-borne diseases. In the particular case of foodborne illnesses, rodents are essential as reservoirs and transmitters of various bacterial zoonoses, including *Salmonella enterica* and *E. coli* pathogens, which are responsible for foodborne diseases (Jahan *et al.,* 2021).

Foodborne diseases (FBD) result from consuming contaminated or naturally harmful food or drink with pathogenic microorganisms (Rakshna *et al.,* 2020). Foodborne diseases are mainly spread to people by eating contaminated food that has come into contact with rodent excrement and urine, pests (including rodents), human hands, or water and soil infested by rodents (Torgerson *et al.,* 2015). Many FBDs are zoonotic since they can be spread from animals to humans (Chlebicz, 2018); some are also novel diseases or illnesses that change in their hosts, locations, or effects (Cissé, 2019). Rodents have been linked to the spread of some foodborne diseases commonly found in humans, as stated by the World Health Organization (Kadariya *et al.,* 2014). Different rodent species are highly significant to public health because they are reservoirs of pathogens, such as *Salmonella* spp. and *E. coli* (Hardgrove *et al.,* 2021). According to Jemilehin *et al.* (2016), rodents feed on stored food and contaminate it with urine and feces that may contain harmful pathogens and carriers of resistant strains of *Salmonella* spp. and *E. coli*. These bacteria are excreted in feces and are known to cause significant illnesses in humans, like salmonellosis and gastroenteritis (Ma *et al.,* 2019; Pimentel et al., 2020). *Salmonella* spp poisoning is one of the most common and widely distributed diseases in the world, estimated to cause 1.3 billion cases of gastroenteritis and three million deaths worldwide (Ohud, 2012). *E.*

coli frequently contaminates animals and humans compared to other microbes and is a reliable indicator of feces contamination (Samet-Bali *et al.,* 2013). Also, *E. coli* is mainly abundant in the intestinal tract of most mammalian species, including humans and rodents (Fairbrother, 2006). Most *E. coli* are commensals, but some are known to be harmful or pathogenic bacteria, causing severe intestinal and extra-intestinal diseases in humans (Wasiński, 2019). Previous studies conducted by different researchers in Tanzania showed that rodents harbored numerous bacteria species. In Karatu District, 79.2% of *E. coli* isolated from 101 rodents revealed that the intense interaction between humans, animals, and rodents can cause epidemics (Sonola *et al.,* 2021). The study conducted in Ngorongoro District reported a high prevalence of *Salmonella enterica* bacteria, with 43.75% in wild rodents compared to dogs and humans (Issae *et al.,* 2023). The same study reported a lack of knowledge of rodent-borne zoonosis; only 28.13% of participants were aware, while 77.27% of them believed that rodents are animals that destroy crops and do not spread diseases; however, those attitudes may be the risk factors of *Salmonella* spp and *E. coli* (Issae *et al.,* 2023). In Morogoro, the most common diseases are Typhoid, Diarrhea, Dysentery, and Cholera, which are caused by eating contaminated food, as Ndunguru *et al.* (2020) found that 67.1% of ready-to-eat foods were contaminated with bacteria associated with rodents and shrews. However, in the studies conducted in Morogoro, the available information did not show the source and transmission of bacteria responsible for foodborne diseases.

Therefore, this study aimed to establish the prevalence of *Salmonella enterica* and *E. coli* from house rodents and shrews in Morogoro municipality, Tanzania, by (1) determining the prevalence of *Salmonella enterica* and *E. coli* Bacteria in rodents captured from house and food markets, and (2) evaluating the risk factors of *Salmonella enterica* and *E. coli* associated with the rodents in Morogoro urban. The findings of this study will be helpful to the decision-makers in the public health sector to know the strategies

that must be used to reduce the transmission of foodborne pathogens through rodent and shrews management.

MATERIAL AND METHODS

Study area

The study was conducted in five wards of Morogoro municipality: Magadu, Mzinga, Bigwa, Kiwanja cha Ndege/ Mawenzi market, and Mji mpya. Kiwanja cha Ndege/ Mawenzi market and Mji mpya were selected to represent food markets. Magadu, Mzinga, and Bigwa were chosen to represent urban farming in Morogoro, as shown on a map (Fig. 2.1)

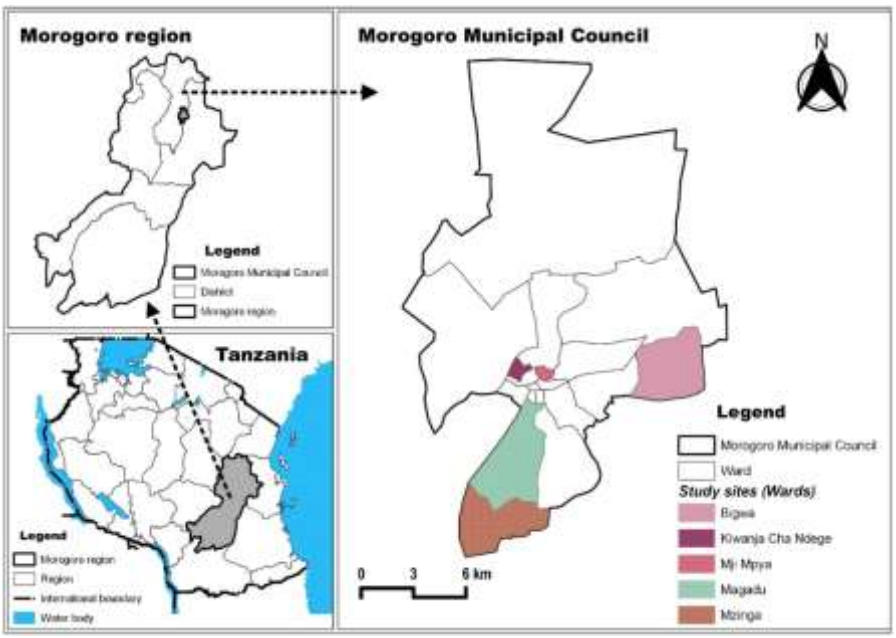

Figure 2.1: **Map of Morogoro Region and Morogoro Municipal Council showing wards where the study was done. The map was developed using QGIS software version 3.26.1 and shapefiles from DIVA-GIS (https://www.diva-gis.org/Data), which are freely accessible by the researcher.**

Research design

Within the framework of the cross-sectional study design, the approach of purposive sampling was used to select households and

food markets. Sample size calculated by following formula **n= Z^2 P (1-P)/d^2**, Z= 1.96, d= desired level of precision =0.05, P= Prevalence of 10.8% of AMR in rodents described by Sonola *et al*. (2021), $n = \frac{1.96^2 \times 0.108(1-0.108)}{0.05^2}$= 148. A total of 148 rodents and shrews trapped,148 household's representatives who voluntarily decided to participate in the study were contacted when collecting samples, and they were given a consent form to sign before conducting any sampling; the representatives of each household were interviewed to obtain information on the risk factors associated with foodborne diseases caused by rodents (Zheng, 2015; Mweshi, 2020). The trapping sites included areas such as the surroundings of restaurants, inside houses, and peri-domestic and food markets; rodents were trapped using locally made wire cages; Havarhart and Sherman's traps were placed randomly inside and outside the selected premises. The traps were baited with Peanut Butter mixed with maize bran, Tomatoes, sweet potatoes, Avocado, Green maize, and ripened bananas. A minimum number of three traps were used on each premise. The traps were kept for three consecutive nights and checked and re-baited every morning. Any rodents and shrews caught were taken to the laboratory at the SUA so that they could be identified, and fecal samples collected.

Collection of Rodent Feces

Each captured rodent was anesthetized using Diethyl Ether ¸ the gastrointestinal tracts were sliced and opened to remove all the contents from the small intestine to the caeca of the rodents and shrews. According to the methods of Nkogwe *et al.* (2011), the feces were collected and stored in a sterile container at 4^0C and transported at the Microbiology Laboratory of the Department of Microbiology, Parasitology, and Biotechnology at Sokoine University of Agriculture (SUA) for further analysis by using Cary Blair Transport Medium(Nagata *et al*., 2019).

Questionnaires survey

A pretested structured questionnaire was administered to the households and shop owners. A total of 148 households from markets, restaurants, and farmers have been interviewed. The questionnaire captured issues related to the risk factors of foodborne diseases associated with house rodents and shrews. Questions were focused on the consumption and management of food eaten by rodents or contact with rat feces if it is rejected or consumed by humans. All participants signed the consent form before interviewing them (Länsimies-Antikainen *et al*., 2010).

Isolation and Identification of *Salmonella* spp. and *E. coli*

Collected Fecal samples were pre-enriched using buffered peptone water (BPW) and were incubated at 37°C for 18–24 h. Using aseptic techniques, 100 µL of the enriched sample was transferred to 10 mL of warmed Rappaport-Vassiliadis Soya (RVS) broth (Oxoid) as selective enrichment of *Salmonella* spp. and incubated at 41.5°C for 21-27 hrs preferred water bath. Following incubation, RVS broth was inoculated onto XLD (Xylose Lysine Deoxycholate) plate agar and incubated at 37°C for 21–27 hrs. All XLD plates were observed for *Salmonella* spp.–like colonies, and the positive colonies, which showed red with or without black centers, were sub-cultured to obtain pure colonies. The enriched samples with buffered peptone water in aseptic procedures were also inoculated onto Mac Conkey agar and incubated for 18–24 hrs at 37^0C. After incubation, plates were observed for colonies typical of *E. coli*. The positive colonies, which showed pink, were sub-cultured to obtain pure colonies; further biochemical confirmation was done (Himsworth *et al.,* 2015).

All bacterial colonies were studied morphologically and suspected *Salmonella* spp. and *E. coli* were confirmed biochemically using the Triple sugar iron (TSI) test, the (IMViC) test, the SIM test, and the Urea test (Iyer *et al.,* 2013; Shahryari *et al.,* 2018). Isolates that passed all biochemical tests for *Salmonella* spp. and *E. coli* were transferred and cultivated on Nutrient agar (N.A.) for molecular confirmation (Nkogwe *et al.,* 2011).

Molecular analysis of *Salmonella* spp. and *E. coli*

DNA extraction

The genomic DNA was isolated from an overnight-growth bacterial colony using the boiling method. Briefly, the colonies were taken using sterile swabs from a petri dish containing pure *Salmonella* spp. and *E. coli* culture, then transferred in an Eppendorf tube containing 100µl of the nuclease-free water and boiled in a water bath at 95 °C for 5 min and immediately transferred to -20 ^{0}C Freezer for 10 min purposely to stress the bacterial cell to release out internal components. This procedure was repeated, and the suspension was centrifuged at 12,000 rpm for 1 minute. Eighty microliters of the supernatant were taken using a micropipette for further processing. The concentration and quality of the extracted DNA were checked by gel electrophoresis (1.5% agarose gel) to look at the intactness of the band and spectrophotometrically quantified using a Nanodrop Spectrophotometer to examine the quality and quantity of extracted DNA. All extracted DNA was stored at -20 for further analysis (Dashti, 2009; Khosravi *et al.,* 2012; Bagus *et al.,* 2017).

Molecular identification of bacterial species

All bacterial colonies presumptively identified based on biochemical and phenotypic characteristics were subjected to molecular identification using a thermal cycler. The primers (forward and reverse primers) were designed to give a product of approximately 585 base pairs targeting *Escherichia Coli,* and 796 base pairs targeting *Salmonella* spp*.* were used for Polymerase chain reaction (PCR) amplification. PCR was performed using a master mix (Bioneer premix-Korea). The primers used are described in Table 2.1 below (James, 2010; Silva *et al.,* 2011; Nanteza *et al.,* 2020).

The PCR amplification For *Salmonella* spp*.* and *E. coli* was done under the following conditions respectively: Initial denaturation steps at 95 °C for 5 minutes, final denaturation at 94°C for the 30s, annealing at 58 °C, 55^{0}C for 30 seconds, and extension at 72°C for 30 second followed by final extension at 72°C for 10 min the reaction was run for 35 cycles and final cooling were maintained at

4^0C. The agarose gel (1.5%) stained with ethidium bromide was used to analyze PCR products (Amplicons) by gel electrophoresis. The ultraviolet trans-illumination machine visualized positive bands (Rahayuningtyas *et al.,* 2020; Alshaheeb *et al*., 2023).

Table 2.1: Primers for amplification of *Salmonella* spp. and *Escherichia Coli*

Bacteria	Primer name	Primer sequence	Size of the PCR product	References
E. coli	16s Forward 16s Reverse	5′GACCTCGGTTTAGTTCACAGA 3′ 5′CACACGCTGACGCTGACCA 3′	585bp	(James, 2010; Moawad *et al.,* 2017)
Salmonella Enterica	*InvA* Forward *InvA* Reverse	5′CGGTGGTTTTAAGCGTACTCTT3’ 5′CGAATATGCTCCACAAGGTTA 3′	796bp	(Silva *et al.,* 2011 ; Paião et *al.,* 2013;)

Sequencing and Phylogenetic Analysis

All the PCR products were cleaned up using Zymo Kit (Quick-DNA^{TM} Miniprep Plus Kit) according to the manufacturer's instructions, and the Macrogen company in Korea did the sequencing on commercial considerations. Raw sequences of the forward primer and reverse primer amplicons were imported into Bioedit software, then trimmed to remove noises, and finally combined to create a consensus sequence for each sample. Sequence similarity of the consensus sequences was compared with published sequences in the GenBank database using the nucleotide BLAST program. Isolates were identified at the species level based on ≥ 95% for *E. coli* and ≥ 94% for *Salmonella enterica* sequence, which determines the strains or reported strains (Altschup *et al.,* 1990). The ClustalW Program within the Mega11 software version 11.0 was used to carry out multiple alignments (Kumar *et al.,* 2021). The neighbor-joining method was utilized to infer the phylogenetic trees (Gascuel, 2006), and the estimated reliability of a phylogenetic tree was done by the bootstrap method. It was decided to delete any missing alignment

gaps or data, and the tree was rooted with *Proteus* species for *E. coli* and *Pseudomonas* species for *Salmonella enterica* (Hall, 2013).

Data analysis

Prevalence of *Salmonella enterica* and *E. coli* in house rodents and shrews was calculated per site, species, and habitat. Data were analyzed using Microsoft Excel (2016) and Statistical Package for Social Sciences (SPSS) Program Version 29.0 (2022) (https://www.ibm.com). Chi-square was used to assess the significance of the prevalence of *Salmonella enterica* and *E. coli* rate between distinct species of rodents and shrews, site, and habitat. Chi-square was also used to compare the prevalence of observations made at the various sampling sites. Differences were considered significant at <0.05 (Hanson *et al.,* 2002; Hosein *et al.*, 2008).

RESULTS

The total number of Rodents and Shrews captured.

A total of 148 rodents grouped into three species named *Rattus rattus*, *Mus* spp., *Cricetomys* spp, and *Shrews* spp*.* were trapped in different areas and were used to assess the *Salmonella* spp and *E. coli*. *Rattus rattus* (51.4%) and *Mus* spp. (41.2%) were more abundant than other species captured. The rodents were found more prevalent inside households (53.4%) and in the open market (28.4%) than in other habitats; the distribution of captured animals per their sex was not too different, as shown in Tables 2.2 and 2.3.

Table 2.2: Total number of rodents and shrews captured.

Rodent species & Shrews	Frequency	Percent (%)
Cricetomys spp	8	5.4
Mus spp.	61	41.2
Rattus rattus	76	51.4
Shrew spp.	3	2.0
Total	**148**	**100.0**

Table 2.3: Sex of Rodents and Shrews and their distribution

	Distribution	Frequency	Percent (%)
SEX	Female	76	51.4
	Male	72	48.6
	Total	**148**	**100**
Habitat	Inside household	79	53.4
	Open market	42	28.4
	Outside household	11	7.4
	Shops	16	10.8
	Total	**148**	**100**

Isolation and Biochemical Identification

A total of 148 Feces samples from house rodents and shrews were analyzed; 77.7% (115 samples) of these samples suspected *E. coli* and *Salmonella* spp positive after using the culture method. A total of 115 bacteria isolates were kept from whole samples for further identification with a Biochemical test. These isolates were identified as *Escherichia coli* with 58.1% (86 samples). In comparison, *Salmonella* spp. was 2.7% (4 samples) after being confirmed biochemically by using the Triple sugar iron (TSI) test, the (IMViC) test, the SIM test, and the Urea test. Accordingly, these data showed *Escherichia coli* has a high prevalence compared with *Salmonella enterica.*

Molecular Detection of *Salmonella enterica* and *E. coli*

A total of 90 gram-negative bacterial isolates were amplified using universal primers targeting the *16S rRNA* gene. Results showed that 36.5% (54 samples) of *E. coli* were positive, and 2% (3 samples) were *Salmonella enterica.* All positive amplicons that appeared on the 585 bp marker were *E. coli,* as shown in Figure

2.1, and the positive isolates for *Salmonella enterica* were located at 796 bp, as shown in Figures 2.2 & 2.3.

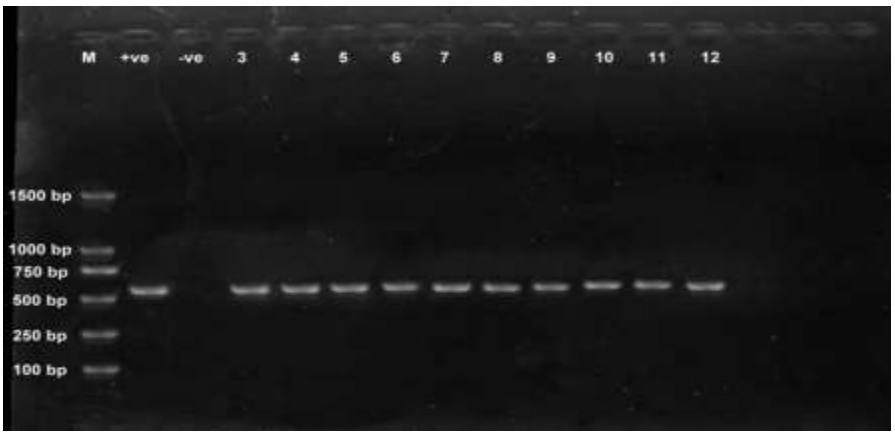

Figure 2.2: **PCR amplification of *E. coli* species, Where M is a DNA molecular marker and lanes 3-12 are samples, where lanes 3-12 are positive samples located at 585bp and lanes number 1 and 2 are positive and negative controls, respectively. For all isolates confirmed *E. coli* positive, seven isolates were from *Cricetomys* spp, 24 were *Mus* spp, 20 were *Rattus rattus,* and three isolates were from *shrews*.**

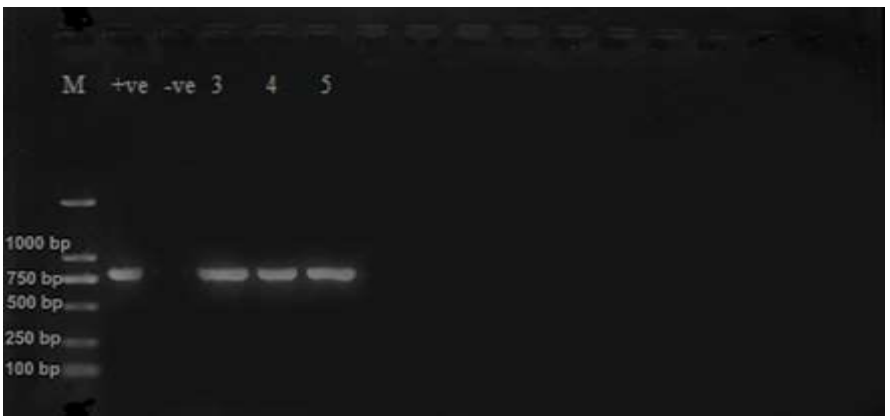

Figure 2.3: **PCR amplification of *Salmonella enterica* Where M is a DNA molecular marker, lanes 3-5 are samples, lanes 3-5 are positive samples located at 796bp, and lanes 1 and 2 are positive and negative controls, respectively. These three isolates confirmed *Salmonella enterica* ; lanes 3 and 5 were isolated from *Mus* spp., and lane 4 was from *Rattus rattus*.**

Sequencing analysis

The obtained nucleotide sequence *16S rRNA* gene and *InvA* gene of all bacterial Isolates were analyzed according to the targeted gene by BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) for identification of the close reference sequences available in GenBank (Figure 2.4 & 2.5). The BLAST analysis showed the similarity of *Salmonella enterica* and *E. coli* from this study to the others from the GenBank database. The results of the sequenced PCR product confirm the results obtained in previous tests (Culture and Biochemical) in the current study; 3 (2%) were *Salmonella enterica,* and 54 (36.5%) were *E. coli* samples.

Neighbor-joining phylogenetic trees based on *16S rRNA* gene partial sequences of *Escherichia Coli* (Bolded) obtained from this study clustered with the closely related genera of the family Enterobacteriaceae retrieved from the GenBank database. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Proteus species was used as an outgroup to root up the tree.

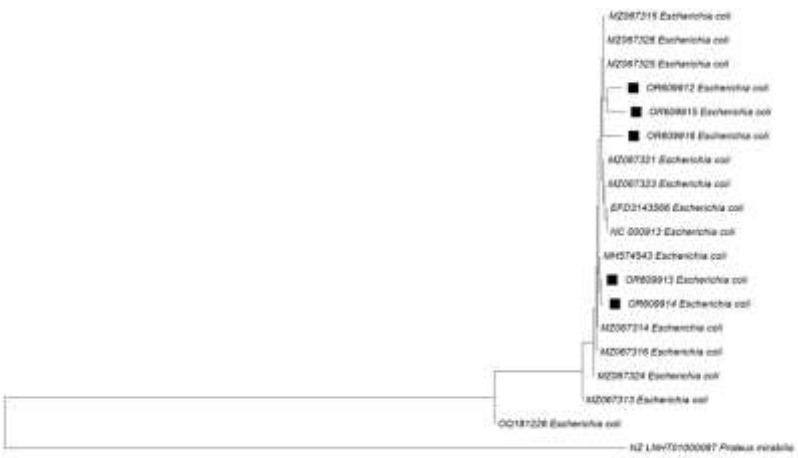

Figure 2.4: Phylogenetic tree of *E. coli*

Neighbor-joining phylogenetic trees based on *InvA* gene partial sequences of *Salmonella Enterica* (Bolded) obtained from this study clustered together with the closely related genera of the family Enterobacteriaceae retrieved from the GenBank database (Fig. 2.5). Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Pseudomonas species was used as an outgroup to root up the tree.

Figure 2.5: Phylogenetic tree of *Salmonella Enterica*

Prevalence of *Salmonella enterica and E. coli* among rodent species captured

Among 148 feces samples from captured mammals, 54 out of 148 samples tested positive for *E. coli,* representing 36.5% of the rodents. This bacterium was more prevalent in *Mus* species with 16.2%, followed by *Rattus rattus* with 13.5% (Table 2.4). Also, the results showed that three rodents were positive for *Salmonella enterica representing* 2% of the total sampled rodents and shrews. The most infected rodents with *Salmonella Enterica* were found in *Mus* spp., with 1.3% of the total samples (Table 2.4). Data analysis showed a statistically significant difference in the prevalence of *E. coli* tested under different species (p-value <0.05) using Pearson Chi-Square. On the contrary, there was no statistical significance between the prevalence of *Salmonella enterica* tested in *Mus* spp. and *Rattus rattus*.

Table 2.4: Prevalence of *Salmonella enterica* and *E. coli* per rodent and shrew species

Pathogens	Results	Species				Total
		***Criceto mys* spp**	***Mus* spp.**	***Rattus rattus***	***Shrew* spp.**	
	Total samples tested	8	61	76	3	148
E. coli	Mammals infected	7(4.7%)	24(16.3%)	20(13.5 %)	3(2.0%)	54(36.5 %)
	% within species	7(87.5%)	24(39.3%)	20(26.3 %)	3(100.0 %)	
Salmonella enterica	Mammals infected	0(0%)	2(1.3%)	1(0.7%)	0(0%)	3(2%)
	% within species	0(0%)	2(3.3%)	1(1.3%)	0(0%)	

Prevalence of *Salmonella enterica and E. coli* in rodents in selected wards

The greater prevalence of *E. coli* positive was found in the Mji Mpya market, with 15.5% of 148 samples tested, followed by the Mawenzi market with 9.5%. *Salmonella Enterica,* 1.3% of 148 samples tested were found positive from Mawenzi market and 0.7% from Mzinga. The prevalence of both bacteria was also observed in different wards of Morogoro Municipality, as shown in Table 2.5 below. Data analysis showed a statistically significant difference in the prevalence of *E. coli* tested under various sites selected (p-value <0.05) using Pearson Chi-Square. However, there was no statistical significance between the prevalence of *Salmonella enterica* tested in Kiwanja cha Ndege/Mawenzi and Mzinga wards.

Table 2.5: Prevalence of *Salmonella enterica and E. coli.* per ward

Pathogens	Results	Wards					
				i	ι		
	Total samples tested	L(7.4%)	6(10.8%)	55(37.2%)	6(24.3%)	30(20.3%)	18(100%)
E. coli	Mammals infected	6(4.1%)	5(3.4%)	14(9.5%)	8(15.5%)	6(4.0%)	4(36.5%)
	% within wards	54.5%)	6(31.2%)	14(25.4%)	8(66.6%)	6(20 %)	
Salmonella enterica	Mammals infected	0(%)	0(%)	2(1.3%)	0(%)	1(0.7%)	3(2 %)
	% within wards	0(%)	0(%)	2 (66.7%)	0(%)	1(33.3 %)	

Prevalence of *Salmonella enterica and E. coli* per habitat

High Prevalence of *E. coli* was observed in the open market at 16.9%, followed by inside households at 13.5%, outside households at 4.1%, and 2.0% in the shops, while the most significant prevalence of *Salmonella enterica was* observed inside households at 1.3 % and 0.7% in the open market as shown in Table 2.6. The results showed a statistically significant difference in the prevalence of *E. coli* tested under different habitats of rodents and shrews (p-value <0.05) using Pearson Chi-Square. On the contrary, there was no statistical significance between the prevalence of *Salmonella enterica* tested inside households and in the open market.

Table 2.6: Prevalence of *Salmonella enterica* and *E. coli* per habitat

Pathogens	Results	Habitat				Total
		Inside household	Open market	Outside household	Shop	
	Total samples tested	79	42	11	16	148
E. coli	Mammals infected	20(13.5%)	25(16.9 %)	6(4.1%)	3(2%)	54(36.5 %)
	% within habitat	20(25.3%)	25(59.5 %)	6(54.5 %)	3(18.7 %)	
Salmonella enterica	Mammals infected	2(1.3%)	1(0.7%)	0(0%)	0(0%)	3(2%)
	% within habitat	2(2.5%)	1(2.4%)	0(0%)	0(0%)	

Risk factors of foodborne diseases associated with house rodents.

A total of 148 participants were interviewed during the survey of this study; each participant represented one household. Results of the questionnaire used during data collection found that out of 148 interviewers,140 (94.6%) of them within selected households claimed the presence of rodents and shrews in their houses. This survey showed that rodents caused problems, including eating food in storage facilities, fruits, and maize bran used to feed livestock. Also, this study revealed the risk factors that were associated with *Salmonella enterica* and *E. coli* from rodents and shrews, as shown in Table 2.7 below. In some households, 24.3% did not have food

stores; this increased rodent invasion and transmission of pathogens harbored by them.

Similarly, 10.8% of households did not cover food before and after cooking food, and 83.70% of respondents found feces of rodents in uncooked or cooked food. Furthermore, the results indicated that many participants were not aware of the linkage between rodents, shrews, and foodborne diseases. Also, they do not know pest management strategies and foodborne control, as illustrated in Table 2.7. Within households selected, 27% of participants said that they were diagnosed with Diarrhea, which may have a linkage with pathogens from house rodents. This study revealed that the main risk factors of foodborne diseases were using food contaminated with rodent feces and urine or eaten by them and spoiling foods.

Table 2.7: Risk factors of *Salmonella enterica and E. coli* associated with rodents and shrews.

Variables	Households and shops interviewers (n= 148), %= Percentage		
	Yes	No	Percentage of risk factors
Presence of rodents and shrews at home	140 (94.6%)	8(5.4%)	94.60%
Problems of rodents and shrews at home (eating food in storage, human toes, limbs, clothes, maize bran, fruits, Flour, papers…)	138(93.2%)	10(6.8%)	93.20%
If they have a food store	112(75.7%)	36(24.3%)	24.30%
Cover food before and after cooking	132(89.2%)	16(10.8%)	10.80%
Feces of rodents and shrews in uncooked or cooked food	124(83.7%)	24(16.3%)	83.70%
Feces removed by bare hand	33(22.3%)	115(77.7%)	22.30%
washing hands after handling rodents and shrews or their feces	129(87.2%)	19(12.8%)	12.80%
Feces of rodents and shrews in the water storage	45(30.4%)	103(69.6%)	30.40%
Did you find food eaten by a rodent and shrews	138(93.2%)	10(6.8%)	93.20%
Consummation of food contacted with feces or eaten by rodents and shrews	99(66.9%)	49(33.1%)	66.90%
Diarrhea in the last three months	40(27%)	108(73%)	27%

Discussion

In all mammals trapped, 54 were confirmed positive for *E. coli,* representing *36.5%* of all captured rodents and shrews, while only three were confirmed positive for *Salmonella enterica,* they were representing 2% of all captured rodents and shrews. The prevalence of 36.5% of *E. coli* found in rodents and shrews in the current study is slightly higher than the 30% reported for peri domestic rodents in Madagascar in Ambatolahy village (Bublitz *et al.,* 2014). The low incidence of *Salmonella enterica* in these rodents and shrews could indicate that rodents are less prone to harboring *Salmonella enterica* than *E. coli*. Laboratory investigations on *Salmonella enterica* in rodents revealed that infection is temporary, with fecal shedding being sporadic and short-lived in the absence of repeated exposure (Himsworth *et al.,* 2015). In this study, Rattus *rattus* and *Mus* spp. were reported as potential rodents that may pose a risk in the zoonosis of *E. coli* infections and salmonellosis (Islam *et al.,* 2021). *E. coli* is a type of fecal *Coli*form bacteria found in sewerage, contaminated food, and water (Edberg *et al.,* 2000). *Rattus rattus* and *Mus* spp mostly live in house sewerage pathways or animal farms from where they can pick *E. coli* by eating contaminated feed, drinking water, wastes through poultry and cattle excreta, and also from infected herbage (Mushtaq-ul-Hassan *et al*., 2008). *E. coli* in the present study remained the most prevalent bacteria isolated and identified from the fecal matter of *Rattus rattus* and *Mus* spp, which is in agreement with Antoniou *et al.* (2010), who reported that 470/625 (75.2%) of rodents stool samples were found positive for *E. coli* in the Republic of Cyprus. Contrary, *Salmonella enterica* is a group of bacteria that can cause diarrheal illness (salmonellosis) in humans (Chiu et al., 2004). They are usually transmitted to *Rattus rattus* and *Mus* spp through eating contaminated feed and wastes of infected humans, animals, raw food of animal origin, contaminated water, milk, and sewerage wastes (Hilton *et al.,* 2002).

The study's results indicated that the high prevalence of *E. coli* and *Salmonella Enterica* bacteria were in *Mus* spp. and *Rattus rattus*.

The same results have been observed in *Rattus rattus* in the study conducted by Kimwaga et al. (2022) in Tanzania, Kilosa district, who reported a higher prevalence of *E. coli* and *Salmonella* in *Rattus rattus* than other species.Similarly, the study conducted by Sonola et al. (2021) in Karatu District reported a high prevalence of *E. coli* in rodents captured indoors and per domestic habitat, as the study of Issae et al. (2023) in Ngorongoro District reported a high prevalence of *Salmonella* in wild rodents. The *Mus* spp and *Rattus rattus* are known to be more abundant where there is food, meaning that the transmission of the pathogens to humans is high when consuming the contaminated food and water attacked by those rodents (Meerburg *et al.,* 2009). These two species have been researched and have been found to serve as carriers for numerous human diseases (Hill, 2011; Murray *et al.,* 2020). According to Jahan et al. (2021), it has been observed that *Mus* spp and *Rattus rattus* can be effectively harboring *E. coli* and *Salmonella* spp*.*, which can persist for a duration exceeding ten months. In the current study, *E. coli* exhibited a high prevalence, accounting for approximately 36.5% of the total species composition in the feces samples. However, the low prevalence of *Salmonella enterica* does not mean that it cannot have a negative impact on human health (Jahan *et al.,* 2021). According to Jahan *et al.* (2021), just one rodent in a house or food-producing facility can introduce around 23 million *Salmonella* pathogens into production pipelines within 24 hours.

The high prevalence of *Salmonella enterica* and *E. coli* in inside households and open markets found in this study was influenced by the rodents captured in these premises compared to others. Besides that, rodents prefer to live where they can easily find food and leave behind pellets containing *E. coli* and *Salmonella* spp bacteria. Those bacteria can be transmitted from rodents to other animals and humans (Phifer-Rixey, 2015). Therefore, food markets and households are particularly vulnerable to *E. coli* and *Salmonella enterica*. (Damborg *et al.,* 2016; Ribas *et al.,* 2016). Hence, rodents and shrews can act as reservoirs of several pathogens, and

controlling them effectively leads to control of these pathogens and the diseases they cause (Meerburg *et al.,* 2007).

Molecular approaches for microbial identification and characterization outperform biochemical procedures that use phenotypic features (Franco-Duarte *et al.,* 2019). The *16S rRNA* for *E. coli* and the *InvA* for *Salmonella enterica* were molecular detection tools because they were accurate and sensitive in detecting bacterial isolates (Barletta *et al.,* 2013). In this study, isolates of *E. coli* and isolates of *Salmonella* spp were sequenced after DNA extraction, and with BLAST online program and multiple sequence alignment of sequenced isolates showed all isolates of *E. coli* from the current study were 95% similar to *E. coli*. In comparison, isolates of *Salmonella* spp were 94% identical to *Salmonella enterica* in the gene bank. Using *16S rRNA* and *InvA* partial sequences, the evolutionary tree was constructed and showed that all isolates from the study were almost similar as they clustered together. This could be due to the presence of conserved and variable regions in their genomes (Mukhtar *et al.,* 2018) and also due to geographical and ecological similarities, as all the isolates were obtained from rodents and shrews and the same environment (Macrae, 2000).

The Physical presence of rodents and shrews in houses and peri domestic found in this study have linked to the 93.2% physical damage they cause like eating food in the store, maize bran, clothes, eating human toes and limbs, which showed the high possibility of foodborne pathogens transmission between human, animal, and environment. The study conducted by Hamidi (2018) reported that the signs of rodent infestations are physical damage to food and properties like electricity wires, crops, plants, clothes, and other packaging products; at the same time, they leave behind their contaminated excrement, which has important significance to public health.

Rodents and shrews are reported as carriers and transmitters of numerous zoonotic diseases (Jahan *et al.,* 2021). They carry different pathogens on their skins and in their digestive system, such as Borrelia spp., Campylobacter spp., Clostridium spp., Cryptosporidium parvum, and *E. coli, Leptospira* spp., *Listeria* spp., *Mycoplasma* spp., *Salmonella* spp., *Staphylococcus* aureus, *Streptobacillus moniliformis*, *Toxoplasma* spp., *Trichinella* spp., *Francisella tularensis*, *Yersinia pestis*, and Hantaviruses (Ogunniyi *et al.,* 2014; Dahmana *et al.,* 2020). They also cause infectious diseases such as campylobacteriosis, ascaridiasis, Bubonic plague, capilariasis, and *Coli*. Bacillosis, hemorrhagic enteritis, hymenolepiasis, leptospirosis, listeriosis, Lyme disease, mycoplasmosis, pasteurellosis, rat bite fever (RBF), or Haverhill fever, salmonellosis, toxoplasmosis, trichinosis, tularemia and Hantavirus pulmonary syndrome (HPS)(Bordes *et al.,* 2015).

This study has assessed the possible risk factors associated with the *Salmonella Enterica* and *E. coli,* where 27% of the respondents mentioned having Diarrhea after eating food contaminated by feces and urine of rodents and shrews or eaten by these mammals. These pathogens are the main threat to public health as they cause human diseases such as Diarrhea, typhoid, urinary tract infection, salmonellosis, and gastroenteritis (Tawyabur *et al.,* 2020a). The risk factors identified in this study are related to the results from the study conducted by Alfred-Chengula *et al.* (2015) in Morogoro municipality, who found several diseases, such as Diarrhea, dysentery, cholera, typhoid, worm diseases, and different bacteria, whereby the root causes were *Salmonella typhimurium* (16.7%) and *E. coli* (8.3%) frequency of isolation from solid waste disposal in Morogoro municipality, with direct linkage with rodents and shrews.

The results in Table 7 illustrate the risk factors by which foodborne *E. coli* and *Salmonella enterica* may transmitted from rodents and shrews to humans. In brief, human exposure to foodborne *E. coli* and *Salmonella enterica* in rodents and shrews can occur through

various pathways. These include the feces and urine of rodents in uncooked or cooked food, feces in water storages, and eating food eaten by rodents (Islam *et al.,* 2022). Additionally, consumption of edible rodents and associated foods contaminated with *Salmonella enterica* and *E. coli*, particularly when not properly cooked, can lead to exposure to foodborne diseases (Greig *et al.,* 2015; Heredia *et al.,* 2018). Consequently, individuals who possess *E. coli* and *Salmonella enterica* derived from rodents can contribute to its spread within the environment, among family members, and throughout the community (Winfield *et al.,* 2003). Nevertheless, there is a lack of empirical evidence about the role and relative contribution of the several channels through which humans are exposed to aspects of foodborne *E. coli* and *Salmonella enterica* (Torgerson *et al.,* 2015b). Consequently, until now, most literature reviews about foodborne disease pathogens and their impact on human health have failed to adequately consider the contribution of rodents and shrews regarding transmission and associated risks (Jahan *et al.,* 2021; Islam *et al.,* 2022).

The problem of identifying and estimating the detrimental human health implications of foodborne diseases caused by rodents and shrews, such as illness rates and outbreaks, has been acknowledged (Meerburg *et al.,* 2007; Jahan *et al.,* 2021). This phenomenon can be attributed, in part, to a convergence of methodological constraints and a dearth of the crucial data needed for risk assessment. Strategies in rodent and shrews management and foodborne control methods are required to reduce foodborne diseases that burden public health.

Conclusion and Recommendations

The findings of this study indicate that there is a significant difference between *E. coli* under the habitat, species, and Sites. On the contrary, there was no statistically significant prevalence of *Salmonella enterica* between rodent species, their habitats, and

trapped sites. Also, Human beings are at high risk of foodborne pathogens because they are close to rodents and shrews.

Nevertheless, these investigations widely acknowledged that rodents and shrews carry human pathogens, leading to various human infections on a global scale upon their interaction with food. The study focused on the prevalence of foodborne *E. coli* and *Salmonella enterica* and their risk factors for human health. It was assumed that the presence of foodborne pathogens in the rodents and shrews captured inside the house, in the open food markets, outside the house, and in the food shops indicated their potential to transport pathogens and subsequently contaminate food or food-related utensils, posing a risk to human health. Consequently, the rodents that typically reside close to people are the primary agents responsible for transmitting foodborne *E. coli* and *Salmonella enterica* to humans through different channels. However, future research endeavors must examine the extent to which the factors mentioned in this study contribute to human exposure to foodborne diseases, pathogens, and genes.

Extensive research is needed on transmitting foodborne diseases from rodents to humans. Therefore, it is advisable to conduct comprehensive investigations to determine the extent to which house rodents and shrews serve as vectors and indicators of foodborne disease transmission. These investigations must incorporate rigorous pathogens control methods, rodent and shrews management. Rodents and shrews can move and traverse diverse settings, potentially carrying foodborne pathogens. Moreover, the degree of risk posed to humans regarding foodborne *E. coli* and *Salmonella enterica* found in rodents and shrews remains uncertain.

Ethical considerations

The animal-related procedures conducted in this study followed the ethical standards and guidelines for the care and use of animals in research. The research protocol involving animals was reviewed and

approved by the Sokoine University of Agriculture under the ethical review board with reference and publication committee reference number SUA/DPRTC/186 VOL IV/82. The Sokoine University of Agriculture gave an approval letter for conducting this study (Ref. No. SUA/ADM/R.1/8/995; 16th January 2023). Also, the local administrative authorities TAMISEMI Dodoma (Ref. No. AB.307/323/01/191; 16th February 2023) and Morogoro Municipality (Ref. No. R.10/MMC-24/32; 10th March 2023) provided permission too before starting data collection on field. The study adhered to all relevant national and international regulations for the animal and ethical treatment of animals. All efforts were made to minimize the number of animals used and to ensure their well-being throughout the study.

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Authors' contributions

Chantal M. Uwanyirigira: Methodology design, data collection, laboratory work, data analysis, and interpretation, as well as the drafting of the manuscript

Ginethon G. Mhamphi: Laboratory work and critical review of the manuscript

Nelly E. Bapfakurera: Statistical analysis contributes to the discussion and critical manuscript review.

Elisa Mwega: Collaborated in the analysis of laboratory data and critical review of the manuscript.
Sharadhuli Iddi Kimera: Supervisor, overall research management, statistical analysis, data interpretation, writing, and critical manuscript review.
Abdul A.S. Katakweba: Supervisor, overall research management, statistical analysis, data interpretation, and writing, and critical manuscript review.

Conflict of interest

The authors declare that no conflicts of interest are associated with this research study. No financial, personal, or professional relationships with other people or organizations could potentially influence the work or the interpretation of its results. This includes, but is not limited to, employment, consultancies, honoraria, funding, grants, or other forms of payments. The authors affirm that the research was conducted with the highest integrity and impartiality.

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CHAPTER THREE
Manuscript Two

ANTIMICROBIAL RESISTANCE PROFILE OF *SALMONELLA ENTERICA* AND *E. COLI ISOLATES* FROM RODENTS AND SHREWS IN MOROGORO MUNICIPALITY, TANZANIA.

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Abstract

Background: Antimicrobial resistance (AMR) is a pressing issue in global public health, impacting both human and animal health. Small mammals such as rodents and shrews excrement constitute a significant reservoir of zoonotic pathogens, including resistant bacterial strains. To date, there is a scarcity of documented research

specifically addressing resistant strains derived from small mammals' feces.

Methods: A total of 148 fecal samples from small mammals were examined to assess the prevalence of *E. coli* and *Salmonella enterica*, both bacteria isolated through culture methods and identified by biochemical tests. Fifty isolates were subjected to antibiotic susceptibility testing using the disc diffusion method on Muller Hinton agar, a commonly used antibiotic according to the Clinical and Laboratory Standards Institute guideline. Genotypic analysis through PCR confirmed the bacterial strains and their pattern resistance genes. This study conducted from march to November 2023.

Results: Two bacteria were confirmed in 57(38.5%) fecal samples from rodents and shrews. *E. coli* and *Salmonella Enterica* were 54 (36.5%) and 3(2%), respectively. Antimicrobial susceptibility testing revealed substantial resistance percentages, particularly in *Rattus rattus,* and site-specific resistance rates varied, highlighting the localized nature of resistance patterns. Molecular confirmed that *Salmonella enterica* harbors more resistance genes than *E. coli*. Specific resistance genes, including *Sul 1* and *Sul 2*, were identified, constituting 15.4% of the total resistant genes. The prevalence of *β-lactamases* genes was notable, with a higher occurrence in *Salmonella enterica.*

Conclusion: In relation to human health, the implications of the rodents and shrews serving as carriers and transmitters of different pathogens are highlighted. In view of the possibility of unexpected zoonosis arising from the bacteria found in this investigation, these findings contribute to the understanding of AMR in the context of bacterial infections in animals and humans, referencing extensive prior research in Tanzania and globally.

Keywords: Rodents, shrews, foodborne, *Salmonella enterica*, *E. coli,* AMR, Morogoro Municipality.

INTRODUCTION

The World Health Organization (WHO) has categorized *Enterobacteriaceae*, including *E. coli* and *Salmonella enterica*, as pathogens of critical priority for AMR investigation (Mogasale et al., 2021). Indeed, *E. coli* and *Salmonella enterica* bacteria are the primary cause of foodborne illness (Alshaheeb et al., 2023). The studies indicated that *Salmonella enterica* is the leading cause of different diseases in both humans and Animals, including salmonellosis, typhoid fever, and paratyphoid fever, and *E. coli* causes illnesses that are sometimes severe, such as diarrhea, urinary tract infections, respiratory illness, and bloodstream infections (Akbar *et al.,* 2011; Ssemanda *et al.,* 2018). The types of *E. coli* that cause diarrhea are spread through contaminated food or water and contact with animals or humans (Fairbrother, 2006; Ercumen *et al.,* 2017). Therefore, many bacteria extracted from rodents and shrews have been reported to be the causal agents of AMR, particularly *Salmonella enterica* and *E. coli* (Nkogwe *et al.,* 2011).

Rodents and shrews, which are known as small mammals, are recognized as reservoir organisms for disseminating many zoonotic diseases to humans and other animals (Hill *et al.,* 2011; Damborg et *al.,* 2016; Mustapha *et al.,* 2019). Whereby they have been identified as the carriers of many microorganisms, including *E. coli*, *Salmonella* spp., *Campylobacter* spp., *Leptospira* spp, *Proteus mirabilis,* and *Hantavirus* (Böge *et al.,* 2021; Ndakidemi *et al.,* 2022). All those bacteria have the potential to be transmitted to humans by several routes, including the ingestion of food that rodents and shrews have contaminated through their feces and urine, the inhalation of aerosols containing infectious agents, direct bites from rodents and shrews, and transmission through arthropod vectors that have come into contact with rodents and shrews (Chlebicz, 2018; Hamidi, 2018; Jahan *et al.,* 2021). Fecal matter is a notable reservoir for pathogens, such as *E. coli and Salmonella spp.*, which

can induce intestinal illnesses in humans and animals *(Nkogwe et al.*, 2011).

Several studies have demonstrated the presence of AMR *E. coli* and *Salmonella* spp in rodents and shrews inhabiting both urban and rural environments (Katakweba, 2014; Kimwaga *et al.,* 2022; Ndakidemi *et al.,* 2022). Most of the prior investigations have examined the resistance phenotype of *E. coli* and *Salmonella* spp strains obtained from rodents (Ball *et al.,* 2019). Therefore, rodents and shrews residing in proximity to human settlements have the potential to participate in the dissemination of AMR genes (Benavides *et al.,* 2021). Recently, research has shifted towards studying AMR genes in conjunction with the resistant phenotype (Benavides *et al.,* 2021; Sonola *et al.,* 2022). While some discrepancies may arise between phenotypic and genotypic results, a broader analysis of gram-negative bacteria allows for more accurate predictions (Sonola *et al.,* 2022). From a zoonotic standpoint, the emergence of AMR *Escherichia Coli* and *Salmonella* strains from rodents and shrews raises noteworthy concerns (Skarżyńska et al., 2020; Sonola *et al.,* 2021). Recent studies on zoonotic *E. coli* have revealed a significant presence of AMR *E. coli* strains, including those that produce extended-spectrum *beta-lactamases* (ESBLs) and *E. coli* strains carrying virulence genes (Sonola *et al.,* 2022).
The Antimicrobial resistance found in *E. coli* and *Salmonella enterica* can be horizontally transferred to diverse bacterial populations (Ripanda *et al.,* 2023). This phenomenon poses a possible risk of dissemination to both humans and the environment, exacerbating AMR (Nelson et al., 2008). Therefore, this study was conducted to examine the occurrence of AMR *E. coli* and *Salmonella enterica* and their related resistance genes in rodents and shrews captured in Morogoro Municipality.

Materials and Methods

Study area

The investigation took place in Morogoro Municipality wards at 6°85'S and 37°65'E. Morogoro Municipality is 196 km west of Dar es Salaam, Tanzania's main city and economic center, and 260 km east of Dodoma, the capital. The study area has a tropical climate with more summer rain than winter. December has the highest average temperature of 26.9 °C, while July has the lowest at 21.5 °C. Yearly temperature variations are 5.4 °C. The wettest and driest months differ by 178 mm in precipitation (Katakweba, 2008). (Fig. 3.1)

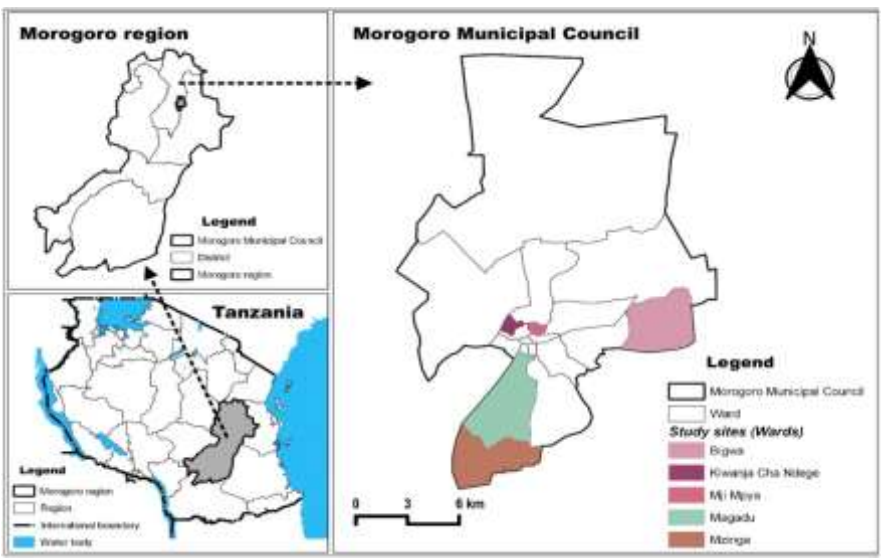

Figure 3.1: Map of Morogoro Region and Morogoro Municipal Council showing wards where the study was done. (By the researcher using QGIS)

Collection of feces samples from rodents and shrews

Diethyl Ether was used to anesthetize rodents and shrews. The gastrointestinal tracts were incised and opened to extract all the contents from the small intestine to the caeca of the rodents. As per the techniques described by Nkogwe *et al.* (2011), the feces were gathered and placed in a sterile container at a temperature of 4^0C.

They were transported to the Microbiology Laboratory of the Department of Microbiology, Parasitology, and Biotechnology at Sokoine University of Agriculture (SUA) for subsequent analysis. Cary Blair Transport Medium was used for this purpose (Nagata *et al*., 2019).

Isolation and Biochemical Identification

To isolate and identify bacteria present in the feces of rodents and shrews, the samples were pre-enriched with buffered peptone water (BPW) and incubated at a temperature of 37°C for a duration of 18–24 hours. As a selective enrichment of *Salmonella* spp., 100 L of the enriched sample was aseptically transferred to 10 mL of warmed Rappaport-Vassiliadis Soya (RVS) broth (Oxoid) and incubated at 41.5°C with preferred water for 21-27 hours. RVS broth was inoculated onto Xylose Lysine Deoxycholate (XLD) plate agar after incubation for 21–27 hours at 37°C. All XLD plates were checked for colonies that looked like *Salmonella* spp. The positive colonies, which had red centers with or without black centers, were sub-cultured to get pure colonies. The samples enriched with buffered peptone water were also put on Mac Conkey agar and left to sit at 37^0C for 18–24 hours. After that, the plates were checked for *E. coli* colonies. The positive colonies, which were pink, were subcultured to get pure colonies (Himsworth *et al.,* 2015). All Isolated bacteria were confirmed biochemically using the Triple sugar iron (TSI), Indole, Methyl Red, Voges Proskauer and Citrate (IMViC), Sulfur, Indole, Motility (SIM), and the Urea tests (Nkogwe *et al.,* 2011).

Antimicrobial Susceptibility Testing of *E. coli* and *Salmonella* spp

All confirmed isolates were subjected to antibiotic susceptibility testing using the disc diffusion method on Muller Hinton agar (Oxoid Ltd.). Colonies of each sample were lightly touched with a wire loop and inoculated in a tube containing sterile Buffered Peptone Water until the suspension became slightly turbid and matched with the 0.5 Mac Farland turbidity standards. The inoculum was transferred onto

well-dried Mueller Hinton agar plates. Bacteria were uniformly spread on top of Müller–Hinton agar using a sterile swab and were exposed to the antibiotic diffusing from antibiotic-impregnated paper disk into the agar medium. Based on the antibiotics commonly used in humans in Tanzania, the most used antimicrobials for treating different diseases in humans and animals were selected at the concentrations shown: Penicillins (Ampicillin 10 µg), Beta-lactam (Amoxicillin 10 µg), Quinolone (Ciprofloxacin 5µg), Cephalosporins (Ceftriaxone 30 µg, cefotaxime 30 µg) and Sulfonamides (Sulphamethoxazole/Trimethoprim 25 µg) (Oxoid, Basingstoke, U.K.). The plates were incubated at 37 °C for 24 hrs (overnight). The results were interpreted according to the Clinical and Laboratory Standards Institute guideline 2021. Isolates are classified as susceptible, intermediate, or resistant according to the interpretation of the zone diameter as recommended by CLSI guidelines (CLSI, 2021). A multidrug-resistant (MDR) strain was defined as a strain resistant to more than one different class of antimicrobials (Magiorakos *et al.,* 2012). The breakpoints of each antibiotic have been considered, as shown in Table 3.1.

Table 3.1: Breakpoints of Antibiotics used

BREAKPOINTS	**ANTIBIOTICS**					
	CRO: Ceftriaxone	**SXT: Sulphamethoxazole/Trimethoprim**	**AML: Amoxicillin**	**CTX: Cefotaxime**	**CIP: Ciproflo xacin**	**AMP: Ampicillin**
S: Susceptible	≥23	≥16	≥17	≥26	≥26	≥17
I: Intermediate	20-22	11-15	14-16	23-25	22-25	14-16
R: Resistance	≤19	≤10	R ≤13	≤22	≤21	R ≤13

Genotypic Analysis

DNA Extraction

The genomic DNA was extracted from an overnight-grown bacterial colony using the boiling procedure. The colonies were extracted from a Petri dish containing a pure *E. coli* and *Salmonella* spp culture using sterile swabs. They were then transferred into an Eppendorf tube containing 100 µl of nuclease-free water. The tube

was subjected to boiling in a water bath at 95 °C for 5 minutes, followed by freezing at -20 °C for 10 minutes.

After performing this technique, the suspension was centrifuged at 12,000 rpm for one minute. Eighty microliters of the supernatant were taken using a micropipette for further processing. The concentration and quality of the extracted DNA were checked by electrophoresis (1% agarose gel) and spectrophotometrically quantified using a Nanodrop Spectrophotometer. All isolated DNA was kept at -20^{0}C until the PCR was done(Dashti *et al.,* 2009; Khosravi *et al.,* 2012; Bagus *et al.,* 2017).

Molecular identification of bacterial species

Thermal cycler molecular identification was performed on all isolates presumed to be biochemically and phenotypically recognized. The forward and reverse primers were designed to give a product of approximately 585 base pairs targeting *E. coli,* which are complementary to conserved regions of *16S rRNA* genes, and 796 base pairs targeting *Salmonella* spp., which targeted the *InvA* gene. A master mix was used for PCR (Bioneer premix-Korea) (James, 2010; Silva *et al.,* 2011; Nanteza *et al.,* 2020).

Identification of resistance genes

All bacterial colonies that indicated phenotypical resistance were screened by PCR to detect various recognized resistance genes to different antibiotics (Moura *et al.,* 2012). Resistance genes were tested using both positive and negative controls. Although, it was impossible to source positive controls for some screened genes. Optimized and previously published primers and PCR protocols were used without positive controls. The *Sul*1 and *Sul*2 genes were amplified under the following conditions: 95^{0}C for 5 minutes, 35 cycles of 94^0 C for 30 s, 55^0 C for 1 minute for *Sul 1* and 58^{0}c for *Sul* 2, then 72^{0}C for 2 minutes, and final extension at 72^{0}C for 10 min. The following PCR amplification settings were used to identify *Bla SHV*, *Bla TEM,* and *Bla CTXM*: Initial denaturation processed at

95^0C for 5 min; 35 cycles of denaturation at 94^0C for 30 and annealing at 50^0C for *Bla TEM* and 58^0C for *Bla SHV* and *Bla CTXM* for 30 s. Extension at 72^0C for 2 minutes, then the final extension step for 10 minutes at 72^0C. The agarose gel (1.5%) stained with ethidium bromide was used to analyze PCR products (Amplicons) by gel electrophoresis. The ultraviolet trans-illumination machine visualized positive bands *(Rahayuningtyas et al.*, 2020).

Table 3.2: The Primers were used to detect the AMR gene.

Gene	Primer (5' -3')	size	Annealing	Reference
Sul 1	F -CGGCGTGGGCTACCTGAACG R -GCCGATCGCGTGAAGGTTCCG	450	55^0C	(Zou *et al.,* 2014)
Sul 2	F- GCGCTCAAGGCAGATGGCATT R- GCGTTTGATACCGGCACCCGT	625	58^0C	(Zou *et al*., 2014)
Bla SHV	F - ATGCGTTATATTCGCCTGTG R - AGCGTTGCAGTGCTCGATC	862	58^0C	(Tofteland *et al.,* 2007)
Bla CTXM	F - SCSATGTGCAGYACCAGTAA R - CCGCRATATGRTTGGTGGTG	554	58^0C	(Ejaz *et al*., 2021)
Bla TEM	F -ATGAGTATTCAACATTTCCG R- CCAATGCTTAATCAGTGAGG	858	50^0C	(Ejaz *et al.,* 2021)
16 s rRNA	F-GACCTCGGTTTAGTTCACAGA R-CACACGCTGACGCTGACCA	585	55^0C	(James, 2010 ; Moawad *et al*., 2017a)
InvA	F-CGGTGGTTTTAAGCGTACTCTT R-CGAATATGCTCCACAAGGTTA	796	58^0C	(Silva *et al.,* 2011 ; Paião *et al.,* 2013;)

Percentage calculation of Antimicrobial Resistance Genes

The percentage of resistance genes of *E. coli* and *Salmonella enterica* have been calculated following formula: **%= N′*n′/ 100** whereby, **N′=N*n**, **N′=** The total number of antibiotic resistance genes tested for each bacteria, **n′=** The total number of bacteria showed resistance on targeted gene per each species of bacteria**, N =**Number of bacteria tested, n= number of bacteria showed resistance on antimicrobial targeted on each gene.

Results

Isolation and Biochemical Identification

The isolates identified as *E. coli* were 54(36.5%) samples; in comparison, *Salmonella enterica* was identified in 3(2%) samples after using biochemical tests such as the Triple Sugar Iron (TSI) test, the (IMViC) test, the SIM test, and the Urea test. Accordingly, these data showed *E. coli* was more prevalent than *Salmonella enterica*. All isolates' bacteria were kept in a freezer under - 8^0C for molecular confirmation.

Phenotypic Antimicrobial Susceptibility Testing

Percentage of AMR of bacterial isolates from small mammals

For all bacteria identified of *E. coli* and *Salmonella enterica*, only 50 isolates of bacteria were selected randomly, 47 isolates were *E. coli,* and 3 isolates were *Salmonella enterica*, whereby 19 isolates were from *Rattus rattus*, 24 *Mus* spp., 5 *Cricetomys,* and two from *shrews* spp. The selected isolates were tested to determine their antimicrobial susceptibility pattern. The high percentage of AMR of isolates recovered from *Rattus rattus* species for Amoxicillin (89.5%), ampicillin (74%), sulphamethoxazole/trimethoprim (31.6%) and Ciprofloxacin (10.5%) respectively. In *Mus* spp*.,* the isolates bacteria showed high resistance to Amoxicillin at 87.5%, followed by Ampicillin (62.5%); in *Cricetomys*, isolates bacteria were resistant to Amoxicillin and Ampicillin with 80% for each antibiotic and 100 (%) of isolates from *shrews* spp. were resistant to Amoxicillin (Table 3.3)

Table 3.3: Percentage of AMR of bacterial isolates from rodents and shrews

Antimicrobial	Rodents species and Shrews			
	R. Rattus (n= 19)	*Mus* spp (n=24)	*C. ansorgei* (n=5)	*Shrews* spp (n=2)
CRO	0	0	0	0
SXT	6 (31.6%)	0	0	0
AML	17 (89.5%)	21(87.5%)	4 (80%)	2 (100%)
CTX	0	0	0	0
CIP	2 (10.5%)	0	0	0
AMP	14 (74%)	15 (62.5%)	4 (80%)	0

CRO: Ceftriaxone, SXT: Sulphamethoxazole/Trimethoprim, AML: Amoxicillin, CTX: Cefotaxime, CIP: Ciprofloxacin, AMP: Ampicillin

Percentage of AMR of bacterial isolates per Sites

Among 50 isolates detected antimicrobial resistance phenotypically in *E. coli* and *Salmonella enterica*, resistance to antimicrobials was observed in Mji Mpya and Mzinga for Amoxicillin, with 100% resistance in both cases. For Ampicillin, resistance was observed in Mji Mpya (77.3%) and Mzinga (100%). *E. coli* exhibited high resistance to Sulphamethoxazole/trimethoprim in Mzinga at 66.7%, followed by Magadu at 50%. Additionally, resistance to Ciprofloxacin was observed in Mzinga at 33.3%. The antimicrobial resistance testing results for *Salmonella enterica* indicated high resistance in Mzinga for Ciprofloxacin, Ampicillin, and Sulphamethoxazole/ trimethoprim, all at 100%. Multidrug resistance (MDR) was observed in Mzinga, Mawenzi, and Magadu for *E. coli.* For *Salmonella enterica*, MDR was observed in Mzinga (Tables 3.4 and 3.5).

Table 3.4: Percentage AMR of *E. coli* isolates per Sites

Antimicrobial	**Sites**				
	Bigwa(n=5)	Magadu (n=4)	Mawenzi market (n=13)	Mji mpya market (n=22)	Mzinga (n=3)
CRO	0	0	0	0	0
SXT	0	2(50%)	1(7.7%)	0	2(66.7%)
AML	3(60%)	3(75%)	12(92.3%)	22(100%)	3(100%)
CTX	0	0	0	0	0
CIP	0	0	0	0	1(33.3%)
AMP	4(80%)	1(25%)	6(46.2%)	17(77.3%)	3(100%)

Table 3.5: Percentage of *Salmonella enterica* isolates per site.

Antimicrobial	**Sites**				
	Bigwa (n=0)	Magadu (n=0)	Mawenzi market (n=2)	Mji mpya market (n=0)	Mzinga (n=1)
CRO	0	0	0	0	0
SXT	0	0	0	0	1(100%)
AML	0	0	1(50%)	0	0
CTX	0	0	0	0	0
CIP	0	0	0		1(100%)
AMP	0	0	1(50%)	0	1(100%)

Resistance rates of bacterial isolates identified from small mammals.

An antimicrobial susceptibility test was performed for *Salmonella enterica* and *E. coli*. The results showed that *Salmonella enterica* (n=3) had strains resistant to Sulphamethoxazole/Trimethoprim with 1(33%),1 (33%) to Amoxicillin, 1(33%) to Ciprofloxacin, and 2 (67%) to Ampicillin, respectively.

Also, *Escherichia Coli* (n=47) had strains resistant to Sulphamethoxazole/Trimethoprim with 5(11%), 43(92%) to Amoxicillin, 1(2%) to Ciprofloxacin, and 31(66%) to Ampicillin (Table 3.6).

Table 3.6: Resistance rates of bacterial isolates identified from small mammals.

Antibiotic & Breakpoints	**CRO**	**SXT**	**AML**	**CTX**	**CIP**	**AMP**
	≤19	≤10	≤13	≤22	≤21	≤13
Microorganisms						
Salmonella enterica (n=3)	0	1(33%)	1(33%)	0	1(33%)	2(67%)
Escherichia Coli (n=47)	0	5(11%)	43(92%)	0	1(2%)	31(66%)

Prevalence of Multidrug resistance isolated from small mammals.

These results showed that among 50 isolated bacteria tested AMR, *E. coli* harbored multidrug resistance in *Rattus rattus* species with 3(16%); *Salmonella enterica* showed resistance in *Rattus rattus* and *Mus* spp with 1(5%) and 1(4.1%) respectively.

Table 3.7: Prevalence of Multidrug resistance isolated from small mammals.

Isolates	Rodents' species and Shrews			
	R. Rattus (n= 19)	*Mus* spp (n=24)	*Cricetomys* (n=5)	Shrews spp (n=2)
Escherichia Coli	3 (16%)	0	0	0
Salmonella spp	1(5%)	1(4.1%)	0	0

Genotypic analysis

Molecular identification of bacterial species

PCR amplified *E. coli species,* Where M is a DNA molecular marker and lanes 3-12 are samples, where lanes 3-12 are positive samples

located at 585bp and lanes number 1 and 2 are positive and negative controls, respectively (Fig. 3.2a). PCR also amplified *Salmonella enterica* Where M is a DNA molecular marker, lanes 3-5 are samples, lanes 3-5 are positive samples located at 796bp, and lanes 1 and 2 are positive and negative controls, respectively (Fig. 3.2b).

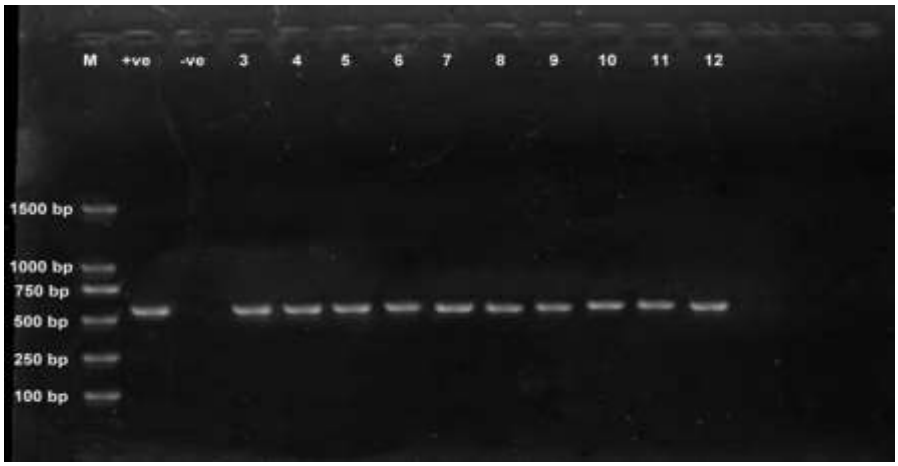

Figure 3.2a: PCR amplification of *16S rRNA* for *E. coli*

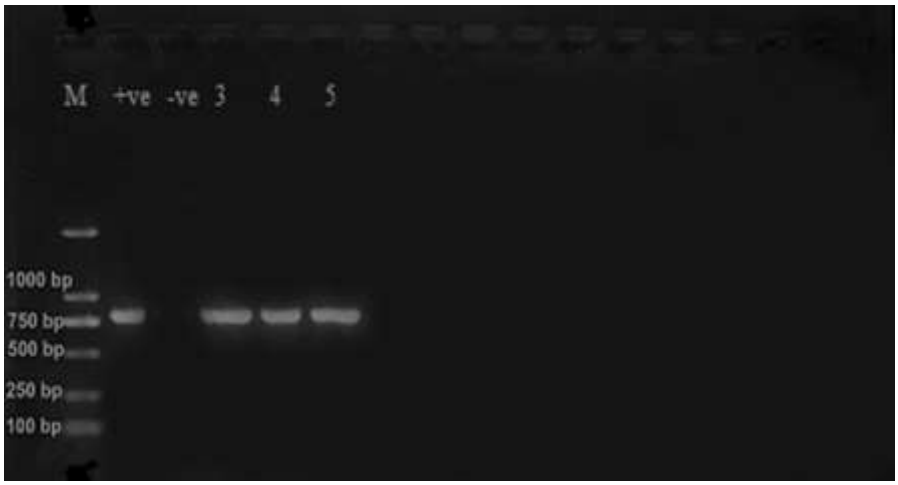

Figure 3.2b: PCR amplification of *InvA* for *Salmonella* spp

Identification of resistance genes

Among 13 bacterial isolates that detected AMR genes, the results showed that *Salmonella enterica* harbored more resistance genes (20%) followed by *E. coli* (12%) (Table 3.5). Two (2) isolates out of 13 contained sulphonamide-resistant genes as follows: *Sul 1*(n=1) and *Sul 2* (n=1), both representing 15.4% of the total resistant gene analyzed in this study (Fig. 3.3 (a–c) and Table 8). *β-lactamases* (*Bla TEM*, *Bla SHV*, *Bla CTXM*) were found in 7 isolates (53.8%), with *Salmonella enterica* harboring more resistance genes compared to *E. coli* (Table 3.8).

Table 3.8: Antibiotic-resistant genes detected on *Salmonella enterica* and *E. coli.*

Bacterial species	Number (N)	*Sul 1* (n)	*Sul 2* (n)	*SHV* (n)	*TEM* (n)	*CTXM* (n)	MDR Genes (%)
Salmonella enterica	3	1	0	0	0	2	20
E. coli	10	0	1	2	2	1	12
% Resistance genes		7.7	7.7	15.4	15.4	23	

(a)

(b)

(c)

Figure 3.3 (a): PCR amplification of *E. coli* on *Bla TEM* and *Sul 2* resistance gene. M was a 100bp marker, and lanes 1 and 2 were positive and negative control, respectively, for *Bla TEM* and lanes number 3 and 4 were positive samples of *Bla TEM* located at 858bp, and lanes 5 and 6 were positive and negative control, respectively for *Sul 2* where lane number 7 was positive sample located at 625bp. (b): **PCR amplification of *E. coli on Bla SHV* and *Bla CTXM* resistance gene. M was a 100bp marker, and lanes 1 and 2 were positive and negative control, respectively, for *Bla SHV,* whereby lanes 3 and 3,4 were positive samples located at 862bp, and lanes 5,6 positive and negative control, respectively for *Bla CTXM* where lane number 7 was positive sample located at 554bp.** (c): **PCR amplification of *Salmonella enterica* on *Bla CTXM* and *Sul 1* resistance genes. M was a 100 bp marker, lanes 1 and 2 were positive and negative control for *Bla CTXM,* and lanes 3 and 4 were positive samples for *Bla CTXM* gene located at 554 bp. Lanes 6 and 7 were positive control and negative control, respectively, for *Sul 1,* and lane 8 was a positive sample for the *Sul*1 gene located at 450 bp.**

Discussion

The study has confirmed that rodents and shrews captured during data collection were harboring *E. coli* and *Salmonella enterica* with 36.5% and 2% respectively, indicating a higher prevalence of *E. coli Rattus rattus* exhibited notable resistance percentages, particularly against Amoxicillin (89.5%), Ampicillin (74%), Sulphamethoxazole/ Trimethoprim (31.6%), and Ciprofloxacin (10.5%). Site-specific resistance rates varied, with 100% resistance to Amoxicillin in Mji Mpya and Mzinga. *Salmonella enterica* strains displayed diverse resistance profiles, with 33% resistance to Sulphamethoxazole/ Trimethoprim, Amoxicillin, and Ciprofloxacin and 67% resistance to Ampicillin. *E. coli* strains exhibited resistance to Sulphamethoxazole/ Trimethoprim (11%), Amoxicillin (92%), Ciprofloxacin (2%), and Ampicillin (66%). Multidrug resistance (MDR) was observed in *E. coli* (16% in *Rattus rattus*) and *Salmonella enterica* (5% in *Rattus rattus* and 4.1% in *Mus* spp). The genotypic analysis confirmed molecular identification with *Salmonella enterica* harboring more resistance genes (20%) than *E. coli* (12%). Specific resistance genes, such as *Sul 1* and *Sul 2*, constituted 15.4% of the total resistant genes. β-lactamases (*Bla TEM , Bla SHV, Bla CTXM*) were found in 53.8% of isolates, with a higher prevalence in *Salmonella enterica.*

Antimicrobial resistance in the context of bacterial infections in animals and humans has been extensively investigated and documented in Tanzania and several other regions worldwide. Our findings are consistent with previous studies on rodent isolates (Jemilehin *et al.,* 2016; Skarżyńska *et al.,* 2020; Zhong *et al.,* 2020; Sonola *et al.,* 2021, 2022). According to Semakula *et al*. (2015), the findings of this study suggest that the occurrence of *Salmonella* and *E. coli* in rodents can be attributed to environmental interactions and shared characteristics in the food chain with humans and animals, including livestock and pets. This, in turn, facilitates the dissemination of AMR traits among different species coexisting in the ecosystem alongside humans (Kimwaga *et al.,* 2022). The

antimicrobials tested in this study have been commonly used in veterinary medicine for the treatment of animals, as evidenced by studies conducted by Katakweba *et al.* (2012), Kissinga *et al.* (2018), and Kimera *et al.* (2020). Furthermore, Kissinga et al. (2018) reported the use of Tetracyclines, Ampicillin, Amoxicillin, and sulphonamides in humans in Morogoro Municipality. Therefore, the presence of humans and animals in the Morogoro region has implications for small mammals living in the same ecosystem(Rebecca *et al.,* 2012), such as the search for feeding in the animals' and humans' houses. These small mammals get access to human and animal wastes where the resistant bacteria can be easily accessed.

The prevalence of high ampicillin resistance in *Salmonella enterica* and Amoxicillin in *E. coli* was the predominant resistance observed in the wild small-mammal isolates, and its occurrence was strongly linked to the study areas, which are close to human living (Ndakidemi *et al.,* 2022). The prevalence of Amoxicillin and Ampicillin is not surprising since it is often used as the first antimicrobial in treating the bacteria in Morogoro, both in humans and animals (Rebecca *et al.,* 2012). Given that AMR can be induced by the presence of antimicrobials in human and animal wastes, it is plausible that certain strains of *E. coli* and *Salmonella enterica* found in small mammals were directly subjected to selective pressure due to the consumption of human and animal wastes containing antimicrobials, including Amoxicillin and Ampicillin (Skarżyńska *et al.,* 2020). Furthermore, Small rodents and shrews gain access to antimicrobials in improperly disposed dumping areas containing expired or unfit antimicrobials for human and animal consumption. The study conducted by Kissinga *et al.* (2018) reported the disposal of expired and unused antimicrobials in Morogoro Municipality through dumps, latrines, and burning. These methods of antimicrobial disposal facilitate good access for small mammals to get access to those antimicrobials.

Additionally, these rodents engage in the consumption of chicken feces and occasionally human and children feces from individuals lacking access to latrines that have resistant bacterial or undigested antimicrobials.The molecular examination utilizing the *Sul* gene revealed that only 15.4% of isolates showed resistance (Park et al., 2012). This finding was unsurprising given that sulphonamides are a frequently used antibiotic in animal populations (Katakweba *et al.,* 2014; Katakweba *et al.,* 2018). Consequently, these compounds are commonly detected as contaminants within humans and animals(Hassell *et al.,* 2019). Therefore, this characteristic renders them prevalent environmental pollutants dispersed in water and assimilated by all creatures inhabiting the given ecosystems (Grudlewska-Buda *et al.,* 2023). As a result, this phenomenon significantly influences the evolutionary forces driving the development of resistant (Katakweba, 2014).

Ciprofloxacin has been used for over four decades as a therapeutic approach to treating salmonellosis (Ong *et al.,* 2020). The present study revealed that 33% resisted Ciprofloxacin, as determined by disc diffusion testing. The resistance observed to Ciprofloxacin could be attributed to the widespread use of Ciprofloxacin as a standard therapy for *Salmonella* pathogens (Kira, 2015). The isolates were additionally exposed to disk diffusion to assess the resistance level to Ampicillin. It was found that only 67% of *Salmonella enterica* and 66% of *E. coli* of the isolates exhibited resistance (Kimwaga et al., 2022). In addition, the isolates underwent molecular assessment for resistance by examining the presence of the *Bla TEM* gene (Sonola et al., 2022). As a result, 53.8% tested positive for the *Bla TEM* gene. The nonexistence of this gene in isolates that exhibited favorable phenotypes suggests that the specific gene targeted by this antibiotic may vary (James, 2010). Examples of such genes include *SHV*, *TEM*, and *CTX-M*, among others. The disk diffusion method also demonstrated higher sensitivity but lacked specificity in detecting AMR (Benavides *et al.,* 2021). This finding is not only expected but also raises concerns, as a prior study conducted in this

region has already reported the presence of AMR in humans (Oketcho *et al.,* 2012). These findings can be corroborated by a study that was conducted in Tanzania by Katakweba *et al*. (2018) and Munuo *et al.* (2022), who extracted the *Sul II* genes from feces samples obtained from animals.

The presence of *Sul 1* and *Sul 2* genes in both Ampicillin and sulfamethoxazole-trimethoprim-resistant and susceptible isolates indicates the potential existence of "silent" antimicrobial agents within bacterial populations, as reported by Grudlewska-Buda *et al.* (2023). This finding suggests a potential risk, as dormant genes can become active within a living organism in response to antimicrobial agents (Kariuki *et al.,* 2018). Furthermore, these genes can also be transmitted to other microorganisms in the intestines and the surrounding environment (Zhang *et al.,* 2015).

The observed resistance to the antimicrobials described above may also be attributed to many other factors, such as inadequate disposal of unused antimicrobials, inappropriate utilization of antimicrobials, frequent use of chemoprophylaxis therapy, and high increase in livestock management (Katakweba *et al.,* 2012; Gatabazi, 2013; Kissinga *et al.,* 2018). Furthermore, this resistance might also be attributed to the widespread availability and affordability of antimicrobial drug shops, the common practice of self-medication with over-the-counter medications, and repeated exposure to different antimicrobials before obtaining prescriptions from healthcare professionals(Katakweba, 2014). Furthermore, Sonola et al. (2021) propose that the cohabitation of humans and small mammals in a shared environment can result in the transfer of resistance genes between the two species, which may then be deposited in the landscape. The intricate nexus between heavy metal exposure, foodborne pathogens, particularly *Salmonella enterica* and *E. coli*, and antimicrobial resistance (AMR) is underscored by multifaceted interdependencies. Environmental contamination with heavy metals from anthropogenic activities

infiltrates the food chain, leading to plant bioaccumulation and subsequent livestock uptake, thereby culminating in human exposure. Concurrently, exposure to heavy metals fosters adaptive responses in bacterial populations, potentially co-selecting for genes associated with both heavy metal and antimicrobial resistance. This co-selection phenomenon amplifies the risk of AMR dissemination in foodborne pathogens, exemplifying the intricate dynamics wherein environmental factors interplay with microbial genetics, thereby contributing to the broader landscape of antimicrobial resistance (Ngwewa *et al.,* 2022).

The phenomenon of multidrug resistance (MDR) refers to the ability of microorganisms, such as bacteria or viruses, to the virulence of *Salmonella* isolates is found to be much higher in comparison to non-multi-drug resistant strains, as indicated by previous studies.(Magiorakos *et al.,* 2012; Katakweba *et al.,* 2018; Tawyabur *et al.,* 2020). *Salmonella enterica* isolates in this study were shown to possess multiple drug-resistant genes, with some isolates harboring 20% resistance genes among the 20% of isolates examined (Tawyabur *et al.,* 2020). The presence of multidrug resistance in *Salmonella* spp has been documented as a cause of sickness in both humans and animals across various countries, such as the United States and Denmark (Frank, 2007), Italy (Dionisi *et al.,* 2008), Eastern China (Lu *et al.,* 2014), and Vietnam (Vo *et al.,* 2010). Therefore, there was a significant prevalence of isolates that exhibit resistance to multidrug, including Amoxicillin, Ampicillin, Ciprofloxacin, and Sulphonamides. These antimicrobial drugs are frequently employed in human and veterinary medicine (Katakweba *et al.,* 2012; Sonola *et al.,* 2022). As indicated by Bosco et al. (2012), and Walusans (2007), those antibiotics are commonly prescribed for the treatment of gastroenteritis, salmonellosis, and colibacillosis in animals, which is a severe worry to human and animal health.

Limitations of the study

The study encounters several limitations in assessing antimicrobial resistance in rodents and shrews. The representativeness of the sampled populations may be compromised, as trapping efforts may not comprehensively capture the diversity and distribution of these species across different environments. Establishing a direct causal link between observed antimicrobial resistance in rodents and specific sources proves challenging due to complex ecological interactions and potential transmission routes. Additionally, the study's scope may not fully encompass the diverse spectrum of antimicrobials used in households and food markets, potentially leading to the oversight of specific agents contributing to resistance. Moreover, the limited number of antimicrobials tested, in comparison to the broad range available in Tanzania, may constrain the comprehensive evaluation of resistance patterns. Lastly, the presence of other animals on-site during rodent and shrew sampling introduces potential confounding factors that may influence the observed resistance rates in bacterial isolates. These limitations emphasize the need for careful interpretation and consideration of the study's findings within the broader context of antimicrobial resistance dynamics.

Conclusions

The study comprehensively assesses AMR profiles in small mammals, highlighting the prevalence, site-specific patterns, and genotypic characteristics of resistance. These findings underscore the importance of integrated strategies to mitigate the spread of AMR and inform public health interventions. This study's results indicated that most of the foodborne *Salmonella enterica* and *E. coli* isolates that infected the rodents and shrews have both phenotypic and genotype AMR characteristics. The prevalence of AMR *Salmonella enterica* and *E. coli* in isolates obtained from rodents and shrews in Morogoro municipality indicated the potential for extensive dissemination of both resistance genes and bacteria throughout the study areas. This raises concerns regarding the potential for the

emergence of challenging-to-treat diseases. The antimicrobials used in this study are extensively used in the study areas for treating humans and animals, indicating the high dissemination between them. Moreover, the spread of AMR *Salmonella enterica* and *E. coli* through the feces and urine dropped by rodents and shrews may lead to environmental pollution. This contamination can subsequently facilitate the transmission of this feature to other dangerous bacteria, such as *Salmonella enterica* and *E. coli*, as well as unexpected bacterial species.

Consequently, this poses a significant public health risk. Hence, it is imperative to implement comprehensive interventions that adopt a one-health approach to manage the issue effectively. These findings provide a better understanding of the role of rodents and shrews in transmitting and maintaining AMR *Salmonella enterica* and *E. coli*. This *Salmonella enterica* and *E. coli* strain has the potential to be passed on to humans through the consumption of food products.

Ethical

Ethical considerations

The research protocol involving animals was reviewed and approved by the Sokoine University of Agriculture under the ethical review board with reference and publication committee reference number SUA/DPRTC/186 VOL IV/82. The Sokoine University of Agriculture gave an approval letter for conducting this study (Ref. No. SUA/ADM/R.1/8/995; 16 January 2023). Also, the local administrative authorities TAMISEMI Dodoma (Ref. No. AB.307/323/01/191; 16 February 2023) and Morogoro Municipality (Ref. No. R.10/MMC-24/32; 10 March 2023) provided permission too before starting data collection on field.

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Authors' contributions

Chantal M. Uwanyirigira: Methodology design, data collection, laboratory work, data analysis, and interpretation, as well as the drafting of the manuscript

Ginethon G. Mhamphi: Laboratory work and critical review of the manuscript

Nelly E. Bapfakurera: Statistical analysis contributes to the discussion and critical manuscript review.

Elisa Mwega: Collaborated in the analysis of laboratory data and critical review of the manuscript.

Sharadhuli Kimera: Supervisor, overall research management, statistical analysis, data interpretation, writing, and critical manuscript review.

Abdul A.S. Katakweba: Supervisor, overall research management, statistical analysis, data interpretation, and writing, and critical manuscript review.

Conflict of interest

The authors declare that no conflicts of interest are associated with this research study. No financial, personal, or professional relationships with other people or organizations could potentially influence the work or the interpretation of its results. This includes, but is not limited to, employment, consultancies, honoraria, funding, grants, or other forms of payments. The authors affirm that the research was conducted with the highest integrity and impartiality.

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CHAPTER FOUR

4.0 General Discussion, Conclusions, and Recommendations

4.1 General Discussion

The current study was conducted to determine the prevalence and antimicrobial resistance profile of *Salmonella enterica* and *E. coli* from rodents and shrews in Morogoro municipality, Tanzania. A total of 148 small mammals were captured, whereby 145 were rodents grouped into three species named *Rattus rattus*, *Mus* spp., *Cricetomys*, and 3 were *shrews* spp. In all 148 mammals, 54 were confirmed positive for *E. coli,* representing *36.5%* of all captured rodents and shrews, while only three were confirmed positive for *Salmonella enterica,* representing 2% of all captured.

The study's results indicated the prevalence of *E. coli and Salmonella enterica* bacteria in *Mus* spp. and *Rattus rattus*. These results are similar to what has been observed in the study conducted by Kimwaga *et al.* (2022), in Tanzania, Kilosa district, who reported a higher prevalence of *E. coli* and *Salmonella* spp in *Rattus rattus* than other species. Similarly, the study conducted by Sonola et al. (2021), in Karatu District reported a high prevalence of *E. coli* in rodents captured indoors and per domestic habitat, as the study of Issae *et al.* (2023), in Ngorongoro District reported a high prevalence of *Salmonella* spp in wild rodents. The *Mus* spp and *Rattus rattus* are known to be more abundant where there is food, meaning that the transmission of the pathogens to humans is high when consuming the contaminated food and water attacked by those rodents (Meerburg *et al.,* 2009). According to Jahan *et al.* (2021), it has been observed that *Mus* spp and *Rattus rattus* can be effectively infected by *E. coli* and *Salmonella* spp., which can persist for a duration exceeding ten months. The high prevalence of *Salmonella enterica* and *E. coli* in inside households and open markets found in this study was influenced by the high number of rodents captured in these premises compared to others. Besides that, rodents prefer to live where they can easily find food and leave behind pellets

containing *E. coli* and *Salmonella* spp bacteria and those bacteria can be transmitted from rodents to other animals and humans (Phifer-Rixey, 2015). Therefore, food markets and households are particularly vulnerable to *E. coli* and *Salmonella enterica* (Damborg *et al.,* 2016; Ribas *et al.,* 2016). Hence, rodents and shrews can act as reservoirs of several pathogens and controlling them effectively leads to control of these pathogens and the diseases they cause Meerburg, 2007).

This study has assessed the possible risk factors associated with the *Salmonella Enterica s* and *E. coli,* these pathogens can be transmitted from rodents and shrews to human through feces and urine of those small mammals in uncooked or cooked food, feces in water storages, eating food eaten by rodents (Islam *et al.,* 2022). In current study 27% of the respondent mentioned having Diarrhea after eating food contaminated by feces and urine of rodents or eaten by rodents. These pathogens are the main threat to public health as they cause human diseases such as Diarrhea, typhoid, urinary tract infection, salmonellosis, and gastroenteritis (Tawyabur *et al.,* 2020). The risk factors identified in this study are related to the results from the study conducted by Alfred-Chengula *et al.* (2015), in Morogoro municipality, who found several diseases, such as Diarrhea, dysentery, cholera, typhoid, worm diseases, and different bacteria, whereby the root causes were *Salmonella typhimurium* (16.7%) and *E. coli* (8.3%) frequency of isolation from solid waste disposal in Morogoro municipality, with direct linkage with rodents and shrews. Nevertheless, there is a lack of empirical evidence about the role and relative contribution of the several channels through which humans are exposed to aspects of foodborne *E. coli* and *Salmonella* spp (WHO, 2015). Consequently, until now, most literature reviews about foodborne disease pathogens and their impact on human health have failed to adequately consider the contribution of rodents and shrews regarding transmission and associated risks (Jahan *et al.,* 2021; Islam *et al.,* 2022).

The problem of identifying and estimating the detrimental human health implications of foodborne diseases caused by rodents and shrews, such as illness rates and outbreaks, has been acknowledged (Meerburg *et al.,* 2009; Jahan *et al.,* 2021). This phenomenon can be attributed, in part, to a convergence of methodological constraints and a dearth of the crucial data needed for risk assessment. Strategies in rodent management and foodborne control methods are required to reduce foodborne diseases that burden public health.

Identifying various populations animals as reservoirs of AMR is crucial for understanding and managing the transmission routes of AMR and pathogens (Moawad *et al.,* 2017). This knowledge will contribute to the prevention of potential dangers and implications for both human and animal healt (Rebecca *et al.,* 2012) h. AMR in the context of bacterial infections in animals and humans has been extensively investigated and documented in Tanzania and several other regions worldwide (Katakweba, 2014; Sonola *et al.,* 2021, 2022). This research encompasses a diverse range of rodents and shrews from various habitats in connection with humans.

This study revealed that rodents and shrews were found to carry antimicrobial-resistant *E. coli* and *Salmonella enterica.* The results suggested that the strain resistance of *Salmonella enterica* was high to Ampicillin followed by Sulphamethoxazole/Trimethoprim, Amoxicillin, and Ciprofloxacin. At the same time, the strain resistance of *E. coli* was high to Amoxicillin followed by Ampicillin, Ciprofloxacin, Sulphamethoxazole/Trimethoprim and Ciprofloxacin (Table 2.3). The phenotypes resistant were 100% in shrews, followed by *Rattus rattus and Mus* spp on Amoxicillin. Therefore, the AMR to Ampicillin was more frequently in *Rattus rattus* and *Mus* spp. The results on multidrug resistance indicated that the multidrug resistance in *Rattus rattus* was 16% on *E. coli* and 5% on *Salmonella enterica,* at the same time, *Mus* spp. was 4.1% on *Salmonella enterica*. Among 13 bacterial isolates that were detected

in AMR genes, the results showed *Salmonella enterica* has more resistance genes (20%) as compared to *E. coli* (12%) (Table 2.6). The sulphonamide-resistant genes (*Sul 1* and *Sul 2*) were 15.4% of the total resistant genes analyzed in this study (Fig 2.3 and Table 2.6). At the same time, β-lactamases (*Bla TEM, Bla SHV*, *Bla CTXM*) were 53.8% whereby *Salmonella enterica* harbored more resistance genes compared to *E. coli* (Table 2.6). These findings are consistent with previous studies on rodent isolates (Katakweba, 2014; Jemilehin *et al.,* 2016; Skarżyńska *et al.,* 2020; Zhong *et al.,* 2020). According to Semakula *et al.* (2015), the findings of this study suggest that the occurrence of *Salmonella enterica* and *E. coli* in rodents can be attributed to environmental interactions and shared characteristics in the food chain with humans. This, in turn, facilitates the dissemination of AMR traits among different species coexisting in the ecosystem alongside humans (Kimwaga *et al.,* 2022). The antimicrobials tested in this study have been commonly used in veterinary medicine for the treatment of animals, as evidenced by studies conducted by Katakweba *et al.* (2012), Kissinga *et al.,* (2018), and (Kimera *et al.* (2020). The same antimicrobials were also found in use by humans in Morogoro (Kissinga *et al.,* (2018). Therefore, the presence of humans and animals in the Morogoro region has implications for small mammals that live in the same ecosystem (Rebecca *et al.,* 2012).

The molecular examination utilizing the *Sul* gene revealed that only 15.4% of isolates showed resistance (Park *et al.,* 2012). This finding is unsurprising given that sulphonamides are a frequently used antibiotic in animal populations (Katakweba, 2014). Consequently, these compounds are commonly detected as contaminants within humans and animals (Hassell *et al.,* 2019). In addition, the isolates underwent molecular assessment for resistance by examining the presence of the *Bla TEM* gene(Sonola *et al.,* 2022). As a result, 53.8% tested positive for the *Bla TEM* gene. The nonexistence of this gene in isolates that exhibited favorable phenotypes suggests that the specific gene targeted by this antibiotic may vary (James,

2010). Examples of such genes include *SHV*, *TEM*, and *CTX-M*, among others. The disk diffusion method also demonstrated higher sensitivity but lacked specificity in detecting AMR (Benavides *et al.,* 2021). This finding is not only expected but also raises concerns, as a prior study conducted in this region has already reported the presence of AMR in humans (Oketcho *et al.,* 2012). These findings can be corroborated by a study that was conducted in Tanzania by Katakweba *et al.* (2018), Kissinga *et al.* (2018), and Sonola et al. (2021), who extracted the *SuI* genes from feces samples obtained from animals. The presence of Sul1 and Sul2 genes in both Ampicillin and sulfamethoxazole-trimethoprim-resistant and susceptible isolates indicates the potential existence of "silent" antimicrobial agents within bacterial populations, as reported by Grudlewska-Buda *et al.* (2023). This finding suggests a potential risk, as dormant genes can become active within a living organism in response to antimicrobial agents (Kariuki *et al.,* 2018). Furthermore, these genes can also be transmitted to other microorganisms in the intestines and the surrounding environment (Zhang *et al.,* 2015).

The observed resistance to the antimicrobials described above may also be attributed to many other factors, such as inadequate disposal of unused antimicrobials, inappropriate utilization of antimicrobials, frequent use of chemoprophylaxis therapy, and high increase in livestock management (Gatabazi, 2013). Furthermore, this resistance might also be attributed to the widespread availability and affordability of antimicrobial drug shops, the common practice of self-medication with over-the-counter medications, and repeated exposure to different antimicrobials before obtaining prescriptions from healthcare professionals (Katakweba, 2014). Furthermore, Sonola *et al.* (2021) proposed that the cohabitation of humans and small mammals in a shared environment can result in the transfer of resistance genes between the two species, which may then be deposited in the landscape.

The phenomenon of multidrug resistance (MDR) refers to the ability of microorganisms, such as bacteria or viruses, to the virulence of *Salmonella* isolates is found to be much higher in comparison to non-multi-drug resistant strains, as indicated by previous studies conducted by (Magiorakos *et al.,* 2012; Tawyabur *et al.,* 2020). *Salmonella Enterica isolates* in this study were shown to possess multiple drug-resistant genes, with some isolates harboring 20% resistance genes. The presence of multidrug resistance in *Salmonella enterica* has been documented as a cause of sickness in both humans and animals across various countries, such as the United States and Denmark (Frank (2007), Italy (Dionisi *et al.,* 2008), Eastern China (Lu *et al.,* 2014), and Vietnam (Vo *et al.,* 2010). Therefore, there was a significant prevalence of isolates that exhibit resistance to multidrug, including Amoxicillin, Ampicillin, Ciprofloxacin, and Sulphonamides. These antimicrobial drugs are frequently employed in human and veterinary medicine (Sonola *et al.,* 2022). As indicated by Bosco *et al.* (2012) and Walusansa, (2007) those antibiotics are commonly prescribed for the treatment of salmonellosis, which is a severe worry to human and animal health.

4.2 Conclusions

In conclusion, this study revealed that there is a significant difference between *E. coli* under the habitat, species, and Sites. On the contrary, there was no statistically significant prevalence of *Salmonella enterica* between rodent species, their habitats, and trapped sites. Also, Human beings are at high risk of foodborne pathogens because they are close to rodents and shrews.

This study focused on the prevalence of foodborne *E. coli* and *Salmonella enterica and* their risk factors for human health. It was assumed that the presence of foodborne pathogens in the rodents and shrews captured inside the house, in the open food markets, outside the house, and in the food shops indicated their potential to

transport pathogens and subsequently contaminate food or food-related utensils, posing a risk to human health.

Rodents can move and traverse diverse settings, potentially carrying foodborne diseases and pathogens. Moreover, the degree of risk posed to humans regarding foodborne *E. coli* and *Salmonella* spp found in rodents and shrews remains uncertain.

Also, the results of this study indicated that most of the *Salmonella enterica* and *E. coli* isolates that infected the rodents and shrews have both phenotypic and genotype AMR characteristics. The prevalence of AMR *Salmonella enterica* and *E. coli* in isolates obtained from rodents and shrews in Morogoro municipality indicated the potential for extensive dissemination of both resistance genes and bacteria throughout the study areas. This raises concerns regarding the potential for the emergence of challenging-to-treat diseases. The antimicrobials used in this study are extensively used in the study areas for treating humans and animals, indicating the high dissemination between them. Moreover, the spread of AMR *Salmonella enterica* and *E. coli* through the feces and urine dropped by rodents and shrews may lead to environmental pollution. This contamination can subsequently facilitate the transmission of this feature to other dangerous bacteria, such as *Salmonella* spp and *E. coli*, as well as unexpected bacterial species. Consequently, this poses a significant public health risk. These findings provide a better understanding of the role of rodents and shrews in transmitting and maintaining AMR *Salmonella enterica* and *E. coli*. This *Salmonella Enterica and E. coli* strain has the potential to be passed on to humans through the consumption of food products.

4.3 Recommendations

- Extensive research is needed on transmitting foodborne diseases from rodents and shrews to humans.
- It is advisable to conduct comprehensive investigations to determine the extent to which rodents and shrews serve as vectors and indicators of foodborne disease transmission.
- These investigations must incorporate rigorous infection control methods and rodent management.
- Hygiene and sanitation practices in houses, peri-domestic, and food market areas to eliminate rodent habitats from human surroundings should be encouraged. Implementation of comprehensive interventions that adopt a one-health approach to manage the issue effectively.
- Future studies are recommended on the detection of resistance genes found in bacterial isolates from rodents samples, shrews, humans, animals (sheep, goats, cattle, dogs, cats, pigs), and the environment to understand the interconnection of transmission patterns.
- Identification of bacteria and bacterial genes should be carried out by using current technologies of whole genome sequences

4.4 Limitations of Study

The current study sampled 148 rodents and shrews from Morogoro Municipality, which has limited the generalization of the results to be used as representative of the status of antimicrobial resistance with *E. coli* and *Salmonella enterica* in rodents and shrews in Tanzania. Also, the number of antimicrobials used in the study was few compared to the antimicrobials under use and available in Tanzania. Testing for antibiotic susceptibility of bacteria using disk diffusion test method may be inferior to broth microdilution methods. In spite of that, the presence of other animals on site where rodents and shrews sampling were done, were acted as confounding factors on the resistance rates observed in bacteria isolates from rodents and shrews.

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APPENDICES

Appendix 1: Questionnaire

PART A: Subjective Data

1. Name of interviewer ……..
2. Phone number ……
3. Street / Village /Wards……...
4. Identification of interviewer

a) Sex: Female ☐ Male ☐
b) Age in years: 15-25 ☐ 26 - 35 ☐ 36 - 45 ☐ 46 – 60 ☐ 60 + ☐
c) Date of birth: Date ☐ ☐ Month ☐ year ☐
d) Level of education: Illiterate ☐ Primary ☐ Secondary ☐ Advanced ☐ College and above ☐
e) Marital status: Single ☐ Married ☐ Divorced ☐ Widowed ☐
5. How many are you in the house? (specify numbers)

Adults from 65 years –up ☐

Adults from 31 - 64 years ☐

Adults from 20 – 30 years ☐

Adolescents 13-19 years ☐

Children 5- 12 years ☐

Infants under 5 years ☐

6. What do you do for a living?

Farming ☐ Self-employed ☐ ormal employment ☐ unemployed ☐
Others ☐
(Specify)……
……

PART B General Knowledge on house rodents

7. Did you see rodents in your area (inside the house or outside)? Yes No

8. What is the importance of rodents to your livelihood?
……
……
……

9. Do you have problems with rodents? Specify (e.g.; Storage or health)
……
……

10. Do you have outbreaks of rodents in your area? Yes ☐
No ☐

11. If response of 10 is yes, which months do you have outbreaks in your area?

a. January to March ☐
b. April to June ☐
c. July to September ☐
d. October to December ☐

12. What rodent species do you have in your area?
……
……

PART C: Risk factors of food borne pathogens associated with house rodents

13. Do the rodent invade in your house? Yes ☐ No ☐

14. How do you assess for invasion in your house? Through

a. Droppings ☐

b. Urine ☐
c. Damage on household materials
d. Their sight ☐
15. Do you eat rodents? ☐ Yes ☐ No ☐
16. If yes, which rodents species preferably eaten in their place?
……
…….
17. How do you prepare the rodents for consumption?
a. Boiling ☐
b. Roasting ☐
c. others (specify)
……
18. Do you have a place for keeping food (store)? Yes ☐
No ☐
19. Do you cover your food before or after cooking? Yes ☐
No
20. Did you see the feces of rodents in uncooked or cooked food?
Yes ☐ No ☐
21. Did you see the food eaten by rodents in your place?
Yes ☐ No ☐
22. If yes on 20 and 21, how do you manage the feces of rodents and food contaminated?
a. Removed by hands ☐
b. Removed by using gloves ☐
c. Removed with another material (toilet paper, broom….) ☐
d. Not removed ☐
23. Do you wash your hands after handling a rodents or its feces?
Yes ☐ No ☐
24. Do you consume food eaten by rodents or contact with their feces at home? Yes ☐ No ☐
25. If yes on 24, do you cook before eating them?
Yes ☐ No ☐
26. If yes on 24, do you wash them before eating?
Yes ☐ No

27. Do you ever find rodents drinking from the water kept inside the house? Yes ☐ No ☐

28. Did you see the feces of rodents in the water storage or containers? Yes ☐ No ☐

29. If yes on the 27 and 28, how do you manage the water in contact with rodents and their feces?

A. Not use the water ☐

B. Used without treatment ☐

C. Boiling before used ☐

D. Filtering

E. Commercially

30. Did you have diarrhea in the last 3 months? Or any person has it in the last 3 months in your home? Yes ☐ No ☐

31. If yes above, after how long did the diarrhea stop?

A. For more than a month ☐

B. a month ☐

C. a week

D· others (specify) …… ☐

32. Did he/she complain of any other gastrointestinal discomfort (abdominal cramps, nausea, vomiting, and blood in stool) in the last 3 months? Yes ☐

No ☐

33. Did you go to the hospital during the last problem?

Yes ☐ No ☐

34. Was she/he diagnosed with any intestinal bacteria worm?

Yes ☐ No ☐

35· Did you take an antibiotic drug? Yes ☐ No ☐

36· If you take medicine where do you get the antibiotic drug?

A· hospital or clinic

B· Private pharmacy ☐

C· Supermarket ☐

D· others (specify) …….

37· Did you finish your dosage? Yes ☐ No ☐

Appendix 2: Ethical Clearance Permit

JAMHURI YA MUUNGANO WA TANZANIA

OFISI YA RAIS
TAWALA ZA MIKOA NA SERIKALI ZA MITAA

Anuani ya Simu "TAMISEMI" DODOMA
Simu Na: +255 26 2321607
Nukushi: +255 26 2322116
Barua pepe:ps@tamisemi.go.tz
Unapojibu tafadhali taja:-

Mji wa Serikali – Mtumba,
Mtaa wa TAMISEMI,
S.L.P. 1923,
41185 DODOMA.

Kumb. Na. AB.307/323/01/191

16 Februari, 2023

Katibu Tawala wa Mkoa,
Ofisi ya Mkuu wa Mkoa,
S.L.P 650,
MOROGORO.

Yah: KIBALI CHA KUFANYA UTAFITI KUHUSU PREVALENCE AND ANTI-MICROBIAL RESISTANCE PROFILE OF FOOD BORNE SALMONELLA AND E-COLI TRANSMITTED BY HOUSEHOLD RODENT IN MOROGORO URBAN

Tafadhali rejea somo tajwa hapo juu.

2. Ofisi ya Rais –TAMISEMI imetoa kibali kwa **Bi. Maria C. Uwanyirigira, Mwanafunzi** kutoka Chuo Kikuu cha Kilimo Sokoine (SUA) kwa ajili ya kufanya utafiti tajwa katika Halmashauri ya Manispaa ya Morogoro.

3. Muda wa kufanya utafiti huu ni kati ya mwezi Januari, 2023 na mwezi Juni, 2023. Ofisi ya Rais -TAMISEMI kwa kushirikiana na Taasisi nyingine za Serikali itafanya ukaguzi wakati wowote kujiridhisha na utekelezaji sahihi wa kibali hiki. Takwimu zitakazokusanywa kutokana na utafiti huu ni kwa ajili ya matumizi ya ndani tu na iwapo zitatakiwa kuchapishwa na kusambazwa kibali kutoka Mamlaka husika kitapaswa kuombwa.

4. Kwa barua hii, tafadhali muelekeze Mkurugenzi wa Halmashauri tajwa ili kutoa ushirikiano utakaohitajika na kukamilisha utafiti huu kama uilivyokusudiwa. Kazi hii isimamiwe na Mtakwimu wa Mkoa na Halmashauri husika na kutoa taarifa ya utekelezaji.

Ninakushukuru kwa ushirikiano wako.

Prof. Riziki S. Shemdoe
KATIBU MKUU

Nakala: Katibu Mkuu Kiongozi,
Ofisi ya Rais,
IKULU,
1 Barabara ya Julius Nyerere,
Chamwino,
S. L. P. 1102,
40400 DODOMA. *(Aione RSO wa Mkoa wa Morogoro).*

Makamu Mkuu wa Chuo,
Chuo Kikuu cha Kilimo Sokoine (SUA),
S. L. P 3000,
Barua Pepe: vc@sua.ac.tz,
MOROGORO. *(Rejea barua yenye Kumb Na. SUA/ADM/R.1/8/995)*

Bi. Maria C. Uwanyirigira,
Chuo Kikuu cha Kilimo Sokoine (SUA),
S. L. P 3000,
MOROGORO. *(Nakala ya taarifa ya utafiti iwasilishwe Ofisi ya Rais - TAMISEMI na Ofisi husika ya Mkuu wa Mkoa na Halmashauri. Kibali kinaweza kufutwa muda wowote endapo kutakuwa na ukiukwaji wowote au sababu nyingine yoyote)*

JAMHURI YA MUUNGANO WA TANZANIA

OFISI YA RAIS

TAWALA ZA MIKOA NA SERIKALI ZA MITAA

HALMASHAURI YA MANISPAA MOROGORO

Unapojibu tafadhali taja:

Kumb. Na: R.10/MMC-24/32 Tarehe: 10/03/2023

Bi. Maria C. Uwanyirigira,
Mwanafunzi,
Chuo Kikuu cha Kilimo cha Sokoine (SUA),
S. L. P. 3000,
MOROGORO

Yah: KIBALI CHA UTAFITI

Nakiri kupokea barua kutoka kwa Katibu Tawala Mkoa wa Morogoro ikinielekeza kutoa kibali kwa ajili ya utafiti kuhusu ***"Prevalence and Anti. Microbial Resistance Profile of food borne Salmonella and E- Coli Transmitted by Household rodent".***

2. Kwa barua hii, kibali **kimetolewa** kwako ili uweze kufanya utafiti katika Kata ya **Mzinga, Bigwa, Magadu, Kiwanja Ndege na Mji Mpya** zilizopo Manipsaa ya Morogoro kuanzia mwezi Machi hadi Juni, 2023.

3. Nakutakia utafiti mwema.

Martha N. Kipanga
Kny: MKURUGENZI WA MANISPAA
MOROGORO

Nakala:- Katibu Tawala Mkoa,
Ofisi ya Mkuu wa Mkoa,
Boma Road,
S. L. P. 650,
67117 MOROGORO

- Mtendaji wa Kata ya Mzinga, Bigwa, Magadu, K/Ndege na Mji Mpya
Halmashauri ya Manispaa,
S. L. P. 166,
MOROGORO – Tafadhali toa ushirikiano wa karibu

CLEARANCE PERMIT FOR CONDUCTING RESEARCH IN TANZANIA

UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY.

SOKOINE UNIVERSITY OF AGRICULTURE
OFFICE OF THE VICE-CHANCELLOR

P.O Box 3000, CHUO KIKUU, MOROGORO, TANZANIA.
Phone: +255 (023) 2640006/7/8/9, Direct Line: +255 (023) 2640015,
E-mail:vc@sua.ac.tz, Website: https://www.sua.ac.tz

Please refer to:
Our Ref: SUA/ADM/R.1/8/995 **Date:** 16th January, 2023

Permanent Secretary,
President's Office,
Regional Administration and Local Government,
P.O. Box 1923, Mji wa Serikali,
41185 DODOMA.
Email: ps@tamisemi.go.tz

RE: UNIVERSITY STAFF, STUDENTS AND RESEARCHERS CLEARANCE

The Sokoine University of Agriculture was established by University Act No. 7 of 2005 and SUA Charter, 2007 which became operational on 1st January 2007 repealing Act No. 6 of 1984. One of the mission objectives of the University is to generate and apply knowledge through research. For this reason the staff and researchers undertake research activities from time to time.

2. To facilitate the research function, the Vice Chancellor of the Sokoine University of Agriculture (SUA) is empowered to issue research clearance to staff, students, research associate and researchers of SUA on behalf of the Tanzania Commission for Science and Technology.

3. The purpose of this letter is to introduce to you **Ms. Marie C. Uwanyirigira** a bonafide **MSc. (Public Health and Pest Management)** student with Registration number **MPP/D/2021/0102** of SUA. By this letter **Ms. Marie C. Uwanyirigira** has been granted clearance to conduct research in the country. The title of the research in question is ***"Prevalence and Anti-microbial resistance profile of food borne salmonella and E- Coli transmitted by household Rodent in Morogoro Urban".***

CLEARANCE PERMIT FOR CONDUCTING RESEARCH IN TANZANIA

4. The period for which this permission has been granted is from **January, 2023** to **June, 2023**. The research will be conducted in **Morogoro Region (Morogoro Municipality).**

5. Should some of these areas/institutions/offices be restricted, you are requested to kindly advice the researcher(s) on alternative areas/institutions/ offices which could be visited. In case you may require further information on the researcher please contact me.

6. We thank you in advance for your cooperation and facilitation of this research activity.

Yours sincerely,

VICE CHANCELLOR
SOKOINE UNIVERSITY OF AGRICULTURE
P.O.BOX 3000
MOROGORO, TANZANIA

Prof. Maulid W. Mwatawala
FOR: VICE-CHANCELLOR

c.c. Director, DPRTC, SUA. - To note in file.

c.c. Student – **Ms. Marie C. Uwanyirigira**

SECTION P : FOR OFFICIAL USE

(i) APPROVAL

Date received : Click here and arrow to enter a date. 28/12/2023	Received by: Click here to type names. LUCIA MADALLA
Date of approval: Click here and arrow to enter a date. 02/1/2024 **Name:** PROF J APHET KASHAIGILI **Title:** DIRECTOR DPRTC. Approving authority in capital letters (example: SRPC, Departmental /College/Centre R&PC Click here to enter the name of approving authority.	Approval reference number: Click here to enter number. SUA/DPRTC/186 VOL IV/82 Approval is valid from Click here and arrow to enter a date. 02/01/2024 To: Click here to enter a date.

*All undergraduate studies shall be evaluated and/or approved by the College/Centre R&PC and Reports submitted to the chair Research Ethics Committee, DPRTC

(ii) NOT APPROVED

☐ The applicant is required to revise the application by addressing reviewer's concerns (Reviwer's comments are provided to the applicant)

☐ Other reasons (Describe briefly)

Click here to enter text.

Director
Postgraduate studies, Research, Technology Transfer and Consultancy
Sokoine University of Agriculture
P. O. Box 3151, Morogoro
TANZANIA