

Antimicrobial Profiles of Pathogenic Enteric Bacteria Isolated from Commensal Rodents and Cockroaches from Morogoro Region in Tanzania: An Environmental Vehicle for Resistance Transmission to Human and Animals

Bernadetha Richard Kimwaga ^{1*} Dr. Alexandra Mzula ² Prof. Ladslaus L. Mnyone ³

1. Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3015, Chuo Kikuu, Morogoro, Tanzania
2. Department of Veterinary Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, P. O Box 3015, Chuo kikuu Morogoro, Tanzania
3. Institute of Pest Management, Sokoine University of Agriculture, P.O.BOX 3110, Chuo Kikuu, Morogoro, Tanzania

*E-mail of the corresponding author: bernadetharichardkimwaga@gmail.com

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Abstract

The increase in antimicrobial resistance (AMR) is becoming the global health concern and implicated to be a silence pandemic. It reduces the effectiveness of antimicrobials and threatening the health of human and animals. Rodents and cockroaches can play a significant role in the dissemination of resistance bacteria between the environment and humans. This study aimed at determining antimicrobial resistance profile of enteric bacteria isolated from cockroaches and house rats. The study was cross-sectional in design, the target population included house rats (*Rattus rattus*) and cockroaches (*Periplaneta americana*). A total of 114 house rats and 57 cockroaches were trapped. Cockroaches were manually trapped with surgical gloves and sealed in sterile plastic containers where by house rats were trapped with wire cages and transported to microbiology laboratory. Bacteria were isolated following standard Microbiological techniques and antimicrobial susceptibility testing was performed using Kirby Bauer diffusion technique. Data analysis was done using Graphpad Prism version 9. A total of 157 isolates of three targeted bacterial species were isolated from the gut of 114 house rats and 57 cockroaches. Among isolated bacteria only 83 were subjected to antimicrobial susceptibility test. Out of the tested antimicrobials; Amoxicillin, Ciprofloxacin and Tetracycline showed the highest resistance. Moreover Multidrug-resistance (MDR) was also observed for 66.3%. Generally, these findings indicate that cockroaches and house rats play a significant role of harboring and disseminating pathogenic resistance bacteria, therefore necessary control measures need to be taken against the infestation of rodents and cockroaches around households so as to minimize transmission of pathogenic and resistant bacteria.

Keywords: Antimicrobial resistance (AMR), Enteric bacteria, Rodents and Cockroaches, Multidrug-resistance (MDR)

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1.0 INTRODUCTION

The house rat, *Rattus rattus*, and cockroach, *Periplaneta americana*, are among the most prolific and widespread urban pest species in the world. Both pests are found within and around human and animal habitats. In Africa and most other developing countries, overcrowding and poor sanitary conditions, have been reported to be the major cause of increasing infestation by rodents and cockroaches (Arif *et al.*, 2017; Kassir *et al.*, 2014; Pant-may *et al.*, 2016).

Both the commensal rodents and cockroaches are prominent sources of disease causing agents including viruses, bacteria, protozoa, fungi and helminthes (Omudu & Akosu, 2013; Oyeyemi *et al.*, 2016; Tachbele *et al.*, 2006). Most of these are responsible for human morbidity and mortality in Tanzania and many other parts of the world. Most importantly, rodents and cockroaches are increasingly reported as prominent sources of multidrug resistant strains of bacteria (Donkor, 2019; Gwenzi *et al.*, 2021; Mpuchane *et al.*, 2006; Nkogwe *et al.*, 2011; Solomon *et al.*, 2018; Wannigama *et al.*, 2014). Examples of resistant-bacteria isolated from commensal rodents and cockroaches include *Salmonella* spp, *Shigella* spp, *Campylobacter* spp, *Escherichia coli*, *Klebsiella* spp and *Staphylococcus aureus* (Gwenzi *et al.*, 2021; Moges *et al.*, 2016).

The resistant and disease causing agents carried by rodents and cockroaches can be found on various part of their body including body surface, gastrointestinal tract (GIT) and visceral organs (Moges *et al.*, 2016; Saengthongpinit *et al.*, 2019). Both house rats and cockroaches are likely to be found in dark rooms, kitchens,

bathrooms, food handling and food storage rooms. And due to their ability of moving from one part to another they are easily able to come in contact with garbage, feces, stored food, sewage and biological wastes. So, a close association to human dwelling and environment influence the emergence and spread of pathogenic and most likely antimicrobial resistance bacteria (Gwenzi *et al.*, 2021; Islam *et al.*, 2016; Meerburg and Kijlstra, 2007).

Many studies worldwide, are increasingly reporting the role of commensal rodents and cockroaches in transmitting and/or harbouring pathogenic bacteria and most likely the antimicrobial resistance bacteria to humans and animals (Ayyal *et al.*, 2019; Moges *et al.*, 2016), which are recognized to be the cause of diarrhea, dysentery, typhoid and food poisoning. This brings difficulties in treating bacterial infections (Amin, 2019; Naher *et al.*, 2018; Solomon *et al.*, 2018). Despite this increasing risk, little is known about the role of such pests in transmitting pathogenic and antimicrobial resistant bacteria in Tanzania. This study was designed to assess pathogenic enteric bacteria isolated from cockroaches and house rats and determine their antimicrobial susceptibility patterns in Morogoro region, Tanzania, in order to have a snapshot on the role of the two pests as potential resistance transmission vehicle in home settings in the country Tanzania.

2.0 MATERIAL AND METHODS

2.1 Description of the study area

This study was conducted in Morogoro Municipal and Kilosa district, in Morogoro region. Morogoro Municipal covers an area of 260 km². The district has an average minimum and maximum temperature of 16°C and 33°C respectively. The annual rainfall ranges from 821-1505 mm (Ernest *et al.*, 2017). The main economic activities include subsistence and commercial farming, small scale enterprises and trade.

Kilosa district cover an area of 14,245km² and experience rainfall from November to May. The dry season occurs from June to October. The average annual temperature is 24.6°C (Chipwaza *et al.*, 2015). The main economic activities include agriculture and livestock keeping. Both districts experience high infestation of both rodents and cockroaches.

2.2 Study design

The study followed a cross-sectional study design. The study was conducted from January 2022 to April 2022. Laboratory analyses for both cockroaches and rodents were largely conducted in laboratories within Sokoine University of Agriculture.

2.3 Sampling procedures

Cockroaches and house rats' collection

Cockroaches and house rats were collected from four randomly selected wards, two from each district. Cockroaches and house rats were trapped from 62 randomly selected house hold, 25 from Morogoro Municipal and 37 from Kilosa District. In aseptic conditions cockroaches were manually trapped and stored in sterile containers while house rats were trapped with wire cages.

Cockroaches and house rats' sample processing

Each of trapped cockroaches and rats were identified based on their morphological features with the guidance of identification keys (Kingdon *et al.*, 2013; Picker *et al.*, 2004). Both cockroaches and house rats were sacrificed with chloroform. Cockroaches were individually placed in tubes with 5ml of normal saline (0.85% NaCl) and shaken manually to dislodge bacteria from its body surface; thereafter soaked in 90% ethanol for 5 minutes and dried to further decontaminate their external body surface. They were then rewashed with sterile normal saline to remove traces of ethanol. Both Cockroaches and house rats were dissected aseptically using scissors to remove the hind gut. The hind gut contents were collected in sterile tubes containing maximum recovery diluent media and stored in sterile cool box with ice cubes and transported to Sokoine University of Agriculture for further laboratory analysis.

Bacterial isolation and identification

The hind gut of cockroaches and house rats were separately dissected in aseptic condition and a homogenous suspension of hind gut was prepared in peptone buffered water and incubated at 37°C for 24 hours for enrichment. A loopful of each sample were streaked on differential and selective media (MacConkey) agar (Oxoid Ltd, Basingstoke, UK) and Xylose lysine deoxycholate agar (X.L.D) agar (Oxoid Ltd, Basingstoke, UK) and incubated overnight at 37°C. Single suspected colonies of *E. coli*, *Klebsiella* spp and *Salmonella* spp were subcultured on respective selective media and incubated at 37°C for 24 hours. Pure colonies were then confirmed by a series of biochemical test such as Triple Sugar Iron Agar (TSI) Test, Citrate test and IMViC.

Antimicrobial susceptibility testing for *E. coli*, *Salmonella* spp and *Klebsiella* spp

All confirmed isolates were subjected to antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method. Colonies of each sample were lightly touched with a wire-loop and inoculated in a tube containing sterile normal saline until the suspension became slightly turbid and matched with the 0.5 Mac Farland turbidity

standards. Using a sterile cotton swab, the entire surface of dried Muller Hinton agar (Oxoid Ltd, Basingstoke, UK) plates were streaked by the inoculum. The inoculated plates were left to dry for about fifteen minutes and by using sterile forceps five antibiotic discs namely Tetracycline (TE) (30µg), Gentamicin (CN) (10 µg), Ciprofloxacin (CIP) (5µg), Ampicillin (AMP) (10 µg)and Sulfamethoxazole-Trimethoprim(STX) (25 µg) were applied and then plates were incubated overnight at 37°C. After the incubation, the inhibition zone diameters were measured using a transparent plastic ruler and interpreted according to the zone diameter interpretive chart of Clinical and Laboratory Standards Institute CLSI (2021).

2.4 Data management and Statistical analysis

The collected raw data were entered into an Excel sheet, cleaned, imported and analyzed by using GraphPad Prism 9. Descriptive statistics were used to analyze percentage, proportions and prevalence of bacterial isolates. The Chi-Square test was used to compare the prevalence of bacterial carriage and the proportion of antibiotic resistance between the two host species and collection sites. Differences were considered to be significant at the level $p < 0.05$.

3.0 RESULTS

3.1 Bacterial isolates

A total of 114 house rats and 57 cockroaches were trapped from Morogoro Municipal and Kilosa district. From Morogoro municipal; 45 rats and 16 cockroaches were from Mzinga, and 9 rats and 6 cockroaches were from Kauzeni wards. Kilosa district had 28 rats and 27 cockroaches from Ruaha, 32 rats and 8 cockroaches were from Kidodi wards (Table 1). All rats from both districts (100%) and 75.4% (43, n=57) of the cockroaches collected from the districts were positive for targeted bacterial species. Figure 1 show the distribution of bacterial isolates from four selected wards. A summary of three different microorganism isolated were shown in Table 2. Of 157 isolates, *Escherichia coli* 120 (76.4%) was the leading isolate among the other bacterial isolates from both rats and cockroaches and from two districts sampled, followed by *Klebsiella* spp 33 (21%) and *Salmonella* spp 6 (3.8%). There was no significant difference in prevalence of bacteria spp isolated from *Periplaneta americana* and *Rattus rattus* except for *Escherichia coli* ($P = 0.0004$). furthermore, there was no statistically pronounced difference in prevalence of all bacterial isolates between Morogoro municipal and Kilosa district.

Table 1: Two collected host species in two selected study sites.

Location	Morogoro municipal		Kilosa District	
	Mzinga	Kauzeni	Ruaha	Kidodi
Host				
<i>Ratus ratus</i>	45	9	28	32
<i>Periprinata americana</i>	16	6	27	8

Table 2: Prevalence of bacterial isolates n (%) identified from *Rattus rattus* and *Periplaneta americana*

AREA	MOROGORO MUNICIPAL		KILOSA DISTRICT		TOTAL(N=171)
	RR (N=54)	PA (N=22)	RR (N=60)	PA (N=35)	
ISOLATES					
<i>Escherichia coli</i>	38(70.4)	12(54.5)	52(86.6)	18(51.4)	120(70.2)
<i>Klebsiella</i> spp	16(29.6)	3(13.6)	5(8.3)	9(25.7)	33(19.3)
<i>Salmonella</i> spp	1(1.9)	1(1.8)	2(3.3)	0 (0)	4(2.3)
TOTAL	55(101)	16(72.7)	61(101.6)	27(77.1)	157(91.8)

Key; RR; *Rattus rattus*, PA; *Periplaneta americana*.

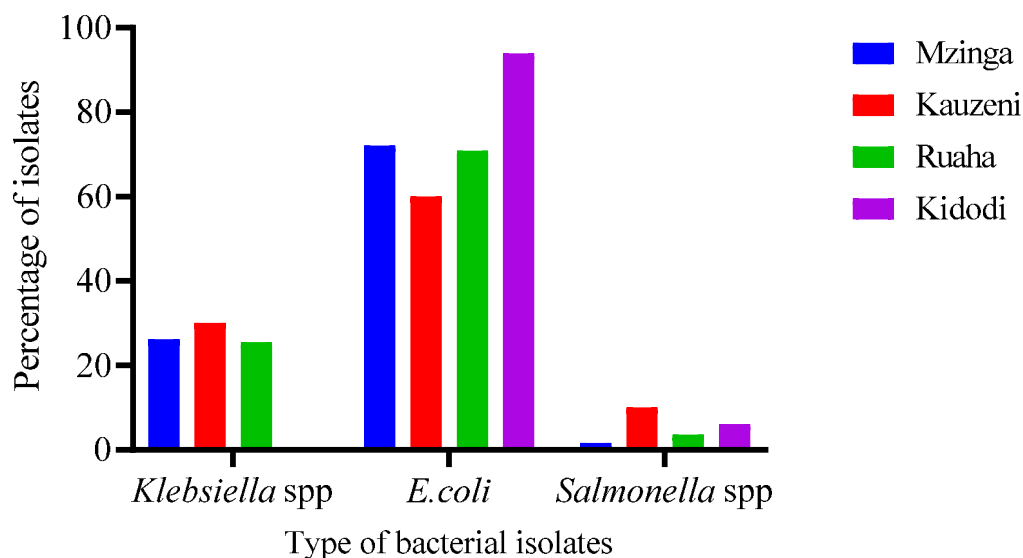


Figure 1; Percentage of bacterial isolates from four selected wards.

3.2 Antibiotic susceptibility pattern

Among all isolated bacteria only 83 isolates (52 from house rats and 31 from cockroaches) were tested to determine their antimicrobial susceptibility pattern. The highest percentage of resistance from both cockroaches and house rats' isolates were observed for Ampicillin (100% and 98.1% respectively), Ciprofloxacin (80.6% and 63.5% respectively) and Tetracycline (77.4% and 50% respectively). As it has been shown in figure 2, cockroaches appeared to harbor more resistant isolates to almost all tested antimicrobials than house rats. The resistance against Gentamycin ($P=0.045$), Trimethoprim/ sulfamethoxazole ($P=0.003$) and Tetracycline ($P=0.013$) were significantly different between *Periplaneta americana* and *Rattus rattus*. Table 3 shows that isolates from *Rattus rattus* indicated no significant variation in resistance by location against all tested antimicrobials except for Ampicillin ($P=0.027$). For *Periplaneta americana* there were no significant variations by location in resistance against all tested antimicrobials. Table 4 highlights the overall resistance rates of the isolates. All bacterial isolates showed high resistance rates to Ampicillin 98.8%, followed by Ciprofloxacin 69.9% and Tetracycline 60.2% and low resistance rates were observed to Gentamycin 18.1% and Sulfamethoxazole-Trimethoprim 34.9%. Species specific resistance rates are also presented in Table 4; where by *Escherichia coli* and *Klebsiella* spp showed 100% resistance rates to Ampicillin and fortunately no resistance was observed to *Salmonella* spp against Sulfamethoxazole-Trimethoprim.

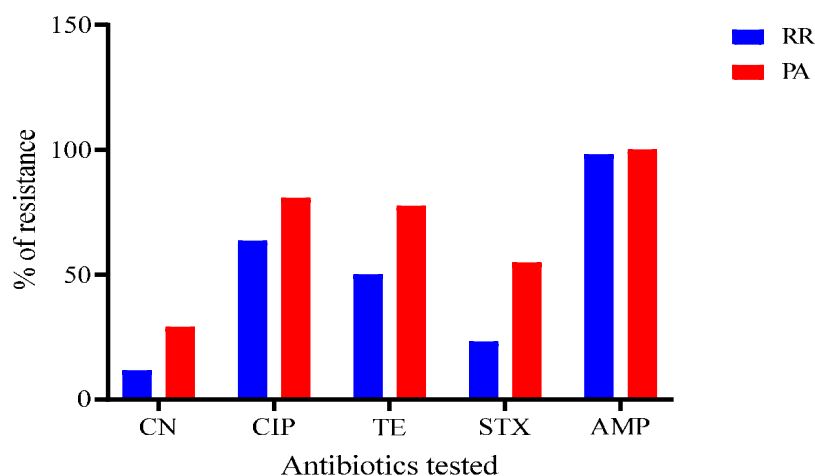


Figure 2: Percentage of antibiotic resistance from the house rats and cockroach isolates. Key CN, Gentamycin; CIP, Ciprofloxacin; TE, Tetracycline; SXT, Trimethoprim/ sulfamethoxazole; AMP, Ampicillin. PA, *Periplaneta americana*; RR, *Rattus rattus*

Table 3: Percentage of antibiotic resistance of bacterial isolates from *Periplaneta americana* and *Rattus rattus* by Districts

Location	Morogoro Municipal		Kilosa District	
	PA (N=15)	RR (N=43)	PA (N=16)	RR (N=9)
Antimicrobial				
CN	9(60%)	6(13.9%)	0	0
CIP	12(80%)	28(65.1%)	13(81.3%)	5(55.6%)
TE	12(80%)	23(53.5%)	12(75%)	3(33.3%)
SXT	4(26.7%)	15(34.9%)	8(50%)	2(22.2%)
AMP	15(100%)	43(100%)	16(100%)	8(88.9%)

Key; CIP, Ciprofloxacin; TE, Tetracycline; CN; Gentamycin; STX; Trimethoprim/sulfamethoxazole; AMP, Ampicillin; PA, *Periplaneta americana*; RR, *Rattus rattus*

Table 4: Resistance rates of bacterial isolates identified from *Rattus Rattus* and *Periplaneta americana*

Isolates	n	Antimicrobials				
		CN (%)	STX (%)	CIP (%)	TE (%)	AMP (%)
<i>Escherichia coli</i>	55	8(14.5)	21(38.2)	38(69.1)	33(60)	55(100)
<i>Klebsiella spp</i>	22	5(22.7)	8(36.4)	18(81.8)	16(72.7)	22(100)
<i>Salmonella spp</i>	6	2(33.3)	0	2(33.3)	1(16.7)	5(83.3)
Total	(N=83)	15(18.1)	29(34.9)	58(69.9)	50(60.2)	82(98.8)

Key; CIP, Ciprofloxacin; TE, Tetracycline; CN; Gentamycin; STX; Trimethoprim/sulfamethoxazole; AMP, Ampicillin.

Moreover, the isolates were also assessed for multi-drug resistance (MDR) which is a resistance to more than two classes of antibiotics. The overall MDR in all isolated bacterial species was 66.3 % (n=55). Table 5 summarizes the proportions of MDR bacteria. *E. coli* isolates (44.6%), showed the highest MDR followed by *Klebsiella spp* (19.3%) and *Salmonella spp* (2.4%). High rate of MDR isolates was observed in isolates from *Periplaneta americana* 77.4% (24/31) than isolates from *Rattus rattus* 59.6% (31/52) but it was not statistically significant proven (P=0.248). On the other hand, the rate of MDR isolates was significantly higher in Morogoro municipal 48.2% (40/83) than in Kilosa District 18.1% (15/83) (P=<0.0001). Table 6 shows the MDR pattern of the isolates obtained from *Periplaneta americana* and *Rattus rattus*. The most common resistance pattern observed were TE/ CIP/ AMP (12 *E. coli* and 6 *Klebsiella* isolates) and STX/ TE/ CIP/AMP (8 *E. coli* isolates).

Table 5: The proportions of MDR isolates from *Rattus rattus* and *Periplaneta americana* at Morogoro Municipal and Kilosa District

Location	Morogoro Municipal		Kilosa District		TOTAL (N=83)
	PA (N=15)	RR (N=43)	PA (N=16)	RR (N=9)	
<i>Escherichia coli</i>	10(66.7%)	18(41.9%)	8(50%)	1(11.1%)	37(44.6%)
<i>Klebsiella spp</i>	2(13.3%)	8(18.6%)	3(18.8%)	3(33.3%)	16(19.3%)
<i>Salmonella spp</i>	1(6.7%)	1(2.3%)	0	0	2(2.4%)

Key; RR; *Rattus rttus*, PA; *Periplaneta americana*.

Table 6: Multidrug resistance pattern of bacterial isolates isolated from *Rattus rattus* and *Periplaneta americana*

Bacterial isolates	No of classes	Antimicrobial combination	No. of isolates	Prevalence (%)	
<i>Escherichia coli</i>	3	TE/ CIP/ AMP	12	14.5	
		STX/ TE/ AMP	7	8.4	
		STX/ CIP/ AMP	1	1.2	
		CN/ STX/ AMP	1	1.2	
		CN/ TE/ AMP	2	2.4	
		4	STX/ TE/ CIP/ AMP	8	9.6
<i>Klebsiella spp</i>	3	CN/ TE/ CIP/ AMP	3	3.6	
		5	CN/ STX/ TE/ CIP/ AMP	2	2.4
		3	TE/ CIP/ AMP	6	7.2
		3	STX/ TE/ AMP	3	3.6
		4	STX/ TE/ CIP/ AMP	2	2.4
		3	CN/ TE/ CIP/ AMP	3	3.6
<i>Salmonella spp</i>	4	STX/ TE/ CIP/ AMP	1	1.2	
		5	CN/ STX/ TE/ CIP/AMP	2	2.4
		3	CN/ TE/ AMP	1	1.2
		4	CN/ TE/ CIP/ AMP	1	1.2

Key; CIP, Ciprofloxacin; TE, Tetracycline; CN; Gentamycin; STX; Trimethoprim/sulfamethoxazole; AMP, Ampicillin.

4.0 DISCUSSION

Cockroaches and house rats are nuisance pests which are increasingly reported to carry and transmit various pathogenic microorganism with negative impacts to human health. Previous studies from different areas keep on reporting about pathogenic microorganism isolated from these two pests which were trapped from various places like hospital, homes, markets and cafeteria (Adenusi *et al.*, 2018; Moges *et al.*, 2016; Ofukwu *et al.*, 2011; Vahabi *et al.*, 2015). Results from this study show that nearly all of these two pests collected from human residents carry pathogenic bacteria in their gut and thus act as potential mechanical vector of pathogens to humans. About 157 bacterial isolates were identified from the gut of these pests. Three bacterial species were identified and they included *E. coli* (76.4%) which was the leading isolate from both the two host species and the two sampled district. followed by *Klebsiella* spp (21%) and *Salmonella* spp (3.8%). All of the isolated bacteria from these two pests have public health threat as they cause various diseases to humans including diarrhea, urinary tract infections, typhoid, gastroenteritis and food poisoning (Ifeanyi & Odunayo, 2015). Other previous studies have also reported about the above-mentioned bacterial pathogens to be isolated from cockroaches and house rats (Tilahun, 2012).

Since both of these pests are waste scavengers and they freely move and inhabit different places including dirty and dark places their parasite carrying capacity and transmission rate increases (Amin, 2019; Zurek & Ghosh, 2014). Different findings have been suggesting that the most convenient route of pathogens transmission could be through contamination of food due to a great interaction between these pests and humans (Oyeyemi *et al.*, 2016; Williams *et al.*, 2018). This is because they disseminate pathogens by leaving their droppings to food and food materials. This is strongly supported as both of these pests have been reported to be found on food handling establishments like kitchens and places where foods are stored. Their unlimited movement enables them to acquire and disseminate various pathogens from different areas to humans (Akinjongunla *et al.*, 2012; Abbas, 2017; Ribas *et al.*, 2016). Their body morphology also facilitates pathogen carriage as reported by several studies which showed the number of bacterial pathogens isolated from body surface of cockroaches and house rats (Brown & Alhassan, 2014; Gwenzi *et al.*, 2021). Interaction between domesticated and wild animals have also been reported to facilitate their carrying and transmission of pathogenic bacteria (Islam *et al.*, 2022; Meerburg & Kijlstra, 2007; Umali *et al.*, 2012).

According to various findings cockroaches and house rats are the main sources of bacterial pathogens but are also reported to carry and disseminate resistant bacteria strains (Adekule *et al.*, 2012; Moges *et al.*, 2016). Findings from the present study demonstrated the high resistance rate of bacterial isolates from both cockroaches and house rats against the tested antimicrobials. It was shown that the isolated bacteria were resistant to almost all of the tested antimicrobials. The highest resistance rate was observed for Ampicillin, Ciprofloxacin and Tetracycline. Among the two host species sampled, cockroaches were observed to harbor more resistant isolates compared to house rats, this is perhaps due to their small body size which enable them to move on different dirty places including toilets (Ifeanyi & Odunayo, 2015). Various studies reported that the possible source of antimicrobial resistance could be due to over use and misuse of antimicrobials both from medical and veterinary field (Adeleke *et al.*, 2012; Katani *et al.*, 2014; Moges *et al.*, 2016; Williams *et al.*, 2011), however, the spread and amplification of resistance can also be contributed by these pests.

Findings from the present study demonstrated also the occurrence of Multi Drug Resistance (MDR) of some bacterial isolates. About 66.3% of bacterial isolates showed multiple drug resistance (resistance to more than two classes of antibiotics). As reported by Gwenzi *et al.*, 2021; Moges *et al.*, 2016; Radhouani *et al.*, 2014 the MDR strain could arise due to accumulation of resistant genes in a single bacterial cell or expression of genes that codes for multidrug efflux pump.

5.0 CONCLUSION AND RECOMMENDATION

Since both cockroaches and house rats have been reported to carry and transmit pathogens including resistant bacterial pathogens which result to the difficultness in treatment or ineffectiveness of these therapeutic agents, proper measures are recommended to be taken like enhancing sanitary conditions in household, hospitals and food handling establishments so as to void or minimize the occurrence of these pest and transmission of bacterial pathogens. Also, pest control measures like the use of pesticides and insecticides should be implemented so as to reduce their population if not to completely eliminate them.

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