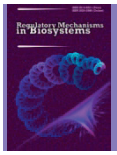

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Characterization and metagenomics analysis of the oral microbiome of *Pteropus medius*: Insights from next-generation sequencing

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The present study was planned to characterize the oral microbiome of *Pteropus medius* using next-generation sequencing and to evaluate its potential zoonotic implications. Six specimens were sampled from rural and urban sites namely Kasur, Okara and Lahore, Punjab-Pakistan. The comparative metagenomic analysis revealed significant differences in the microbial composition between rural and urban roosting sites. Bats that were captured from the rural sites had dominance of Proteobacteria (81%) followed by Firmicutes (16%) and Actinobacteria (3%). The bacterial genera such as *Escherichia* (76%), *Streptococcus* (8%) and *Staphylococcus* (5%) were identified. In contrast, urban sampling sites showed lower relative abundance of Proteobacteria (68%) followed by Actinobacteria (9%). The bacterial genera including *Cellvibrio* (51%), *Sphingobacterium* (11%), and *Stenotrophomonas* (9%) were identified. The identification of pathogenic genera viz., *Escherichia*, *Streptococcus*, and *Staphylococcus* highlights the potential zoonotic threat posed by *Pteropus medius*. It can be concluded that there is a need for continuous monitoring and assessment of the microbial communities in *P. medius* populations mostly in the areas with high human-bat interactions. The small sample size and restricted geographical scope is the limitation of the present study so future research with larger sample sizes and more diverse sites could provide a more detailed understanding of the environmental and ecological factors influencing the *P. medius* microbiome. Moreover, functional analysis of the identified bacterial taxa such as their role in metabolism, immunity and pathogen resistance could provide detailed insights into the health implications and help understand the mechanisms of pathogen transmission from bats to humans.

Keywords: fruit bats; 16S rRNA; Indian flying fox; Chiroptera; Kasur.

Introduction

Wildlife plays a part in the maintenance of ecosystems and spread of infections, particularly those that cause endemic diseases. Wildlife has long been a significant source of infectious diseases, comprising 71.8% of emerging and recurring zoonotic illnesses. Zoonotic diseases represent a serious health concern to humans and livestock worldwide as most of the pathogens have an animal origin (Munyua et al., 2016; Allen et al., 2017). In recent times, new emerging zoonotic diseases viz., brucellosis, Zika, Ebola, H_1N_1 influenza and SARS spread rapidly (Fowlkes et al., 2014; Bagre et al., 2022). This rise in zoonotic diseases is due to many anthropogenic hazards, including population growth, expansion of cities, habitat degradation, agricultural intensification and climate change (Baker et al., 2022). Fungi, parasites, bacteria and viruses are prevalent and can cause zoonosis at high frequency in the human population (Aguirre, 2017). Tropical and subtropical areas are more prone to zoonotic infectious diseases because of high richness in species diversity. Although, most of the current research focuses on the detection of new viral pathogens most of the infectious diseases are bacterial in origin (Kumar et al., 2020; Magouras et al., 2020; Rees et al., 2021).

As interactions between wild animals and humans become more frequent, we can expect an increase in zoonotic diseases (Webster et al., 2016; Ferreira et al., 2021). However, the diagnosis, description and distribution of new zoonotic pathogens remains a challenge for public health. Conventional methods including culturing of bacteria, DNA extraction, single primer amplification and Sanger sequencing are well established and are widely used. However, none of these techniques allow us to fully understand the microbiota of animals. In contrast, metagenomics-based approaches such as Next-Generation Sequencing (NGS) techniques enable a complete characterization of an animal's microbial composition (Woods et al., 2019; Ghosh et al., 2019; Nelson et al., 2019; Thongsripong et al., 2021). Bats (Chiroptera) are nearly cosmopolitan in distribution and are globally represented by over 1,460 species. Bat species of the world: A taxonomic and geographic database). In Pakistan, bats are represented by 53 species of 23 genera (Roberts 1997; Mahmood-ul-Hassan et al., 2006; Attaullah et al., 2022), including three genera of Old-World fruit bats (Pteropodidae), *Rousttus*, *Pteropus* and *Cynopterus* with four species, *Rousettus aegyptiacus*, *Rousettus leschenaultia*, *Pteropus medius* – formerly *P. giganteus* and *Cynopterus sphinx* respectively (Korine, 2016; Bates et al., 2019; Tsang, 2020; Bouillard et al., 2021). Bats have been implicated in the epidemiology of many zoonotic diseases (Mühldorfer, 2017; Cláudio et al., 2018). They are carriers of many types of microorganisms including parasites, viruses, fungi and bacteria (Galicia et al., 2014; Veikkolainen et al., 2014; Sun et al., 2020; Federici et al., 2022). Their physiology and ecology make bats important reservoirs and distributors of pathogens (Mühldorfer, 2013; Szentivanyi et al., 2023). Bats use many human-dominated areas such as fruit orchards, feed for insects around streetlights and roost in buildings. This proxymity to humans ultimately increases the chance of disease transmission to humans and domestic animals as well as livestock (Chomel et al., 2023).

Bats can be used as models to investigate microbial evolution. Hence, studies of the oral microbiota of bats could help us to better understand host-microbe evolution, ecology and the impact these animals may have on human health (Ingala et al., 2018). With their unique ability of flight, bats can easily explore large and diverse geographic ranges, thus facilitating the spread of emerging infectious diseases (EIDs). In addition, other characteristics such as low body mass, generation of B-cells in different organs, high metabolism and body temperature gives them certain immunological characteristics that could favor microorganisms (Guerrero-Chacón et al., 2018; Voigt et al., 2020). Bats are also reservoirs of antibiotic-resistant bacteria.

However, information is still limited (Pandian et al., 2022). It is important to know what types of bacteria are part of the normal microbiome of bats as compared to causative agents of diseases in their natural and urban habitats that can affect humans and animals both in rural and urban areas. While recent studies have explored bacterial communities within the intestines, colon, feces, and urine of bats, there remains a gap in research regarding the bacterial composition of their oral cavities (Federici et al., 2022).

The Indian flying fox (*Pteropus medius*) is widely distributed in Pakistan, particularly in the southern and eastern regions, including Sindh and Punjab. These bats roost in large colonies in tall trees, often near human settlements, and play a key ecological role in seed dispersal and pollination. They feed primarily on fruits and nectar, contributing to forest regeneration and biodiversity. The coexistence of humans and Indian Flying foxes has raised environmental concerns, particularly regarding the transmission of pathogens and the risk of antimicrobial resistance spillover, posing health hazards. Despite advancements in metagenomics techniques, there have been limited studies conducted in Pakistan. Therefore, the aim of the present study was to characterize the oral microbiome of the Indian flying fox (*Pteropus medius)*, comparing urban and rural roosting sites in the districts of Lahore, Kasur and Okara, Punjab, Pakistan.

Materials and methods

The study presented here was conducted between August 2023 to July 2024 in six sites located within the districts Okara, Kasur and Lahore (Fig. 1).

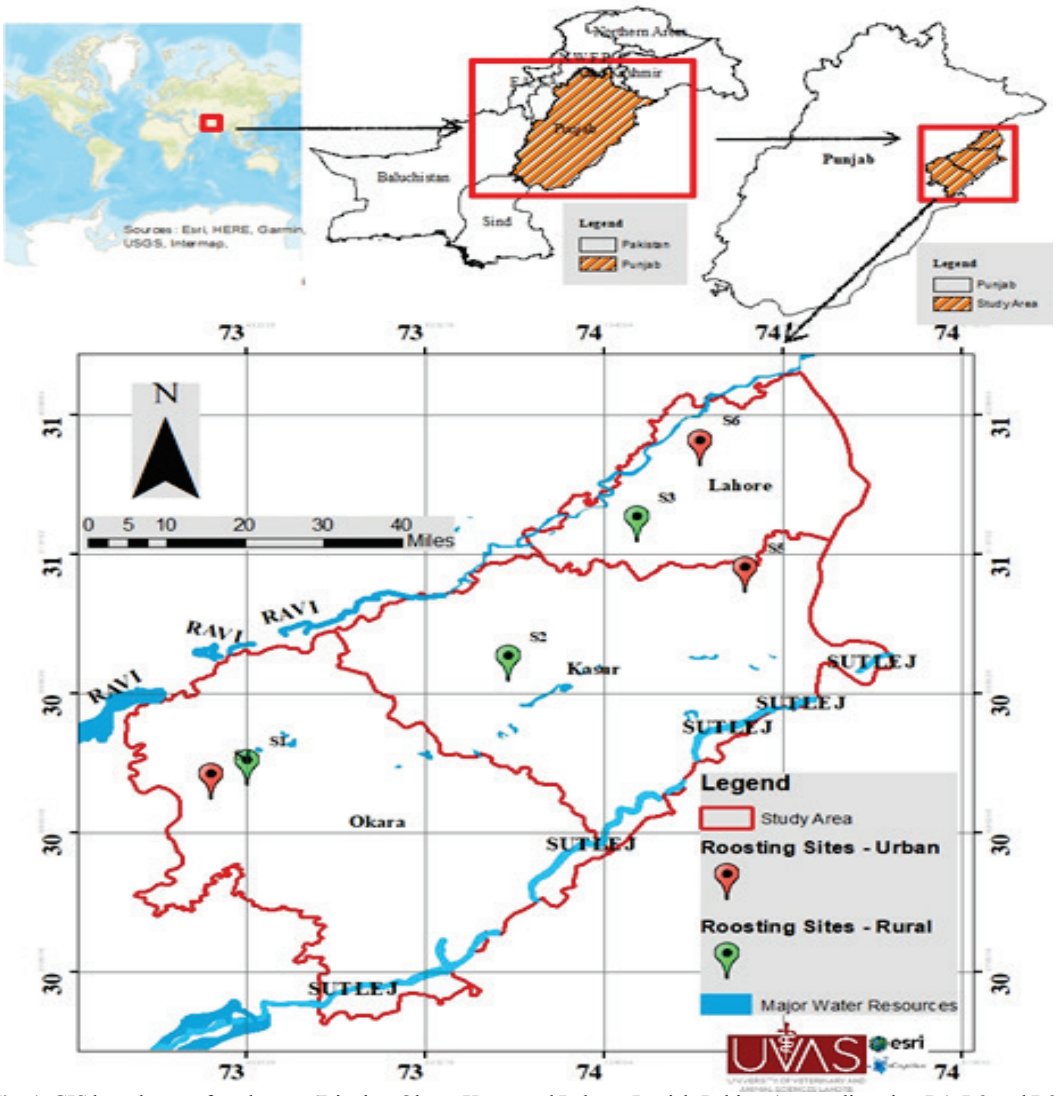

Fig. 1. GIS based map of study area (Districts Okara, Kasur and Lahore, Punjab-Pakistan): sampling sites R1, R2 and R3 were roosting sites in rural areas (green pins) while U1, U2 and U3 were roosting sites in urban areas (red pins)

The study area cover a diverse range of habitats from fertile agricultural plains and forested areas to urban landscapes. Okara covers an area of 4,377 km^2, and is characterized by an extreme climate, with temperatures ranging from 1 °C in winter to 47 °C in summer. The average annual rainfall is 468 mm. Kasur has an area of 3,995 km^2 and is located between the Ravi river in the northwest and the Sutlej river in the southeast (Fig. 1). It experiences a local steppe climate, characterized by minimal rainfall throughout the year. The average temperature is 23.9 °C and annual precipitation approximately 424 mm. Lahore covers an area of 1,772 km^2 and has a semi-arid climate. The hottest month is June, the wettest month is July and January is the coldest month accompanied by dense fog. As sub-adult male of *Pteropus medius* species traveled a maximum distance of 42 km in a single night (Murugavel et al., 2023) so six known roosting sites of *P. medius* were selected and categorized into urban (present in major cities) and rural (present in natural vegetation minimum 8 km away from a major city).

Bat sampling and identification. Overall, six specimens of *P. medius* were captured from rural and urban locations in the study area. The specimens were captured according to the ethical guidelines provided by Ethical Review Committee (ERC) of University of Veterinary and Animals Sciences, Lahore, Pakistan.At each site, three mist nets (12.0 x 2.5 m) were installed at height of the roosting trees (minimum at 20 m) with bamboo sticks on alternative months of the year and six specimens of *P. medius* were randomly selected, one specimen from each site during every attempt. The details of roost types,

description and GPS coordinates of roosting sites are given in Table 1. The captured specimens were brought to the PG Lab, Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore.

Table 1
Roost types, description and GPS coordinates of roosting sites of *Pteropus medius*

Site No.	Area	Site area description	GPS Coordinates
R1	Rural	Sikandar Road, Okara	30°49'56.40"N 73°29'33.66"E
R2		Changa Manga Forest, Kasur	31° 4'4.70"N 73°58'25.70"E
R3		Safar Zoo and Wildlife Park, Lahore	31°22'52.74"N 74°12'40.38"E
U1	Urban	Near DPO Office, Okara	30°48'4.91"N 73°25'31.92"E
U2		Mustafaabad, Kasur	31°16'5.21"N 74°24'44.75"E
U3		Jinnah Garden, Lahore	31°33'15.00"N 74°19'44.16"E

Sterile cotton swabs were used to collect saliva samples from the captured specimens. The bats were allowed to bite the swab for a few minutes to obtain a substantial amount of saliva. All individuals were released in their natural habitat after sampling. Swabs were stored in a bovine albumin 1 viral transport medium to reduce the risk of contamination. Oral swabs were stored at –10 ^{o}C until DNA extraction was done.

In microfuge tubes, each cotton swab was cut in 1% PBS solution and incubated at 80 ^{o}C for 2 hours. DNA was extracted using QIAamp DNA Microbiome kit and quantified using Nano-Drop One. The quality of extracted DNA samples was checked through 1% agarose gel. The primer set 27F (5-AGAGTTTGATCCTGGCTCAG-3) and BS-R1407 (5-GACGGGCGGTGWGTRC-3) was used to amplify the variable regions (V) V1 to V8 of 1380 bp of the 16S rRNA gene (Klindworth et al., 2013; Rodriques et al., 2019; Hussain et al., 2024). PCR reaction was done in 25 µL reaction mixture following Hussain et al. (2024). The Next-Generation Sequencing of PCR products was done from Macrogen, Korea through Alpha Genomics lab, Islamabad, Pakistan.

The preprocess analysis was done using PKSSU4.0 taxonomy databases of the prokaryotic 16S rRNA gene and Kraken2 (Lu & Salzberg, 2020). Operational Taxonomic Units (OTU) based on valid DNA reads were picked for further analysis (Hussain et al., 2024). FASTQ from the valid DNA reads were imported in Qiime2 version 2021.4 software (Brown et al., 2017). Paired-end reads for all samples were imported using the manifest file method. This approach facilitated the construction of a fragment library for paired-end sequencing. Following read trimming and filtering, OTU clustering, species annotation, and abundance analysis were performed to determine the species composition within the samples (Magurran, 2003). The bacterial communities at the genus and species level were grouped. OTU clustering based on closed references was done using the VSEARCH tool. A FASTA file containing the sequences was used as a reference in combination with QIIME2's. The SILVA database (www.arb-silva.de/download/archive/qiime) for 16S rRNA gene was used as the reference database (Quast et al., 2012). Taxonomies were assigned to each DNA read using a Naïve Bayes classifier with the Q2 feature classifier plugin. The classifier was trained on DNA sequences with 97% similarity from the reference databases (Wang et al., 2007). All the non-bacterial OTUs sequences were filtered out using the feature table filtering method in Qiime2. All the valid reads were grouped into OTUs at a 97% sequence similarity threshold to check the species diversity within each sample.

Bar plots were constructed to visualize the taxonomic composition and relative abundance of different bacterial taxa at seven classification levels. The relative abundance of different bacterial groups was visualized through a KRONA plot (Ondov et al., 2011) and Sankey plots were generated in Pavian (https://github.com/fbreitwieser/pavian). The circles from inside to outside in a KRONA plot represents the different classification levels and the relative abundance of different OTUs.

Results

The details of external body measurements and weight of all the captured specimens are given below in Table 2.

Table 2
External body measurements and weight of *Pteropus medius* specimens captured from selected sites in Punjab, Pakistan

Parameters	Roosting sites in rural areas			Roosting sites in urban areas		
	R1	R2	R3	U1	U2	U3
Weight, g	820	980	950	890	1010	940
Wingspan, mm	1118	1172	1146	1136	1202	1140
BL, mm	204	218	210	208	220	209
SL, mm	33	35	35	34	36	34
FA, mm	195	190	198	210	201	194
E, mm	38	36	38	35	33	36
LFC, mm	41	44	44	43	44	43

Note: weight: entire organism's weight; wingspan: both wings length; BL: body length; SL: snout length; FA: forearm length from elbow to thumb; E: length of the ear; LFC: length of foot claw; all measurements taken were based on Khan (2020); R: rural sites, U: urban sites.

The DNA extraction from the oral swabs was done according to standard protocols. After DNA extraction, the quality of the DNA was checked with 1.2% agarose gel. Agarose gel electrophoresis showed the high integrity of DNA samples with distinct DNA bands (Fig. 2). The purity of the DNA was checked through Nano-Drop ONE (Table 3).

Identified phyla and their relative abundance of bacteria taken from oral swabs of *P. medius* captured from rural roosting sites were as follows; Proteobacteria 81% > Firmicutes and Actinobacteria were 16% and remaining were Patescibacteria and Bacteriodetes. Bacterial classes identified include Bacteroidia, Saccharimonadia, Negativicutes, Actinobacteria, Bacilli and Gammaproteobacteria. The order of abundance was Gammaproteobacteria 81% > Bacilli 15% > Actinobacteria 3%. Similarly, Saccharimonadales, Xanthomonadales, Betaproteobacteriales, Pseudomonadales, Selenomonadales, Pasteurellales, Corynebacteriales, Bacillales, Lactobacillales were different bacteria orders identified in the saliva of *P. medius*. The order of abundance was as follows Enterobacteriales and Lactobacillales 10% > Bacillales 6%. The identified families were Enterobacteriaceae 79% > Streptococcaceae 8% > Corynebacteriaceae 3%> Pasteurellaceae 1% while genera including *Actinobacillus*, *Pseudomonas*, *Veillonella*, *Weissella*, *Pantoea*, *Staphylococcus*, *Streptococcus* and *Escherichia* were identified. The percentage of *Escherichia* were 76% > *Streptococcus* 8% > *Staphylococcus* 5% > *Pantoea* 2% > and *Weissella* was 1%. The relative abundance of *Staphylococcus aureus* was 3%.

Similarly, identified bacterial phylums and their relative abundance from oral swabs of *P. medius* captured from urban roosting sites. were as follows Proteobacteria 68% > Actinobacteria 9% and others were Firmicutes and Bacteroidota. Bacterial classes included Gammaproteobacteria 66%, Bacilli 11%, Bacteroidia 11% and Actinobacteria 9% and the other was Alphaprotobacteria. Similarly, Xanthomonadales, Pseudomonadales were 56%, Burkholderiales, Caulobacterales, Staphylococcales, Lactobacillales, Sphingobacterials, Propionibacterials were 3%, Micrococcales and Corynebacteriales were 4%. The percentages of identified bacterial families were Cellvibrionaceae 51%, Sphingobacteriaceae 11%, Xanthomonadaceae 9%, Staphylococcaceae 8%, Streptococcaceae 8%, Pseudomonadaceae 4%, Nocardioidaceae 3%, Caulobacteraceae 2%, Brevibacteriaceae 2% and Corynebacteriaceae 1%. The percentages of bacterial genera were *Cellvibrio* 51%, *Sphingobacterium* 11%, *Stenotrophomonas* 9%, *Streptococcus* 8%, *Pseudomonas* 4%, *Jeotgalicoccus* 3%, *Nocardioides* 3%, *Brevundimonas* 2%, *Brevibacterium* 2% and *Corynebacterium* was 1%. Figure 3 shows the hierarchical taxonomy and relative abundances of different bacterial taxa across different taxonomic levels. The analysis of the oral microbiome of *P. medius* from

urban and rural roosting sites discovered many overlapping bacterial taxa. At the phylum level, both environments were mainly composed of Proteobacteria, Firmicutes and Actinobacteria. These phyla are commonly associated with mammalian oral microbiota, playing crucial roles in the overall health of the animals. At the class level, Gammaproteobacteria, Bacilli, and Actinobacteria were identified in both sampling sites. Gammaproteobacteria, in particular dominated the bacterial community in the both sites. Furthermore at the family level, Streptococcaceae and Corynebacteriaceae were identified in both sites representing their adaptability to varied environmental conditions. These two families include the genera viz., *Streptococcus* and *Corynebacterium*, which are reported from diverse environments. The genus level analysis showed the presence of *Streptococcus* and *Pseudomonas*. These genera are often related to various metabolic and pathogenic processes.

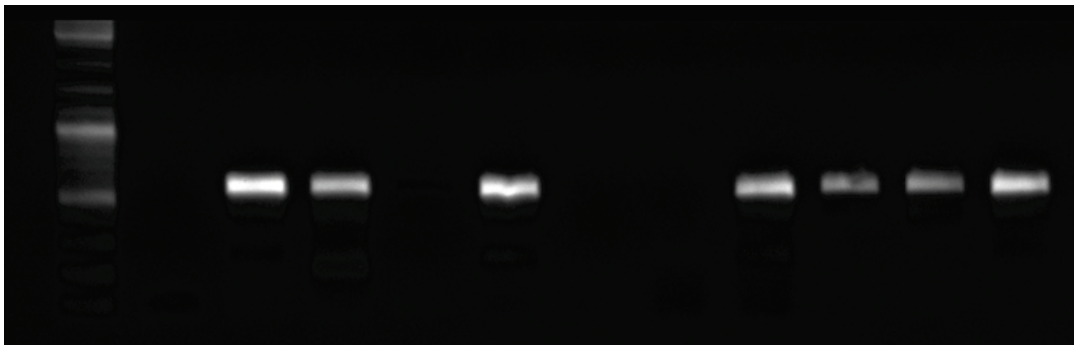

Fig. 2. Successful extracted DNA samples were checked on 1.2% agarose gel against 1KB ladder

Table 3
DNA quantification using NanoDrop

Species	Nucleic acid, ng/uL	A260/A280	A260/A230	A260	A280	Nucleic acid factor	Baseline correction, nm	Baseline absorbance
Rural sites								
R1	458.560	1.620	0.818	9.441	5.489	49	339	0.507
R2	460.511	1.654	0.840	9.167	5.710	51	336	0.498
R3	457.380	1.680	0.860	9.251	5.523	50	338	0.510
Urban sites								
U1	262.60	1.647	0.890	3.88	2.620	49	338	0.163
U2	259.50	1.680	0.851	4.06	2.575	50	340	0.174
U3	257.40	1.629	0.872	3.99	2.550	49	337	0.169

Notable differences were observed in the diversity and the relative abundance of different bacterial taxa showing the impact of environmental factors on oral microbial composition. In the rural roosting sites the oral microbiome of *P. medius* was dominated by Proteobacteria (81%) representing a less diverse bacterial community compared to urban samples. *Escherichia* was the most abundant genus (76%), *Staphylococcus* (5%) and *Pantoea* (2%) were also identified. The relative abundance of Enterobacteriaceae was 79%, which further highlighted the predominance of gut-associated bacteria in rural bats, possibly due to dietary habits or habitat-specific factors.

However, urban roosting sites showed a reduction in Proteobacteria (68%). The genus *Cellvibrio* was highly abundant (51%). This genus is commonly found in soil and plant matter, may indicate a shift in microbial diversity due to urban environmental exposures. Furthermore the relative abundance of *Sphingobacterium* was 11%, followed by *Stenotrophomonas* 9%. These genera are often associated with environmental resilience indicating adaptation to urban habitats. Two unique genera viz., *Jeotgalicoccus* and *Nocardioides* (3% each) were present in urban sites but absent in rural samples. These taxa are associated for their role in environmental and nutrient cycling, indicating the influence of urban ecological niches on the oral microbiome of fruit bats. The relative abundance of families such as Cellvibrionaceae was 51% and Sphingobacteriaceae was 11% and these which are less common in the rural samples.

Discussion

The present study provides valuable insights into the oral microbiota composition of *P. medius* captured from urban and rural roosting sites from Punjab, Pakistan. The significant differences in bacterial taxa between urban and rural roosting sites suggested that environmental factors viz., habitat, diet and human activities significantly influence the oral microbiome of *P. medius*. The higher relative abundance of bacteria reported in the urban sites may have resulted from exposure to a broader range of environmental bacteria potentially offering urban bats an advantage in adapting to changing environmental conditions. In contrast the dominance of *Escherichia* and other gut-associated bacteria in rural bats indicated a closer link between oral and gut microbiota in less anthropogenically influenced habitat.

The results indicated that while there is a core microbiome present in both urban and rural bats, the diversity and relative abundance of specific bacterial taxa differ significantly. Proteobacteria, Firmicutes, and Actinobacteria were consistently found in both environments, suggesting that their fundamental role in the oral ecosystem. However, the dominance of Proteobacteria in rural samples (81%) compared to the urban samples (68%) points to environmental and dietary influences in shaping microbial communities. Our analysis of the oral microbiota in *P. medius* from rural and urban roosting sites highlights distinct bacterial community compositions, underscoring potential zoonotic risks and environmental influences. This dominance is consistent with findings in the oral microbiomes of various bat species, including those studied by Luna et al. (2024), who reported Proteobacteria at 64.3% as a major component in *Phyllostomus* and *Carollia* species. Ingala et al. (2018) further suggested that the prevalence of Proteobacteria in bats may reflect their unique feeding and roosting habits, which expose them to diverse environmental microbes. Kolodny et al. (2019), who reported colony-wide shifts in microbiomes in response to environmental changes in Egyptian fruit bat (*Rousettus aegyptiacus*).

Our study also identified habitat-specific differences, with rural bats showing higher proportions of Enterobacteriaceae (79%), Streptococcaceae (8%), and Corynebacteriaceae (3%). In comparison, urban bats exhibited a unique composition dominated by Cellvibrionaceae (51%) and Sphingobacteriaceae (11%). Such shifts are aligned with the dietary and environmental adaptation theories proposed by Banskar et al. (2016), who documented similar community shifts in *Rousettus leschenaultia*.

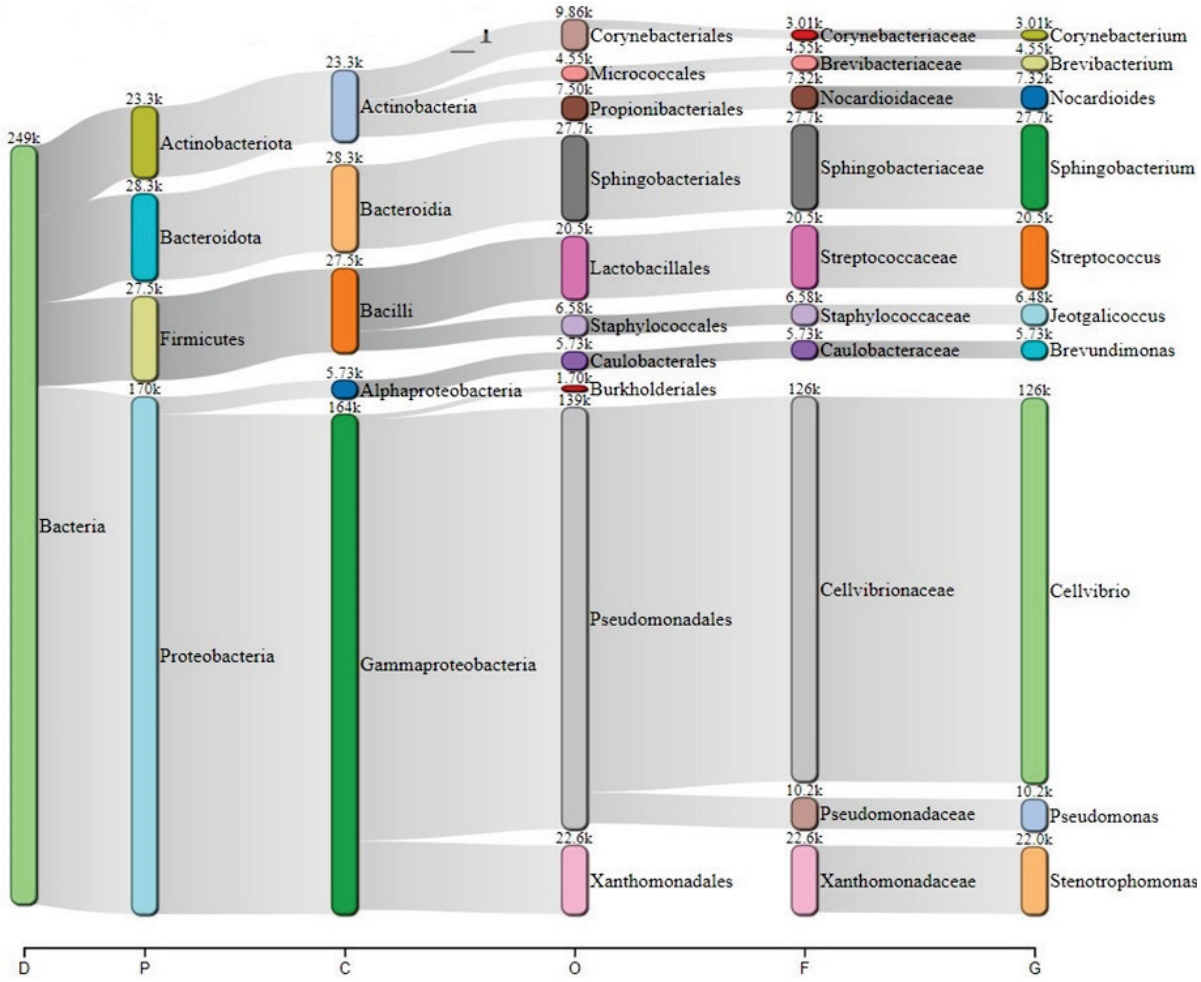

Fig. 3. The Sankey chart representing the relative abundance of different bacterial taxa in saliva of *Pteropus medius* captured from urban roosting sites

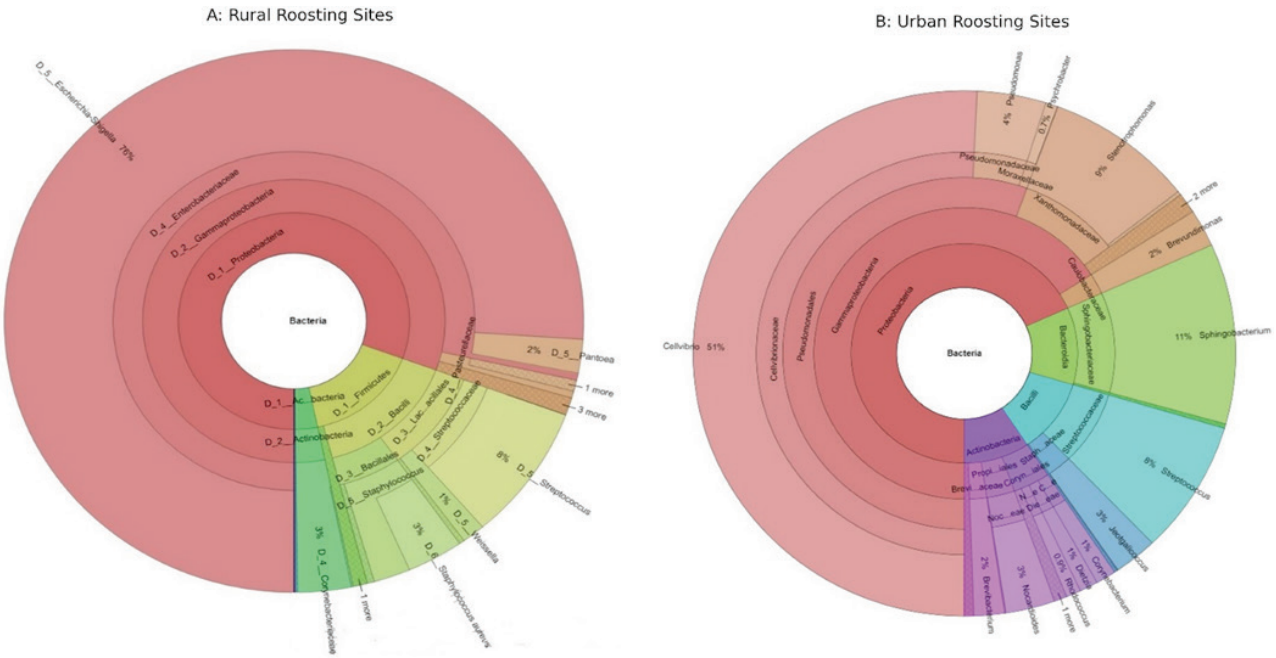

Fig. 4. The KRONA plot showing the relative abundance of different bacterial groups

In terms of bacterial genera, *Escherichia* (76%) was notably abundant in rural bats, followed by *Streptococcus* (8%) and *Staphylococcus* (5%). This is significant given the zoonotic potential of these genera, which have been identified as potential reservoirs for pathogens in various studies, such as Attaullah et al. (2022), who associated *Staphylococcus aureus* with potential zoonotic risk in bat populations. Similarly, Uddin (2018) found high levels of *Escherichia coli* in free-ranging bats, raising concerns about antimicrobial resistance and its implications for zoonotic transmission. Our findings align with these studies, suggesting that *P. medius* could be a source of zoonotic bacteria.

Urban bats, however, showed a significant presence of *Cellvibrio* (51%) and *Stenotrophomonas* (9%) genera known for environmental

adaptability. The presence of *Streptococcus* (8%) and *Staphylococcus* (8%) in urban bats is also noteworthy, reflecting similar bacterial profiles found in human-impacted environments, as suggested by Carrillo-Araujo et al. (2015). These findings also resonate with Klausenstock (2016), who found that captive bats exhibited a greater diversity of pathogenic *Staphylococcus* bacteria compared to wild bat s within the same species *Dermanura tolteca* and *Myotis keaysi*.

In contrast, the urban samples displayed a more diverse bacterial community. The predominance of *Cellvibrio* (51%) and the presence of environmental taxa such as *Sphingobacterium* and *Stenotrophomonas* highlight the influence of urban environmental factors, including exposure to human activities, pollution, and varied dietary inputs. These findings align with previous studies suggesting that urban environments promote microbial diversity due to increased exposure to heterogeneous microbial sources.

The distinct microbial profiles observed between the two environments underscore the adaptability of *P. medius* to different ecological niches. Urban bats, exposed to a wider range of environmental bacteria, may benefit from a more diverse microbiome, potentially enhancing their resilience to environmental changes and pathogen colonization. The presence of genera such as *Jeotgalicoccus* and *Nocardioides* in urban bats further supports this hypothesis, as these taxa are known for their environmental versatility and stress tolerance.

On the other hand, the rural bats' microbiome, dominated by gut-associated bacteria, may reflect a more specialized or constrained microbial community, potentially linked to their specific dietary and habitat preferences. This could have implications for their susceptibility to infections or environmental changes, as a less diverse microbiome might limit their adaptability.

The oral microbiome serves as a gateway to both the respiratory and digestive systems, making its composition crucial for overall health. The dominance of specific taxa such as *Escherichia* in rural bats may have implications for their susceptibility to gastrointestinal or systemic infections. Conversely, the diverse microbiome in urban bats could provide a protective effect by preventing the colonization of pathogenic bacteria.

Furthermore, the presence of potential opportunistic pathogens such as *Streptococcus* and *Staphylococcus* in both rural and urban sites raised questions about their role in *P. medius* health and their potential role to act as reservoirs for zoonotic diseases. Understanding these dynamics is particularly significant given the role of *P. medius* in the transmission of pathogens to other species including humans.

Conclusion

The comparative metagenomic analysis revealed the dynamics of the oral microbiome in *P. medius*, which is shaped by ecological factors and roosting sites. The findings of the current study provide significant insights into how urbanization and habitat variations influence microbial diversity and the health of these animals. The identification of pathogenic genera viz., *Escherichia*, *Streptococcus*, and *Staphylococcus* highlights the potential zoonotic threat posed by *P. medius*. It can be concluded that there is a need for continuous monitoring and assessment of the microbial communities in *P. medius* populations mostly in areas with a high level of human-bat interactions. The restricted sample size and geographical scope is the limitation of the present study so future research with larger sample sizes and more diverse sites could provide a more detailed understanding of the environmental and ecological factors influencing the *P. medius* microbiome. Moreover, functional analysis of these identified bacterial taxa, such as their role in metabolism, immunity and pathogen resistance could provide detailed insights into the health implications and may help better understand the mechanisms of pathogen transmission from bats to humans.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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