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Research article

Occurrence and antimicrobial resistance pattern of *Escherichia coli* and *Salmonella* species isolated from domestic and peridomestic rodents

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Abstract

E. coli and *Salmonella* species are bacterial pathogens contributing to opportunistic infections, food poisoning, and urinary tract infections. Recently, research has shown that pathogens have repeatedly been found in rodents. Environmental interaction between human, livestock, and rodents is possibly disseminating these bacteria and their antimicrobial resistance. Therefore, this study aimed to determine the prevalence and antimicrobial resistance patterns of *E. coli* and *Salmonella* spp. isolated from domestic and peri-domestic rodents in Iringa municipality, Tanzania. A total of 153 rodents were captured from January to March 2023 and clarified at the species level using identification keys. Rectal swabs were collected. *E. coli* and *Salmonella* spp. were isolated by culture methods and identified by biochemical tests and conventional PCR. The antimicrobial susceptibility test was done by disk diffusion method using five antimicrobials, including tetracycline, ciprofloxacin, gentamicin, ceftriaxone, and trimethoprim-sulfamethoxazole. Seven resistant genes were tested by PCR, which were *blaCTX-M, blaSHV, sul1, sul2, tet*A, *acrA,* and *aac(3)-1.* 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* spp. were isolated. All 17 isolates were susceptible to gentamycin and resistant to sulphamethoxazole/trimethoprim (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, tet*A, *acrA,* 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*, *acrA*, 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 observing regular antimicrobial resistance screening and improving rodent management and control programs in the studied area.

Keywords: Rodent, *E. coli*, *Salmonella,* Antimicrobial-resistance, Resistance gene, MDR

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Introduction

Zoonotic pathogens typically originate from wildlife sources, contributing to roughly 60-70% of all emerging human infections (Azim et al., 2021). *Escherichia* coli (*E. coli*) holds notable significance in the realm of public health, primarily contributing to opportunistic infections and outbreaks within healthcare settings. Additionally, they stand as the leading culprits behind hematogenous and ascending urinary tract infections in human populations (Mirzaei et

al., 2019). 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 within the normal flora (Singleton et al., 2003). This pathogen frequently emerges as one of the most frequently isolated microorganisms in various clinical diarrheal diseases affecting individuals. 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*, a zoonotic bacterial genus, encompasses both typhoidal and non-typhoidal strains predominantly associated with human foodborne infections (Stanaway et al., 2019). Non-typhoid salmonellosis 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, diarrhea, nausea, and sporadic vomiting. While most non-typhoid fevers can be effectively treated, 10-15% of cases may take a severe turn (Crump et al., 2010). 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, on the other hand, stand out as the most extensive group among small mammals, with 2277 known species (Ssuuna et al., 2020; Mlowe et al., 2023). This remarkable order encompasses 29 families, including mice, rats, voles, squirrels, and even beavers, chipmunks, and guinea pigs (Eisen et al., 2018). Their remarkable adaptability allows them to thrive in diverse terrestrial habitats across the globe, excluding only Antarctica (Ssuuna et al., 2020).

A study published by Sonola et al. (2021) and Issae et al. (2023) found a high prevalence of zoonotic bacterial pathogens in rodents in Tanzania. This suggests that rodents could be a reservoir for these bacteria, which can be transmitted to humans. The study identified several bacterial species, including *Borrelia* spp., which can cause Lyme disease in humans (Issae et al., 2023).

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). The multifaceted tapestry of ecological landscapes inhabited by rodents profoundly influences the fluctuating degrees of contamination and the associated risks of transmitting these pathogens to proximate human populations (Tiller et al., 2023). A noteworthy facet of this transmission mechanism lies in the direct deposition of rodent urine and faecal pellets onto consumables 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.

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 sectors, including human health, veterinary medicine, and agriculture, aiming at disease control, enhanced productivity, and growth promotion in food animals (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 are widespread and 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 harboring and transmitting *E. coli* to humans and animals (Sonola et al., 2021). *E. coli* and *Salmonella* spp. are commonly associated with various diseases, with the most well-known being gastrointestinal infections. *E. coli* and *Salmonella* spp. cause food poisoning, leading to symptoms such as diarrhea, 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 *Salmonella* spp-related bacterial infections depends on several factors, including the specific strain of its antimicrobial susceptibility and the severity of the infection (Mdegela et al., 2021). Commonly used antimicrobials for treating *E. coli* and *Salmonella* spp. 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* and *Salmonella* spp infections, especially when dealing with more severe cases (Rahman et al., 2020). Furthermore, sulfonamides, including trimethoprim-sulfamethoxazole (TMP-SMX), is a combination antimicrobial used for treating *E. coli* and *Salmonella* spp. infections, particularly for urinary tract infections. However, carbapenems, including Imipenem and meropenem, are powerful antibiotics reserved for treating highly resistant strains of *E. coli* and severe infections (Paul et al., 2022).

It's essential to note that antimicrobial resistance (AMR) 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* and *Salmonella* spp. is worrisome, and it is increasingly resistant to several classes of antimicrobials. Beta-lactam-resistant *E. coli* and *Salmonella* spp. 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 *amp*C beta-lactamase-producing *E. coli* and *Salmonella* spp. are of particular concern. It is important to note that resistant *E. coli* and *Salmonella* spp. strains are not limited to healthcare settings but are increasingly being found in the community, minimizing treatment options (Rahman et al., 2020).

The rising prevalence of antimicrobial-resistant strains of *E. coli and Salmonella* spp. in food animals is now a globally growing concern in public health, where infection from such strains is difficult to treat, leading to serious illness, prolonged hospitalizations, and increased mortality (Azabo et al., 2019). These resistant *E. coli* and *Salmonella* spp. strains seem to possess intrinsic resistance traits in combination with externally acquired ones, enhancing their virulence (Otigbu et al., 2018). Multiple studies in Tanzania have indicated that antimicrobials, particularly tetracycline, are widely used to treat livestock, and it is associated with the emergence of AMR zoonotic bacteria (Azabo et al., 2022). The widespread irrational use of antimicrobials in veterinary settings creates an environment for AMR. Resistant bacteria can then spread between animals, and potentially even to humans, through direct contact or the food chain (Mdegela et al., 2021).

Unless we take adequate measures to address this problem, the economic and clinical impacts attributable to AMR will be enormous. By 2050, approximately 10 million deaths yearly and an average annual loss of approximately 3 trillion dollars are expected. 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).

Although findings by Sonola et al. (2022) have revealed the presence of these pathogens in rodents and other animal species in Tanzania, the role of these reservoirs as potential drivers that might spread resistant enteric pathogens and contribute to enteric infections remained limited in Iringa. This study, therefore, aimed at assessing the occurrence and respective antimicrobial resistance patterns of *E. coli* and *Salmonella* spp. as zoonotic agents that might be

contributing to gastroenteritis conditions to residents in Iringa Municipal. The results of this study hold significance in producing evidence-based information that contributes to the surveillance of AMR originating from environmental factors, thereby supporting the One Health initiative.

Material and methods

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 (NBS, 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 favorable environment for rodent habitation. Eight wards were selected via purposive sampling based on high population density and reports of rodent infestations coupled with agricultural practices to conduct the study. The selected wards for sampling included Nduli, Kihesa, Mtwivila, Kitwiru, Isakalilo, Kitanzini, Makorongoni, and Mkwawa, as shown in Figure 1. The research sites were documented and marked using a GPS device.

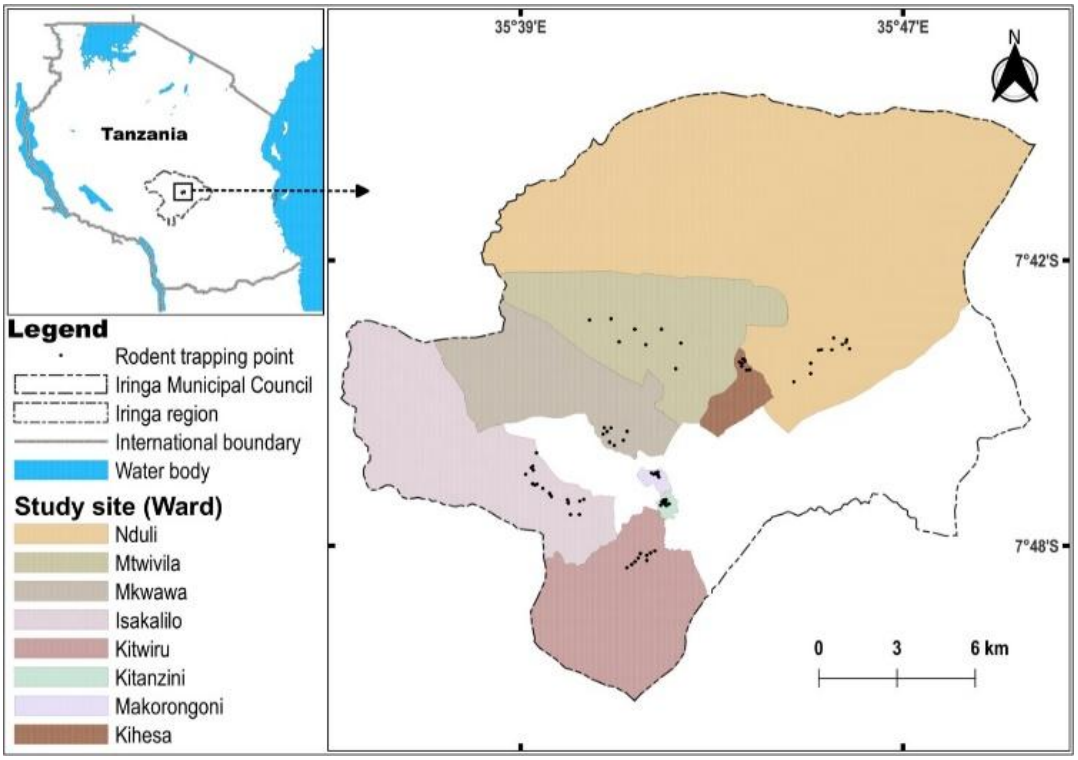

Figure 1: Map of Iringa municipality showing sampling ward. Source: QGIS 3.24.0. CRS EPSG: 4326 Version.

Ethical statement

Ethical permission was acquired by the Sokoine University of Agriculture Directorate of Research, Technology Transfer and Consultancy review board with reference and publishing committee reference number SUA/ARTC/R/186VoLIV-66.

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.

Strategy for capturing rodents

Trapping in households and storage facilities

In each of the eight wards purposefully selected, eight candidate households/storage facilities were recruited 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 household, determined by the house's size and cues indicating rodent presence provided by the household members. These traps were enticingly baited with tomatoes, a blend of peanut butter, and small fish. The traps were strategically placed at 18:00 hrs and examined at 07:00 hrs over three consecutive nights to enhance our capture rate (Mlowe et al., 2023).

Trapping in various agricultural settings

To capture rodents in these diverse environments, particularly maize and tomato farms, we utilized Sherman live traps, which are known as large folding aluminum traps (LFA), employing a bait mixture of peanut butter and maize bran (Mlowe et al., 2023). Two sampling sites were purposively selected in each of the eight wards. We deployed 100 traps each night 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., 2013). Traps were set at 16:00 hrs and diligently checked at 07:00 hours for three consecutive nights to optimize our capture yield.

Field-based rodent identification

The captured rodents underwent anesthesia 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., 2013). Additionally, morphometric data, including body weight (rounded to the nearest gram), head and 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 (Mlowe et al., 2023).

Collection, processing, and transportation of laboratory specimens

To collect samples for laboratory analysis, the abdominal cavity of each anesthetized rodent was sterilized by the 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 milliliters of maximum recovery transportation media and stored at a temperature of 4°C for preservation, following the method outlined by (Ndakidemi et al., 2023). All collected samples were stored at the Tanzania Veterinary Laboratory Agency (TVLA Laboratory before being transported to the Sokoine University of Agriculture microbiology laboratory, still at 4C, for isolation of *E. coli* and *Salmonella* spp.

Bacterial isolation, identification, and biochemical characterization

Isolation of *Escherichia coli*

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

Candidate bacterial colonies were observed to reveal relevant macroscopic features on primary cultures, 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.

Isolation of *Salmonella* spp.

The isolation of *Salmonella* spp. was performed by adding 3 ml of enriched broth to tetrathionate broth, which was then incubated for 24 hrs at

37°C. Subsequently, loops were inoculated onto Xylose Lysine Deoxycholate (XLD) agar (Hardy Diagnostics supplies, California, USA) 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 (Southern Group Laboratory, Corby, UK) and the other to a 10 mL Rappaport Vassiliadis Soy broth (RVSB) tube (Southern Group Laboratory, Corby, UK), both of which were incubated for 24 hrs at 37°C. Finally, one loop of broth culture positive from SFB was later inoculated and incubated on XLD at 37°C for 24 hrs.

Suspected *Salmonella* spp. colonies were transferred into cryopreservation vials for further confirmation through biochemical assays, including Triple Sugar Iron (TSI), Indole, Urease, Simon's citrate, and MR-VP tests (Thomas Scientific, New Jersey, USA).

Bacterial DNA extraction and PCR

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™ Proflex™ 3*32-well (Thermo Fisher Scientific from California, USA). 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 1 (Azim et al., 2021). 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.

Table 1: Primers used for amplification of *E. coli* and *Salmonella* ssp. (Azim et al., 2021).

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

Detection of post-PCR products through agarose gel electrophoresis

The final step involved separating PCR amplicons on an agarose gel using 1.5% tris Tris-Borate-Ethylenediaminetetraacetic acid (EDTA) 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, California, USA).

Phenotypic antimicrobial susceptibility testing using Kirby – Bauer disk diffusion method

Kirby Bauer disk diffusion method was employed to test the phenotypic antimicrobial susceptibility pattern. Five antimicrobial agents representing important antimicrobial classes commonly used in public health for treating various bacterial diseases in humans in the study area were employed, namely: ciprofloxacin (CIP, 5µg), gentamicin (CN, 10µg), tetracycline (TE, 30µg), ceftriaxone (CRO, 30µg), and sulphamethazole/trimethoprim (SXT, 25µg) 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 (1.5×10^8 Colony Forming Units (CFU)/mL). Then, inoculums were inoculated onto Muller Hinton agar (Oxoid Ltd, UK) plates by using sterile swabs. Five commercial antimicrobial discs were placed on top of the medium and then subsequently incubated at 37°C for 24 hrs. Bacteria growth was then observed on plates initially inoculated by *E. coli,* followed by measuring and recording the zone of inhibition diameter in millimeters with the aid of a metric

ruler. The results obtained were classified as susceptible, intermediate, or resistant based on the Clinical Laboratory Standard Institute guidelines of 2022 (Table 2). Inference of the *E. coli* phenotypic antimicrobial susceptibility results based on breakpoints of disk diffusion for *Enterobacteriaceae* groups.

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

DNA was extracted from the bacteria using the thermal extraction/boing method. Briefly, the collected colonies were emulsified in 200µL of nuclease-free water and boiled at 95°C in a water bath before thawing at -20°C for 5 minutes. This process was repeated three times, and then the content was centrifuged at a high speed of 13,000g to collect the supernatant. The extracted DNA was subjected to conventional PCR to detect and confirm *E. coli* with a specific pair of primers targeting the 585bp segment of the 16srRNA. The primers used were F5′-GACCTCGGTTTAGTTCA CAGA-3′ and R5′-CACACGCTGACGCTGACCA-3′ as forward and reverse primers, respectively. The amplification reaction mix constituted a final volume of 25µL 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 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.

Table 2: Zone diameter breakpoints for antimicrobial susceptibility test used in this study according to CLSI 2022.

Antimicrobial tested	Antimicrobial code	Disc drug concentration (µg)	Breaking point (mm)		
			Susceptible	Intermediate	Resistance
Ciprofloxacin	TIP	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
Sulfamethoxazole /trimethoprim	SXT	25	≥16	11-15	≤10

The DNA was further used to detect five antimicrobial resistance genes *tet*A, *bla*$_{CTX-M}$ *bla*$_{SHV}$, *aac (3)-1, acrA), Sul1,* and *Sul2*. The PCR amplification followed the same regimen above with the exception of annealing temperature, where specific resistance genes had their annealing temperature, as shown in Table 3.

All PCR reactions were carried out in a GeneAmp® PCR system 9700 (Applied Biosystems, California, USA). The final PCR products for each targeted antimicrobial resistance gene were run on a 1.5% Agarose gel stained with Gel red at 80 volts for 40 minutes, and the results were viewed using an ultraviolet trans-illumination machine.

Table 3: Primers used to detect antibiotic resistance genes during the PCR amplification method (Ripanda et al., 2023).

Antibiotics	Gene	Gene sequence (5'-3')	Size(bp)	Annealing temperature ˚C
Tetracycline	*Tet*A	F-GGTTCACTCGAACGACGTCA R-CTGTCCGACAAGTTGCATGA	576	58
Ceftriaxone	*bla CTX-M*	F-SCS ATG TGC AGY ACC AGT AA R-CCG CRA TAT GRT TGG TGG TG	554	58
	bla SHV	F-ATG CGT TAT ATT CGC CTG TG R-AGC GTT GCC AGT GCT CGA TC	882	58
Gentamycin	*aac(3)-1*	F-ACCTACTCCCAACATCAGCC R-ATATAGATCTCACTACGCGC	169	60
Ciprofloxacin	*acrA*	F-CTCTCAGGCAGCTTAGCCCTAA R-TGCAGAGGTTCAGTTTTGACTGTT	106	58
Sulfonamides	*Sul1*	F-CGGCGTGGGCTACCTGAACG R-GCCGATCGCGTGAAGTTCCG	450	55
	Sul2	F-GCGCTCAAGGCAGATGGCATT R-GCGTTTGATACCGGCACCCGT	625	58

Data analysis

The raw data were cleaned and entered into Microsoft Excel spreadsheets. Descriptive statistics were computed using 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 using the total counting method to provide respective species composition. Descriptive statistical analysis was used to present the prevalence of *E. coli*-resistant genes.

Results

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 in Table 4.

Table 4: Rodent species composition and abundance in selected habitats.

Types	Genus/Species	Household	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

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 5. The result summarized in Table 6 revealed that there was no statistically significant variation in the prevalence of all *E. coli* among rodent species (p>0.005).

Table 5: Prevalence of *E. coli from* three species of rodents captured.

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

Table 6: Association between *E. coli* prevalence and rodent species, habitats, and sex of rodents.

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			

Phenotypic antimicrobial susceptibility pattern of *E. coli*

All (100%) isolates were susceptible to gentamicin. However, 23.5%, 29.4%, and 23.5% of the isolates were resistant to ciprofloxacin, tetracycline, and ceftriaxone, respectively. The isolates displayed intermediate resistance to ciprofloxacin and ceftriaxone only. At least 50%

of isolates were susceptible to each of the antimicrobials employed in the assay (Table 7).

Phenotypic detection of multidrug-resistant (MDR) isolates

Four out of seventeen *E. coli* isolates were found resistant to more than two different classes of antimicrobial agents (Sonola et al., 2022). The majority (75%) of MDR isolates were obtained from a similar study site (Isakilo ward). All of these isolates showed resistance to ciprofloxacin, sulfamethoxazole/trimethoprim, and ceftriaxone. However, one of these isolates showed resistance to four antimicrobials, whereas tetracycline was also resisted by these particular isolates (Table 8).

Molecular detection of antimicrobial resistance genes in *E. coli*

PCR detection of antimicrobial resistance genes revealed six genes among the 17 *E. coli* isolates assessed. The *acr*A gene was the most abundantly detected in all isolates (100%). Genes conferring resistance to ceftriaxone were the least abundant, whereas *bla*-CTXM was detected in only 5.8% of the *E. coli* isolates. Genes conferring resistance to sulphonamides and gentamycin were found in 30% of 17 isolates, as displayed in Table 9 and Figure 2.

Co-occurrences of resistance genes in *E. coli* isolates

Nine out of 17 (52.9%) isolates displayed co-occurrence of resistance genes. The *acrA* gene could show frequent co-occurrence with other genes in these isolates. The *bla-CTXM* gene was found in only 1 (11%) of the isolates. However, six of the assayed genes, excluding *sul1*, were simultaneously detected in only one of the 9 isolates, as displayed in Table 10.

Table 7: The antimicrobial susceptibility results of the five antimicrobials tested.

Antimicrobial	Pattern (n=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 (25µg)	8	47.1	0	0	9	52.9

S=susceptible I=intermediate R=resistance n= number of isolates

Table 8: 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

CIP= ciprofloxacin SXT= sulphamethoxazole/trimethoprim CRO= ceftriaxone TE= tetracycline

Table 9: Targeted resistant gene abundance in (%) from *E. coli* isolates.

Bacteria	targeted antibiotic-resistance genes						
	bla-CTXM	***bla-SHV***	***sul1***	***sul2***	***tetA***	***acrA***	***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 %)

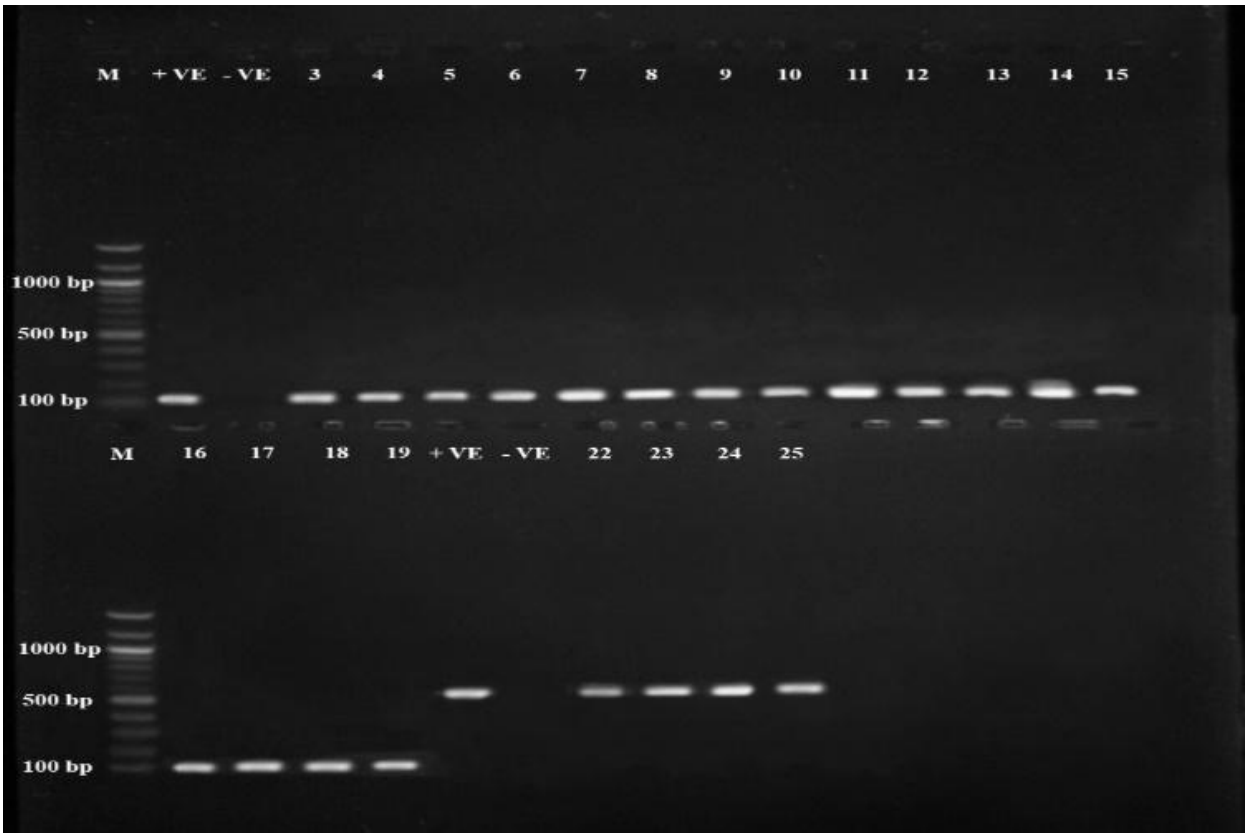

Figure 2: PCR amplification of ciprofloxacin *(acrA) and* bla-CTXM resistance genes. Where M is a 100bp marker and lanes 1 and 2 are positive and negative controls, respectively. Lanes 3-19 are positive samples located at 106bp. Lanes 20 and 21 are positive and negative controls for bla-CTXM, respectively, whereas lane numbers 22-25 are samples located at 554bp.

Table 10: Occurrence of resistance genes among the 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	Kitanzini	*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*

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 urinary tract infection (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 and 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 the 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 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: $bla_{\text{CTX-M}}$, *tetA*, *sul1*, *sul2*, *acrA*, 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 *acrA* 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 harbor 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.

Conclusion

The research in Iringa municipality, Tanzania, found rodents harboring 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.

Article Information

Author contribution. All authors contributed equally.

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