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Rodent reservoirs: unraveling spectrum of zoonotic and pathogenic bacteria

Agnes A. Mpinga*[1,2], Rudovick R. Kazwala[1], Happiness H. Kumburu[3], Coletha Mathew[1]

Abstract

Background: Zoonotic diseases are the major public health threat, with over 70% originating from wildlife. Rodents, while beneficial to the environment, transmit many zoonotic diseases such as hemorrhagic fevers, plague, tularemia, and leptospirosis, mainly due to increased agriculture and land use changes. Understanding rodent-borne pathogens is essential for effective intervention. Therefore, this study aimed to identify pathogenic and zoonotic bacteria in rodents and identify rodent species in the study area.

Methods: A total of 116 rodents achieved samples (101 oral-pharyngeal and 15 rectal swabs) collected from Kibondo, Uvinza and Kyerwa were used in this study. Total RNA (Ribonucleic Acid) was extracted from each swab sample and then pooled based on rodent species, location and swab types to make twelve pools. A portion of pooled swabs were polyadenylated and used for metagenomics sequence libraries preparation. A 16S rRNA (ribosomal Ribonucleic Acid) metagenomics sequencing was performed on 12 pools by using MinIon platform in order to identify microbial diversity.

Results: A total of 13 different microbial communities including bacteria were identified; where, 15 families of potentially pathogenic, zoonotic and bacteria of unknown zoonotic potential were also identified. These families included Mycobacteriacea, Helicobacteriacea, Enterobacteriacea, Vibrionacea, Staphylococcaceae, Nocardiaceae, Bacillaceae, Pasteurellaceae, Streptococcaceae, Campylobacteraceae, Leptospiraceae, Brachyspiraceae, Moraxellaceae, Enterococcaea, Flavobacteriacea. Potentially zoonotic pathogenic bacteria including Mycobacterium tuberculosis, Vibrio cholerae, Helicobacter pylori and Vibrio parahaemolyticus are reported in this study.

Conclusion: This study identifies several bacteria of public and veterinary importance, highlighting the possibility of increased risk of human infection and risk of cross-transmission between rodents, humans, and animals given the proximity between rodents, humans and animals. While no concrete evidence of rodent-to-human transmission was found, we hypothesize that rodents are a potential infection source, especially in resource-poor areas with close rodent-human contact.

Keywords: Zoonoses, Rodents, 16S rRNA metagenomics, Families, Bacteria, Tanzania

Correspondence: Agnes A. Mpinga (agnerashampinga108@gmail.com)
[1]College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O. Box 3021, Chuo Kikuu, Morogoro, Tanzania; [2]African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE IRPM and BTD) of the Sokoine University of Agriculture, Morogoro, Tanzania.

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Background

Emerging infectious diseases that pose public health and economic threats are mainly zoonoses, with over 70% estimated to originate in wildlife [1,2]. Zoonotic diseases impose a major morbidity and/or mortality burden worldwide [3]. Rodents are the most specious and diverse group of mammals and they perform several beneficial roles in environment [4,5]. Despite their benefits, they are also sources of zoonotic diseases [2]. Over decades, wild and commensal rodents have been cited as a major reservoir of evolving zoonotic pathogens which cause diseases in humans [6]. Rodents are considered reservoir of several diseases including leptospirosis [7,8], plague [9,10], toxoplasmosis [11] and hemorrhagic fevers [12–14]. They have also been reported to harbor several complex bacteria like Mycobacterium tuberculosis, Mycobacterium microti, and Escherichia coli [15]. Agricultural intensification, urbanization, and industrialization throughout the globe, has contributed to a significant increase in rodent borne zoonotic diseases [16,17]. Rodents can transmit pathogenic agents to humans and animals via direct contact, or through contamination of human food and water with rodents' stool and/or urine [1]. Ectoparasites carried on the skin of most rodents are also able to transmit zoonotic pathogens [18]. Occupations associated with rodent population handling, animal trade and large-scale traveling are among the risk factors associated with rodent-human pathogens transfer [19]. The fact that diseases can be transmitted between rodents and humans highlights the risks associated with the close contact between humans and commensal or peri-domestic rodents in Tanzania [20]. Rodents have caused human disease outbreaks

in the past, and they will certainly continue to do so in the future. However, it has been widely acknowledged that dealing with the problem of zoonotic infections is a task that is beyond medical and public health specialists alone, but rather it should include veterinary and environmental parameters, together with understanding of human social behavior [21]. Information about the prevalence of various infections in rodents is essential in estimation of the risk for humans. Previous studies in rodents have been able to provide useful information through identification of several agents of public health importance, however most of these studies have used traditional/conventional methods of identification. In this study we employed the high throughput Next generation metagenomics sequencing employing oxford nanopore MinIon platform to identify the microbial diversity in rodents.

Metagenomics is able to analyze multiple genomes of bacterial species [22]; it also allows the identification of bacteria genomes directly from samples without culture and can reveal information related to the diversity of microbes that circulate in hosts [23]. On the other hand, Nanopore sequencing is a third-generation sequencing method with two significant advantages over second-generation technologies: it produces longer sequence reads and allows for real-time sequence analysis [24]. Long-read sequencing significantly improve the contiguity of metagenomic assemblies due to its ability to provide more accurate, complete, and high-resolution data [25-27]. Therefore, this study focused on screening and identification of various pathogenic and zoonotic bacteria circulating in rodents by using Nanopore MinIon 16S rRNA metagenomic sequencing.

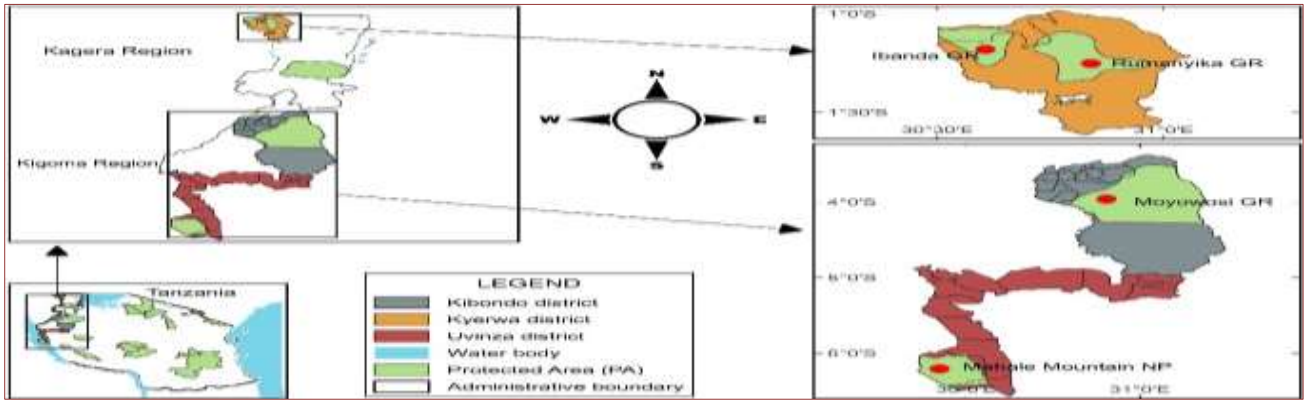

Figure 1: Map of the study area and surrounding wild life areas (game reserves and national park) (Sources: QGIS Version 3.24 "Tisler" retrieved on April 2024)

Methods

Study design and participants

The study employed rodent samples that were collected in 2018 in connection to another project and archived in SUA (Sokoine University of Agriculture) laboratory. Samples were collected in human-wildlife interfaces around three districts in two regions: Kyerwa district in the Kagera region as well as Kibondo and Uvinza districts in the Kigoma region as indicated in (Figure 1). In Kyerwa samples were collected from Murongo ward, which is located at latitude 1° 3' 47" South, longitude 30° 40'13" East in Kyerwa district. Murongo is bordered to the north by Uganda and to the west by Rwanda and within the Ibanda and Rumanyika Game reserves. This is a high-risk interface characterized by the high transboundary movement of both humans and livestock. There is also land use change due to agricultural intensification and mining resulting in close contact between humans and wildlife including rodents. Kibondo district is located at latitude 3° 35' 11" South, longitude 30° 43' 13" East in the western part of Tanzania. The district is bordered to the North-West by Burundi which facilitates cross-border trade at Kumsenga and Mkarazi markets near the Burundi-Tanzania border. High demands for charcoal and firewood as well as illegal hunting in the Moyowosi game reserve pose risk for infection spillover and spread. Uvinza district is located at latitude 5° 6' 7.80" South, longitude 30° 23' 16.79" East in Kigoma region, where Illegal wildlife hunting and consumption in the Uvinza open area could facilitate zoonotic pathogen transmission.

RNA Extraction

A total of 116 swab samples (101 oropharyngeal swabs and 15 rectal swabs) collected from 101 rodents were used in this study. Total RNA was extracted by using Direct-zol RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA) as per the manufacturer's instructions. In short, the extraction processes involved a series of centrifugation and filtration by using pre-wash and wash buffers. Elution of RNA from the silica membrane was done by using 50ul nuclease-free water. University of Agriculture HALi Laboratory, and then dry shipped to the Kilimanjaro Clinical Research Institute-Biotechnology Laboratory (KCRI-BL) for metagenomics Next generation sequencing Extracted RNA was aliquoted and stored in a -800C freezer for a few days at the Sokoine

Pooling of samples

In the laboratory RNA samples were pooled according to the type of swabs (Oral-pharyngeal or rectal swabs) rodent species (Crocidura spp., Lemniscomys spp., Mastomys natalensis and Rattus rattus and Mus muscullus combined as domestic rats) and location (Kibondo, Kyerwa, Uvinza) to make twelve pools. Oral-pharyngeal swabs made eleven pools (1-11) and rectal swabs made a single pool (the twelfth). Each district had four pools, with each pool comprising one of the rodent species i.e., a pool of Mastomys natalensis, Crocidura spp., Lemniscomys spp., and domestic rats (Mus muscullus and Rattus rattus) from Kibondo, Uvinza, and Kyerwa (with exception of the twelfth pool that comprised rectal swabs from all of the collected rodent species from Kyerwa). Each pool carried a different number of samples (a minimum of 1 and a maximum of 34) based on sample proportions; and hence different final volumes in the pools. Rodent species from Kibondo including, domestic rats (Rattus rattus+ Mus muscullus), Mastomys natalensis, Lemniscomys spp. and Crocidura spp. were included in pools 1, 2, 3, and 4 respectively. On the other hand, pools 5, 6, 7, and 8 included domestic rats (Rattus rattus+ Mus muscullus), Mastomys natalensis, Lemniscomys spp., and Crocidura spp. respectively from Uvinza. Pool 9, 10, and 11 were domestic rats (Rattus rattus+ Mus muscullus), Mastomys natalensis, and Crocidura spp. from Kyerwa respectively. Pool 12 were the rectal swabs from all of the species (domestic rats (Rattus rattus+ Mus muscullus), Mastomys natalensis, and Crocidura spp.) collected in Kyerwa. Pooling was done by taking 10µl of RNA (where samples were more than one), and 20µl (where there was only a single sample) from each species across three locations/districts, ending up with four pools from each location (Table 1).

Table 1: Description of the pooling procedure for Metagenomics sequencing

Pool ID	Number of Rodent swab samples	Pooling Volume (ul) per Sample	Total Volume (ul) per Pool	Swab type
1	3	10	30	Oral-pharyngeal
2	34	10	340	Oral-pharyngeal
3	4	10	40	Oral-pharyngeal
4	9	10	90	Oral-pharyngeal
5	2	10	20	Oral-pharyngeal
6	30	10	300	Oral-pharyngeal
7	2	10	20	Oral-pharyngeal
8	2	10	20	Oral-pharyngeal
9	1	20	20	Oral-pharyngeal
10	10	10	100	Oral-pharyngeal
11	4	10	40	Oral-pharyngeal
12	15	10	150	Rectal swab

Poly (A) Tailing

A total of 20µl of the pooled RNA samples from each of the final pool was taken for Polyadenylation (a process of adding ≥150 bases poly (A) tail to RNA transcripts), by using enzyme E. coli Poly (A) Polymerase (E-PAP) and ATP at 370C, 60 minutes incubation.

Reverse transcription and strand switching

To obtain ≥1µg of metagenomics complementary DNA (cDNA) for the library required for the Nanopore sequencing protocol, randomly amplified cDNA was generated using a primer-extension pre-amplification method according to the Protocol (ONT) provided with PCR-cDNA Barcoding kit (SQK-PCB 109); as previously described by [25]. A single-cycle PCR at 42°C for 90 minutes was performed for reverse transcription and strand switching. Polyadenylated RNA were reverse transcribed by using Maxima H Minus Reverse Transcriptase (Thermo Fisher Scientific), and amplified by random primers (VNP Primers). Strand switching was done by using Strand-Switching primer (Oxford Nanopore Technologies). Labeling of the samples was done by adding barcodes to the reverse transcribed RNA (cDNA) sample in a Barcoding PCR that used barcode Primers (Oxford Nanopore Technologies) and LongAmp Taq 2x Master Mix (New England BioLab).

Preparation of Nanopore sequencing libraries

Barcoded cDNA from the PCR reaction mixture was purified using AMPure XP beads (Beckman Coulter, Brea, CA). 1µg cDNA was used as input into Oxford Nanopore SQK-PCB 109 kit for generation of MinION Oxford Nanopore-compatible libraries following manufactures protocol as described by Joyon [28]. Briefly, to each reaction tube, 1µl 20 Exonuclease 1 (New England BioLabs) was added and incubated on HulaMixer and eluted in 12 µl elution buffer (EB) on a magnet as per instructions of the kit. After elution, quantity measurement was carried out on Qubit 4 Fluorometer (Invitrogen) using Qubit™ dsDNA HS Assay Kit (Thermofisher Scientific). Subsequently, 1µl from each sample was pooled together in one reaction tube making the final volume of the pooled cDNA library 12 µl. Ligation to the protein-linked adapter was done by adding 1µl of Rapid Adapter (RAP) to the cDNA library. Again, libraries were quantified by using qubit fluorimeters and quality assessment was done by using gel electrophoresis, where observation of smear was an indication of good quality libraries.

Nanopore sequencing

Sequencing of the cDNA on the MinION device was performed in the R9.4.1 Flow cell (FAO17147). Before loading, the flow cell was washed with Flow Cell Wash Kit according to the protocol (ONT). Priming and loading the MinION Spot on the flow cell was done following the instructions of the PCR-cDNA Barcoding kit (SQK-PCB 109). The standard MinKNOW protocol script was used for the sequencing. The run time of the MinION device was set to 36 hours in 190 voltages without base-calling and the quality score cut-off was set to 7.

Bioinformatics analysis

MinION reads were basecalled with ONT Guppy version 6.4.2, using the 9.4.1_450bps_SUP model. Basecalls were demultiplexed with ONT Guppy barcorder 6.4.2. Reads were screened for human and vector contaminants by using FastQscreen v0.14.1 with GRCh38 and UniVec_Core. Basic QC metrics (read count, base count, Q score, N counts) were obtained using fastq-stats from fastq-utils 1.3.0 Taxonomic classification was performed with Kraken2 v2.1.2 using the Kraken2 “standard” databases, constructed from NCBI RefSeq data retrieved between 15-18 November 2022. Moreover, the sequencing reads were processed by trimming the sequencing adapters using Porechop version 0.2.4. The quality of the trimmed sequencing reads was checked using nanoplot version 1.41.0. Afterwards, the data were uploaded and run in Kaiju [29] for metagenomics classification overview.

Results

A total of 116 (101 oral-pharyngeal and 15 rectal swabs) samples were used in the present study. In a metagenomics analysis 44 species of bacteria were detected. Where by, 10 (22.7%) of the bacteria species including H. pylori and other Helicobacter species of were also detected in the rectal swab pool which included (domestic rats (Rattus rattus+ Mus muscullus), Mastomys natalensis, and Crocidura spp.) from Kyerwa district; the remaining 34 (77.3%) of the detected bacteria species was distributed among oral-pharyngeal swabs pools (Figure 2). Bacteria species were detected in eight pools (1, 2, 4, 5, 6, 10, 11 and 12). Whereas, in pools 3, 7, 8 and 9 there was no any detected bacteria species (Figure 3). A total of 13 Microbial families with diverse characteristics, which play essential roles in various ecosystems were identified in a metagenomics analysis (Figure 4). A total of 15 bacterial families including groups of pathogenic, zoonotic and bacteria of unknown zoonotic potential were identified in a metagenomics analysis as indicated in (Figure 5) below.

Discussion

Several microbial families and numerous potentially pathogenic, zoonotic and bacteria of unknown zoonotic potential are reported in this study (Figure 5). Bacteria of public health and animal health importance were identified in a metagenomics analysis.

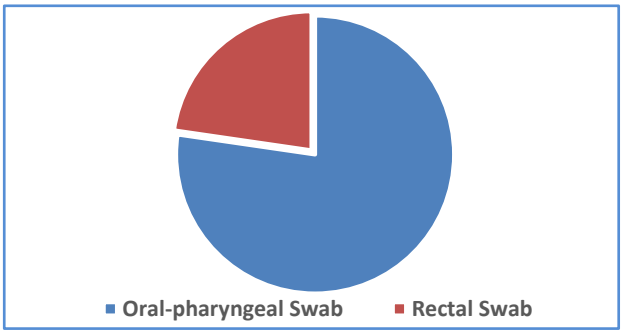

Figure 2: Proportion of bacteria detected across swab types collected from rodent species in Kagera and Kigoma regions, Tanzania

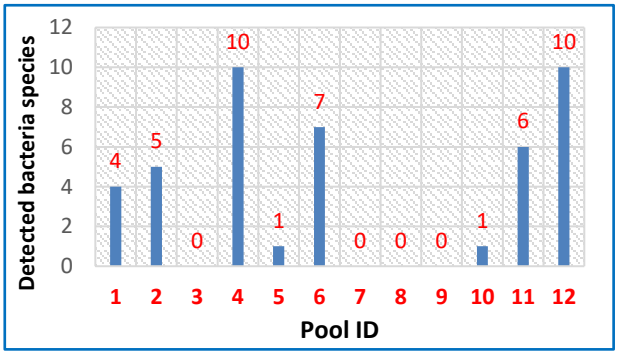

Figure 3: Number of bacteria specie detected in different pools of rectal and oropharyngeal swabs from rodent species obtained from Kagera and Kigoma regions, Tanzania

These detected bacteria have various transmission methods including, airborne, direct contact, through contaminated food products and other fomites; indicating the possibility of cross-transmission of diseases between human, animals and rodents. In rural settings, rodents exist in large population where they live and feed in close proximity to humans than many other mammal species. As urbanization continue most successful synanthropic species are likely to assume significance role in zoonotic disease transmission as the complexity of the human-animal-rodents interface increase. This study is an important first step toward understanding the risk of zoonotic disease transmission posed by rodents. This study was unable to track tangible evidence of tuberculosis transmission by rodents. However, it was found that rodents are potential reservoir of Mycobacterium tuberculosis; suggesting the circulation of the pathogen between humans and animals in the area. Although rodents have been extensively used as animal models for tuberculosis [30.31] and have been used in sniffing studies to detect tuberculosis [32]; there are scarce of information regarding natural infection of rodent with M. tuberculosis. To the best of our knowledge this is the first report of natural infection of rodents with M. tuberculosis which indicates the possibilities of cross-transmission. The life styles of the people in these communities, close contact between animals, humans and rodents and/or the habit of consuming raw animal products, are the possible factors for transmission of M. tuberculosis between human, animal and rodents; consequently, impacting on the tuberculosis (TB) control programs in human. In Tanzania, human TB control program have been widely implemented, however, the role of rodents in the transmission of the causative agent has been neglected which could be one of the challenges for an effective control program. The findings of this study together with previous studies that suggest rodents as a reservoir of Mycobacterium microti - a member of the M. tuberculosis complex [33-35]; necessitates the need for integrating animal TB control as an effective element of TB control for both human and animal using One Health approach.Detection of Helicobacter pylori (H. pylori) in rodents may be an indication of environmental contamination and circulation of the bacteria in an ecosystem. One possibility is that, rodents acquire the bacteria from the environment but also rodents can be reservoirs host with the potential of transmitting infections to human and other animals. Moreover, because humans live in close association with rodents and over the decades human have been cited as the only natural host of H. pylori there are possibilities that rodents have acquired these bacteria from humans. Weather and how rodents naturally harbor or have acquired the bacterium from humans and surrounding environment is still a subject to research. To date, there is still no any reported case of clinical condition caused by H. pylori in rodents; however, this is the first report of natural infection of rodents with H. pylori. Therefore, the presence of this bacteria in rodents is of more concern to human health as it indicates circulation of the bacteria and the possible increase in the chances of human infection; especially in rural settings as in the study area and many other resources poor environments, where there is normally a constant close association between humans, animals and rodents. Assuming the fecal-oral mode of transmission and the proximity of rodents to human settlement in a way that rodent even defecates in human foods, presence of this bacteria in rodent not only indicate increased risks to human health but also the increase in antibiotic resistance of H. pylori which is mentioned among the list of WHO antibiotic resistance priority pathogen.

Previous studies have also revealed several other environments where H. pylori have been detected. For instance, in 1997, Grubel et al. demonstrated that housefly has the potential to transmit H. pylori mechanically, and thus fly excreta might also contaminate human foods. In Chile, consumption of uncooked vegetables that had been irrigated with water contaminated with untreated sewage was associated with H. pylori seropositivity [36]. These hypotheses may be of the most significant in areas of the world with poor sanitation and close association between rodents and humans which is the case in our study area. In this study, although the dose of H. pylori, required to cause infection in humans is unknown, and we don't know if the amount released by rodents is enough to cause infection in humans; due to their diversity and the proximity to human settlements, rodents cannot be ruled out as a potential reservoir and vector of H. Pylori pathogen found in the present study. The reports of H. pylori detection in various biotic and abiotic environments such as in surface water, waste water and drinking water, in flies and now in rodents calls for ONE HEALTH effort to strengthen surveillance and detection. Moreover, in this study rodents appear to harbor Vibrio cholerae; a causative agent of Cholera. Cholera affects both children and adults and can kill within hours if untreated, it is a global threat [37]. Therefore, detection of V. cholerae in rodents in the study area, sadly where the communities are poor, with lack of social improvements and poor sanitation, suggest increased risks of human infections. The presence of V. cholera cDNA in rodents indicates the possibility that bacteria are shed into the environment through feces and potentially infecting people. Nonetheless, recently in Tanzania (April 2022) there was a report of Cholera outbreak in Tanganyika and Uvinza districts, the latter is one of the study sites. As a regard to the nature of the areas, Uvinza for instance, where there are challenges to attain access to safe and clean drinking water, along with inadequate sanitation and a close proximity between human and rodents as a result of environment fragmentation and agriculture intensification rodents cannot be ruled out as the possible sources of human infection. Despite success in control and containment of the outbreak, in order to strengthen surveillance and preparedness it is essential to look on the other potential reservoir of V. cholerae including rodents. Moreover, the detection of the none-routinely

investigated Vibrio parahaemolyticus; a causative agent of cholera-like diarrhea associated with consumption of seafoods [38] should be considered as a serious public health concern as it can lead to an unpredictable impact on populations. Therefore, this calls for a unified efforts between public, animal and environmental health which will help in preventing and controlling outbreaks and incidences of diarrhea. Furthermore, several other bacteria of zoonotic and unknown zoonotic potential were detected. These includes, Streptococcus mutans a pathogen for dental caries, and a known cause of bacteremia and infective endocarditis [39]. Chlamydia psittaci responsible for avian chlamydiosis (psittacosis) in birds. In human psittacosis can cause mild illness or pneumonia [40], Campylobacter sputorum which causes gastrointestinal infections through consumption of contaminated food or contact with infected animals [41]. Acinetobacter species (Acinetobacter baumannii, Acinetobacter pitti) the most common causes of bacteremia and nosocomial pneumonia. Haemophilus influenzae which is reported to cause pneumonia, bacteremia, meningitis, epiglottitis, cellulitis and infectious arthritis [42]. Moreover, Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus were also identified in a metagenomics analysis. Escherichia coli, Staphylococcus aureus and Haemophilus influenzae were detected at least in two pools each, indicating a relatively higher recovery rate of these bacteria in rodents; although very few strains of these bacteria are known to cause diseases in animals and humans it is essential to follow up and identify the specific strains harbored in rodents so as to identify risks in humans. On the other hand, some genera including Streptococcus, Campylobacter, Acinetobacter and Helicobacter reported in this study their role in causing diseases should not be overlooked and this should alarm the scientific community; as the possible dissemination and amplification in the environment may continue the transmission cycle and exacerbate antibiotic resistance problem in both humans and animals. Therefore, the findings of this study are in line with several other studies that have cited rodents as potential reservoir of zoonotic pathogens [1,2,4,8,43].

Conclusion

We hypothesize that rodent carriers can facilitate pathogen spread and maintain disease transmission cycles, especially in regions lacking adequate sanitation infrastructure. Rodents can be potential source of human infection as we have seen in the present study that they carry a number of potentially pathogenic and zoonotic bacteria. However, despite the high potential for zoonotic transmission, the interactions among humans, animals, and rodents are still somewhat understudied. Since no molecular characterization has been done in the present study, therefore this study is not conclusive of the several pathogenic bacteria being present in rodents; hence, we recommend follow up studies to further characterize these bacteria identified as being either pathogenic or non-pathogenic strains. Moreover, to further understand the occurrence, transmission dynamics and characterize risks so as to develop effective prevention and control plans. Yet, the public has to ensure proper hygiene and food safety practices are improved in order to minimize the risk of zoonotic infections.

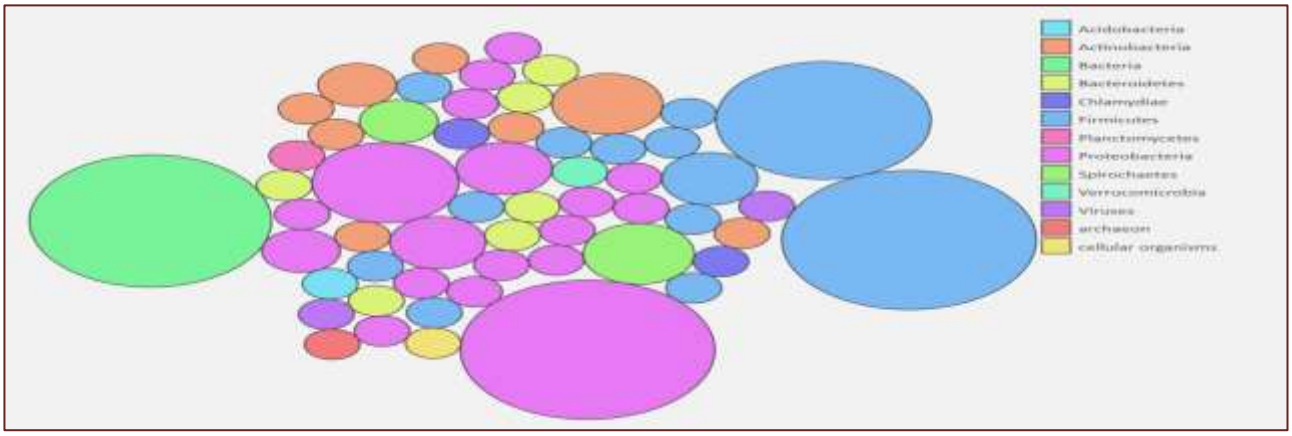

Figure 4: Metagenome overview of oropharyngeal and rectal swabs collected from rodents in human-wildlife interfaces in Kigoma and Kagera regions, Tanzania

Abbreviation

Technology Development; cDNA: complementary DNA; IRPM: Innovative Rodent Pest Management; HALI; Health for Animal and Livelihood Improvement; KCRI-BL: Kilimanjaro Clinical Research Institute-Biotechnology Laboratory; RAP: Rapid Adapter; RNA: Ribonucleic Acid; rRNA: ribosomal Ribonucleic Acid; SUA: Sokoine University of Agriculture; Kilimanjaro Clinical Research Institute-Biotechnology Laboratory.

Declaration

Acknowledgment

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Availability of data and materials

Data will be available by emailing agnerashampinga108@gmail.com

Authors' contributions

Agnes A. Mpinga (AAM) designed the project, conducted laboratory analysis, drafted the manuscript, reviewed and edited the manuscript. Coletha Mathew (CMM) participated in the designing of the project, supervising the laboratory analysis, reviewing and editing the manuscript. Happiness H. Kumburu (HHK) supervised laboratory analysis, reviewed and edited the manuscript. Rudovick R. Kazwala (RRK) participated in acquisition of funds, design of the project, supervise the project, and review the manuscript. All authors except RRK approve the final version of the manuscript.

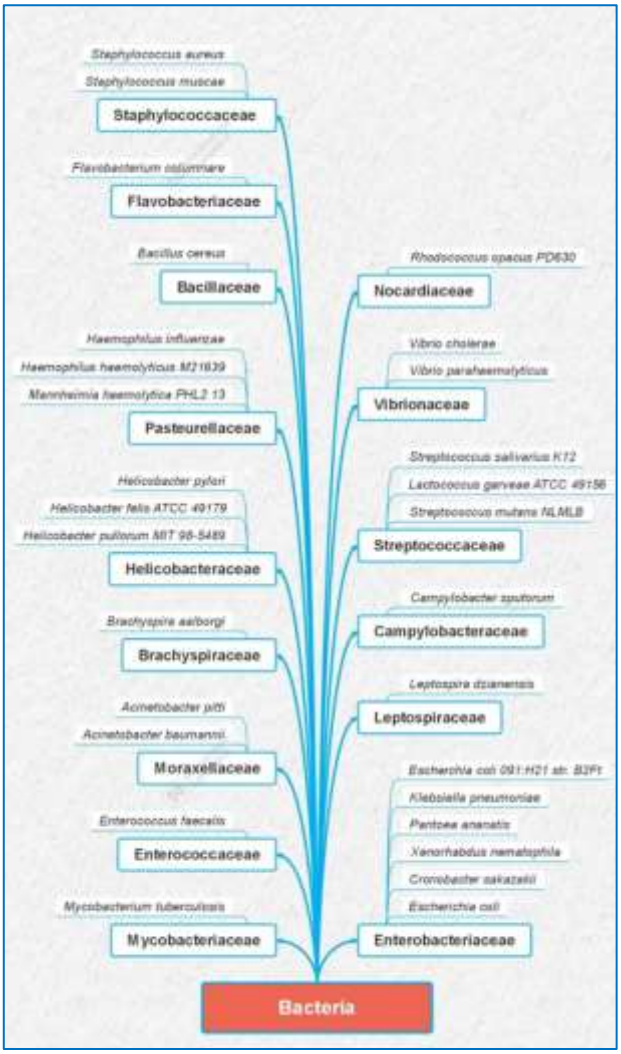

Figure 5: Tree diagram showing families and species of bacteria detected in a metagenomics analysis of oropharyngeal and rectal swabs collected from rodent species from Kigoma and Kagera region, Tanzania

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. The ethical approval was obtained from the Ethical approval was obtained from the COSTECH and TAWIRI ethical review committee, along with the research approval from the Sokoine University of Agriculture; [Ref. No. 2022].

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

Author Details

[1]College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O. Box 3021, Chuo Kikuu, Morogoro, Tanzania. [2]African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE II IRPM & BTD) Sokoine University of Agriculture, Morogoro, Tanzania. 3Kilimanjaro Clinical Research Institute, Kilimanjaro, Tanzania.

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