JAVeST Journal Of Applied Veterinary Science and Technology

Journal of Applied Veterinary Science and Technology

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Research Reports

Antimicrobial Resistance Profile of Foodborne *Salmonella spp.* and *E. coli* Isolates from Rodents and Shrews in Morogoro Municipality, Tanzania

Profil Resistensi Antimikroba Isolat *Salmonella spp.* dan *E. coli* dari Rodensia dan Celurut di Morogoro Municipality, Tanzania

Marie Chantal Uwanyirigira[1*], Elias Nelly Bapfakurera[2], Eliza Mwega[3], Sharaduli Kimera[4], Abdul A.S. Katakweba[5,6]

[1]Sokoine University of Agriculture, Morogoro- Tanzania
[2]College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda, Kigali-Rwanda
[3]Department of Vet. Microbiology, Parasitology and Biotech., College of Vet. Med. and Biomed. Sci., Sokoine University of Agriculture, Morogoro-Tanzania
[4]Department of Vet. Medicine and Public Health, College of Vet. Med. and Biomed. Sciences, Sokoine University of Agriculture, Morogoro-Tanzania
[5]Africa Centre of Excellence for Innovative Rodent Pest Manag. and Biosensor Tech. Development, Sokoine University of Agriculture, Morogoro-Tanzania
[6]Institute of Pest Management, Sokoine University of Agriculture, Morogoro-Tanzania

ABSTRACT

Background: Antimicrobial resistance (AMR) is a pressing global public health issue that affects both human and animal health. Small mammals, such as rodents and shrews, represent a significant reservoir of zoonotic infections. However, there is limited documented research specifically addressing *Escherichia coli* and *Salmonella spp.* resistant strains from small mammals. **Purpose:** This study was conducted to examin the occurrence of AMR E. coli and Salmonella spp. and their associated resistance genes in rodents and shrews. **Methods:** A total of 148 fecal samples from rodents and shrews were examined for *E. coli* and *Salmonella spp.* using culture methods and biochemical tests. Fifty isolates were subjected to antibiotic susceptibility testing using the disk diffusion method on Muller-Hinton agar. Genotypic analysis by PCR was used to confirm the bacterial strains and their resistance pattern genes. **Results:** Two bacteria were confirmed in 57 (38.5%) of the fecal samples. Specifically, *E. coli* and *Salmonella spp.* were identified in 54 (36.5%) and 3 (2%) fecal samples, respectively. Antimicrobial susceptibility testing revealed significant resistance in *Rattus rattus*. Molecular analysis confirmed that *Salmonella spp.* carry a larger proportion of resistance genes than *E. coli* . Specific resistance genes, including Sul1 and Sul2, were identified, constituting 15.4% of the total resistance genes. The prevalence of β-lactamase genes was remarkable, with a higher occurrence in *Salmonella spp.* **Conclusion:** In terms of human health, the implications of rodents and shrews serving as carriers and transmitters of various pathogens were highlighted. Given the possibility of unexpected zoonoses arising from the bacteria found in this study, these findings contribute to the understanding AMR in the context of bacterial infections in animals and humans.

ARTICLE INFO

Received: 26 December 2023
Revised: 19 March 2024
Accepted: 24 April 2024
Online: 30 April 2024

***Correspondence:**
Marie Chantal Uwanyirigira
E-mail: muwachantos@gmail.com

Keywords: Antimicrobial Resistance; *Escherichia coli*; Foodborne; Rodents; *Salmonella spp.* ; Shrews

ABSTRAK

Latar Belakang: Resistensi antimikroba (AMR) adalah masalah kesehatan masyarakat global yang mendesak yang mempengaruhi kesehatan manusia dan hewan. Mamalia kecil, seperti tikus dan tikus, merupakan reservoir infeksi zoonosis yang signifikan. Namun, ada penelitian terdokumentasi terbatas yang secara khusus menangani *Escherichia coli* dan *Salmonella spp.* strain resisten dari mamalia kecil. **Tujuan:** Penelitian ini dilakukan untuk menguji terjadinya AMR *Escherichia coli* dan *Salmonella spp.* dan gen resistensi terkait mereka pada tikus dan celurut. **Metode:** Sebanyak 148 sampel tinja dari tikus dan celurut diperiksa untuk mendeteksi *Escherichia coli* dan *Salmonella spp.* menggunakan metode kultur dan tes biokimia. Lima puluh isolat menjadi sasaran pengujian kerentanan antibiotik menggunakan metode difusi disk pada agar Muller-Hinton. Analisis genotip dengan PCR digunakan untuk mengkonfirmasi strain bakteri dan gen pola resistensi mereka. **Hasil:** Dua bakteri dikonfirmasi pada 57 (38,5%) sampel tinja. Secara khusus, *Escherichia coli* dan *Salmonella spp.* diidentifikasi masing-masing pada 54 (36,5%) dan 3 (2%) sampel tinja. Uji kerentanan antimikroba menunjukkan resistensi yang signifikan pada *Rattus rattus*. Analisis molekuler menegaskan bahwa *Salmonella spp* membawa proporsi gen resistensi yang lebih besar daripada *Escherichia coli* . Gen resistensi spesifik, termasuk Sul1 dan Sul2, diidentifikasi, merupakan 15,4% dari total gen resistensi. Prevalensi gen β-laktamase sangat tampak, dengan kejadian yang lebih tinggi pada *Salmonella spp.* **Kesimpulan:** Dalam hal kesehatan manusia, implikasi dari hewan rodensia dan celurut yang berfungsi sebagai pembawa dan penyebar berbagai patogen disorot. Mengingat kemungkinan zoonosis tak terduga yang timbul dari bakteri yang ditemukan dalam penelitian ini, temuan ini berkontribusi pada pemahaman AMR dalam konteks infeksi bakteri pada hewan dan manusia.

Cite This Article:
Uwanyirigira, M.C., Bapfakurera, E.N., Mwega E., Kimera, S., Katakweba, A.A.S., 2024. ***Antimicrobial Resistance Profile of Foodborne Salmonella spp. and E. coli Isolates from Rodents and Shrews in Morogoro Municipality, Tanzania.*** Journal of Applied Veterinary Science and Technology. 5(1): 48-59. https://doi.org/10.20473/javest.V5.I1.2024.48-59

Kata kunci: Celurut; *Escherichia coli* ; *Foodborne*; Resistensi Antibiotik; Rodensia

doi 10.20473/javest.V5.I1.2024.48-59

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INTRODUCTION

The World Health Organization (WHO) has categorized Enterobacteriaceae, including *E. coli* and *Salmonella spp.*, as pathogens of critical priority for antimicrobial resistance (AMR) investigation (Mogasale *et al.*, 2021). In fact, Escherichia coli and Salmonella spp. are the leading causes of foodborne illness (Alshaheeb *et al.*, 2023). Studies have shown that *Salmonella spp.* is the leading cause of various illnesses in both humans and animals, including salmonellosis, typhoid fever, and paratyphoid fever. Meanwhile, *E. coli* causes illnesses that are sometimes severe, such as diarrhea, urinary tract infections, respiratory illnesses, and bloodstream infections (Akbar and Anal, 2011; Ssemanda *et al.*, 2018). The types of *E. coli* that cause diarrhea are spread through contaminated food or water and through contact with animals or humans (Fairbrother, 2006; Ercumen *et al.*, 2017). Therefore, many bacteria from rodents and shrews have been reported as causative agents of AMR, particularly *Salmonella spp.* and *Escherichia coli* (Nkogwe *et al.*, 2011).

Rodents and shrews, which are known as small mammals, are recognized as reservoir organisms for the spread of many zoonotic diseases to humans and other animals (Hill and Brown, 2011; Damborg *et al.*, 2016; Mustapha *et al.*, 2019). They have been identified as the carriers of many microorganisms, including *Escherichia coli, Salmonella spp., Campylobacter spp., Leptospira spp., Orientia tsutsugamushi, Proteus mirabilis*, and Hantavirus (Böge *et al.*, 2021; Ndakidemi *et al.*, 2023). All of these bacteria have the potential to be transmitted to humans by several routes, including the ingestion of food contaminated by rodents and shrews through their feces and urine, inhalation of aerosols containing infectious agents, direct bites from rodents and shrews, and transmission by arthropod vectors that have come into contact with rodents and shrews (Chlebicz and Slizewska, 2018; Hamidi, 2018; Jahan *et al.*, 2021). Fecal matter is a notable reservoir for pathogens, such as *E. coli* and *Salmonella spp.*, which can cause intestinal illnesses in humans and animals (Nkogwe *et al.*, 2011).

Several studies have demonstrated the presence of AMR *Escherichia coli* and *Salmonella spp.* in rodents and shrews inhabiting both urban and rural environments (Katakweba, 2014; Kimwaga *et al.*, 2022; Floramanka *et al.*, 2023). The majority of previous studies examined the resistant phenotype of *Escherichia coli* and *Salmonella spp.* strains obtained from rats (Ball *et al.*, 2019). Therefore, rodents and shrews living nearhuman settlements have the potential to participate in the spread of AMR genes (Benavides *et al.*, 2021). Recently, research has shifted towards studying AMR genes in relation to the resistant phenotype (Benavides *et al.*, 2021; Sonola *et al.*, 2022). While some discrepancies may arise between phenotypic and genotypic results, a broader analysis allows for more accurate predictions (Sonola *et al.*, 2022). From a zoonotic perspective, the emergence of AMR *Escherichia coli* and Salmonella are of particular concerns (Skarżyńska *et al.*, 2020; Sonola *et al.*, 2021). Recent studies on zoonotic E. coli have revealed a significant presence of AMR *E. coli* strains, including those producing extended-spectrum beta-lactamases (ESBLs) and *E. coli* strains carrying virulence genes (Sonola *et al.*, 2022).

The AMR found in *Escherichia coli* and *Salmonella spp.* can be horizontally transferred to different bacterial populations (Ripanda *et al.*, 2023). This phenomenon poses a potential risk of spreading to both humans and the environment, exacerbating AMR (Nelson *et al.*, 2008). Therefore, this study was conducted to examine the occurrence of AMR *Escherichia coli* and *Salmonella spp.* and their associated resistance genes in rodents and shrews captured in Morogoro Municipality.

MATERIAL and METHOD

Study Area

This study was conducted in Morogoro Municipality at 6°85'S and 37°65'E. Morogoro Municipality is located 196 km west of Dar es Salaam, the main city and economic center of Tanzania, and 260 km east of Dodoma, the capital. The study area has a tropical climate with more rainfall in summer than in winter. December has the highest average temperature of 26.9 °C, while July has the lowest at 21.5 °C. The annual temperature variation is 5.4 °C. The wettest and driest months differ by 178 mm in precipitation (Katakweba, 2008) (**Figure 1**).

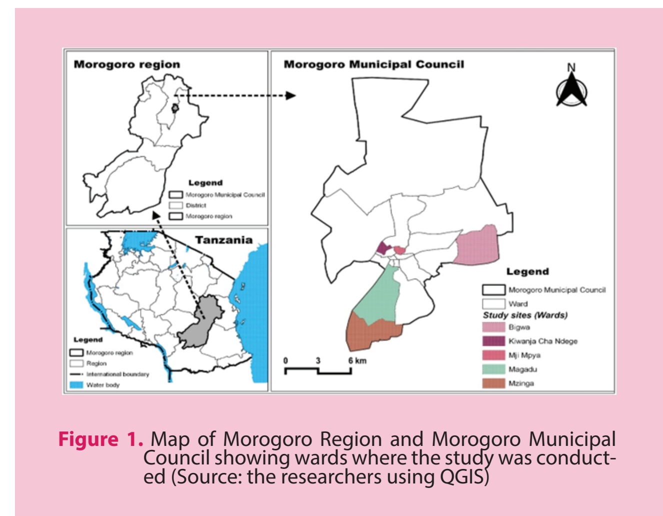


Figure 1. Map of Morogoro Region and Morogoro Municipal Council showing wards where the study was conducted (Source: the researchers using QGIS)

Collection of Fecal Samples from Rodents and Shrews

The trapping sites were selected randomly. Baited traps were placed inside houses, peri-domestic areas, and food markets for three consecutive nights and checked every morning. The captured rodents and shrews were anesthetized using diethyl ether. The gastrointestinal tracts were cut and opened to remove all contents from the small intestine to the caeca of the rodents. Following the methods of Nkogwe *et al.*, (2011), the feces were collected and placed in a sterile container at a temperature of 40°C. They were then transported to the Microbiology Laboratory of the Department of Microbiology, Parasitology, and Biotechnology at Sokoine University of Agriculture (SUA) for subsequent analysis. Cary Blair Transport Medium was used for this purpose (Nagata *et al.*, 2019).

Isolation and Biochemical Identification

For isolation and identification of bacteria from the feces of rodents and shrews, samples were pre-enriched using buffered peptone water (BPW) and incubated at 37°C for18 to 24 h. Using aseptic techniques, 100 µL of the enriched sample was transferred to 10 mL of warmed Rappaport-Vassiliadis soya (RVS) broth (Oxoid) for selective enrichment of Salmonella spp and incubated at 41.5°C for 21 to 27 h. Following incubation, RVS broth was inoculated onto Xylose Lysine Deoxycholate (XLD) agar plate and incubated at 37°C for 21 to 27 h. All XLD plates were observed for Salmonella-like colonies. Positive colonies, which showed red with or without black centers, were sub-cultured to obtain pure colonies. The enriched samples with buffered peptone water in aseptic procedures were also inoculated onto Mac Conkey agar and incubated for 18 to 24 h at 370°C. After incubation, the plates were observed for colonies typical of *E. coli*. Positive colonies, which showed pink, were sub-cultured to obtain pure colonies (Himsworth *et al.*, 2015). All isolated bacteria were confirmed biochemically using the Triple Sugar Iron (TSI), Indole, Methyl Red, Voges Proskauer and Citrate (IMViC), Sulfur, Motility (SIM), and the Urea tests (Nkogwe *et al.*, 2011).

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

Due to financial limitations, the number of isolates that could be included in this study was limited. Therefore, a subset of isolates was randomly selected for the analysis of antimicrobial susceptibility testing. The total number of isolates tested was 50, as this was the maximum capacity of the one antimicrobial susceptibility testing kit. The selected isolates were subjected to antimicrobial susceptibility testing using the disk diffusion method on Muller-Hinton agar (Oxoid Ltd.). Colonies from each sample were lightly touched with a wire loop and inoculated into a tube containing sterile Buffered Peptone Water until the suspension became slightly turbid and matched to the 0.5 Mac Farland turbidity standards. The inoculum was transferred onto well-dried Mueller-Hinton agar plates. Bacteria were spread evenly on top of the Muller-Hinton agar using a sterile swab and were exposed to the antimicrobial diffusion from the antimicrobial impregnated paper disk into the agar medium. In Tanzania, the most commonly used antimicrobials for treating various diseases in humans and animals were selected at the following concentrations: Penicillins (Ampicillin 10 µg), Beta-lactam (Amoxicillin 10 µg), Quinolone (Ciprofloxacin 5µg), Cephalosporins (Ceftriaxone 30 µg, Cefotaxime 30 µg) and Sulfonamides (Sulphamethoxazole/Trimethoprim 25 µg) (Oxoid). The plates were incubated at 37 °C for 24 h (overnight). The results were interpreted according to the guidelines from the Clinical and Laboratory Standards Institute 2021. Isolates were classified as susceptible, intermediate, or resistant according to the interpretation of the zone diameter as recommended by CLSI guidelines (CLSI, 2020). The breakpoints of each antibiotics were considered, as shown in **Table 1.** A multidrug-resistant (MDR) strain was defined as resistant to more than one class of antimicrobials (Magiorakos *et al.*, 2012).

DNA Extraction

The boiling method was used to extract genomic DNA from a bacterial colony that had grown overnight. The colonies were collected from a Petri dish that contained pure cultures of *E. coli* and *Salmonella spp.* using sterile swabs, transferred to an Eppendorf tube containing 100 µl of the nuclease-free water, boiled in a water bath at 95°C for five minutes, and then transferred to a freezer at -20°C for 10 minutes. After this procedure, the suspension was centrifuged at 12,000 rpm for one minute. Eighty microliters of the supernatant were collceted using a micropipette for further processing. The concentration and quality of the extracted DNA were checked by electrophoresis (1% agarose gel) and spectrophotometrically quantified using a NanoDrop Spectrophotometer. All isolated DNA was stored at -200°C until PCR (Dashti *et al.*, 2009; Khosravi *et al.*, 2012; Bagus *et al.*, 2017).

Table 1. Breakpoints of antibiotics used

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

Molecular Identification of Bacterial Species

All isolates presumptively identified based on biochemical and phenotypic characteristics were subjected to molecular identification using the thermal cycler method. The primers (forward and reverse primers) were designed to give a product of approximately 585 base pairs targeting *Escherichia coli*, which are complementary to conserved regions of 16S rRNA genes used for PCR amplification, and 796 base pairs targeting Salmonella spp. used for PCR amplification. InvA gene was also targeted for Salmonella spp. PCR was performed using a master mix (Bioneer PreMix, Korea) (James, 2010; Silva *et al.*, 2011; Nanteza *et al.*, 2020).

The PCR amplification for *Salmonella spp.* and *E. coli* was performed under the following conditions respectively: initial denaturation at 95 °C for five minutes, final denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension

at 72°C for 10 minutes. The reaction was run for 35 cycles and final cooling was maintained at 40°C. The agarose gel (1.5%) stained with ethidium bromide was used to analyze the PCR products (Amplicons) by gel electrophoresis. The ultraviolet transillumination machine visualized the positive bands (Rahayuningtyas *et al.,* 2020).

Identification of Resistance Genes

All bacterial colonies showing phenotypic resistance were screened by PCR to detect several recognized resistance genes to different antibiotics (Moura *et al.,* 2012). Resistance genes were tested using both positive and negative controls. However, it was impossible to obtain positive controls for some screened genes. Optimized and previously published primers and PCR protocols were used without positive controls. The Sul1 and Sul2 genes were amplified under the following conditions: 95°C for five minutes, 35 cycles of 94°C for 30 seconds, 55°C for one minute for Sul1 and 58°C for Sul2, 72°C for two minutes, and final extension at 72°C for 10 minutes. The following PCR amplification settings were used to identify Bla SHV, Bla TEM, and Bla CTXM: initial denaturation at 95°C for five minutes, 35 cycles of denaturation at 94°C for 30 seconds, and annealing at 50°C for Bla TEM and 58°C for Bla SHV and Bla CTXM for 30 seconds (**Table 2**). The extension was at 72°C for two minutes, and the final extension step was 10 minutes at 72°C. The agarose gel (1.5%) stained with ethidium bromide was used to analyze the PCR products (Amplicons) by gel electrophoresis. The ultraviolet transillumination machine was used to visualize positive bands (Rahayuningtyas *et al.,*2020).

Table 2. Primers used to detect AMR genes

Gene	Primer (5' -3')	Size	Annealing	Reference
Sul 1	F -CGGCGTGGGCTACCTGAACG R -GCCGATCGCGTGAAGGTTCCG	450	55⁰C	(Zou *et al.*, 2014)
Sul 2	F- GCGCTCAAGGCAGATGGCATT R- GCGTTTGATACCGGCACCCGT	625	58⁰C	(Zou *et al.*, 2014)
Bla SHV	F - ATGCGTTATATTCGCCTGTG R - AGCGTTGCAGTGCTCGATC	862	58⁰C	(Tofteland *et al.*, 2007)
Bla CTXM	F - SCSATGTGCAGYACCAGTAA R - CCGCRATATGRTTGGTGGTG	554	58⁰C	(Ejaz *et al.*, 2021)
Bla TEM	F -ATGAGTATTCAACATTTCCG R- CCAATGCTTAATCAGTGAGG	858	50⁰C	(Ejaz *et al.*, 2021)
16 s rRNA	F-GACCTCGGTTTAGTTCACAGA R-CACACGCTGACGCTGACCA	585	55⁰C	(James, 2010; Moawad *et al.*, 2017a)
InvA	F-CGGTGGTTTTAAGCGTACTCTT R-CGAATATGCTCCACAAGGTTA	796	58⁰C	(Silva *et al.*, 2011, Paião *et al.*, 2013)

Percentage Calculation of Multi-Drug Resistance Genes

The percentage of resistance genes of *E. coli* and *Salmonella spp.* was calculated using the following formula: % = N′*n′/100 where N′ is N*n, N′ is the total number of bacteria tested for antibiotic resistance for each gene, n′ is the total number of bacteria showing resistance on the targeted gene per each species of bacteria, N is the number of bacteria tested, and n is the number of bacteria showing resistance on the antimicrobial targeted on each gene.

RESULTS

Isolation and Biochemical Identification

A total of 148 fecal samples from small mammals were analyzed, of which 77.7% (115 samples) were suspected for being positive for *E. coli* and *Salmonella spp.* using the culture method. A total of 115 bacterial isolates from the whole samples were retained for further identification with biochemical tests. Of these isolates, 36.5% (54 samples) were identified as *Escherichia coli*. In comparison, *Salmonella spp.* identified in 2% (3 samples) using the Triple Sugar Iron (TSI), IMViC, SIM, and urea tests. Accordingly, these results showed that Escherichia coli was more prevalent than *Salmonella spp.* All bacterial isolates were stored in a freezer below -80°C for molecular confirmation.

Percentage of AMR in Bacterial Isolates

Of 50 bacterial isolates identified as *E. coli* and *Salmonella spp.*, 47 isolates were *E. coli*, and three isolates were *Salmonella spp.* In addition, 19 isolates were from *Rattus rattus*, 24 isolates from *Mus spp.,* five isolates from Cricetomys, and two isolates from shrews. The selected isolates were tested to determine their antimicrobial susceptibility pattern. The high percentage of AMR was observed in *Rattus rattus* species for amoxicillin (89.5%), ampicillin (74%), sulphamethoxazole/trimethoprim (31.6%), and ciprofloxacin (10.5%). In *Mus spp.,* the bacterial isolates showed high resistance to amoxicillin at 87.5%, followed by ampicillin at 62.5%. In Cricetomys, the bacterial isolates were resistant to amoxicillin and ampicillin, each at 80%. Finally, 100% of isolates from shrews were resistant to amoxicillin (**Table 3**).

Table 3. Percentage of AMR of bacterial isolates from rodents and shrews

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

Percentage of AMR in Bacterial Isolates per Sites

Among 50 isolates phenotypically resistant to antimicrobials in *E. coli* and *Salmonella spp.,* resistance to antimicrobials was observed in Mji Mpya and Mzinga for amoxicillin, with 100% resistance in both cases. For ampicillin, resistance was observed in Mji Mpya (77.3%) and Mzinga (100%). *E. coli* showed high resistance to sulphamethoxazole/trimethoprim in Mzinga at 66.7%, followed by Magadu at 50%. Additionally, resistance to ciprofloxacin was observed in Mzinga at 33.3%. The results of antimicrobial resistance testing for *Salmonella spp.* showed a high percentage of resistance in Mzinga for ciprofloxacin, ampicillin, and sulphamethoxazole/trimethoprim, all at 100%. Multidrug resistance (MDR) was observed in Mzinga, Mawenzi, and Magadu for *E. coli.* For *Salmonella spp.,* MDR was observed in Mzinga (**Tables 4 and 5**).

Table 4. Percentage of AMR *E. coli* isolates per site

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

Table 5. Percentage of *Salmonella spp.* isolates per site

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

Resistance Rates of Bacterial Isolates of Small Mammals

Antimicrobial susceptibility testing was performed for *Salmonella spp.* and *E. coli.* The results showed that *Salmonella spp.* (n = 3) had strains resistant to sulphamethoxazole/trimethoprim (33%), amoxicillin (33%), ciprofloxacin (33%), and ampicillin (67%). In addition, *Escherichia coli* (n = 47) had strains resistant to sulphamethoxazole/trimethoprim (11%), amoxicillin (92%), ciprofloxacin (2%), and ampicillin (66%) (**Table 6**).

Table 6. Resistance Rates of Bacterial Isolates From Small Mammals

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

Prevalence of Multidrug Resistance

These results showed that among 50 isolated bacteria tested for AMR, *E. coli* showed multidrug resistance in *Rattus rattus* species (16%), while *Salmonella spp.* showed resistance in Rattus rattus and Mus spp. at 5% and 4.1%, respectively (**Table 7**).

Table 7. Prevalence of Multidrug Resistance Isolated from Small Mammals

Isolates	Species of Rodents and Shrews			
	R. Rattus (n = 19)	*Mus spp.* (n = 24)	*C. ansorgei* (n = 5)	Shrews (n = 2)
Escherichia coli	3 (16%)	0	0	0
Salmonella spp.	1 (5%)	1 (4.1%)	0	0

Molecular Identification of Bacterial Species

PCR amplified *E. coli*, where M is a DNA molecular marker, and lanes 3 to 12 are samples, where lanes 3 to 12 are positive samples at 585 bp, and lanes number 1 and 2 are positive and negative controls, respectively (**Figure 2a**). PCR also amplified *Salmonella spp.,* where M is a DNA molecular marker, lanes 3 to 5 are samples, lanes 3 to 5 are positive samples at 796 bp, and lanes 1 and 2 are positive and negative controls, respectively (**Figure 2b**).

Identification of Resistance Genes

Among 13 bacterial isolates in which AMR genes were detected, the results showed that *Salmonella spp.* had more resistance genes (20%), followed by *E. coli* (12%) (Table 5). Two isolates out of 13 contained sulfonamide-resistance genes, namely Sul1 (n = 1) and Sul2 (n = 1), both representing 15.4% of the total resistance genes analyzed in this study (**Figure 3a, 3b, and 3c and Table 5**). In addition, β-lactamases (BlaTEM, BlaSHV, BlaCTXM) were found in seven isolates (53.8%) with *Salmonella spp.* having more resistance genes compared to *E. coli* (Table 8).

Table 8. Antibiotic-Resistant Genes Detected in *Salmonella spp.* and *E. coli*

Bacterial Species	Number (N)	*Sul1* (*n*)	*Sul2* (*n*)	*SHV* (n)	*TEM* (n)	*CTXM* (n)	Total number (N')	Total resistance (n')	MDR Genes (%)
Salmonella spp.	3	1	0	0	0	2	15	3	20
E. coli	10	0	1	2	2	1	50	6	12
% Resistance genes		7.7	7.7	15.4	15.4	23			

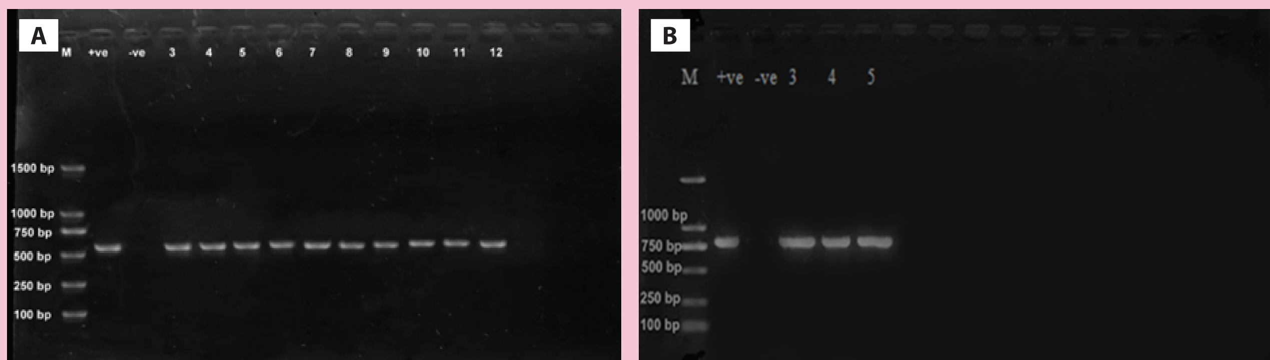
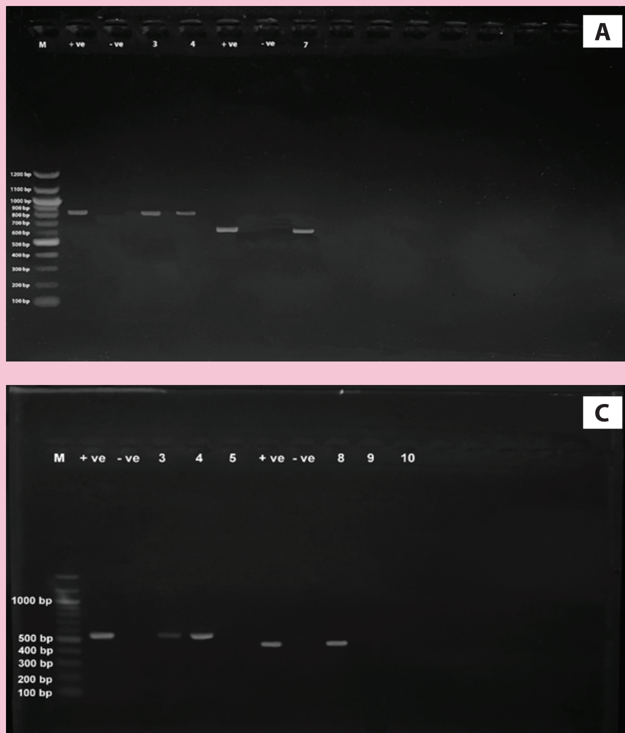
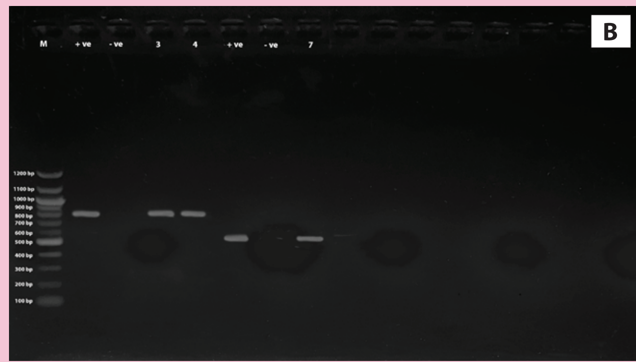


Figure 2. **(A)**. PCR amplification of 16S rRNA for *E. coli*; **(B)**. PCR amplification of InvA for *Salmonella spp.*





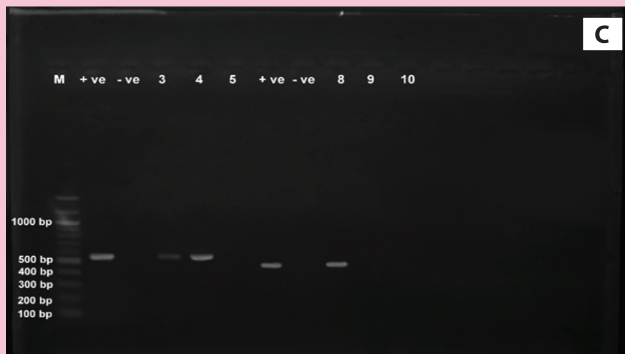


Figure 3. (A): PCR amplification of *E. coli* on Bla TEM and Sul2 resistance gene. M was a 100 bp marker, and lanes 1 and 2 were positive and negative control, respectively, for Bla TEM and lanes number 3 and 4 were positive samples of Bla TEM located at 858 bp, and lane 5 and 6 were positive and negative control, respectively for Sul2 where lane number 7 was positive sample located at 625 bp. **(B)**: PCR amplification of *E. coli* on Bla SHV and Bla CTXM resistance gene. M was a 100bp marker, and lanes 1 and 2 were positive and negative control, respectively, for Bla SHV, whereby lanes 3 and 3,4 were positive samples located at 862 bp, and lanes 5,6 positive and negative control, respectively for Bla CTXM where lane number 7 was positive sample located at 554 bp. **(C)**: PCR amplification of *Salmonella spp.* on Bla CTXM and Sul1 resistance genes. M was a 100 bp marker, lanes 1 and 2 were positive and negative control for Bla CTXM, and lanes 3 and 4 were positive samples for Bla CTXM gene located at 554 bp. Lanes 6 and 7 were positive control and negative control, respectively, for Sul1, and lane 8 was a positive sample for the Sul1 gene located at 450 bp.

DISCUSSION

The study revealed that 77.7% of the samples were suspected positive for *E. coli* and *Salmonella spp.* Biochemical tests identified 36.5% as *E. coli* and 2% as *Salmonella spp.,* indicating a higher prevalence of *E. coli. Rattus rattus* showed remarkable resistance rates, especially to amoxicillin (89.5%), ampicillin (74%), sulphamethoxazole/trimethoprim (31.6%), and ciprofloxacin (10.5%). Site-specific resistance rates varied, with 100% resistance to amoxicillin in Mji Mpya and Mzinga. *Salmonella spp.* strains showed varying resistance profiles, with 33% resistance to sulphamethoxazole/trimethoprim, amoxicillin, and ciprofloxacin and 67% resistance to ampicillin. *E. coli* strains showed resistance to sulphamethoxazole/trimethoprim (11%), amoxicillin (92%), ciprofloxacin (2%), and ampicillin (66%). Multidrug resistance (MDR) was observed in *E. coli* (16% in *Rattus rattus*) and *Salmonella spp.* (5% in *Rattus rattus* and 4.1% in *Mus spp*). The genotypic analysis confirmed the molecular identification with *Salmonella spp.* having more resistance genes (20%) than *E. coli* (12%). Specific resistance genes, such as Sul1 and Sul2 represented 15.4% of the total resistance genes. In addition, β-lactamases (Bla TEM, Bla SHV, Bla CTXM) were found in 53.8% of isolates, with a higher prevalence in *Salmonella spp.*

Antimicrobial resistance associated with bacterial infections in animals and humans has been extensively investigated and documented in Tanzania and several other regions worldwide. The findings of this study are consistent with previous studies on rodent isolates (Jemilehin *et al.,* 2016; Skarżyńska *et al.,* 2020; Zhong *et al.,* 2020; Sonola *et al.,* 2021, 2022). According to Semakula *et al.,* (2015), the presence of *Salmonella spp.* and *E. coli* in rodents can be attributed to environmental interactions between and shared characteristics of the food chain in humans and animals, including livestock and pets. This, in turn, facilitates the spread of AMR traits among different species coexisting in the ecosystem with humans (Kimwaga *et al.,* 2022). The antimicrobials tested in this study have been commonly used in veterinary medicine for the treatment of animals, as evidenced by studies conducted by Katakweba *et al.,*(2012), Kissinga *et al.,* (2018), and Kimera *et al.,* (2020). Furthermore, Kissinga *et al.,* (2018) reported the use of tetracyclines, ampicillin, amoxicillin, and sulfonamides in humans in Morogoro Municipality. Therefore, the presence of humans and animals there has implications for small mammals that are living in the same ecosystem (Oketcho *et al.,* 2012b), such as the search for feed in the homes of animals and humans. These small mammals gain access to human and animal wastes, where resistant bacteria exist.

The prevalence of high ampicillin resistance in *Salmonella spp.* and amoxicillin in *E. coli* was the predominantly observed in the wild small mammal isolates, and its occurrence was strongly associated with the study areas, which are close to humans (Ndakidemi *et al.,* 2022). The prevalence of amoxicillin and ampicillin was not surprising because they are often used as first-line antimicrobials to treat the bacteria in Morogoro, both in humans and animals (Oketcho *et al.,* 2012b). Given that AMR can be induced by the presence of antimicrobials in human and animal wastes, it is plausible that certain strains of *E. coli* and *Salmonella spp.* found in small mammals were directly subjected to selective pressure due to the consumption of human and animal wastes

containing antimicrobials, including amoxicillin and ampicillin (Skarżyńska *et al.,* 2020). Furthermore, small rodents and shrews gain access to antimicrobials in improperly disposed landfills containing expired or unused antimicrobials for human and animal consumption. The study conducted by Kissinga *et al.,* (2018) reported the disposal of expired and unused antimicrobials in Morogoro Municipality through dumps, latrines, and burning. These methods of antimicrobial disposal allow small mammals to gain easy access to those antimicrobials. In addition, these rodents consume chicken feces and occasionally human and child feces from people who do not have access to latrines that contain resistant bacteria or undigested antimicrobials.

The molecular testing using the Sul gene revealed that only 15.4% of isolates showed resistance (Park *et al.,* 2012). This finding was not surprising given that sulfonamides are commonly used antibiotics in animal populations (Katakweba *et al.,* 2014, 2018). Consequently, these compounds are commonly detected as contaminants in humans and animals (Hassell *et al.,* 2019). Therefore, this characteristic makes them prevalent environmental pollutants, which are dispersed in water and assimilated by all living organisms in the respective ecosystems (Grudlewska-Buda *et al.,* 2023). As a result, this phenomenon significantly influences the evolutionary forces driving the development of resistance (Katakweba, 2014).

Ciprofloxacin has been used for more than four decades as a therapeutic approach for the treatment of salmonellosis (Ong *et al.,* 2020). This study revealed that 33% of the isolates were resistant to ciprofloxacin based on the results of the disk diffusion testing. This could be attributed to the widespread use of ciprofloxacin as a standard therapy for *Salmonella spp.* infections (Kira, 2015). The isolates were additionally subjected to disk diffusion to assess the resistance to ampicillin. It was found that only 67% of *Salmonella spp.* and 66% of *E. coli* isolates showed resistance (Kimwaga *et al.,* 2022). In addition, the isolates underwent molecular assessment for resistance by examining the presence of the BlaTEM gene (Sonola *et al.,* 2022). As a result, 53.8% tested positive for the BlaTEM gene. The absence of this gene in isolates with favorable phenotypes suggested that the specific gene targeted by this antibiotic may vary (James, 2010). Examples of such genes include SHV, TEM, and CTX-M. The disk diffusion method also demonstrated higher sensitivity, but lacked specificity in detecting AMR (Benavides *et al.,* 2021). This finding is not only expected but also raises concerns, as a previous study conducted in this region has already reported the presence of AMR in humans (Oketcho *et al.,* 2012a). These findings are supported by studies conducted in Tanzania by Katakweba *et al.,* (2018) and Munuo *et al.,* (2022), who extracted the Sul II genes from fecal samples obtained from animals.

The presence of Sul1 and Sul2 genes in both ampicillin and sulfamethoxazole-trimethoprim-resistant and susceptible isolates indicates the potential existence of "silent" antimicrobial agents within bacterial populations, as reported by Grudlewska-Buda *et al.,* (2023). This finding suggested a potential risk, as dormant genes can become active within a living organism in response to antimicrobial agents (Kariuki *et al.,* 2018). Furthermore, these genes can be transferred to other microorganisms in the intestines and the surrounding environment (Zhang *et al.,* 2015).

The observed resistance to the antimicrobials described above can also be attributed to many other factors, such as inadequate disposal of unused antimicrobials, inappropriate utilization of antimicrobials, frequent use of chemoprophylaxis therapy, and high increase in livestock production (Katakweba *et al.,* 2012; Gatabazi, 2013; Kissinga *et al.,* 2018). Furthermore, this resistance can be attributed to the widespread availability and affordability of antimicrobial drug shops, common practice of self-medication with over-the-counter medications, and repeated exposure to different antimicrobials before obtaining prescriptions from healthcare professionals (Katakweba, 2014). Moreover, Sonola *et al.,* (2021) suggested that the cohabitation of humans and small mammals in a shared environment can result in the transfer of resistance genes between the two species, which may then be deposited in the landscape. The complex relationship between heavy metal exposure, foodborne pathogens, particularly *Salmonella spp.* and *E. coli*, and antimicrobial resistance (AMR) is underscored by multifaceted interdependencies. Environmental contamination with heavy metals from anthropogenic activities infiltrates the food chain, leading to bioaccumulation in plants and subsequent uptake by livestock, thereby culminating in human exposure. At the same time, exposure to heavy metals fosters adaptive responses in bacterial populations, potentially co-selecting for genes associated with both heavy metal and antimicrobial resistance. This co-selection phenomenon amplifies the risk of the spread of AMR in foodborne pathogens, exemplifying the complex dynamics in which environmental factors interact with microbial genetics, thereby contributing to the broader landscape of antimicrobial resistance (Ngwewa *et al.,* 2022).

The phenomenon of multidrug resistance (MDR) was found to be much higher in Salmonella isolates compared to non-multidrug resistant strains, as indicated by previous studies (Magiorakos *et al.,* 2012; Katakweba *et al.,* 2018; Tawyabur *et al.,* 2020). *Salmonella spp.* isolates in this study were shown to possess multi-drug resistance genes, with some isolates having 20% resistance genes among the 20% of theisolates examined (Tawyabur *et al.,* 2020). The presence of multidrug resistance in *Salmonella spp.* has been documented as a cause of sickness in both humans and animals across various countries, such as the United States, Denmark (Aerestrup *et al.,* 2007), Italy (Dionisi *et al.,* 2009), Eastern China (Lu *et al.,* 2014), and Vietnam (Vo *et al.,* 2010). Therefore, there was a significant prevalence of isolates that exhibit resistance to multidrug agents, including amoxicillin, ampicillin, ciprofloxacin, and sulphonamides. These antimicrobial drugs are commonly used in human and veterinary medicine (Katakweba *et al.,* 2012; Sonola *et al.,* 2022). As indicated by Bosco *et al.,* (2012) and Walusansa, (2007), these antibiotics are commonly prescribed for the treatment of gastroenteritis,

salmonellosis, and colibacillosis in animals, which is a serious concern for human and animal health.

Limitations of the Study

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

CONCLUSION

The study provides a comprehensive assessment of AMR profiles in small mammals, highlighting the prevalence, site-specific patterns, and genotypic characteristics of resistance. These findings underscore the importance of integrated strategies to mitigate the spread of AMR and inform public health interventions. The results of this study indicated that most of the *Salmonella spp.* and *E. coli* isolates that infected the rodents and shrews have both phenotypic and genotypic characteristics of AMR. The prevalence of AMR *Salmonella spp.* and *E. coli* in isolates obtained from rodents and shrews in Morogoro Municipality indicated the potential for extensive spread of both resistance genes and bacteria throughout the study areas. This raises concerns regarding the potential for the emergence of difficult-to-treat diseases. The antimicrobials used in this study are extensively used in the study areas for treating humans and animals, indicating the high spread between them. Moreover, the spread of AMR *Salmonella spp.* and *E. coli* through the feces and urine shed by rodents and shrews can lead to environmental contamination. This contamination may subsequently facilitate the transmission of this feature to other dangerous bacteria, such as *Salmonella spp.* and *E. coli*, as well as unexpected bacterial species.

Consequently, this represents a significant public health risk. Therefore, it is necessary to implement comprehensive interventions that adopt a one-health approach to manage the issue effectively. These findings provide a better understanding of the role of rodents and shrews in transmitting and maintaining AMR *Salmonella spp.* and *E. coli*, which has the potential to be transmitted to humans through food

ACKNOWLEDGEMENT

The authors would like to thank the African Centre of Excellence for Innovative (ACEII-IRPM and BTD) at the Sokoine University of Agriculture in Morogoro, Tanzania, for supporting this scientific study. The authors also extend their gratitude to all of the local authority leaders, communities, and government officials in the Morogoro Municipality for their approval of conducting this study. Finally, the authors wish to thank the field technicians, as well as all the laboratory technicians from the Institute of Pest Management, Department of Veterinary Microbiology, Parasitology and Biotechnology, and Genome Center, who have provided helpful support in the laboratory work.

CONFLICT of INTEREST

The authors declare no conflicts of interest associated with this study.

RESEARCH FUNDING

The African Centre of Excellence for Innovative (ACEII-IRPM and BTD) at the Sokoine University of Agriculture in Morogoro, Tanzania, provided financial support for this scientific study with a certificate number 5799-TZ.

ETHICAL APPROVAL

The research protocol involving animals was reviewed and approved by the ethical review board of the Sokoine University of Agriculture with a certificate number SUA/DPRTC/186 VOL IV/82.

AUTHORS' CONTRIBUTIONS

MCU: methodology design, data collection, laboratory work, data analysis, and interpretation, as well as the drafting of the manuscript, ENB: statistical analysis, discussion, and critical manuscript review, EM: laboratory methodology and critical review of the manuscript, SIK: supervisor, overall research management, statistical analysis, data interpretation, writing, and critical manuscript review, AASK: supervisor, overall research management, statistical analysis, data interpretation, writing, and critical manuscript review.

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