Sokoine University of Agriculture

MSc Dissertation

Antimicrobial Resistance Pattern of *Escherichia Coli* and *Salmonella* Species, Isolated from Domestic and Peridomestic Rodents in Iringa Municipality, Tanzania

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ANTIMICROBIAL RESISTANCE PATTERN OF *ESCHERICHIA COLI* AND *SALMONELLA* SPECIES, ISOLATED FROM DOMESTIC AND PERIDOMESTIC RODENTS IN IRINGA MUNICIPALITY, TANZANIA

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

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EXTENDED ABSTRACT

Escherichia coli (*E. coli*) and *Salmonella* species are two of the most common causes of foodborne illness worldwide. *E. coli* is a Gram-negative bacterium that is found in the intestines of humans and animals. Most strains of *E. coli* are harmless, but some strains can cause serious infections, such as food poisoning and urinary tract infections. *Salmonella* species is a gram-negative bacterium that is found in the environment and in the intestines of animals. *Salmonella* species can cause a variety of infections, including gastroenteritis, typhoid fever, and paratyphoid fever. Antimicrobial resistance is a major concern in the treatment of *E. coli* and *Salmonella* infections. When *E. coli* and *Salmonella* species become resistant to antimicrobials, they are more difficult to treat, which can lead to more severe infections and even death.

Rodents are known to be reservoirs of *E. coli* and *Salmonella* spp. bacteria. Rodents can transmit *E. coli* and *Salmonella* spp. to humans and other animals through their faeces, urine, and saliva. The aim of this study was to determine the prevalence and antimicrobial resistance pattern of *E. coli* and *Salmonella* spp. isolated from domestic and peridomestic rodents in Iringa, Tanzania.

A cross-sectional study design was used in this study. The study sites possessing favourable rodents habitats were purposively selected. A total of eight wards (sampling sites) were selected. The target study population was rodents, a total of 153 rodents were trapped in Iringa municipality, Tanzania. The rodents were trapped in both domestic and peridomestic settings. Following euthanization, rats were identified to genus/species level using morphological and morphometric features. Faecal swab samples were collected from the rodents and pre-enriched in buffered peptone water, followed by culture and biochemical tests for identification of *E. coli* and *Salmonella* spp. Molecular confirmation tests were done using 16RrRNA PCR identification methods. The antimicrobial susceptibility test was performed by disk diffusion method

comprising five antimicrobials, including tetracycline (30µg), ciprofloxacin (5µg), gentamicin (10µg), ceftriaxone (30µg), and trimethoprim-sulfamethoxazole (25µg). Seven resistant genes were analyzed by PCR, which were *blaCTX-M, blaSHV, sul1, sul2, tetA, acr(A),* and *aac(3)-1* in each isolate. Three rodent species, *Rattus rattus* (75.2%), *Mastomys natalensis* (23.5%), and *Mus musculus* (1.3%), were captured. 17 (11.1%) *E. coli* were detected, and no *Salmonella* species were isolated. All 17 isolates were susceptible to Gentamycin, while Sulphamethaxazole/Trimethoprim was highly resistant (52.9%). Four *E. coli* isolates exhibited multidrug resistance (MDR), whereby 75% of these MDR isolates originated from the same area. Six resistant genes were detected: *bla*CTX-*M, sul1, sul2, tetA, acr(A), and aac (3)-1,* where the *acr(A)* resistant gene was the most abundant. There were co-occurrences of the resistant genes per isolate such as *sul2*, *acr(A)*, and *aac(3)-1*. This study reveals the antimicrobial resistance of *E. coli* isolated from rodents, providing preliminary data on the prevalence and antimicrobial resistant features of these pathogens in their respective reservoirs in Iringa municipality. The study recommends observation of regular antimicrobial resistance screening and improvement in rodent management and control programs in the studied area.

Keywords: Rodent, *E. coli*, *Salmonella* spp., antimicrobial-resistance, resistance gene, multidrug resistance

IKISIRI KUU

Escherichia coli (*E. coli*) na Salmonella spishi ni mojawapo ya sababu za kawaida za magonjwa yanayotokana na chakula ulimwenguni. E. coli ni bakteria wa Gram hasi ambayo hupatikana katika utumbo wa binadamu na wanyama. Zaidi ya mikunjufu ya *E. coli* ni isiyo na madhara, lakini baadhi ya mikunjufu inaweza kusababisha maambukizo mabaya, kama vile kuhara chakula na maambukizo ya njia ya mkojo. Salmonella spishi ni bakteria wa Gram hasi ambayo hupatikana katika mazingira na katika utumbo wa wanyama. Salmonella spishi inaweza kusababisha aina mbalimbali za maambukizo, ikiwa ni pamoja na na magonjwa ya tumbo(homa ya typhoid, na homa ya paratyphoid). Upinzani wa antibayotiki ni wasiwasi mkubwa katika matibabu ya maambukizo ya *E. coli* na Salmonella spishi. Wakati *E. coli* na Salmonella spp zinapokuwa na upinzani wa antibayotiki, ni vigumu zaidi kuzitibu, ambayo inaweza kusababisha maambukizo makali zaidi na hata kifo.

Panya wanajulikana kuwa vyanzo kimoja vya bakteria wa *E. coli* na Salmonella spishi. Panya wanaweza kuambukiza *E. coli* na Salmonella spishi kwa binadamu na wanyama wengine kupitia kinyesi chao, mkojo, na mate. Lengo la utafiti huu lilikuwa kutambua wingi na mifumo ya upinzani wa antibayotiki wa *E. coli* na Salmonella spishi. zilizotengwa kutoka kwa panya wa ndani na karibu na nyumba huko Iringa, Tanzania.

Utafiti huu ulitumia muundo wa utafiti wa aina ya kukusanya data kwa muda mmoja. Maeneo ya utafiti yenye makazi mazuri ya panya yalichaguliwa kwa makusudi. Jumla ya kata nane (maeneo ya kuchunguza) zilichaguliwa. Idadi ya panya waliochunguzwa ilikuwa 153 katika manispaa ya Iringa, Tanzania. Panya walichunguzwa katika mazingira ya ndani na karibu na nyumba. Baada ya kufanya mauti, panya walitambuliwa kulingana na jenasi/spishi kwa kutumia sifa za morfolojia na morfometriki. Sampuli za njia ya haja kubwa

zilichukuliwa kutoka kwa panya na kisha kuchunguzwa katika maji yenye peptoni iliyotengenezwa, ikifuatiwa na upimaji wa utamaduni na vipimo vya kibayokemikali kwa kutambua *E. coli* na Salmonella spishi. Vipimo vya molekuli vilifanywa kwa kutumia mbinu ya PCR ya 16RrRNA kwa kutambua. Jaribio la ushikivu wa antibayotiki lilifanywa kwa njia ya kufyonzwa kwa diski kwa kutumia dawa tano za antibayotiki, ikiwa ni pamoja na tetracycline (30µg), ciprofloxacin (5µg), gentamicin (10µg), ceftriaxone (30µg), na trimethoprim-sulfamethoxazole (25µg). Majeni saba yenye upinzani yalipimwa kwa PCR, ambayo ni blaCTX-M, blaSHV, sul1, sul2, tetA, acr(A), na aac(3)-1 katika kila utambuzi. Spishi tatu za panya, Rattus rattus (75.2%), *Mastomys natalensis* (23.5%), na *Mus musculus* (1.3%), zilichukuliwa. *E. coli* 17 (11.1%) ziligunduliwa, na hakuna spishi ya Salmonella iliyotengwa. Sampuli zote 17 zilikuwa na uwezo wa kustahimili Gentamycin, wakati Sulphamethaxazole/Trimethoprim ilikuwa na upinzani mkubwa (52.9%). Sampuli nne za *E. coli* zilionyesha upinzani wa dawa nyingi (MDR), ambapo 75% ya sampuli hizi za dawa nyingi(MDR) zilitokea eneo moja . Majeni sita yenye upinzani yaligunduliwa: *blaCTX-M*, *sul1*, *sul2*, *tetA*, *acr*(A), na *aac* (3)-1, ambapo jeni ya upinzani ya *acr*(A) ilikuwa yenye kawaida zaidi. Kulikuwa na ushirikiano wa majeni yenye upinzani kwa kila sampuli, kama vile *sul2, acr*(A), na *aac*(3)-1. Utafiti huu unafunua upinzani wa antibayotiki wa *E. coli* uliotengwa kutoka kwa panya, ukitoa data ya awali juu ya wingi na sifa za upinzani wa antibayotiki wa vimelea hivi katika vyanzo vyao husika katika manispaa ya Iringa. Utafiti unapendekeza ufuatiliaji wa kawaida wa upinzani wa antibayotiki na kuboresha mipango ya usimamizi na udhibiti wa panya katika eneo lililochunguzwa.

Neno kuu: Panya, *E. coli*, Salmonella spishi, upinzani wa antibayotiki, jeni ya upinzani, upinzani wa dawa nyingi

DECLARATION

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

_____ _____

Jackson Chrispin Mkopi Date
(MSc. Candidate)

The above declaration is confirmed by;

_____ _____

Dr. James Mushi Date
(Supervisor)

_____ _____

Dr. Alexanda Nzula Date
(Supervisor)

LIST OF MANUSCRIPT

Manuscript 1: *Occurrence of Escherichia coli and Salmonella species isolated from domestic and peridomestic rodents in Iringa municipality, Tanzania*

Manuscript 2: *Detection of antimicrobial resistance pattern of Escherichia coli isolated from domestic and peri-domestic rodents in Iringa municipality, Tanzania*

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DEDICATION

This thesis is dedicated to my late parents, Chrispin Mkopi and Zawadi Iddi. They did not only raise and mature me but also taxed themselves dearly over the years for my education and intellectual development.

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ABBREVIATIONS AND ACRONYMS

ACEIRPM	African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development
AFLP	Amplified fragment length polymorphism
AMR	Antimicrobial Resistance
Bp	base pairs
CRISPR	Clustered regularly interspaced short palindromic Repeat
BTD	Biosensor Technology Development
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ESBL	Extended Spectrum Beta Lactamases
gDNA	Genomic Deoxyribonucleic Acid
GIT	Gastrointestinal tract
GPS	Global Positioning System
MDR	Multi Drug Resistance
M.M	*Mus musculus*
µm	Microlitre
WHO	United Nations World Health Organization
SUA	Sokoine University of Agriculture
CVMBS	College of veterinary medicine and biomedical
XLD	sciences
GIS	Xylose Lysine Deoxycholate agar
USA	Geographic Information System
	United states of America
MLST	Multilocus sequence typing
mm	Millimeter
NCBI	National Center of Biotechnology Information
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
pH	Potential for hydrogen
RFLP	Restriction fragment length polymorphism

RR	*Rattus rattus*
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
TAE	Tris acetate EDTA buffer
TVLA	Tanzania Veterinary Laboratories Agency
URT	United Republic of Tanzania
UTI	Urinary Tract Infection
UV	Ultraviolet

CHAPTER ONE

1.0 General Introduction

The concept of zoonosis pertains to infectious diseases that cause suffering to humans but originate from pathogens shared with other vertebrate animals (Keesing and Ostfeld. 2021). Throughout history, zoonotic bacterial pathogens have posed significant threats to human lives on a global scale (Chlebicz and Śliżewska. 2018). Zoonotic pathogens typically originate from wildlife sources, contributing to roughly 60-70% of all emerging human infections (Azimi *et al*., 2021).

Escherichia coli (*E.coli*) and *Salmonella* spp. hold immense medical and veterinary significance as zoonotic bacteria within the *Enterobacteriaceae* family (Hurst, 2018). These bacterial frequently inhabit the gastrointestinal tracts and kidneys of humans, birds, and various other animals. Furthermore, *E. coli* and *Salmonella* spp. are essential ecological microorganisms involved in a variety of infections including urinary tract infections, GIT infections, abdominal and pelvic infections, pneumonia, bacteremia, and meningitis (Sonola *et al*., 2021). Both of these bacterial species have been isolated from diverse sources, including soil, contaminated water sources, sewage systems, human beings, poultry meat, currency notes, bats, and rodents (Lyimo *et al*., 2016).

E. coli a resident of the lower intestines in warm-blooded living organism exists in contradicting contexts. While most strains peacefully coexist within their hosts, contributing to essential gut functions, others possess the potential to cause outbreaks as potent pathogens (Abebe *et al*., 2020; Young, 2006). Understanding this duality demands thorough identification of *E. coli* strains, a process involving their genetic, biochemical, and pathogenic variations (Young, 2006).

Growing *E. coli* in diverse mediums like MacConkey and Blood agar, followed by microscopic examination and biochemical assays, provides valuable insights into their characteristics (Abebe *et al*., 2020; Timmis *et al*., 2017). However, the advent of molecular techniques like PCR and DNA sequencing has revolutionized *E. coli* identification, allowing for precise differentiation and targeting of specific virulence genes (Ayyal *et al*., 2019; Tilevik *et al*., 2022).

Despite their beneficial role in the gut, pathogenic *E. coli* strains occur in polluted water and animal excrement, posing a significant threat to human health through contaminated food and water sources (Gambushe *et al*., 2022; Chekabab *et al*., 2013; Sonola *et al*., 2021). The epidemiology of these strains varies considerably, with environmental factors influencing their prevalence and impact. For instance, *E. coli* O157:H7 is famous for causing global foodborne outbreaks (Chotinun *et al*., 2014; Figler *et al*., 2016).

Managing *E. coli* infections necessitates a multi-pronged approach. Antimicrobial resistance concerns necessitate a cautious approach, favoring improved hygiene, water treatment, and careful food handling practices alongside robust food safety monitoring systems (Feuerstein *et al*., 2021; Arbab *et al*., 2022).

Salmonella spp. belongs to the *Enterobacteriaceae* family and encompasses a diverse array of serotypes within its two main species: *Salmonella bongori* and *Salmonella enterica* (Yoshida *et al*., 2016; Sundaresan *et al*., 2023). These serotypes are distinguished by their specific O and H antigenic profiles, crucial for accurate identification (Mann *et al*., 2021; Rahman *et al*., 2019; Thung *et al*., 2018).

Traditionally, pre-enrichment, selective media cultivation, and serological methodologies aided by Gram staining under a microscope have been the backbone of *Salmonella* spp. identification (Mann *et al*., 2021; Rahman *et al*., 2019). However,

molecular techniques like DNA sequencing and PCR have emerged as powerful tools, offering rapid and specific identification by targeting unique genes or segments within the *Salmonella* spp. genome (Chatterjee *et al*., 2023; Tilevik *et al*., 2022).

These adaptable bacteria thrive in diverse environments, colonizing the digestive systems of humans and animals while readily spreading through contaminated food and animal waste (Ehuwa *et al*., 2021). *Salmonella* spp. infections that primarily originate from contaminated food sources remain a global public health concern, necessitating comprehensive control measures throughout the food production chain (Ehuwa *et al*., 2021; Gebeyehu *et al*., 2022).

Treatment for *Salmonella* spp. infections prioritizes electrolyte replacement and avoids routine antibiotic therapy due to concerns about antibiotic resistance. Instead, stringent food safety measures and robust surveillance systems remain the cornerstones of preventing the spread of these infections (Mann *et al*., 2021; Stanaway *et al*., 2019).

Rodents are the most extensive group among small mammals with 2277 known species (Backhans & Fellström, 2012; Pimsai *et al*., 2014). This remarkable order encompasses 29 families, including like mice, rats, voles, squirrels, and even beavers, chipmunks, and guinea pigs (Eisen *et al*., 2018). Their adaptability allows them to thrive in diverse terrestrial habitats across the globe (Siegfried & Brown, 1992; Makundi *et al*., 2011).

Some populations flourish in slightly disturbed landscapes like cultivated lands, while others exhibit high habitat specificity, thriving in undisturbed environments and vulnerable to human interference (Lema & Magige, 2018). In regions like East Africa, rodent populations fluctuate in response to climatic changes, with factors like species abundance and rainfall patterns influencing their dynamics (Makundi *et al*., 2010).

Distribution patterns reveal intriguing insights into the world of rodents. Forested habitats harbor greater diversity compared to lowland areas, highlighting the influence of habitat complexity on species richness (Mulungu *et al*., 2011). Sub-Saharan Africa showcases this diversity, with rodents found in savanna woodlands, secondary growth, forest clearings, and even cultivated lands (Massawe *et al*., 2010). Understanding rodent population density requires considering past densities, environmental influences, and control measures (Stenseth *et al*., 2003; Makundi *et al*., 2011). One particularly abundant species across much of Sub-Saharan Africa is the Natal multimammate mouse (*Mastomys natalensis*), thriving in lowland cultivated areas with remarkably large litter sizes (Stenseth *et al*., 2003; Makundi *et al*., 2011).

Rainfall, habitat structure, and productivity play crucial roles in shaping rodent populations (Makundi *et al*., 2010; Martín-Regalado *et al*., 2019). Rainfall patterns, especially in tropical and arid regions, significantly impact density, with increased rainfall often leading to population growth (Madsen & Shine, 1999; Massawe *et al*., 2010). Habitat structure, including vegetation types and covers, influences resource availability like food, water, and breeding sites, further impacting rodent populations (Chidodo *et al*., 2020). Rodent productivity, defined by the offspring produced per female during a specific period, also contributes to population dynamics (Makundi *et al*., 2011).

Rodents pose significant public health concerns as reservoirs for approximately 60% of zoonotic bacteria, viruses, and parasites (Katakweba *et al*., 2012; Dahmana *et al*., 2020). Wild rodents, including the Norway rat (*Rattus norvegicus*), act as crucial hosts for numerous bacterial zoonotic pathogens capable of transmission to humans and other vertebrates (Azimi *et al*., 2021). These pathogens, transmitted through rodent excretion and contamination of food and water, have historically contributed to disease outbreaks,

such as the role of the black rat (*Rattus rattus*) in plague transmission during the middle ages (Stenseth *et al*., 2003).

Rodents can also carry and transmit bacteria like *E. coli* and *Salmonella* spp. often without displaying symptoms but posing risks to human and animal health through various transmission routes (Nkogwe *et al*., 2011; Jahan *et al*., 2021). Managing and reducing rodent populations requires a deep understanding of their ecology and biology, especially in regions facing significant challenges. Effective strategies often draw upon ecological principles like improved sanitation and community-level trapping efforts (Makundi & Massawe, 2011).

Urban environments all over the globe are bothered by the ubiquitous presence of rodents (Panti-May. 2016). Primary factors fueling rodent infestations in such environments, particularly in Africa and many other developing nations are population growth and substandard sanitation conditions (Panti-May. 2016). Furthermore, rodents are a prominent subgroup among other groups of small mammals that have been known to harbour zoonotic bacteria, viruses, and parasites of public health significance (Bonwitt *et al*., 2017). These small mammals may serve as reservoirs for numerous zoonotic pathogens and play a crucial role in transmitting them to both humans and other animals (Katakweba *et al*., 2013; Young *et al*., 2017; Dahmana *et al*., 2020).

Even though these small mammals have been documented as potential carriers of various zoonotic pathogens due to the frequent interaction noted between them and sources of human and agricultural waste. Such an interaction further exposes them to environmental antimicrobials, increasing the likelihood of antimicrobial resistance development in the pathogens harboured by these mammals (Furness *et al*., 2017). The rising concern over antimicrobial resistance in all pathogens has become a significant public health issue (Furness *et al*., 2017). However, recent research

conducted by Gwenzi *et al*. (2021) has spotlighted rodents as major sources of multidrug-resistant bacterial strains. Furthermore, studies by (Nkogwe *et al*., 2011; Saengthongpinit *et al*., 2019) have elucidated that household rodents have been found to harbour various antimicrobial-resistant microorganisms, including *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Escherichia* spp., *Klebsiella* spp., and *Staphylococcus aureus*.

Antimicrobial resistance (AMR) has emerged as a critical global health issue, with an estimated 700,000 deaths attributed to it annually. If left unchecked, this figure is projected to escalate to a staggering 10 million by 2050 (Dadgostar, 2019). This concerning phenomenon, particularly prevalent in bacteria, manifests in various forms: innate, acquired, and adaptive (Christaki *et al*., 2020). Innate resistance, inherent in a bacterium's genetic makeup, exemplifies defensive mechanisms like glycoprotein barriers in Gram-negative bacteria (Christaki *et al*., 2020). Acquired resistance, arises through genetic mutations or the acquisition of resistant genes, while adaptive resistance thrives on dynamic environmental cues, such as stress or changing ion concentrations, often employing strategies like biofilm formation (Christaki *et al*., 2020).

Bacteria utilize a diverse arsenal of resistance mechanisms to evade drug action including reduced drug uptake, modification of drugs themselves, alteration of their target sites within the bacterial cell, and rapid efflux through plasma membrane pumps, a strategy employed by both Gram-positive and Gram-negative bacteria (Peterson & Kaur, 2018). The consequences of AMR extend far beyond mortality rates. It significantly impacts common ailments like urinary tract infections and pneumonia, contributing to an enormous economic burden through escalating healthcare costs (Poudel *et al*.,2023) Estimates predict a potential $100 trillion loss in the global economy by 2050 due to AMR (Dadgostar, 2019).

Combating AMR necessitates a multifaceted approach encompassing environmental health, animal welfare, and human well-being. Vigilant prescription practices in both human and veterinary medicine, coupled with strict adherence to regulations, represent crucial first steps (Chandler *et al*., 2023). Continuous monitoring of resistance trends is vital, allowing for the identification of emerging patterns, the localization of resistance hotspots, and the evaluation of intervention effectiveness (Chandler *et al*., 2023). Public awareness campaigns play a pivotal role in educating individuals about the risks of improper antimicrobial use, emphasizing the importance of completing prescribed regimens, and promoting responsible medication disposal (Miyano *et al*., 2022).

The existence of antimicrobial resistance in shared ecosystems has demonstrated significant adverse consequences for the health of both animals and humans (Sonola *et al*., 2021). Current efforts to combat drug resistance primarily focus on controlling the administration of drugs during treatments and implementing usage restrictions to prevent the proliferation of antimicrobial-resistant strains in humans (Wierup *et al*., 2021). However, due to their mobility, rodents frequently come into contact with food wastes, faeces, stored food, sewage, and biological materials. They thus stand out as reservoir species with a more notably significant capacity to contribute to the proliferation and dissemination of potentially antimicrobial-resistant strains of bacteria in human settings (Gwenzi *et al*., 2021; Meerburg *et al*., 2009; Ayyal *et al*., 2019). Therefore rodents residing near human habitats should undergo screening for the presence of resistant strains of bacteria to evaluate their role in disease transmission and compare antimicrobial resistance patterns in the pathogens they harbour and those of both humans and animals (Wakawa *et al*., 2015; Azimi *et al*., 2021).

Although findings by Sonola *et al*. (2022) have revealed the presence of these *E.coli* and *Salmonella* spp. in rodents and other

animal species in Tanzania, our understanding of the ecological consequences of these rodents, particularly those carrying antimicrobial-resistant strains and their role as carriers and transmitters of these pathogens remains limited. This study, therefore, aimed at assessing the occurrence and respective antimicrobial resistance patterns of *E. coli* and *Salmonella* spp. as zoonotic agents of public health significance isolated from a diversity of rodent species in Iringa Municipal. The findings will elucidate the clear distribution of these two species regarding particular rodent species that host them. The information obtained will be used in planning for rodent control and zoonosis management in the area.

1.2 Problem Statement and Study Justification

Antimicrobial resistance remains a globally recognized public health challenge of this century that is growing into a global pandemic that poses a threat to humans and food production animals (Zhou *et al*., 2022). In 2019, Africa and Sub-Saharan Africa had a higher mortality rate (23.3 deaths per 100,000) attributable to AMR compared to other regions of the world (Mendelson and Matsoso, 2015). Most East African nations, including Tanzania, have limited AMR monitoring capability and current data on AMR trends of prevalent pathogenic bacteria (Okeke *et al*., 2007).

E. coli and *Salmonella* spp. have established causative pathogens for food poisoning. In Tanzania, information revealing the prevalence of these pathogens in uncommon reservoirs remains limited. However, only one study documented that rodent carries resistant *E. coli* and *Salmonella* spp. with the potential to be transmitted to humans and livestock in their ecological vicinity (Sonola *et al.*, 2021). Furthermore, other studies have similarly highlighted this possibility, focusing on the same species of bacteria isolated from the same reservoirs (rodents) in other parts of the world (Nkogwe *et al*., 2011; Nhung *et al*., 2015; Ribas *et al*., 2016; Islam *et al*., 2022).

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Although research by Sonola *et al*. (2022) has shown that these pathogens are present in rodents and other animal species in Tanzania, little is known about the ecological effects of these rodents especially those that carry strains of the pathogen that are resistant to antimicrobials, and how they act as carriers and transmitters of the pathogens. Therefore, the purpose of this study was to evaluate the prevalence and corresponding patterns of antimicrobial resistance of *E. coli* and *Salmonella* spp. the two important zoonotic agents for public health that were isolated from a variety of rodent species in Iringa Municipality. The results will clarify the two species' diverse distribution with respect to the specific rodent species that serve as their hosts. Planning for zoonotic disease management and rodent control will make use of the information gathered.

1.3 Research Objectives

1.3.1 General objective

To establish antimicrobial resistance pattern of *E. coli* and *Salmonella* spp. isolates from domestic and peridomestic rodents in Iringa municipality, Tanzania.

1.3.2 Specific objectives

i. To classify species composition of domestic, peridomestic rodents
ii. To determine the occurrence of pathogenic *E. coli* and *Salmonella* spp. from domestic and peridomestic rodents
iii. To determine the antimicrobial pattern of E. coli from domestic and peridomestic rodents

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CHAPTER TWO

Manuscript One

Occurrence of *Escherichia Coli* and *Salmonella* Species Isolated from Domestic and peridomestic Rodents in Iringa Municipality, Tanzania

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Abstract

Escherichia coli and *Salmonella* species are bacterial pathogens whose negative impact on human health is most conspicuous in opportunistic and urinary tract infections. However, the prevalence of pathogenic bacterial species reserved by these mammals remains unclear. This study objective was to determine the prevalence of these bacterial species with reference to rodents as their reservoir. A cross-sectional study was carried out in 8 purposively selected wards of Iringa Municipality. Captured rodents were counted and identified to species level. Rectal swabs were collected for isolation of *Salmonella* spp. and *E. coli*. Primer-specific conventional PCR was employed for the detection of the isolates. The Chi-Square test was employed for statistical inference of variations in occurrence of targeted pathogens within sex, habitat and host species. A total of 153 rats were trapped in selected habitats within the study area. *Rattus rattus* was the most abundant (75.2%) captured rodent species in all selected habitats. However, the least (1.3%) abundance of rodents captured was noted in farmlands. Of 35 isolates of *E. coli* and 2 isolates of *Salmonella* spp. conventionally identified, 17 isolates for *E. coli* and none for Salmonella spp. were confirmed using primer-specific PCR targeting to amplify the 585 bp region. The prevalence of *E. coli* varied by rodent species, habitat, and gender, with no statistically significant difference ($p>0.05$. Majority (77%) of these belonged to *Rattus rattus.* This study reports a prevalence of 11.1% and 0% for *E. coli* and *Salmonella* spp. respectively. We hence speculate a greater risk of transmission due to the close association between the reservoir and susceptible host (humans) in areas with anthropogenic exposure and recommend further studies to elucidate the genotype and molecular characteristics of the detected pathogen. Anticipated information is of epidemiological value and can be utilized in the design of effective control and prevention strategies.

Keywords: Rodent, Prevalence, Zoonosis, *Escherichia*, *Salmonella*

2.1 Introduction

Zoonotic pathogens typically originate from wildlife sources, contributing to roughly 60-70% of all emerging human infections (Azimi *et al.,* 2021). *E. coli* hold notable significance in the realm of public health, primarily contributing to opportunistic infections and outbreaks within healthcare settings. Among the members of this genus, *E. coli*, a Gram-negative bacterium, naturally resides as part of the commensal gut flora in humans, pigs, chickens, and other livestock animals (Young *et al.,* 2022). However, this bacterium takes on a pathogenic role when it infiltrates regions of the gut where it does not typically exist as normal flora (Etienne *et al.,* 2017; Singleton *et al*., 2003). This pathogen frequently emerges as one of the most frequently isolated microorganisms in various clinical diarrheal diseases affecting individuals (Young *et al*., 2022). Moreover, it stands as a prominent causative agent of nosocomial infections, with pathogenic strains often linked to urinary tract infections (Lin *et al.,* 2022).

On a contrasting note, *Salmonella* spp. a zoonotic bacterial genus encompasses both typhoidal and non-typhoidal strains that are predominantly associated with foodborne infections in humans (Stanaway *et al.,* 2019). Non-typhoid salmonellosis, however, remains prevalent, stemming from food contamination or asymptomatic carriers, particularly in animal-derived foods like beef, chicken, and eggs (Crump *et al.,* 2010). Human cases of non-typhoid salmonellosis typically manifest with initial symptoms such as fever, abdominal discomfort, diarrhoea, nausea, and sporadic vomiting. While most non-typhoid fevers can be effectively treated, 10%-15% of cases may take a severe turn (Bhan *et al.,* 2005). Furthermore, these fevers persist as a prominent cause of morbidity and mortality in animals and humans, particularly children and adolescents (Crump *et al.,* 2010). Consequently, non-typhoidal salmonellae (NTS) are internationally recognized as the primary culprits behind foodborne infections, posing a significant public health concern (Stanaway *et al.,* 2019).

Rodents, the free-living diminutive creatures belonging to the Rodentia order, boast a grand total of 2,277 recognized species, constituting a remarkable 42% of the entire spectrum of mammalian species, thus establishing their status as the most substantial group amongst small mammals (Pimsai *et al.,* 2014). They are notably characterized by their penchant for gnawing, a behaviour dictated by their ever-growing incisors (Backhans and Fellström. 2012). The adaptability of most rodent species permits them to thrive across a diverse array of habitats, effectively establishing their presence within their respective ecological niches (Siegfried and Brown 1992). In Tanzania, *Mastomys natalensis* appears to be the most abundant rodent species, predominantly inhabiting cultivated areas and neighbouring human settlements (Makundi *et al.,* 2006). Conversely, on the other hands, *Rattus rattus,* demonstrates a strong commensal relationship with humans and predominantly frequents domestic residences and structures (Katakweba *et al.,* 2013). It's worth noting that the ebb and flow of rainfall patterns is closely linked to the population dynamics of select rodent species, emerging as a pivotal factor influencing their abundance (Makundi *et al*., 2006).

The realm of rodents, intriguingly, serves as a potential reservoir for a myriad of zoonotic pathogens that hold significant public health implications (Katakweba *et al.,* 2012; Dahmana *et al.,* 2020; Jahan *et al.,* 2021). Furthermore, *E. coli* and *Salmonella* spp. have emerged as prime players in the realm of public health threats, harbored by rodents, avian species, and a sundry of other creatures, with the latter being recognized as the principal reservoir for such infections (Adhikari *et al.,* 2002; Tiller *et al*., 2010).The multifaceted tapestry of ecological landscapes inhabited by rodents casts a profound influence on the fluctuating degrees of contamination and the associated risks of transmitting these pathogens to proximate human populations (Meerburg and Kijlstra. 2007). A noteworthy facet of this transmission mechanism lies in the direct deposition of rodent urine and faecal pellets onto food designated for human

consumption, thus serving as a significant conduit for pathogen transmission (Jahan *et al.,* 2021). Furthermore, an investigation undertaken by Katakweba *et al.* (2013) elucidated that the transmission of most diseases from rodents to humans is notably amplified in regions plagued by socio-economic deficiencies, including a lack of proper hygiene, poverty, and overcrowding.

The diversity and abundance of rodents potentially capable of transmitting zoonotic pathogens and the specific type of pathogenic zoonotic agents harbored by such rodent species in Iringa Municipality remained unclear. This study aimed at assessing the prevalence of *E. coli and Salmonella* spp. as zoonotic agents of public health significance isolated from a diversity of rodent species in Iringa Municipal. The findings will elucidate the clear distribution of these two species with reference to particular rodent species that host them. The information obtained will be used in planning for rodent control and zoonosis management in the area.

2.2 Materials and Methods

2.2.1 Study area

The research was carried out within Iringa municipality, Tanzania. Situated at a longitude of 35° 69’ east of the Greenwich Meridian, and latitude of 12.9300’’ south, and 7° 77’ south of the Equator, this area is known for its diverse landscape. Iringa municipality is further divided into eighteen wards, encompassing urban, peri-urban, and rural zones, and hosts a population of approximately 1 192 728 individuals, as reported in the 2022 national census (URT, 2022). Geographically, the municipality shares its boundaries with Iringa Rural District to the north, west, and south, while Kilolo District Council lies to the east. Covering a total surface area of 331.4 km^2, Iringa municipality constitutes 0.9% of the entire regional landmass, thus establishing itself as the council with the smallest land area in the region.

From an administrative standpoint, Iringa Municipal Council consists of a single division, housing 18 wards: Mtwivila, Kihesa, Gangilonga, Ruaha, Mshindo, Mivinjeni, Mlandege, Mwangata, Kwakilosa, Makorongoni, Ilala, Mkwawa, Kitwiru, Isakalilo, Kitanzini, Nduli, Igumbilo, and Mkimbizi. Agriculture plays a significant role in this region, with the cultivation of various crops like vegetables, fruits, potatoes, maize, bananas, and wheat prevalent in the northern and eastern sectors of the municipality, while wheat, maize, and beans are predominant in the southern and western regions. Such agricultural activities create a favourable environment for rodent habitation (Mlyashimbi *et al*., 2020). To conduct the study, eight wards were selected via purposive sampling based on high population density and reports of rodent infestations coupled with agricultural practices. The selected wards for sampling included Nduli, Kihesa, Mtwivila, Kitwiru, Isakalilo, Kitanzini, Makorongoni, and Mkwawa, as shown in Figure 2.1. The research sites were documented and marked using a GPS device.

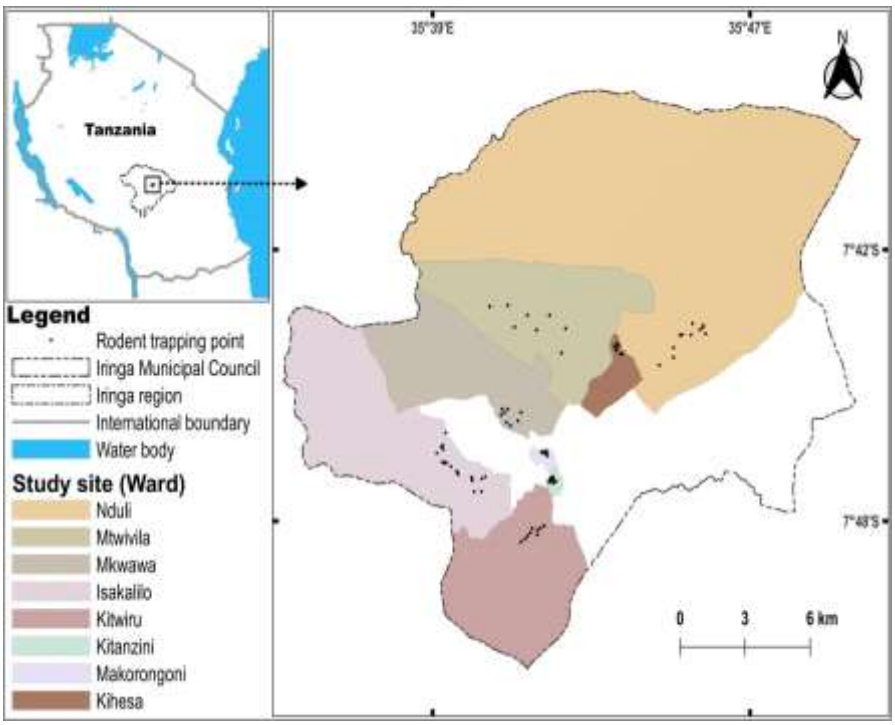

Figure 2.1: Map of Iringa municipality showing sampling ward
Source: QGIS 3.24.0. CRS EPSG: 4326 Version

2.2.2 Sampling strategies

A cross-sectional study design was employed. Rodents were collected from January to March 2023. Study sites possessing rodent habitats; human residences, storage facilities, and agriculture settings in the vicinity of human settlements were purposively selected. Eight wards (sampling sites) were selected.

2.3 Rodent Capturing Strategy

2.3.1 Household and storage facility trapping

In each of the eight wards, purposefully selected 8 candidate houses/storage facilities were for sampling by inquiring with the residents about the presence of rodents within their homes. We employed a total of 40 locally crafted live wire traps per night, with a maximum of five traps being set per house, determined by the

house's size and cues indicating rodent presence provided by the household members. These traps were enticingly baited with tomatoes and a blend of peanut butter as well as small fishes. The traps were strategically placed at 1800 hours and examined at 0700 hours of the following day over three consecutive nights to enhance good capture rate (Mulungu *et al.,* 2008).

2.3.2 Trapping in cultivated, and fallow

To capture rodents in these diverse environments, particularly maize and tomato farms, we utilized Sherman traps which is known as Large Folding Aluminium trap (LFA), employing a bait mixture of peanut butter and maize bran (Mulungu *et al.,* 2008). Two sampling sites were purposively selected in each of the eight wards. Two sampling sites were purposively selected in each of the eight wards, 100 traps in each sampling site, following a grid pattern of 100 meters by 100 meters. This grid consisted of 10 lines, each spaced 10 meters apart, although the orientation was adjusted to accommodate the landscape variations in different habitats (Katakweba *et al*., 2012). Traps were set at 1600 hours and diligently checked at 0700 hours of a following day for three consecutive nights to optimize capture yield.

2.3.3 Identification of captured rodents

Captured rodents were first anaesthesia with absolute ether, which was soaked with cotton wool, and then subjected to species-level identification by an experienced taxonomist as previously described (Katakweba *et al*., 2012). We also meticulously recorded morphometric data, including body weight (rounded to the nearest gram), head to body length, tail length, hind foot measurements, ear size, and the condition of the vagina or the position of the testes were recorded for more identification (Mulungu *et al.,* 2008).

2.3.4 Collection, processing, and transportation of specimens

Faecal samples for laboratory analysis were collected after dissection of animal’s abdominal cavity of each anaesthetized rodent

using sterile surgical instruments and forceps. From the gastrointestinal tract, we obtained deep rectal swabs using sterile microbiology swabs according to Nkogwe *et al.* (2011). Each rectal swab was individually placed in a sterile container containing 5 millilitres of maximum recovery transportation media and stored at a temperature of 4°C for preservation (Richard-Greenblatt *et al.,* 2020). All collected samples were maintained at 4°C, first stored at Tanzania Veterinary Laboratory Agency (TVLA Laboratory in Iringa before being transported to Sokoine University of Agriculture microbiology laboratory, for further analysis.

2.4 Bacterial Isolation, Identification, and Biochemical Characterization

2.4.1 Isolation of *E.Coli*

For isolation and identification of bacteria, collected swabs were first were placed into buffered peptone water and incubated for a duration of 24 hours at a temperature of 37°C. To identify *E. coli*, a loopful of the culture was inoculated onto MacConkey agar and left to grow for 24 hours at 37°C. Subculturing was then performed repeatedly until a pure culture of *E. coli* on MacConkey agar was successfully obtained.

Pure culture colonies were subsequently transferred to sorbitol MacConkey agar (SMAC) mixed with 1 mg/L potassium tellurite, and they were further incubated at 37℃ for a period of 18–24 hours to confirm the presence of pathogenic species. It is noteworthy that all samples produced a pale colony (sorbitol non-fermenters) which was subsequently confirmed to be pathogenic *E. coli* (Sharaf *et al.,* 2017). In order to reveal the relevant macroscopic features (colony colour, texture and size) were observed to detect candidate bacterial colonies observed on primary cultures, and then selected candidates were further subjected to Gram stain for initial microscopy identification. Following this, a series of biochemical assays, including triple sugar iron agar, indole test, methyl red test, Voges-Proskauer test and Citrate test, collectively abbreviated as IMViC

test, urea, mobility test, and xylose, were conducted to characterize *E. coli* strains belonging to the *Enterobacteriaceae* family (Himsworth *et al.,* 2015).

2.4.2 Isolation of *Salmonella* spp.

The isolation of *Salmonella* spp. commenced by adding 3 ml of enriched broth to tetrathionate broth, which was then allowed to incubate for 24 hours at 37°C. Subsequently, loops were inoculated onto XLD agar and incubated overnight at 37°C. The pre-enriched culture was divided into two, with one portion transferred to a 10 ml Selenite F Broth (SFB) tube and the other to a 10 ml Rappaport Vassiliadis Soy broth (RVSB) tube, both of which were incubated for 24 hours at 37°C. Finally, one loop of broth culture positive from Selenite F Broth (SFB) was later inoculated and incubated on Xylose Lysine Deoxycholate (XLD) at 37°C for 24 hours.

Suspected *Salmonella* spp. colonies from XLD were transferred into cryopreservation vials for further confirmation through biochemical assays, including TSI, Indole, Urease, Simon's citrate, and MR-VP tests (Ayyal *et al*., 2019).

2.4.3 Bacterial DNA Extraction

To recover genomic DNA from an overnight-growing bacterial colony, a boiling process was employed. Initially, colonies were extracted using clean swabs from a petri dish containing a pure culture of candidate bacterial species. They were then transferred to an Eppendorf tube along with 100µl of nuclease-free water and subjected to boiling in a water bath at 95°C for duration of 5 minutes. Subsequently, the mixture was placed in a -20°C freezer for 10 minutes to exert pressure on the bacterial cells and facilitate the release of internal components. This process was repeated, and the resulting suspension was subjected to centrifugation for 1 minute at 12,000 rpm (Parvej *et al.,* 2016). Using a micropipette, 80µl of supernatant was extracted for further processing. To assess the content and purity of the obtained DNA, gel electrophoresis (1.5%

agarose gel) was employed. Spectrophotometric analysis using a NanoDrop Spectrophotometer was performed to ensure the quality and quantity of the extracted DNA. All DNA samples were stored at -20°C for future analysis.

2.4.4 PCR Amplification of DNA product for bacterial identification

All bacterial colonies that were presumptively identified based on biochemical and phenotypic characteristics underwent molecular identification through Polymerase Chain Reaction (PCR. This process involved the use of a thermal cycler (Applied Biosystems TM Proflex TM 3*32-well by Thermo Fisher Scientific). Specific primers, forward and reverse, designed to produce a product of approximately 585 base pairs targeting *E. coli* and 796 base pairs targeting *Salmonella* spp., were employed for PCR amplification. PCR was executed using a master mix (Bioneer premix-Korea). Detailed primer information is available in Table 2.1 below. The PCR amplification for *E. coli* was carried out under the following conditions: initial denaturation steps at 95°C for 5 minutes, final denaturation at 94°C for 30 seconds, annealing at 58°C and 550°C for 30 seconds, extension at 72°C for 30 seconds, followed by final extension at 72°C for 10 minutes. The reaction consisted of 35 cycles, with a final cooling step at 40°C. PCR products (Amplicons) were analyzed via agarose gel electrophoresis using a 1.5% gel stained with ethidium bromide. Positive bands were visualized under an ultraviolet transillumination machine (Tan *et al*., 2016).

Table 2.1: Primers used for amplification of *E. coli* and *Salmonella* ssp.

Bacteria	Primer name	Primer sequence	Size of the PCR product
Escherichia	16s Forward	5′GACCTCGGTTTAGTTCACAGA 3′	585bp
	16s Reverse	5′CACACGCTGACGCTGACCA 3′	
Salmonella	16s Forward	5′ CGGTGGTTTTAAGCGTACTCTT 3′	796bp
	16s Reverse	5′ CGAATATGCTCCACAAGGTTAA 3′	

Source: (Azim *et al.,* 2021; Taşkale *et al.,* 2018)

2.4.5 Detection of Post-PCR products through agarose gel electrophoresis

The final step involved separating PCR amplicons on an agarose gel using 1.5% tris EDTA (TBE) buffer. This process was conducted at 120V for 45 minutes, with visualization aided by gel red staining under a UV transilluminator. These procedures were carried out utilizing a gel imaging and documentation system (EZ GelDoc, Bio-Rad, USA).

2.4.6 Data analysis

The raw data were cleaned and entered in Microsoft Excel spread sheets. Descriptive and inferential statistics were computed with the aid of the Statistical Package for the Social Science (SPSS) software version 25, created by IBM Corporation, Armonk, NY, USA in 2017. Prevalence of two species was calculated using the formula below;

$$P = N_o/N_T \times 100\ \%$$

Where P – Prevalence, N_o – number of positive samples and N_T – number of total samples tested. Descriptive statistical analysis was used to illustrate rodent species composition. Inferential statistical tests were run at p-value ≤ 0.05 to test a null hypothesis that there is no significant variation in the prevalence of candidate pathogens between species, sexes and habitats.

2.5 Results

2.5.1 Rodent species composition and abundance in selected habitats

The sexes of the captured rodents were almost equally distributed, with males exceeding females by a mere 51%. A total of 153 hundred rodents belonging to three species were captured. *Rattus rattus* was the most abundant (75.2%) rodent species in all selected habitats. On the other hand, the farmland had the least abundance (1.3%) of rodents captured, irrespective of species. However, *Mastomys natalensis* was the most abundant species captured in farmlands (23.5%), as displayed as displayed in Table 2.2.

Table 2.2: Rodents species composition and abundance in selected habitats

Types	Genus/Species	House hold	Storage facility	Farm area	Abundance (%)
Rodents	Rattus rattus	96	19	0	75.2
	Mastomys natalensis	0	0	36	23.5
	Mus musculus	0	0	2	1.3
Total		96	19	38	100

2.5.2 Isolation and identification of *E. coli* and *Salmonella* spp.

Morphological characteristics on the 153 primary cultures derived from deep rectal swab inoculums, included small and medium pink colonies selected as suspect candidates for *E. coli* while black colonies sizes were suspected to be *Salmonella* spp. A total of 35 primary isolates were subcultured to form pure culture colonies of suspected *E. coli* and the other 2 isolates were suspected to belong to the *Salmonella* spp. *E. coli* were initially subcultured onto sorbitol MacConkey agar to detect pathogenic species where only one out of these was positive (Figure 2.2). On the other hand, the 2 *Salmonella* spp*.* suspect subjected to biochemical tests including TSI and IMViC remained to suggestive to be *Salmonella* spp. All the isolates were archived at a temperature of -20°C for further confirmation using molecular assays.

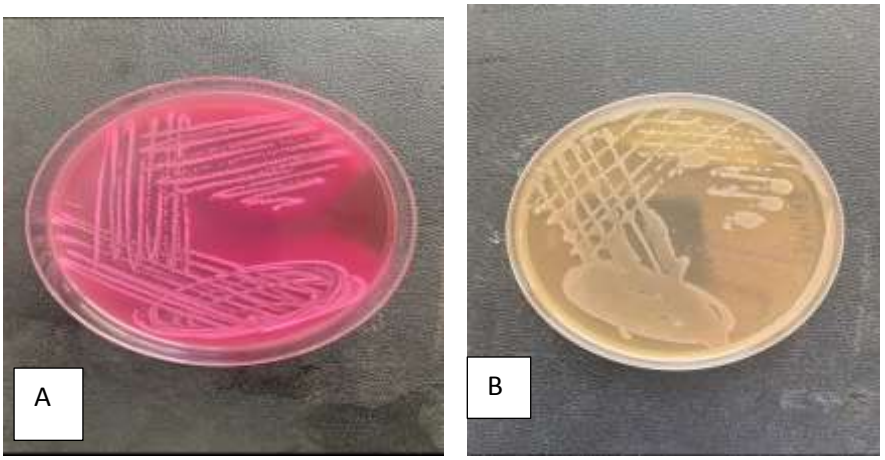

Figure 2.2: The results of *E. coli* fermentation on sorbital McMonkey: None sorbital fermenting colonies is the characteristic of pathogenicity (A) while the pinkish colonies are the characteristic of non-pathogenicity (B)

2.5.3 Molecular identification of the *E. coli* and *Salmonella* spp. suspect isolates

Out of 35 suspected *E*. *coli* isolates, 17 were confirmed to be *E. coli* using primer-specific PCR targeting to amplify the 585 bp region (Figure 2.3). Majority (77%) of them were recovered from *Rattus rattus* while the remaining 5 (33%) were recovered from *Mystomes natanlensis.* On the other hand, none of the 2 *Salmonellas* spp. suspect isolates were confirmed to be positive in molecular assays.

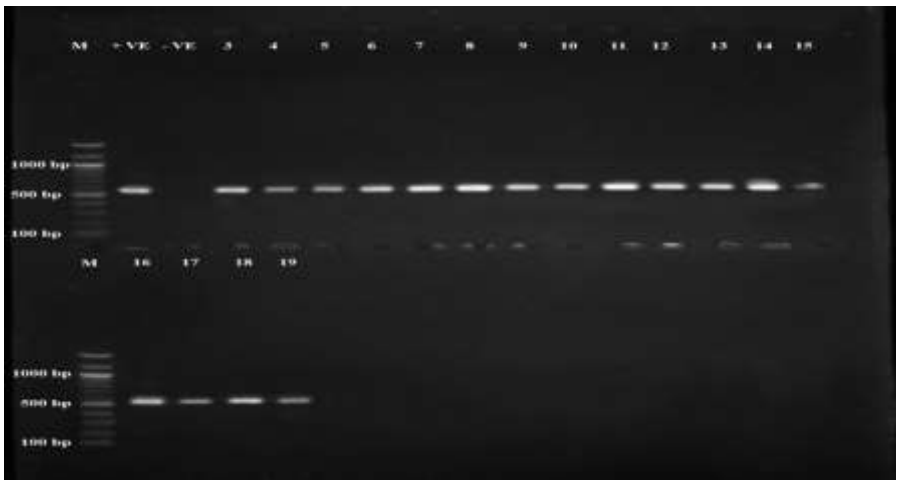

Figure 2.3: Molecular detection of *E coli*; M is a DNA molecular marker, first lane is the positive control followed by negative control. Then lanes 3-19 are positive samples having an expected band size of 585bp.

2.5.4 Prevalence of *E. coli* and *Salmonella* spp. isolated from a diversity of rodents

The results showed a prevalence of 11.1% and 0% for *E. coli* and *Salmonella* spp, respectively. Based on rodent species captured in all wards, *E. coli* was isolated from *Rattus rattus* (12/115), *Mastomys natalensis* (5/36) and *Mus musculus* (0/2) as shown in Table 2.3 The result summarized in Table 2.4 revealed that there was no statistically significant variation in the prevalence of all *E. coli* among rodent species ($p>0.005$).

Table 2.3: Prevalence of *E. coli* from three species of captured rodents

			Positive samples from molecular identification
Captured group	Species captured	No. individuals	*E. coli*
Rodent	*Rattus rattus*	115	12
	Mystomys natalensis	36	5
		0	0
	Mus musculus	2	0
	Total	153	17
	Prevalence (%)	100	11.1

Table 2.4: Association molecular detected positive *E. coli* with selected variable (rodents species, habitats and sex)

Selected variables		*E. coli*		Chi-square	df	p-value
		Positive	Negative			
Rodent species	*Rattus rattus*	12	103	0.585	2	0.747
	Mystomys natalensis	5	31			
	Mus musculus	0	2			
Habitats	Maize farm	5	26	2.683	3	0.443
	Household	11	86			
	Storage facility	0	15			
	Tomato farm	1	9			
Sex	Male	13	63	5.794	2	0.55
	Female	4	73			

2.6 Discussion

The results of this study found that a total of 153 rodents from 3 different species were captured from the selected habitats in all 8 wards. The rodent species captured in the study included *Rattus rattus, Mastomys natalensis and Mus musculus*. Most species of rodents identified in this study were habitat specific with few generalist species. This implies that the diversity and abundance of rodent species varied with respect to habitat structure, as similarly

reported by Chidodo *et al.* (2020). *Rattus rattus* and *Mastomys natalensis* were observed to be the most abundant species, displaying the ability to successfully colonize households and farm areas, respectively. These findings are consistent with the majority of global investigations such as in Nigeria, South Africa, the United Kingdom, Canada, and Trinidad and Tobago, which indicated that *Rattus rattus* was a dominant species in human dwellings (Hilton *et al.*, 2002; Meerburg and Kijlstra, 2007; Nkogwe *et al.,* 2011; Wakawa *et al*., 2015; Ramatla *et al*., 2019).

The prevalence of the habitat generalist species varied between habitats where *Mastomys natalensis* was more prevalent in fallow lands and farmlands while *Rattus rattus* was more prevalent in households compared to other habitats. This finding is similar to that provided in a study conducted by Lema and Magige. (2018), as well as Nkwabi *et al.* (2018), who observed that *Mastomys natalensis* and *Rattus rattus* were the most prevalent rodent species in human-exploited habitats compared to protected areas.

Furthermore, Ndakidemi *et al*. (2023) similarly found that the greatest numbers of *Mastomys natalensis* were captured on peridomestic fields, especially around agricultural field interfaces. However we report *Mus musculus* to be the least (1.3%) abundant species contrary to reports by Ndakidemi *et al.* (2023), who reported a greater (15.8%) abundance of the species in Arusha, a city within the same country as well as Agbonlahor *et al*. (2017), who also reported a greater (39.4%) abundance of the same species in a study conducted in Nigeria.

This study reveals a noticeably low prevalence of 11.1 % *E. coli* isolated from rodents compared to a higher prevalence of (79.2%) of the same pathogen in rodents reported by Sonola *et al.* (2021) in study conducted within the same country. This observation implies that the threat of zoonotic pathogen transmission from rodents to humans is relatively low in Iringa compared to other regions in the

country. However, the varying prevalence observed in the two studies could be resultantly influenced by the varying sample sizes employed in each study. However, another study conducted by Nkongwe *et al.* (2011) found a greater prevalence (83%) of the same pathogen in rodents in Trinidad and Tobago while Khan *et al*. (2022) suspected that variations of these results in such prevalence could be influenced by associated variations in the nucleic acid isolation technique employed and composition of rodent species captured in a particular study.

On the other hand, this study observed none of *Salmonella* spp. isolated from captured rodents. These findings are similar to those reported in other studies by Pocock *et al*. (2001) in the United Kingdom and Kozak *et al*. (2009), who reported the same prevalence of 0% on *Salmonella* spp in rodents sampled in Canada. Contrary to our findings, studies by Himsworth *et al*. (2015) and Camba *et al.* (2020) have reported a relatively higher prevalence of *Salmonella* spp. to be 0.5% and 10% in rodents in Canada and Japan, respectively. The lack of *Salmonella* spp. in the gastrointestinal tracts of rodents might be due to their lack of exposure to contaminated media (Kozak *et al*., 2009).The variation in the prevalence of the *E.coli* among species of rodents, sexes and habitats from the sampled rodents lack statistical significance. This lack of statistical significance could imply that the prevalence of the pathogen is tailored to rodent biology regardless of species, sexes and habitats and faced equal risk of infection.

2.7 Conclusions and Recommendations

The instances of *E. coli* were predominantly found in *Mastomys natalensis* and *Rattus rattus*, out of all species that were captured. Notably, these two species, which have a conspicuously close interaction with humans and domestic animals, are potential carriers of the pathogen. This anticipated information holds epidemiological significance and can be employed in the development of effective preventive and control measures.

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Conflict of interest
None

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CHAPTER THREE

Manuscript Two

Detection of Antimicrobial Resistance Pattern of *Escherichia coli* Isolated from Domestic and Peri-domestic Rodents in Iringa Municipality, Tanzania

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Abstract

Antimicrobial resistance in pathogenic bacterial species is a growing global public health concern. Small mammals are ubiquitous in nature where they intensively interact with human and other domestic animal environments. They hence form a group of small mammals that are highly at risk of exposure to antimicrobials and transmit antimicrobial resistant agents to humans and domestic animals. This study aimed to establish phenotypic and molecular patterns of antimicrobial resistance in *E. coli* isolated from rodents captured in Iringa municipal. A cross-sectional study design was employed whereby various rodents were captured from various selected habitats. Deep rectal swabs were then collected from captured rodents. The pathogens were isolated and identified with the aid of conventional and molecular techniques. Kirby – Bauer disk diffusion method used to identify phenotypic resistance against selected antibiotics. Conventional PCR was used to detect seven antibiotic resistance genes. *Mastomys natalensis*, *Rattus rattus* and *Mus musculus* were identified as species harbouring the pathogen of interest in this study. All (17) isolates appeared to be susceptible to Gentamicin, while almost 23.5%, 29.4% and 23.5% of these isolates were resistant to Ciprofloxacin, Tetracycline and Ceftriaxone respectively. Four *E. coli* isolates were found to resist more than two antimicrobial agents, and the majority (75%) of these ware obtained from a similar study area (Isakililo ward), showed resistance to Ciprofloxacin, Sulphamethaxazole/ trimethoprim and Ceftriaxone. Molecular detection by PCR method revealed the presence of six resistance genes among the 17 *E. coli* isolates assayed, where the *acr(A)* gene was the most abundant resistance gene detected in all (100%) of the isolates. The occurrence of more than one resistance gene in a single isolate was found, where *sul2*, *acr(A)*, and *aac(3)-1* were frequently detected. We recommend observation of regular antimicrobial resistance screening and improvement in rodent management and control programs in the studied area.

Keywords: *E. coli*, antimicrobial resistance, resistance gene, multidrug resistance

3.1 Introduction

Antimicrobial resistance, a term denoting the capacity of infectious microorganisms like bacteria, fungi, and parasites to withstand antimicrobials intended to eradicate them, can either be inherent or acquired through mutations due to prolonged antimicrobial exposure (WHO, 2021). The escalating global public health crisis of antimicrobial resistance is exacerbated by the excessive and improper use of antimicrobials in various sector include human health, veterinary medicine, and agriculture, aiming at disease control, enhanced productivity, and growth promotion in food animals (Furness *et al*., 2017; Dramé *et al*., 2020; Zanardi *et al*., 2020). Over time, bacteria colonizing humans or animals often develop resistance to specific antimicrobials following extended exposure (Allen *et al*., 2016). In the natural environment, antimicrobial resistance emerges from the natural production of antimicrobial substances by microorganisms like bacteria and fungi, horizontal transmission of resistance genes, and exposure to pollutants resulting from everyday human activities involving antimicrobial use (Zanardi *et al.,* 2020).

Small mammals, which are widespread in nature, closely interact with human and other animal environments, especially in agricultural settings where small animals face significant exposure to antimicrobials frequently utilized in farming (Zanardi *et al.,* 2020). Among these small mammals, rodents represent a group capable of potentially harbouring and transmitting *E. coli* to humans and animals (Adhikari *et al.,* 2002; Tiller *et al.,* 2010). *E. coli* is commonly associated with various diseases, with the most well-known being gastrointestinal infections. *E. coli* strains can cause food poisoning, leading to symptoms such as diarrhoea, abdominal pain, and sometimes vomiting (Gambushe *et al*., 2022), while most *E. coli* strains are harmless and even beneficial in the human gut, certain pathogenic strains can cause more severe illnesses, including urinary tract infections, respiratory infections, and in rare cases, bloodstream infections. These infections can vary in severity and are

typically treated with antimicrobials when necessary (Fung *et al*., 2018).

The choice of antimicrobial to treat diseases caused by *E. coli* and related bacterial infections depends on several factors, including the specific strain of *E. coli*, its antimicrobial susceptibility, and the severity of the infection (Ramos *et al*., 2020). Commonly used antimicrobials for treating *E. coli* infections include fluoroquinolones such as Ciprofloxacin and Levofloxacin and are effective against many *E. coli* strains often used for urinary tract infections and other mild to moderate infections. Beta-lactams including penicillins and cephalosporins are commonly used beta-lactam antimicrobials for *E. coli* infections, especially when dealing with more severe cases (Kaur *et al*., 2022). Furthermore, Sulfonamides including Trimethoprim-sulfamethoxazole (TMP-SMX), also known as Bactrim or Septra, is a combination antimicrobial used for treating *E. coli* infections, particularly for urinary tract infections. However, Carbapenems, including Imipenem and meropenem are powerful antimicrobial reserved for treating highly resistant strains of *E. coli* and severe infections (Paul *et al*., 2022).

It's essential to note that antimicrobial resistance is a growing concern, and the choice of antimicrobial should be guided by susceptibility testing to ensure the most effective treatment. The trend of AMR in *E. coli* is worrisome, and it is increasingly resistant to several classes of antimicrobials. Beta-lactam-resistant *E. coli* strains are becoming increasingly resistant to Fluoroquinolones as well as beta-lactam antimicrobials such as penicillins and cephalosporins where extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase-producing *E. coli* are of particular concern. It is important to note that resistant *E. coli* strains are not limited to healthcare settings but are increasingly being found in the community, complicating treatment options (Rahman *et al*., 2020).

The rising prevalence of antimicrobial-resistant strains of *E. coli* in food animals is now a globally growing concern in public health, where infection from such strains is difficult or impossible to treat .The infection from *E. coli* strains leading to serious illness, prolonged hospitalizations and increased mortality (Sobur *et al.,* 2019). These resistant *E. coli* strains seem to pose intrinsic resistance traits in combination with externally acquired ones, enhancing their virulence (Otigbu *et al.,* 2018). Multiple studies have demonstrated a positive correlation between the development of antimicrobial resistance in zoonotic bacteria and the use of the antimicrobials they resist in veterinary medicine and animal production (Wieczorek and Osek.2018; Abd El-Baky *et al.,* 2014; Otigbu *et al.,* 2018). Unless we take adequate measures to address this problem, the economic and clinical impacts attributable to AMR will be enormous with approximately 10 million deaths yearly and an average annual loss of approximately 3 trillion dollars expected by 2050. Furthermore, the impacts will be predominant in the African and Asian regions (WHO, 2021).

Small mammals have demonstrated diverse ways of transmitting antimicrobial-resistant bacteria to humans and domestic animals in different environmental contexts (Zanardi *et al.,* 2020). Despite limited information on AMR patterns of *E. coli* and a lack of sufficient data on the contribution of environmental factors and pests to AMR surveillance, only one study by Sonola *et al*. (2021) has been conducted to reveal AMR patterns on these pathogens isolated from uncommon reservoirs. Therefore, this study aims to investigate the antimicrobial resistance pattern in *E. coli* isolated from rodents in Iringa Municipal. The findings will complement existing findings and provide insights into the antimicrobial profile of these rodents as potential reservoirs in the studied area within the country.

3.2 Materials and Methods

3.2.1 Study area

The research took place in Iringa municipality, Tanzania, situated at 35° 69' east of the Greenwich Meridian, 12.9300'' south of the Equator, and 7° 77' south of the Equator. Iringa municipality consists of urban, peri-urban, and rural areas with a population of 1 192 728 according to the 2022 national census (URT, 2022). The municipality shares borders with Iringa Rural District to the north, west, and south, while Kilolo District Council lies to the east, covering 331.4 km^2. It accounts for 0.9% of the total regional land area, making it the region's smallest council. The municipality is administratively divided into 18 wards, including Mtwivila, Kihesa, Gangilonga, Ruaha, Mshindo, Mivinjeni, Mlandege, Mwangata, Kwakilosa, Makorongoni, Ilala, Mkwawa, Kitwiru, Isakalilo, Kitanzini, Nduli, Igumbilo, and Mkimbizi. Agricultural practices such as vegetable, fruit, potato, maize, banana, and wheat cultivation in the northern and eastern parts and wheat, maize, and bean farming in the southern and western areas create favourable habitats for rodents (Mlyashimbi *et al.,* 2020).

3.2.2 Sampling strategies

A cross-sectional study design was employed. Rodents were collected from January to March 2023. Study sites possessing rodent habitats; human residences, storage facilities, and agriculture settings in the vicinity of human settlements were purposively selected. Eight wards (sampling sites) were selected.

3.3 Strategy for Capturing Rodents

3.3.1 Trapping in households and storage facilities

Within the eight designated wards, eligible households were deliberately identified for inclusion in sampling. The selection process involved consulting residents to confirm the presence of rodents in their homes where 8 houses were purposively selected per ward. A total of 40 locally crafted live wire traps were deployed every night, with a maximum of five traps per house. The allocation

depended on the house's dimensions and cues provided by residents indicating rodent activity. These traps were enticingly loaded with tomatoes and a blend of peanut butter along with small fish. The traps were strategically positioned at 1800 hours and inspected at 0700 hours for three consecutive nights to maximize the capture rate (Mulungu *et al*., 2008).

3.3.2 Trapping in various agricultural settings

To capture rodents in these diverse environments, particularly maize and tomato farms, we utilized Sherman live traps which is known as Large Folding Aluminium trap (LFA), employing a bait mixture of peanut butter and maize bran (Mulungu *et al.,* 2008). Two sampling sites were purposively selected in each of the eight wards. Each night, 100 traps were setup in each sampling location following a grid pattern measuring 100 meters by 100 meters. This grid consisted of 10 lines spaced 10 meters apart, with adjustments made to accommodate landscape variations in different habitats (Katakweba *et al.,* 2012). The traps were positioned at 1600 hours and vigilantly examined at 0700 hours for three consecutive nights to optimize our capture yield.

3.3.3 Field-based rodent identification

The captured rodents underwent anaesthesia with absolute ether, which was soaked with cotton wool, were used and then subjected to species-level identification by a seasoned taxonomist (Katakweba *et al.,* 2012). Additionally, morphometric data, including body weight (rounded to the nearest gram), head to body length, tail length, hind foot measurements, ear size, and the condition of the vagina or the position of the testes were recorded for more identification (Mulungu *et al.,* 2008).

3.3.4 Sample collection, handling and transportation

To collect samples for laboratory analysis, the abdominal cavity of each anaesthetized rodent was sterilized by methylated spirit and dissected using sterile surgical instruments and forceps. From the

gastrointestinal tract, we obtained deep rectal swabs using sterile microbiology swabs (Nkogwe *et al*., 2011). Each rectal swab was individually placed in a sterile container containing 5 millilitres of maximum recovery transportation media and stored at a temperature of 4°C for preservation, following the method outlined by Richard-Greenblatt *et al*. (2020). All collected samples were stored at the Tanzania Veterinary Laboratory Agency (TVLA Laboratory before being transported to Sokoine University of Agriculture microbiology laboratory, still at 4°C, for isolation of *E. coli* and *Salmonella* spp.

3.3.5 Isolation, identification and biochemical characterization of *E. coli*

The process of bacterial isolation and identification centred on the deep rectal swabs initially collected and preserved. These samples were placed into buffered peptone water and incubated for a period of 24 hours at a temperature of 37°C. To identify *E. coli* a loopful of the culture was introduced onto MacConkey agar and allowed to incubate for 24 hours at 37°C. Sub-culturing was subsequently performed repeatedly until a pure culture of *E. coli* on MacConkey agar was successfully obtained.

Pure culture colonies were then transferred to sorbitol MacConkey agar (SMAC) containing 1 mg/L potassium tellurite, and they were further incubated at 37℃ for a period of 18–24 hours to confirm the presence of pathogenic species. It is noteworthy that all samples that produced a pale colony (sorbitol non-fermenters), were subsequently verified as pathogenic *E. coli* (Sharaf *et al*., 2017). To reveal relevant macroscopic features (colony colour, texture, and size) on primary cultures, candidate bacterial colonies were observed, and selected candidates underwent a Gram stain for initial microscopic identification. A series of biochemical assays, including the triple sugar iron agar, indole test, methyl red test, Voges-Proskauer test and Citrate test, collectively abbreviated as IMViC

test, urease and xylose tests were then conducted to characterize *E. coli* isolates (Himsworth *et al*., 2015).

3.3.6 Phenotypic Antimicrobial susceptibility testing using Kirby – Bauer disk diffusion method

Kirby – Bauer disk diffusion method was employed to test the phenotypic antimicrobial susceptibility status. Five antimicrobial agents representing important antimicrobial classes commonly used in public health for treating various bacterial diseases in humans and animals in the study area were employed, namely; Ciprofloxacin (CIP5µg), Gentamicin (CN10µg), Tetracycline (TE30µg), Ceftriaxone (CRO 30 µg), and Sulphamethazole/trimethoprim (SXT25 µg) supplied by Sigma-Aldrich (St. Louis, MO, USA) were assayed against the *E. coli* isolates. Phenotypic antimicrobial susceptibility testing was performed on all 17 isolates.

A loopful of pure *E. coli* colonies was mixed in normal saline to make suspensions which were adjusted to that of 0.5 McFarland standards ($1.5x10^8$ CFU/mL). Then inoculums were inoculated onto well-dried Muller Hinton agar (Oxoid ltd, UK) plates by using sterile swabs (CLSI VETOIS, 2020). Five commercial antimicrobial discs were placed on top of the medium and then subsequently incubated at 37 °C for 24 hours (Onken *et al*., 2015; Sobur *et al*., 2019). Bacteria growth was then observed on plates initially inoculated by *E. coli* followed by measuring and recording the zone of inhibition diameter in millimetres with the aid of a metric ruler (Humphries *et al*., 2021). The results obtained were classified as susceptible, intermediate or resistant based on Clinical Laboratory Standard Institute guidelines (Table 3.1) (CLSI, 2022). Inference of the *E. coli* phenotypic antimicrobial susceptibility results was made based on breakpoints of disk diffusion for *Enterobacteriaceae* groups.

Table 3.1: Zone Diameter Breakpoints for Antimicrobial Susceptibility test used in this study

Antibiotics tested	Antibiotic code	Disc drug concentration (µg)	Breaking point(mm)		
			Susceptible	Intermediate	Resistance
Ciprofloxacin	CIP	5	≥26	22-25	≤21
Tetracycline	TE	30	≥15	12-14	≤11
Ceftriaxone	CRO	30	≥23	20-22	≤19
Gentamicin	CN	10	≥15	13-14	≤12
Sulphamethazole/ trimethoprim	SXT	25	≥16	11-15	≤10

Source: (CLSI, 2022)

3.4 Molecular Detection of Antibiotic Resistance Genes

3.4.1 Bacterial DNA extraction

By utilizing the boiling method, genomic DNA was recovered from overnight-grown *E. coli* colonies. Briefly, sterile swabs were used to pick colonies from Petri dishes and transfer them to an Eppendorf tube with 100µl of nuclease-free water. The suspension was boiled in a water bath at 95 °C for 5 minutes, before being moved to a -20 °C freezer for 10 minutes. This process was repeated, and the suspension was centrifuged for 1 minute at 12,000 rpm (Parvej *et al*., 2016). Thereafter, 80µl of supernatant was then taken from the initially boiled suspension and then transferred to new Eppendorf tubes. The quality and quantity of extracted DNA was determined in 1.5% Agarose gel and NanoDrop Spectrophotometer respectively. All extracted DNA was kept at -20°C for subsequent analysis.

3.4.2 Polymerase chain reaction (PCR) for detection of antimicrobial resistance genes

The initial detection and confirmation of *E. coli* was conducted based on the amplification of the 585bp segment of the 16srRNA gene using F5′GACCTCGGTTTAGTTCACAGA 3′ and R5′CACACGCTGACGCTGACCA 3′ as forward and reverse primers respectively. The extracted DNA was subjected to conventional PCR with a specific pair of primers targeting seven antimicrobial

resistance genes (Table 3.2). A final volume of 25µl was utilized for PCR, which included 12.5µl of premix, 0.5µl of reverse and forward primers, 7.5µl of nuclease-free water, and 4µl of DNA template. PCR amplification for all seven targeted genes was performed based on manufacturer recommendations (Table 3.2).

PCR amplification cycles included an initial denaturation step at 95 ˚C for 15 minutes, 35 cycles of denaturation at 94 ˚C for 30 seconds, annealing at 55 ˚C for 30 seconds, extension at 72 ˚C for 2 minutes, followed by a final extension step at 72 ˚C for 10 minutes. All PCR reactions were carried out in a GeneAmp® PCR system 9700 (Applied Biosystems, USA), and then the final PCR products for each targeted antimicrobial resistance gene were run on a 1.5% Agarose gel stained with ethidium bromide at 80 volts for 40 minutes, and the results were viewed by an ultraviolet trans-illumination machine.

Table 3.2: Primers used to detect antibiotic resistance genes during the PCR amplification method

Antibiotics	Target Gene	Gene sequence 5'-3'	Size (bp)	A/temp ˚C	Reference
Tetracycline	*tet(A)*	F-GGTTCACTCGAACGACGTCA R-CTGTCCGACAAGTTGCATGA	576	58	Zeadan *et al*.,2022
Ceftriaxone	*bla CTX-M*	F-5'-SCS ATG TGC AGY ACC AGT AA R-5'-CCG CRA TAT GRT TGG TGG TG	554	58	Ripanda *et al*., 2023
	bla SHV	F-ATG CGT TAT ATT CGC CTG TG R-AGC GTT GCC AGT GCT CGA TC	882	58	Ripanda *et al*., 2023
Gentamycin	*aac(3)-1*	F-ACCTACTCCCAACATCAGCC R-ATATAGATCTCACTACGCGC	169	60	Kim *et al*., 2020
Ciprofloxacin	*acr(A)*	F-CTCTCAGGCAGCTTAGCCCTAA R-TGCAGAGGTTCAGTTTTGACTGTT	106	58	Kim *et al*., 2020
Sulfonamides	*Sul1*	F-CGGCGTGGGCTACCTGAACG R-GCCGATCGCGTGAAGTTCCG	450	55	Ripanda *et al*., 2023
	Sul2	F-GCGCTCAAGGCAGATGGCATT R-GCGTTTGATACCGGCACCCGT	625	58	Ripanda *et al*., 2023

3.5 Data analysis

The raw data were cleaned and entered in Microsoft Excel spread sheets. Descriptive statistics were computed with the aid of the Statistical Product and Service Solution (SPSS) software version 25, created by IBM Corporation, Armonk, NY, USA in 2017. The abundance of rodents captured from all eight wards was determined by using the total counting method to provide respective species composition. Descriptive statistical analysis was used to present the prevalence of *E. coli* resistant genes.

3.5 Results

3.5.1 Prevalence of *E. coli* from a diversity of rodents

A total of 17 isolates were initially confirmed using primer-specific PCR targeting to amplify the 585 bp region in the *E. coli* genome. The majority (77%) of these 17 *E. coli* PCR-positive isolates were found to belong to *Rattus rattus,* while the remaining 5 (33%) belonged to *Mystomes natanlensis*. None of the 17 PCR-positive isolates belonged to *Mus musculus*. Prevalence of *E. coli* was hence equivalent to 11.1% (17/153) (Table 3.3).

Table 3.3: Prevalence of *E. coli* respective to captured rodent species composition

			Positive samples from molecular identification
Captured group	Species captured	No. individuals	*E. coli*
Rodent	*Rattus rattus*	115	12
	Mystomys natalensis	36	5
		0	0
	Mus musculus	2	0
	Total	153	17
	Prevalence (%)	100	11.1

3.5.2 Phenotypic antimicrobial susceptibility pattern of *E. coli*

All (100%) of the isolates appeared to be susceptible to Gentamicin. It was observed that 23.5%, 29.4% and 23.5% of the isolates were similarly resistant to Ciprofloxacin, Tetracycline and Ceftriaxone respectively. The isolates displayed intermediate resistance to Ciprofloxacin and Ceftriaxone only. At least half of all isolates were completely susceptible to each of the antimicrobials employed in the assay (Table 3.4).

Table 3.4: The antimicrobial susceptibility results of the five antimicrobials tested

Antimicrobial	Pattern =17)					
	S		I		R	
	n	%	N	%	n	%
Ciprofloxacin (5µg)	9	52.9	4	23.5	4	23.5
Tetracycline(30µg)	12	70.6	0	0	5	29.4
Ceftriaxone (30µg)	10	58.8	3	17.7	4	23.5
Gentamicin (10µg)	17	100	0	0	0	0
Sulfamethoxazole /trimethoprim)	8	47.1	0	0	9	52.9

3.5.3 Phenotypic Detection of Multidrug-resistant (MDR) isolates

Four out of seventeen *E. coli* isolates were found resistant to more than two antimicrobial agents, each belonging to separate drug classes. Majority (75%) of MDR isolates were obtained from a similar study site (Isakilo ward). All of these isolates showed resistance to Ciprofloxacin, Sulphamethaxazole/trimethoprim and Ceftriaxone. However, one of these isolates showed resistance to four antimicrobials where Tetracycline was also resisted by this particular isolates (Table 3.5).

Table 3. 5: MDR *E. coli* and their antimicrobial patterns

SN	Sample ID	Collection Site (Ward)	Drugs resisted
1	NDL 6	Nduli	CIP, SXT, CRO
2	IS 86	Isakalilo	CIP, SXT, CRO
3	IS 94	Isakalilo	CIP, SXT, CRO, TE
4	IS 100	Isakalilo	CIP, SXT, CRO

Key: CIP= Ciprofloxacin SXT= Sulphamethaxazole/trimethoprim CRO= Ceftriaxone TE= Tetracycline

3.5.4 Molecular detection of antibiotic resistance genes in *E. coli*

Molecular detection of seven different antimicrobial resistance genes conferring resistance to drugs belonging to various antimicrobial groups by PCR method revealed the presence of 6 resistance genes among the 17 *E. coli* isolates assessed. The *acr(A)* gene was the most abundant resistance gene detected in all (100%) of the isolates. Genes conferring resistance to Ceftriaxone were the least abundant genes where *Bla-CTXM* was detected in only 5.8% of the assessed isolates. Genes conferring resistance to Sulphonamides and Gentamycin were all present in 30% of the 17 isolates assessed (Table 3.6 and Figure 3.1). Of the 17, 9 (52.9%) displaced co-occurrence of resistance genes. The *acr (A)* gene displayed the most frequent co-occurrence with other genes in these isolates. Contrary, the *Bla- CTXM* gene displayed least co-occurrence being detected in only 1(11%) of the isolates. However, six of the assayed genes excluding *Sul1* co-existed in one of these 9 isolates shown Table 3.7.

Table 3.6: Abundance of antimicrobial resistance genes in *E. coli* isolates

Organism	**Targeted antimicrobial resistance genes**						
	Bla-CTXM	*Bla-SHV*	*Sul1*	*Sul2*	*tetA*	*acr(A)*	*aac(3)-1*
***E. coli* n=17**	1(5.8%)	(0)0%	4(23.5%)	6(35.3%)	2(11.8%)	17(100%)	5(29.4 %)

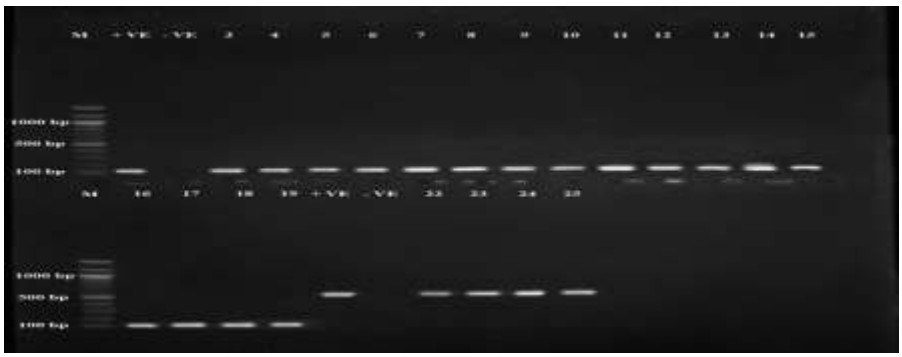

Figure 3.1: Polyacrylamide gel of Ciprofloxacin (*acrA*) and *bla-CTXM* resistance genes. Where M is a 100bp marker and lanes number 1 and 2 are positive and negative control respectively for Ciprofloxacin (*acrA*) whereas lanes number 3-19 are positive samples located at 106bp. Lane numbers 20 and 21 are positive and negative control for *bla-CTXM* respectively whereas lane number 22-25 are samples located at 554bp.

Table 3.7: Co-occurrences of Resistance Genes in *E. coli* Isolates

SN	Sample ID	Collection Site (Ward)	Frequency of Resistance gene
1	MG 121	Makorongoni	*sul1, acr(A)*
2	MK 141	Mkwawa	*sul2, acr(A)*
3	IS 86	Isakalilo	*sul1, acr(A)*
4	MK 143	Mkwawa	*acr(A), aac(3)-1*
5	KZ 101	Kitanzani	*sul2, acr(A)*
6	KT 79	Kitwiru	*tetA acr(A), aac(3)-1*
7	NDL 6	Nduli	*sul2, acr(A), aac(3)-1*
8	MT 37	Mtwivila	*sul1, sul2, acr(A), aac(3)-1*
9	MG 122	Makorongoni	*Bla-CTXM, sul1, sul2, tetA, acr(A), aac(3)-1*

3.6 Discussion

Antimicrobial resistance continues to be a significant public health issue in developing countries, affecting many disease-causing pathogens sourced from diverse origins (Feldgarden *et al*., 2019). The current study was employed to assess various antimicrobial resistance patterns by utilizing phenotypic methods of antibiotic susceptibility testing as well as molecular detection of corresponding

antibiotic resistance genes from *E. coli i*solated from three (*Rattus rattus, Mystomys natalensis* and *Mus musculus)* rodent species in Iringa municipality. This is the first study to determine the antimicrobial susceptibility pattern of this bacterial genus (*Escherichia*) isolated from rodents in this region.

The phenotypic findings of the antimicrobial test performed on *E. coli* isolates showed that all isolates were highly susceptible to Gentamycin while being immediately susceptible to Tetracycline, Ceftriaxone and Ciprofloxacin. Intermediate resistance isolates to Ciprofloxacin and Ceftriaxone are likely to become resistant after prolonged exposure to these antimicrobials., Furthermore, 9/17 (52.9%) of *E. coli* isolates were observed to be resistant to Sulfonamides, these finding was contrary to those obtained by Newberry *et al*. (2021) who showed that 86% of most isolates of the same species isolated from chicken were resistant to the same antimicrobial in Iringa. Rodents are closely interacting with human and other animal environments, especially in agricultural settings where they face significant exposure to antimicrobials frequently utilized in farming (Zanardi *et al.,* 2020). The resistance of isolates obtained from rodents in such settings in this study against this drug might be explained by its comparably more intensified application in Veterinary, Agriculture and Medical field (Uddin *et al*., 2021).

On the other hand, an almost equal 23.5%, 29.4% and 23.5% of the isolates were similarly resistant to Ciprofloxacin, Tetracycline and Ceftriaxone respectively. An average of less than 30% of *E. coli* isolates demonstrated resistance to these antimicrobials. This is different from the findings obtained by Kiiti *et al.* (2021) who reported greater resistance pattern especially for Ceftriaxone (53 %) and Ciprofloxacin (71 %). Likewise high resistance (73%) for tetracycline was reported by Sonola *et al*. (2021) from rodents, chickens, humans and soil. Sulphamethaxazole/trimethoprim was the drug resisted by all multidrug-resistant isolates perhaps highlighting a greater abundance of its respective resistance gene in the bacteria

inhabiting the rodent population in the sampling area. However, incongruence was observed between phenotypic antimicrobial susceptibility patterns to the genotypic antimicrobial patterns of the candidate pathogens observed in this study.

Four out of seventeen *E. coli* isolates were found to resist more than two antimicrobial agents in this study. The detection of multidrug resistant pathogens in rodents has been frequently reported in Tanzania and highlighting the risk of a zoonotic outbreak or pathogen spill in which a multidrug resistant pathogen can cross from candidate reservoir to susceptible species in the studied area (Mshana *et al*., 2013; Ndakidemi *et al*., 2023; Sonola *et al*., 2022). Expectedly, a greater isolation frequency of MDR was noted in study sites occurring in the urban environment where majorities (75%) of multidrug resistant isolates were obtained from a similar study site (Isakilo ward). This finding correlates to data from existing findings which reflects a greater intensity of use or perhaps misuse of the respective resisted antimicrobials, particularly in agricultural and peri-domestic settings where sampling of rodents were conducted (Nhung *et al*., 2015; Ong *et al*., 2020; Sonola *et al*., 2021)**.** A comparative study of AMR patterns between the respective pathogens belonging to separate areas with varying anthropogenic exposure is needed to complement our results on the observed variation in AMR patterns between the two rodent groups. A study by Sonola *et al*. (2021), found just 10.8% of MRD recovered from rodents which is a low occurrence in comparison to what has been obtained in this study. The existence of lower MDR *E. coli* isolates from rodents reflects their lower potential to be primary candidates involved in outbreaks of respective multidrug resistance infections. Furthermore, a lesser isolation frequency of MDR isolates of the same pathogens was also observed in other studies (Guenther *et al*., 2021 Gakuya *et al.,* 2001 Le Huy *et al.,* 2020) conducted in Kenya, Canada, and Vietnam. This variation in the isolation frequency of MDR isolates of the respective pathogens can be attributed to host factors, pathogen factors and human influence.

Molecular detection of seven different antibiotic resistance genes conferring resistance to various antimicrobial groups by PCR method revealed presence of 6 resistance genes among the 17 *E. coli* isolates assayed. The detection of these genes in *E. coli* recovered from rodents hypothesizes the possibility of existing transmission of antimicrobial resistance genes between these reservoir species (rodents) and animal or human sources since these genes have similarly been frequently detected in isolates recovered from the latter (Chen *et al*., 2019; Allen *et al*., 2011; Tate *et al*., 2022).

The *acr(A)* gene was the most abundant resistance gene detected in all (100%) the *E. coli* isolates which coffer to Ciprofloxacin résistance, this could be due to several factors that affect the variety and abundance of antimicrobial-resistant genes in *E. coli*, including the use of antimicrobial agents, environmental variables, spread methods, the nature of the bacterial community, and survival factors (Larsson *et al*., 2022; Tao *et al*., 2022). This is contrary to the study by Sonola *et al*. (2022), which shows the most common resistance gene is *tetA* (46%). It is worth noting that, 14 % of the *E. coli* isolates possessed *aac (3)-1* gene responsible for resistance against gentamicin however, the phenotypic results which characterized these isolates revealed susceptibility to the antimicrobial. This could imply that this particular gene in the bacterium occurs in an operon system that requires an activation signal or factor. Such genes are usually translated by the bacterium under the required physiological conditions that may not have been met in the antimicrobial susceptibility assay. It could also imply that the gene is typically expressed by the bacteria after a typical quorum-sensing event (Cao *et al*., 2019; Li *et al*., 2012)**.** Furthermore, this variation between molecular and phenotypic results could imply that *aac(3)-1* gene is either silent or inactive or that its expression is either incomplete or altered.

In the investigation, a co-occurrence of more than one resistance gene per isolate was discovered in 9 out of 17 isolates which account for 52.9%, where *sul2*, *acr(A)*, and *aac(3)-1* were the most frequently detected, respectively, conferring resistance to trimethoprim/ sulfamethoxazole, ciprofloxacin, and gentamycin. The findings of these *E. coli* isolates with multiple resistance genes obtained from rodents imply that they might transmit these antimicrobial resistance genes to other bacterial species in their respective ecosystems. This transmission becomes of public and veterinary health significance when the bacteria that receive these resistance genes subsequently infect people and animals (Gakuya *et al*., 2001; Aworh *et al*., 2021). Furthermore, this finding aligns with that obtained from a study conducted by Sonola *et al*. (2022) in Arusha, Tanzania, which reported on the co-occurrence of antimicrobial resistance genes in *E. coli* isolates.

Although the antimicrobials with these resistant genes are not used in rodents, the interaction of rodents and the environment where the domestic animals are raised could make these reservoirs harbour these agents, which are essentially used in humans. Many studies have shown the contribution of livestock in accelerating antimicrobial resistance following using antimicrobials in the treatment of animals but also when they are used as prophylaxis and growth promoters. However, this study was limited by the small sample size of rodents captured. Additionally, future studies could also employ other antimicrobials different from the ones used to test AMR patterns of *E*. *coli* isolates in this study.

3.7 Conclusion and Recommendation

The current study reveals antimicrobial resistance patterns of *E. coli* isolated from rodents providing preliminary data on the incidence and drug-resistant features of these pathogens in their respective reservoirs in Iringa municipality. Additionally, the findings from study complements other studies on AMR patterns of *E. coli* isolated from similar reservoir species (rodents), humans and poultry within this

country. We recommend the following as effective measures to prevent possible transmission of resistant bacteria from rodent reservoirs to humans and livestock; (i) proper environmental handling; (ii) close surveillance of AMR bacteria, particularly in possible reservoirs such as rodents; and (iii) increasing public awareness of AMR spreading in our environment.

Ethical statement

Ethical permission was acquired by the Sokoine University of Agriculture's directorate of research, technology transfer, and consultancy review board with reference and publishing committee reference number SUA/ARTC/R/186VoLIV-66.

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Conflict of interest

No conflict of interest was declared by the author.

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CHAPTER FOUR

4.0 General Discussion, Conclusion and Recommendation

4.1 Discussion

Rodents have been important in transmitting pathogens, linking the environment, food chain and humans. It has been noted that they are also carriers of various resistant pathogenic and nonpathogenic bacteria to commonly used antimicrobials. Taking into account that enteric bacteria, which cause enteric diseases and other diseases such as UTI, can be carried by rodents, this study was conducted to assess the occurrence of *E. coli* and *Salmonella* spp and establish their antimicrobial profiles. Based on the results, rodents of two species, *Rattus rattus* and *Mastomys natalensis, were the most prominent. Rattus rattus* was dominant in domestic areas, while *Mastomys natalensis* was dominant in agricultural field areas. These findings are consistent with other reports in Nigeria, South Africa, the United Kingdom, Canada, Trinidad and Tobago (Hilton *et al.*, 2002; Meerburg & Kijlstra, 2007; Nkogwe *et al.,* 2011; Wakawa *et al*., 2015; Ramatla *et al*., 2019). Furthermore, the research findings indicated that the least abundant species was *Mus musculus,* similar to those reported by Ndakidemi *et al. (2023)* in Arusha, Tanzania.

With all the captured rodents, the occurrence of *E. coli was relatively lower (*11.1%) compared to 20% reported by Schaufler *et al*. (2018) in Gabon, West Africa, 34.37% reported by Onanga *et al*. (2020), as well as 79.2% reported by Sonola *et al*. (2021) in Karatu, Tanzania. It was revealed that no *Salmonella* spp (0%) from all species of rodents. The result is consistent with findings documented by Pocock *et al*. (2001) in the United Kingdom and Kozak *et al*. (2009) in Canada. However, some studies have indicated a considerable prevalence of 8.1% in the Democratic Republic of Congo (Falay *et al*., 2022) and 49.1% in Thailand (Ribas *et al*., 2016). Although differences in sample size and sampling strategies play a great role in the variation of prevalence of pathogens, variations in identification approaches for *Salmonella* spp have a considerable

contribution. When using conventional culture identification, bacteria like Proteus and Citrobacter may be accidentally identified as *Salmonella* spp, increasing the prevalence (Sobrinho *et al*., 2021; Sonola *et al*., 2022). The study did not show a substantial difference in pathogen occurrence across rodent species, sexes, or environments, indicating that rodent biology plays a major role in determining pathogen prevalence.

Antimicrobial resistance is a significant public health issue in developing countries, conferred by various disease-causing pathogens. This study assessed antimicrobial resistance patterns in *E. coli* isolated from three rodent species in Iringa municipality. The results showed that all *E. coli* isolates were highly susceptible to Gentamycin while being moderate to Tetracycline, Ceftriaxone, and Ciprofloxacin. Intermediate resistance to Ciprofloxacin and Ceftriaxone was observed in some isolates, while 52.9% of *E. coli* isolates were highly resistant to sulfonamides. The average of less than 30% of *E. coli* isolates demonstrated resistance to Ciprofloxacin, Tetracycline, and Ceftriaxone. Moreover, the results for Tetracycline resistance differ completely from Sonola *et al*. (2021), who reported a relatively higher resistance of 73% obtained from rodents, chickens, humans, and soil. However, incongruence was observed between phenotypic antimicrobial susceptibility patterns and genotypic antimicrobial patterns of the candidate pathogens observed in this study.

Four isolates exhibited multidrug resistance (MDR) against Ciprofloxacin, Sulfamethoxazole/trimethoprim, and Ceftriaxone. The majority (75%) of MDR *E. coli* isolates originated from the same area (Isakalilo ward). This reflects a greater intensity of use of the respective resisted antimicrobial drugs, particularly in agricultural and peri-domestic settings and highlights the potential risk of transmission to the public (Nhung *et al*., 2015; Sonola *et al*., 2021). These findings of MDR isolates of the same pathogens from rodent sources were also observed in other studies by Guenther *et al*.

(2021), Gakuya *et al.* (2001), and Le Huy *et al.* (2020) respectively conducted in Kenya, Canada, and Vietnam. The variation in resistance patterns and occurrences of MDR from the same pathogens (*E. coli*) in this study compared to other studies could be due to host factors, pathogen factors, and human influence (Le Huy *et al*., 2020).

The research reveals the presence of six resistance genes found in all isolates: bla_{CTX-M}, *tetA*, *Sul1*, *Sul2*, *acr(A)*, and *aac (3)-1*. The detection of these genes in *E. coli* recovered from rodents hypothesizes the possibility of existing transmission of antimicrobial resistance genes between these reservoir species (rodents) and animal or human sources since these genes have similarly been frequently detected in isolates recovered from the latter (Allen *et al*., 2011; Tate *et al*., 2022). Moreover, the *acr(A)* gene, which confers resistance to Ciprofloxacin, was the most abundant resistance gene detected in all (100%) E. *coli* isolates. However, this is contrary to the *study by* Sonola *et al*. (2022), which shows that the most common resistance gene is *tetA* (46%). However, it is worth noting that 14 % of the *E. coli* isolates possessed the aac (3)-1 gene responsible for resistance against Gentamicin contrary to phenotypic results, which characterized these isolated as susceptible to the drug. This could be explained by the expression of these putative resistance genes, which may be expressed at any time at a point when pressure is exerted on them. Although the antimicrobials with these resistant genes are not used in rodents, the interaction of rodents and the environment where the domestic animals are raised could make these reservoirs harbour these agents, which are essentially used in humans. Many studies have shown the contribution of livestock in accelerating antimicrobial resistance following using antimicrobials in the treatment of animals but also when they are used as prophylaxis and growth promoters (Rhouma *et al*., 2022). However, few studies have revealed the problem contributed by the environment and its drivers. Therefore, the findings from this study contribute to antimicrobial surveillance data

from the environmental perspective involving rodents, highlighting the potential risks of transmitting these resistant bacteria to humans once they are transmitted through urine and face contaminating foodstuff in Iringa, Tanzania.

4.2 Conclusion

The research in Iringa municipality, Tanzania, found rodents harbouring resistant *E. coli* against commonly used antimicrobials in domestic animals and humans, with MDR attributes. These isolates also possessed resistant genes associated with phenotypic resistance. Rodent control, preventive education, zoonotic disease surveillance, and supporting appropriate sanitation practices are very important in order to block the transmission of resistant *E. coli* to humans in Iringa Municipality.

4.3 Recommendation

The study advises rodent control and preventive education, zoonotic disease surveillance and strengthening control programs through One Health approach, supporting appropriate sanitation practices, and further research on resistance genes.

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APPENDICES

Appendix 1: Research permit from the President Office Regional administrations and local government authorities

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

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

Unapojibu tafadhali taja:-

Kumb. Na. AB.307/323/01

07 Februari, 2023

Katibu Tawala wa Mkoa,
Ofisi ya Mkuu wa Mkoa wa Iringa,
S.L.P 858,
IRINGA.

Yah: **KIBALI CHA KUFANYA UTAFITI KUHUSU DETERMINATION OF ANTIMICROBIAL RESISTANCE PATTERN OF E.COLI AND SALMONELLA SPP, ISOLATED FROM DOMESTIC PERIDOMESTIC RODENTS AND SHREWS IN IRINGA-TANZANIA**

Tafadhali rejea somo tajwa hapo juu.

2. Ofisi ya Rais –TAMISEMI imetoa kibali kwa **Bw. Jackson M. Chrispin, Mwanafunzi** kutoka Chuo Kikuu cha Kilimo Sokoine (SUA) kwa ajili ya kufanya utafiti tajwa katika Halmashauri ya Manispaa ya Iringa.

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 hikl. 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 Iringa).*

Makamu Mkuu wa Chuo,
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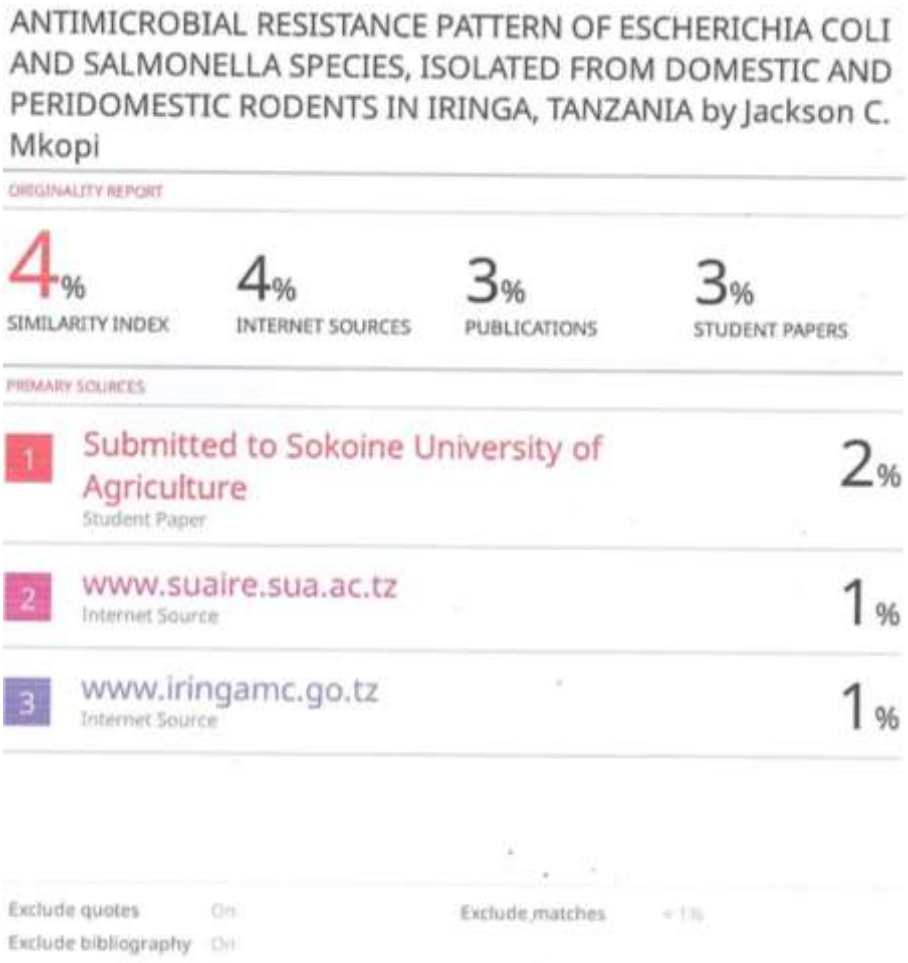
MOROGORO. *(Rejea barua yenye Kumb Na. SUA/ADM/R.1/8/983)*

Bw. Jackson M. Chrispin,
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)*

Appendix 2: Plagiarism report



ANTIMICROBIAL RESISTANCE PATTERN OF ESCHERICHIA COLI AND SALMONELLA SPECIES, ISOLATED FROM DOMESTIC AND PERIDOMESTIC RODENTS IN IRINGA, TANZANIA by Jackson C. Mkopi

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Appendix 3: Research approval statement offered by SUA-DPRTC

STATEMENT OF RESEARCH ETHICAL APPROVAL

1. This project has been considered and has been **Approved/~~Not Approved~~** by the Department/College Research and Publication Committee, Department/College/Unit
   ....
   ....

Signatur[e] .... [Na]me: **Prof. Abdul A.S. Katakweba** Date: **23/6/2023**

(Chairperson, Research & Publication Committee)

2. This project has been considered and has been **Approved/~~Not Approved~~** by the Ethical Committee, DPRTC

Signature: .... Name: DR. Akwilina Mwanri Date: 4/7/2023

(Chairperson, Ethics Committee, DPRTC)

2. This project has been considered and **Approved/Not Approved** by the Committee responsible for Research and Publication, Sokoine University of Agriculture

Signature: .... Name: ErC PRIMPAK Date: 04/07/2023

(Chairperson, SRPC)

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

Kuhusu Tasinifu Hii

Utafiti huu ulilenga kutambua wingi na mifumo ya upinzani wa antibayotiki wa *E. coli* na Salmonella spishi iliyotengwa kutoka kwa panya (153) wa ndani na wa karibu na nyumba katika manispaa ya Iringa, Tanzania. *E. coli* na Salmonella spishi zilitengwa kwa njia ya utamaduni na kutambuliwa kwa vipimo vya kibayokemikali na PCR ya kawaida. Majeni saba yenye upinzani yalipimwa kwa PCR, ambayo ni *blaCTX-M, blaSHV, sul1, sul2, tetA, acr*(A), na *aac(3)-1*. Spishi tatu za panya, *Rattus rattus* (75.2%), *Mastomys natalensis* (23.5%), na *Mus musculus* (1.3%), zilikamatwa. *E. coli* 17 (11.1%) ziligunduliwa, na hakuna Salmonella spishi zilizotengwa. Sampuli zote 17 zilikuwa na uwezo wa kustahimili Gentamycin na upinzani kwa Sulphamethoxazole/Trimethoprim (52.9%). Sampuli nne za *E. coli* zilionyesha upinzani wa dawa nyingi (MDR), ambapo 75% ya sampuli hizi za upinzani wa dawa nyingi (MDR) zilitokea eneo moja. Majeni sita yenye upinzani yaligunduliwa: *blaCTX-M, sul1, sul2, tetA, acr(A),* na *aac(3)-1,* ambapo jeni ya upinzani ya *acr(A)* ilikuwa yenye kawaida zaidi. Kulikuwa na ushirikiano wa majeni yenye upinzani kwa kila sampuli, kama vile *sul2, acr(A*), na *aac(3)-1*. Utafiti huu unafunua upinzani wa antibayotiki wa *E. coli* uliotengwa kutoka kwa panya, ukitoa data ya awali juu ya wingi na sifa za upinzani wa antibayotiki wa vimelea hivi katika vyanzo vyao husika katika manispaa ya Iringa. Utafiti unapendekeza ufuatiliaji wa kawaida wa upinzani wa antibayotiki na kuboresha mipango ya usimamizi na udhibiti wa panya katika eneo lililochunguzwa.