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The Diversity of Hind-Gut Bacterial Microbiome of House Rats and Cockroaches: An indication of Public Health Risk for Residents of Semi-urban and Urban Morogoro, Tanzania

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ABSTRACT

House rats (*Rattus rattus*) and cockroaches (*Periplaneta americana*) are important reservoirs of zoonotic bacterial diseases. Understanding these animals' gut bacteria composition is crucial for monitoring and preventing infections. This study aimed to determine the hindgut bacteria composition and diversity of the two hosts sampled from Kilosa and Morogoro districts, in Tanzania. A cross-sectional study design was employed, A total of 114 house rats and 57 cockroaches were caught. The hind guts of trapped host species were dissected and pooled to obtain four pools (two pools represent the hindgut of *R. rattus* and *P. americana* from Morogoro municipal and two pools for the hindgut of *R. rattus* and *P. americana* from Kilosa district). Genomic DNA was extracted from the pooled samples which was then used in metagenomics sequencing. The observed Shannon and Chao 1 indices indicated higher bacterial species diversity in rats of the Kilosa district and cockroaches of Morogoro municipal. The microbiome diversity in Morogoro municipal was higher for cockroaches than for rats, but it was not statistically significant ($p>0.05$). In Kilosa district, the microbiome diversity was higher for rats than for cockroaches, but it was not statistically significant ($p>0.05$) Proteobacteria and Bacteroidetes were the most abundant phyla in the hindgut samples of both animals. However, abundances differed among the host species and areas sampled. Proteobacteria were the most abundant phylum from *Rattus* rattus from Kilosa district, *P. americana* and *R. rattus* from Morogoro municipal, comprising 48%, 41%, and 40%, respectively. Bacteroidetes were plentiful from *P. americana* from Kilosa district (48%). Bacterial diversity was observed to be higher for *R. rattus* from Kilosa district and *P. americana* from Morogoro municipal. Potential pathogenic bacteria were also observed; the highest relative abundance of pathogenic bacteria was observed from *P. americana* of Kilosa, followed by *R. rattus* from the Morogoro district. Therefore, this study highlights the composition of hindgut bacteria carried by *P. americana* and *R. rattus*, which gives an insight into the different bacteria carried, including the pathogenic ones. This study suggests surveillance of these pests to minimise outbreaks and transmission of zoonotic diseases. Since this study did not focus on factors influencing microbiome composition and diversity, further studies are recommended to be conducted to see the influence of those factors on the gut microbiome.

INTRODUCTION

The house rat (*Rattus rattus*) and cockroach (*Periplaneta americana*) are among the world's most prolific and widespread urban pest species. Both pests inhabit a wide range of habitats including human and animal habitats. The *R. rattus* and *P. americana* gut microbiome comprise horizontally transmitted and vertically transmitted microbes. Both of their guts harbour a variety of microorganisms, which play an important role in the health and fitness of host animals. The microbes contribute positively to the development and growth of the host by participating in food digestion, host nutrition, protection against pathogens and increasing the immune response (Engel and Moran, 2013a; Huang et al., 2013; Yang et al., 2017). They protect the host against pathogens by inhibiting colonisation and enhancing their immunity (Engel and Moran, 2013a). As reported by (Engel and Moran, 2013b; Brune and Dietrich, 2015; Claus et al., 2016) certain toxins such as pesticides can be metabolised by the gut microbiome. Moreover, recent studies have shown that the microbiome can impact different host behaviours, including frequency of social interactions, mate choice, hyperactivity, anxiety, depression and others (Tinker and Ottesen, 2016).

The assemblage and composition of the bacteria community inhabit the three sections of the alimentary canal, but many studies reported that the hindgut has the highest bacterial density and diversity (Cruden and Markovetz, 1987; Schauer et al., 2012; Bauer et al., 2015; Kakumanu et al., 2018). Like in other animals, the gut microbiome in *P. americana* and *R. rattus* is dictated by the interplay of host genetics, early environment, and immediate environment (Tinker and Ottesen, 2016). Typical examples of a host's environment that can cause a major impact on the microbiome include temperature (Sepulveda and Moeller, 2020), diet (Turnbaugh et al., 2009), and/or housing conditions (Ericsson and Franklin, 2015; Caruso et al., 2019). Arguably, the interaction between these and other domestic pests also influences the composition and diversity of the gut microbiome.

Understanding the gut microbial communities is essential as it contributes to understanding their biology and clinical relevance since they are engaged in disseminating pathogens (Kakumanu et al., 2018). Based on the aforementioned, studies have emphasised the urge to acquire an in-depth understanding of host-microbiome-parasites interactions. These interactions are critical for disease transmission. As such, the accrued knowledge will not only establish mechanisms via which gut-microbiome modulates the host's (*P. americana* and *R. rattus*) ability to transmit and/or harbour bacterial and possibly other pathogens but also may reveal symbiotic microbes that may be exploited to achieve transmission blocking in disease vectors/reservoirs. Several symbionts have been identified as potential strategies for reducing disease transmission, for example, *Wolbachia*, *Sodalis*, *Wigglesworthia*, *Rhodococcus* and *Serratia* (Weiss and Akoy, 2011).

This study aimed to assess the composition of the hindgut bacteria microbiome of cockroaches and house rats since little is documented, especially in Tanzania, about the hindgut bacterial composition of these two pests to ascertain their potential for disease transmission.

MATERIALS AND METHODS

The Ethics Statement

The Ethical approval for the study was given by the ethical committees of Sokoine University of Agriculture, Tanzania, with reference No SUA/AMD/R.1/8/763, approved on 5th January 2022.

Description of the study area

This study was conducted in Morogoro Municipal and Kilosa district (Fig. 1), in the Morogoro region, Tanzania. Morogoro Municipal covers an area of 260 km^2. The district has an average minimum and maximum temperature of 16ºC and 33ºC, 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 covers an area of 14,245 km^2 and experiences rainfall from November to May. The dry season occurs from June to October. The average annual temperature is 24.6ºC (Chipwaza et al., 2015). The main economic activities include agriculture and livestock keeping. Both districts experience a high infestation of rodents and cockroaches.

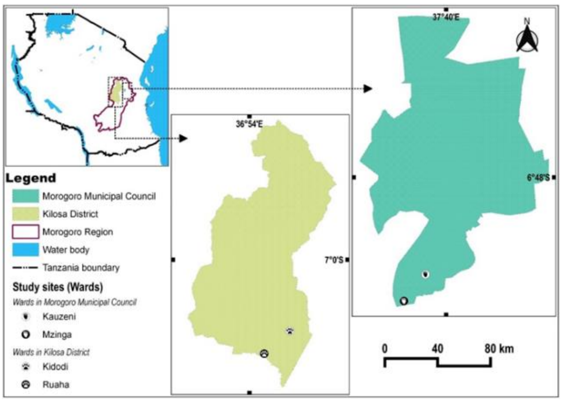

Figure 1. A map showing the selected study sites

Study Design

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

Sampling procedures and sample processing

P. americana and *R. rattus* were collected from four randomly selected wards, two from each district. A total of 57 *P. americana* and 114 *R. rattus* were trapped from 62 randomly selected households, 22 *A. americana* from Morogoro Municipal and 35 from Kilosa District, while 54 *R. rattus* were from Morogoro Municipal and 60 from Kilosa District. *P. americana* were manually caught and stored in sterile containers, while *R. rattus* were trapped in wire cages.

Trapped *P. americana* and *R. rattus* were identified based on their morphological features with the guidance of identification keys (Happold et al., 2013; Picker et al., 2004). Both *P. americana* and *R. rattus* were sacrificed with chloroform. *Periplaneta americana* were individually placed in tubes with 5ml of normal saline (0.9% NaCl) and shacked manually to dislodge bacteria from their body surface; after that 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 *P. americana* and *R. rattus* were dissected aseptically using scissors to remove the hindgut. The hindgut contents were collected in sterile tubes containing maximum recovery diluent media, stored in a sterile cool box with ice cubes, and transported to Sokoine University of Agriculture for further laboratory analysis.

In aseptic conditions, the hindguts of fifty-seven *P. americana* and 114 *R. rattus* from Morogoro municipal and Kilosa district were dissected and pooled. Four pools were obtained (two representing the hindguts of *P. americana* and *R. rattus* from Morogoro municipal, and two representing the hindgut of *P. americana* and *R. rattus* from Kilosa district). The pools have been abbreviated using the following key: PA1; *P. americana* from Morogoro district, PA2; *P. americana* from Kilosa district, RR1; *R. rattus* from Morogoro district, RR2; *R. rattus* from Kilosa district.

DNA extraction

DNA was extracted from the hindgut content stored in buffered peptone water. Genomic DNA was extracted using the Quick-DNA universal extraction kit (Zymo Research, USA) protocol per the manufacturer's instructions. The quantity and quality of gDNA were then measured using a NanoVue Plus spectrophotometer at a wavelength of 260nm (A_{260}/A_{280}). A ratio between 1.8 and 2.0 indicated a high-quality gDNA.

Library preparation and sequencing

The MinION sequencing techniques of Oxford Nanopore technology were used in this study. The MinION sequencing libraries were generated using the DNA sequencing-barcoding kit (SQK-PCB109-Oxford Nanopore Technologies) following the manufacturer's protocol.

Bioinformatics analysis

Firstly, the raw reads were subjected to quality trimming using Cutadapt V3.4 to remove low-quality reads and trim barcode and adopter sequences. The trimmed reads were then assembled using Megahit V1.2.9 to generate contigs representing the genetic material in the metagenomic samples. These contigs were subsequently binned into individual microbial genomes using Metabat2 V2.15 after aligning the reads to assembled contigs using Bowtie2 V2.5.1; Metabat2 utilises sequence composition and coverage information. The resulting metagenomic bins were annotated using Prokka V1.14.6, which predicted and annotated protein-coding genes within the bins by leveraging the Prodigal gene prediction tool and the NCBI non-redundant database. To determine the taxonomic composition of the metagenomic samples, we performed metagenomic taxonomic classification using Kraken2 V2.1.3 with the RefSeq database.

Statistical analysis

R-studio software (V 4.2.3) was used for statistical analysis; whereby descriptive statistics was used to analyse the relative abundances of bacteria. The Phyloseq package was used for diversity analysis. The Chi-square and t-test were used to compare the abundance and diversity of bacteria genera between the host species and areas sampled. Differences were considered to be significant at the level of p<0.05.

RESULTS

The general characteristics of the sequencing data set are shown in Table 1. The data set consisted of 65 633 and 772 874 sequences for *R. rattus* and *P. americana* samples collected from Morogoro municipal, and 437 465 and 50 859 sequences for *R. rattus* and *P. americana* from Kilosa district. A total of 404 575 818 bases were sequenced from four pooled samples, most of which were from *R. rattus* from Kilosa (235 119 094) and fewer from *P. americana* from Kilosa (19 708 309).

Table 1. General characteristics of each sequencing data for each host species collected

Sample	Reads	Bases (bp)	Number of bins
RR1	65633	23120071	9
PA1	772874	126628344	19
RR2	437465	235119094	29
PA2	50859	19708309	7

Key; RR1; Rattus rattus from Morogoro municipal, PA1; Periplaneta americana from Morogoro municipal, RR2; Rattus rattus from Kilosa district, PA2; Periplaneta americana from Kilosa district

Bacterial Community

Taxonomic analysis was performed to identify the hindgut bacterial community of the two host species sampled. A total of 27 bacterial phyla were identified Proteobacteria and Bacteroidetes were the most abundant phyla among all host species sampled. Proteobacteria was abundant from *R. rattus* from Kilosa district (48%) and *P. americana* (41%) and *R. rattus* (40%) from Morogoro municipal. Bacteroidetes were also highly abundant from *P. americana* from Kilosa district (48%) (Fig. 2A & B). To further explore the hind-gut bacterial community of *R. rattus* and *P. americana*, the bacterial community at the genus level was analysed and 806 genera were identified. Fifty-one genera with sequence reads above 15 were presented in Table 2. Five bacteria genera shared by pool samples in the two host species (*R. rattus* and *P. americana*) were *Proteus, Fusobacterium, Escherichia, Citrobacter* and *Bacteroides*.

A chi-square test was performed to determine the significant differences in the number of sequences of bacterial genera that were shared between the two host species (*R. rattus* and *P. americana*). From Kilosa district, the results showed that the number of sequences of *Proteus* (p<0.001), *Fusobacterium* (p<0.001), *Escherichia* (p<0.001), *Citrobacter* (p<0.001) and *Bacteroides* (p<0.001) were significantly higher in *R. rattus* compared to *P. americana*. For Morogoro municipal, the number of sequences of *Bacteroides* (p<0.001), *Citrobacter* (p<0.001) and *Proteus* (p=0.01) were also significantly higher in *R. rattus* compared to *P. americana*. The number of sequences of *Escherichia* (p=0.449) and *Fusobacteria* (p=0.087) showed no significant differences between *R. rattus* and *P. americana*.

The significant differences in sequences of shared genera were also determined between the two districts sampled (Kilosa and Morogoro district). The results showed that the number of sequences of *Bacteroides* (p<0.001), *Citrobacter* (p<0.001), *Escherichia* (p<0.001) and *Proteus* (p<0.001) were significantly higher in *R. rattus* from Kilosa than that from Morogoro district but *Fusobacterium* (p=0.414) showed no significant difference. The number of sequences of *Bacteroides* (p<0.001), *Citrobacter* (p<0.001), *Escherichia* (p<0.001), *Fusobacterium* (p<0.001) and *Proteus* (p<0.001) were significantly higher for *P. americana* from Morogoro district than those from Kilosa district.

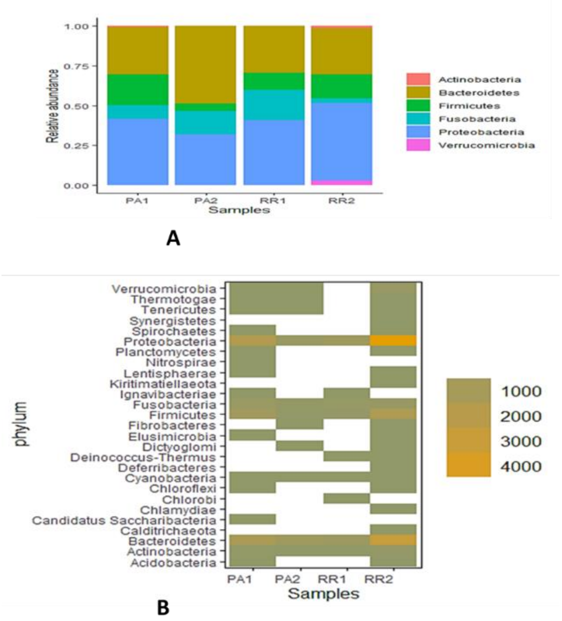

Figure 2. Bacterial composition of different host species (A) and at phylum distribution (B)

Table 2. Taxonomic classification of bacteria at genus level from pools of the two host species sampled

Taxonomy (Phylum; Class; Family; Genus)	**Number of sequences**			
	Kilosa		**Morogoro**	
	RR2	**PA2**	**PA1**	**RR1**
Proteobacteria, Betaproteobacteria, *Alcaligenaceae*, *Achromobacter*	15	-	-	-
Proteobacteria, Gammaproteobacteria, *Moraxellaceae*, *Acinetobacter*	203	-	17	-
Proteobacteria, Gammaproteobacteria, *Aeromonadaceae*, *Aeromonas*	16	-	-	-
Verrucomicrobia, *Verrucomicrobiae*, *Akkermansiaceae*, *Akkermansia*	274	-	-	-
Proteobacteria, Betaproteobacteria, *Alcaligenaceae*, *Alcaligenes*	390	-	60	-
Bacteroidetes, Bacteroidia, *Rikenellaceae*, *Alistipes*	240	-	-	-
Firmicutes, Clostridia, *Lachnospiraceae*, *Anaerotignum*	30	-	-	-
Firmicutes, Bacilli, *Bacillaceae*, *Bacillus*	28	-	26	-
Bacteroidetes Bacteroidia, *Bacteroidaceae*, *Bacteroides*	1838	667	972	456
Firmicutes, Clostridia, *Lachnospiraceae*, *Blautia*	29	-	-	-
Fusobacteria, Fusobacteriia, A*eromonadaceae*, *Bordetella*	73	-	-	-
Proteobacteria, Alphaproteobacteria, *Caulobacteraceae*, *Brevundimonas*	17	-	-	-
Proteobacteria, Gammaproteobacteria, *Enterobacteriaceae*, *Citrobacter*	537	86	263	42
Firmicutes, Clostridia *Comamonadaceae*, *Peptostreptococcaceae*, *Clostridioides*	15	-	-	-
Firmicutes, Clostridia, *Clostridiaceae*, *Clostridium*	135	-	126	18
Proteobacteria, Betaproteobacteria, *Comamonas*	85	-	-	-
Proteobacteria, Deltaproteobacteria, *Desulfovibrionaceae*, *Desulfovibrio*	70	-	25	-
Proteobacteria, Gammaproteobacteria, *Enterobacteriaceae*, *Enterobacter*	239	-	38	17
Firmicutes, Bacilli, *Enterococcaceae*, *Enterococcus*	23	-	62	-
Proteobacteria, Gammaproteobacteria, *Enterobacteriaceae*, *Escherichia*	302	88	178	164
Firmicutes, Clostridia, *Ruminococcaceae*, *Faecalibacterium*	27	-	-	-
Bacteroidetes, Flavobacteriia, *Flavobacteriaceae*, *Flavobacterium*	20	-	-	-
Firmicutes, Clostridia, *Ruminococcaceae*, *Flavonifractor*	75	-	-	-
Fusobacteriota, Fusobacteriia, *Fusobacteriaceae*, *Fusobacterium*	290	210	354	310
Firmicutes, Clostridia, *Peptostreptococcaceae*, *Intestinimonas*	44	-	-	-
Proteobacteria, Gammaproteobacteria, *Enterobacteriaceae*, *Klebsiella*	265	-	60	18
Firmicutes, Bacilli, *Planococcaceae*, *Kurthia*	23	-	-	-
Firmicutes, Clostridia, *Lachnospiraceae*, *Lachnoclostridium*	327	-	41	-
Firmicutes, Bacilli, *Lactobacillaceae*, *Lactobacillus*	272	-	63	30

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district. "- "not found.

Table 3. Continued

Taxonomy (Phylum; Class; Family; Genus)	**Number of sequences**			
	Kilosa		**Morogoro**	
	RR2	**PA2**	**PA1**	**RR1**
Firmicutes, Bacilli, *Streptococcaceae*, *Lactococcus*	18	-	-	-
Firmicutes, Bacilli, *Bacillaceae*, *Lysinibacillus*	109	-	205	43
Proteobacteria, Gammaproteobacteria, *Morganellaceae*, *Morganella*	203	-	70	-
Bacteroidetes, Bacteroidia, *Muribaculaceae*, *Muribaculum*	30	-	-	-
Bacteroidetes, Flavobacteriia, *Flavobacteriaceae*, *Myroides*	225	-	18	-
Bacteroidetes, Bacteroidia, *Odoribacteraceae*, *Odoribacter*	40	-	-	-
Firmicutes, Clostridia, *Oscillospiraceae*, *Oscillibacter*	24	-	-	-
Bacteroidetes, Bacteroidia, *Tannerellaceae*, *Parabacteroides*	250	-	105	-
Proteobacteria, Gammaproteobacteria, Morganellaceae, *Proteus*	396	144	371	306
Proteobacteria, Gammaproteobacteria, *Morganellaceae*, *Providencia*	543	-	139	27
Proteobacteria, Gammaproteobacteria, *Pseudomonadaceae*, *Pseudomonas*	352	-	27	-
Proteobacteria, Gammaproteobacteria, Moraxellaceae, *Psychrobacter*	98	-	-	-
Proteobacteria, Gammaproteobacteria, *Enterobacteriaceae*, *Salmonella*	28	-	19	-
Proteobacteria, Gammaproteobacteria, *Yersiniaceae*, *Serratia*	50	-	53	-
Proteobacteria, Gammaproteobacteria, Shewanellaceae, *Shewanella*	49	-	98	-
Bacteroidetes, Sphingobacteriia, S*phingobacteriaceae*, *Sphingobacterium*	60	-	-	-
Firmicutes, Bacilli, *Staphylococcaceae*, *Staphylococcus*	17	-	-	-
Proteobacteria, Gammaproteobacteria, Xanthomonadaceae, *Stenotrophomonas*	288	70	48	-
Firmicutes, Bacilli, *Streptococcaceae*, *Streptococcus*	16	-	-	-
Actinobacteria, Actinobacteria, Streptomycetaceae, *Streptomyces*	19	-	-	-
Proteobacteria, Gammaproteobacteria, *Vibrionaceae*, *Vibrio*	16	-	-	-
Firmicutes, Clostridia, Peptostreptococcaceae, *Acetoanaerobium*	-	-	66	-

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district. "- "not found.

Potential Pathogenic Bacteria

Potential pathogenic bacteria were identified from the pooled samples of two host species. Only bacteria genera and species with sequence reads above 15 were presented. Twenty-four pathogenic genera were identified with common pathogenic genera, as shown in Table 3. Among pathogenic genera identified, five genera (*Proteus*, *Fusobacterium*, *Escherichia*, *Citrobacter* and *Bacteroides*) were shared by all host species collected from two areas sampled. Relative abundances of pathogenic bacteria carried by the two host species from each sampled site were observed. The highest relative abundance of pathogenic bacteria was observed from *P. americana* from Kilosa followed by *R. rattus* from Morogoro district (Fig 3). Pathogenic bacteria were further explored at the species level, as shown in Table 4, where *Escherichia coli*, *Citrobacter freundii*, and *Proteus mirabilis* were found in all host species. *Klebsiella pneumoneae* was found in all hosts except in *P. americana* from Kilosa. *Salmonella enterica* was also found only in *P. americana* from Kilosa and *R. rattus* from Morogoro district.

Each bar represents the relative abundance of each bacterial taxa (A). (B). A heatmap representing abundances of bacterial taxon depicted by colour intensity (B). Key; PA1; *P. americana* from Morogoro district, PA2; *P. americana* from Kilosa district, RR1; *R. rattus* from Morogoro district, RR2; *Rattus rattus* from Kilosa district.

Table 4. Common potential pathogenic bacteria genera found in the pooled samples of two host species

RR1	**PA1**	**RR2**	**PA2**
Bacteroides	*Bacteroides*	*Bacteroides*	*Bacteroides*
Citrobacter	*Citrobacter*	*Citrobacter*	*Citrobacter*
Clostridium	*Clostridium*	*Clostridium*	*Escherichia*
Enterobacter	*Enterobacter*	*Enterobacter*	*Fusobacterium*
Escherichia	*Enterococcus*	*Enterococcus*	*Proteus*
Klebsiella	*Escherichia*	*Escherichia*	
Fusobacterium	*Klebsiella*	*Klebsiella*	
Proteus	*Proteus*	*Proteus*	
	Pseudomonas	*Pseudomonas*	
	Salmonella	*Salmonella*	
	Fusobacterium	*Fusobacterium*	
		Staphylococcus	
		Streptococcus	
		Vibrio	

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district

Table 5. Some potential pathogenic bacteria species found in the pooled samples of two host species.

RR1	**PA1**	**RR2**	**PA2**
Bacteroides cellulosilyticus	*Citrobacter freundii*	*Citrobacter freundii*	*Citrobacter freundii*
Citrobacter freundii	*Clostridium botulinum*	*Bacteroides cellulosilyticus*	*Escherichia coli*
Escherichia coli	*Escherichia coli*	*Clostridium botulinum*	*Fusobacterium ulcerans*
Klebsiella pneumoniae	*Klebsiella pneumoniae*	*Enterobacter hormaechei*	*Proteus mirabilis*
Proteus mirabilis	*Proteus mirabilis*	*Escherichia coli*	
	Salmonella enterica	*Klebsiella pneumoniae*	
		Proteus mirabilis	
		Pseudomonas aeruginosa	
		Fusobacterium ulcerans	
		Salmonella enterica	

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district.

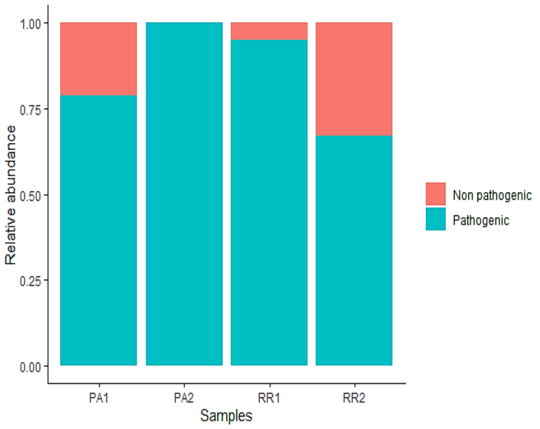

Figure 2. Relative abundances of potential pathogenic bacteria from the pooled samples of two host species. Key; PA1; *Periplaneta americana* from Morogoro district, PA2; *Periplaneta americana* from Kilosa district, RR1; *Rattus rattus* from Morogoro district, RR2; *Rattus rattus* from Kilosa district.

Next, an alpha diversity on all samples was performed. Alpha diversity refers to the diversity of a specific region or ecosystem. The observed Shannon and Chao 1 indices indicated higher species diversity in rats of the Kilosa district and cockroaches of Morogoro municipal (Fig. 4). The microbiome diversity in Morogoro municipal was higher for cockroaches than for rats. However, it was not statistically significant (p>0.05). In Kilosa district, the microbiome diversity was higher for rats than for cockroaches, but it was not statistically significant (p>0.05) (Fig. 5) as well.

In comparing the two districts, the microbiome diversity was high in Kilosa district compared to Morogoro municipal, though the difference was not statistically pronounced (Fig. 6). When beta diversity was assessed. The finding showed that variation of microbial communities between samples existed. However, the composition was similar between cockroaches from Kilosa district and rats from Morogoro municipal (Fig. 7).

The closer the two sample points are, the more similar the bacterial composition of the two samples. Key; PA1; *P. americana* from Morogoro district, PA2; *P. americana* from Kilosa district, RR1; *R. rattus* from Morogoro district, RR2; *R. rattus* from Kilosa district.

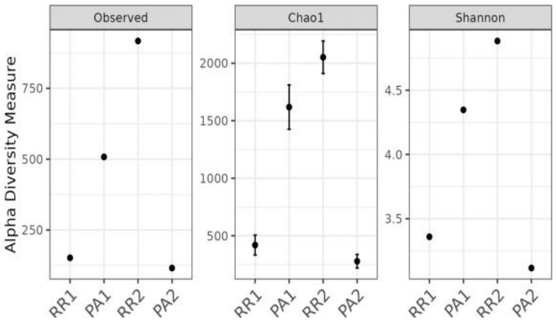

Figure 3: Alpha diversity of collected samples

Key; PA1; *Periplaneta americana* from Morogoro district, PA2; *Periplaneta americana* from Kilosa district, RR1; *Rattus rattus* from Morogoro district, RR2; *Rattus rattus* from Kilosa district.

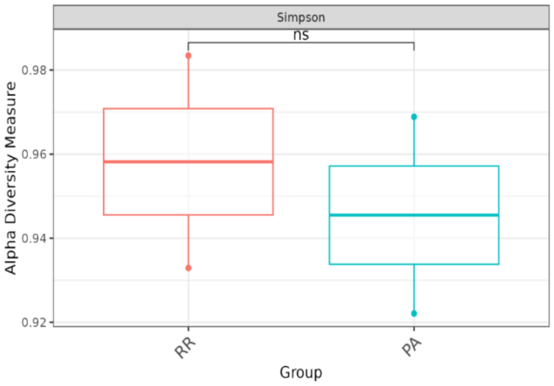

Figure 4: Alpha diversity of collected host species

Key; PA; Periplaneta americana from Morogoro district and Kilosa district, RR; Rattus rattus from Morogoro district and Kilosa district.

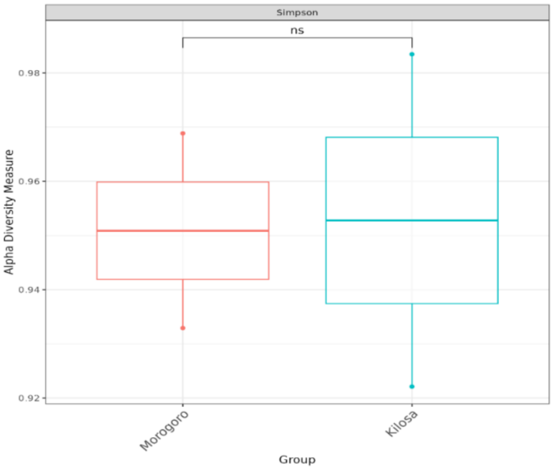

Figure 5: Alpha diversity of collected host species from two sampled areas.

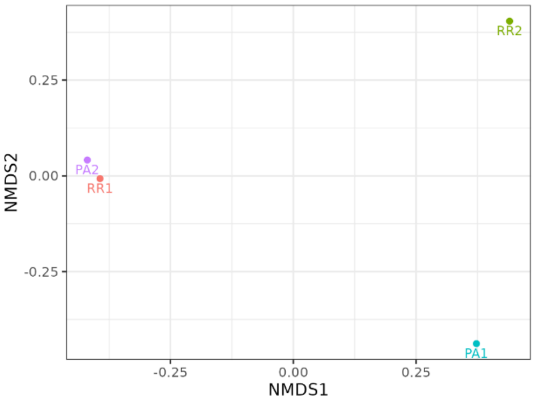

Figure 6: Non metric dimensional scaling (NMDS) using Bray-curtis dissimilarity

DISCUSSION AND CONCLUSION

The animal gut contains many bacterial communities with a complex composition comprising over 500 species. Intestinal microbes are a complex and dynamic ecosystem that coevolves with their host (Han et al., 2020). Previous studies have shown that gut microbiome composition may differ among species based on, diet, environmental factors, genetics and location (Han et al., 2020; Lee et al., 2020). Results showed that the composition of the microbiome did not differ between the hosts collected from the two areas, but their abundances were varied. Proteobacteria and Bacteroidetes were the most abundant phyla compared to other phyla.

Findings from this study regarding the composition of gut microbiome between *P. americana* and *R. rattus* are similar to other previous studies, which showed similar bacteria phyla from *P. americana* and *R. rattus* (Tinker and Ottesen, 2016; Debebe et al., 2017; Han et al., 2020; He et al., 2020). Other previous studies reported on the influence of geographical location on the composition of the gut microbiome (Goertz, Menezes, et al., 2019; Wang, 2022). Findings from this study also revealed that geographical location could influence gut microbiome composition as it was found that Proteobacteria and Bacteroidetes were the most abundant phyla from hosts collected from Kilosa district than that of Morogoro municipal. However, there are other factors reported by several findings which have more influence on the gut microbiome, including species identity (host genetic), sex, diet, gut pH, age and others (Vicente et al., 2016; Kakumanu et al., 2018; Goertz et al., 2019; Wang, 2022; Tinker and Ottesen, 2016;).

Recently, there have been ongoing studies regarding gut microbiomes since they provide insight into both culturable and non-culturable microbiomes. As reported by several previous studies, symbiont bacteria can be used for pest control, several tactics including manipulation of microbial symbionts have been documented, which results in either minimising pest population or reducing their chance of transmitting pathogens (Shapiro-ilan and Gaugler, 2002; Noman et al., 2019; Pan et al., 2020). For instance, Dillon & Dillon, 2004; Arora and Douglas, 2017 reported that symbiont microorganisms may be genetically modified and in turn, become pathogens and induce effects on the targeted pest. Several bacteria, including *Enterobacter* species (Arora and Doglas, 2017), *Klebsiella* species, *Proteus* species and others (Dillon and Dillon, 2004; Noman et al., 2019) were reported to be involved in the control of various insect pests.

The microbiome diversity observed was higher for species from Kilosa district (the rural area) compared to that of Morogoro municipal (urban area); however, it was not statistically supported. The variation in this diversity is perhaps supported by the fact that in our study area, those hosts collected from rural environments had a heterogeneous environment compared to urban environments. These findings are similar to other previous studies, which also found that hosts from rural areas had higher microbiomes compared to those from urban environments (Aimeric et al., 2018; Gurbanov et al., 2022). This is perhaps due to the fact that in rural areas, there is a diversification of food materials, plus the hygienic status of rural areas is not much improved; thus, hosts are likely exposed to more microbes compared to a host of the urban environment.

Findings from this study also showed the diversity within the district sampled whereby in Morogoro municipal, the microbiome diversity was observed to be higher for *P. americana* than *R. rattus*; this is perhaps because of the fact that *P. americana* of urban areas have a wide range of habitats which is supported by their body size compared to *R. rattus* thus enable them to consume a wide variety of food sources (Kakumanu et al., 2018). On the other hand, rats in Kilosa district had a higher microbiome than cockroaches. This may be supported by the fact that the environmental structure of rural areas favours rats more than cockroaches; thus, rats can easily access a wider variety of food.

This study also presents the pathogenic bacteria carried by the host species sampled. The relative abundance of pathogenic bacteria was high in *P. americana* of Kilosa, followed by *R. rattus* of Morogoro district. Some pathogenic bacteria species reported by several previous studies to cause human and animal diseases identified from this study include *E. coli*, *C. freundii* and *P. mirabilis*. Also, other species were *S. enterica*, *K. pneumoneae*, and *C. botulinum*. These findings are similar to previous reports highlighting pathogenic bacteria found in the gut of *P. americana* and *R. rattus* (Kakumanu et al., 2018; He et al., 2020; Gurbanov et al., 2022). Several studies in Tanzania have also demonstrated that rats are potential environmental source of zoonotic bacteria and some isolates having multidrug straits (Kimwaga et al. 2023; Ndakidemi et al., 2023; Mkopi et al., 2024). This is because their biology, behaviours, and habitats contribute to their effectiveness in spreading diseases. These two host species have been numerously reported to be found in indoor environments, and various studies have reported on the potentially carry pathogenic bacteria in their gut and external surfaces. Thus, there is a great chance of these pathogenic bacteria being transmitted to humans and animals through direct contamination and faeces droppings. Controlling their populations is crucial for preventing the spread of infectious diseases. This includes maintaining clean environments, proper waste management, sealing entry points to buildings, and using appropriate pest control methods. Additionally, public awareness and education about the risks associated with these pests are essential for reducing their impact on human health (Kimwaga et al. 2023; Ndakidemi et al., 2023; Mkopi et al., 2024).

Limitation of the Study

The gut microbiome composition observed from this study focused only on the geographical area where the two host species were collected. Other factors, such as species genetics, diet, host parameters, and environmental parameters, which are claimed to impose more influence on the gut microbiome, were not assessed.

This study summarises the composition of the bacteria microbiome between *P. americana* and *R. rattus* collected from two districts (Morogoro municipal, which is an urban area, and Kilosa district, which is a rural area). The bacteria composition was more or less similar to the host species collected from the two areas sampled. Also, the study revealed pathogenic bacteria and symbiont bacteria. This study suggests surveillance of these pests to minimise outbreaks and transmission of zoonotic diseases. Since this study did not focus on factors influencing microbiome composition and diversity, further studies are recommended to be conducted to see the influence of those factors on the gut microbiome.

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Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: BRK., AM., Design: BRC., AM., LM., Data Collection or Processing: BRC., Analysis or Interpretation: BRC., AM., LM., Literature Search: BRC., AM., Writing: BRC., AM., LM.

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