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Preliminary assessment and metagenomic analysis of oral microbiota of the small Indian mongoose *Herpestes auropunctatus* through next-generation sequencing

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A total of 5 small Indian mongoose (*Herpestes auropunctatus* (Hodgson, 1836)) specimens were captured to obtain oral swabs with all specimens being released after sampling. DNA extraction from oral swabs was done using the QIAamp DNA Microbiome and the 16S rRNA gene was amplified targeting variable region V1 to V8 (1350 bp). Next-generation sequencing (NGS) of PCR products was performed from Macrogen, Korea. A KORONA plot was constructed to visualize relative abundance of the top 10 bacterial taxa. The following bacterial phyla were identified; Proteobacteria (58.0%), Bacteroidetes (20.0%), Firmicutes (12.0%), Fusobacteria (7.0%), and Patescibacteria (2.0%). The dominant bacterial classes included Gammaproteobacteria (57.0%), Bacteroidia (20.0%), Bacilli (4.0%), and Alphaproteobacteria (0.8%). The prevalent bacterial orders were Pseudomonadales (19.0%), Bacteroidales (10.0%), Flavobacteriales (9.0%), and Clostridiales (7.0%), with Fusobacteriales and Betaproteobacteriales each at 5.0%, and Lactobacillales, Absconditabacteriales, and Saccharimonadales at 4.0%, 1.0%, and 0.1%, respectively. The identified families and their relative abundances were Pasteurellaceae (29.0%), Weeksellaceae (5.0%), Neisseriaceae (4.0%), Peptostreptococcaceae (3.0%), with Erysipelotrichaceae, Leptotrichiaceae, and Enterobacteriaceae each at 2.0%, and Cardiobacteriaceae and Burkholderiaceae at 1.0% each. Other families included Xanthomonadaceae (0.8%), Carnobacteriaceae (0.5%), and Streptococcaceae (0.2%). The genera identifyed were *Pasteurella*, *Paracoccus*, *Escherichia*, *Shigella*, *Moraxella*, *Stenotrophomonas*, *Neisseria*, *Conchiformibius*, *Bergeyella*, *Capnocytophaga*, *Fusobacterium*, *Oceanivirga*, *Streptococcus*, *Bacillus*, and Defluviitaleaceae. The identification of genera such as *Pasteurella*, *Neisseria*, *Eschrichia* and *Shigella* warrants further investigation into their potential role as reservoirs of zoonotic pathogens especially given the mongoose's invasive nature, close contact with human and animal populations.

Keywords: *Escherichia coli*; *Stenotrophomonas*; *Fusobacterium*; *Bacillus*.

Introduction

The oral cavity of mammals has a complex and dynamic community of microorganisms known as the oral microbiota. The oral microbiota plays a crucial role in maintaining health, help in digestion and protect against pathogenic invasions. The ecological studies are an essential component of integrated wildlife conservation and habitat protection (Zhu et al., 2020). A species' ecology includes a variety of aspects including its geographic distribution, habitat, foraging behavior, innate behavior and physiology (Travelline et al., 2019). The oral and gut microbiome influence the overall health of the host. A great deal of research has been conducted on the microbiomes of relatively rare, endangered or valuable wildlife populations but invasive species are typically overlooked (Bandeira et al., 2016).

These gastrointestinal tract microbes perform a variety of tasks, including vitamin production, breakdown of harmful substances, immune system modulation, and the absorption of nutrients and minerals through fibre fermentation to short-chain fatty acids. Numerous illnesses, including those of the liver, diabetes, inflammatory bowel disease, autoimmune diseases, colon cancer, and central nervous system disorders are closely correlated with the fluctuations in this microbiome. For instance, the gut microbiome composition varies in newborns, adults and elderly; therefore, while evaluating the gut microbiota, a specific age range should be taken into account (West et al., 2019). As a non-invasive method of evaluating the gut microbiome, stool samples are frequently employed. The fact that the mucosal-associated microbiome and the faecal microbiome are grouped differently is significant. Different sequencing methods offer important insights into the function of the gut microbiota in both health and illness. Confounding variables that may affect the gut microbiome in human populations, such as healthy individuals and patients with illnesses, must be taken into account when assessing the data. These variables include food, medicine, age, sex, lifestyle, and so on.

Oral and gut microbiota of animals have been under the attention of researchers in recent times (Cunha et al., 2020a, 2020b). While the first reports had humans as subjects, microbiota studies have been increasingly extended to other domestic and wildlife animal species to understand the significant component of their ecology (Barros et al., 2015). The mammalian oral microbiota is influenced by the host's habitat and feeding ecology. Additionally, many environmental factors and geographical location considerably impact the composition and relative abundance of bacterial taxa in the oral and gut of wild animals (Dijk et al., 2018). These factors collectively play a vital role in the formation of species-specific microbiomes. The family Herpestidae is represented by two subfamilies including Herpestinae and Mungotinae. The family Herpestidae is represented by 34 species (Liccioli et al., 2015). Invasive species can have an intense impact on ecosystem integrity and function in areas where they have been introduced and become established. Globally, invasive animals have served as reservoirs for many pathogens that cause disease to humans. The process may start by introducing novel pathogens to native habitat or by amplifying already available pathogens. *Salmonella* is a prevalent food and waterborne bacterium related to human gastroenteritis. Outbreaks of *Salmonella* that affect humans are often difficult to control (Barun et al., 2011).

The small Indian mongoose is native to India and Southeast Asia but has been introduced to various parts of the world where they often became an invasive species (Bernal et al., 2020). Riverine, open habitats, deep forests, woodlands, and areas near human habitations are among the places where the small Indian mongoose has been spotted. Increased urbanisation has a detrimental effect on the small Indian mongoose's range. In addition, this species is sought for its oil, skin, and hair. Dadu, Thatta, and Tharparkar (province of Sindh), Gujranwala, Gujrat, Sialkot, Jhelum, Khushab, Chakwal, Sargodha, Attock (province of Punjab), Mekran, Turbet (province of Balochistan), and Kotli districts (Azad Jammu and Kashmir) are among the locations in Pakistan where this species is found. The small Indian mongoose has a head to body length of 30–35 cm. The length of the long tapering tail is equal to eighty percent of the head and body length. Its fur coat lacks underwool, making it coarse. Blond and golden buff hair is annulated, giving the impression of "salt and pepper " close up, but dark olive-brown at a distance. Russet red hair surrounds the eyes and muzzle (Roberts, 1997).

In present era Emerging Infectious Diseases (EIDs) are playing an important role in health issues to humans. Tropical and subtropical areas are more prone to zoonotic infectious diseases because of the high richness in species diversity (Berentsen et al., 2017). The small Indian mongoose is an omnivorous animal which primarily preys upon other small vertebrates and fruits, which ultimately affect the composition of its oral microbiota (Bandeira et al., 2018). Although considerable research has been done on their genetics, reproduction and feeding habits, there is still much to know about the oral and gut microbiota of these animals (Whyte et al., 2017). Furthermore, the introduction of the small Indian mongoose into non-native ecosystems has led to significant ecological disruptions (Pieter et al., 2016). As an opportunistic predator, it preys on a wide range of species, including birds, reptiles and small mammals contributing to the decline of native populations (Veron et al., 2007). Numerous native fauna are thought to have gone extinct as a result of the mongoose abundance on these islands growing to invasive levels in recent years (Trick et al., 2017). Similarly, humans are at risk of zoonotic infections from the small Indian mongoose as these animals live in close proximity to humans (Medley et al., 2020). Many factors impact the composition of the oral microbiota including the host's diet, habitat, behavior and ecological interactions (Colston & Jackson, 2016). The mongoose's habitat ranging from forests and grasslands to human altered habitats which may also impact its overall microbiome (Mckenzie et al., 2017). Although current research is mostly concerned with detecting new pathogens, especially viral, in most infectious diseases bacterial microbiomes also play an important role. Next-generation sequencing has revolutionized the study of microbial communities by allowing wide-range analysis of microbial DNA (Weldon et al., 2015). This technique not only provides insights into the diversity and structure of the oral microbiome (Suzuki, 2017) but also helps in understanding the functional roles of these microbes in the host's health and disease (Nelson, 2015; Nikaido et al., 2020). While extensive research has been conducted on the oral microbiota of humans and domestic animals there remains a paucity of information regarding wild species, particularly the small Indian mongoose (*Herpestes auropunctatus*).

Materials and methods

Sahiwal district is almost 100 km from the Faisalabad and 180 km from main city Lahore and lies between Multan and Lahore. The city lies in a densely populated region between the Ravi and Sutlej rivers. Total area of the Sahiwal is 3201 square km lying between (31°58'23'' N, 72°19'32'' E). The major cultivated crops include cotton, wheat, oil seeds and potato. Figure 1 shows the GIS based map of the study area where specimens were captured.

A total of 5 specimens (n = 5) of small Indian mongoose (*Herpestes auropunctatus*) were captured from selected sites in district Sahiwal with the help of live traps using dry fish as bait (Schneider & Kappeler, 2016). For the identification, captured specimens were brought to the Postgraduate lab, UVAS, Ravi Campus. Sterile cotton swabs were used to collected saliva samples. The animals were allowed to bite the swab for a few minutes to obtain a significant amount of saliva. All the specimens were set free afterwards in their natural habitat after sampling. Swabs were stored in a bovine albumin 1 viral transport medium to reduce the risk of contamination (Shriner, 2012). Oral swabs were stored at –80 ºC for further analysis.

DNA extraction from oral swabs was done with the QIAamp DNA Microbiome Kit according to the manufacturer's instructions. The quality of DNA was checked through 1.2% agarose gel (Fig. 2) and DNA was quantified using Nanodrop (Table 1). The variable regions (V) V1 to V8 of 1380 bp of the 16S rRNA gene were amplified using the primers following Hussain et al. (2024). The PCR was done in 25 µL reaction mix using 11 µL PCR Master Mix, 1µL F-primer, 1 µL R-primer, and 10 µL of DDW and 2 µL DNA. PCR conditions were as follows; initial denaturing at 95 °C for 3 minutes, 30 cycles of 95 °C for 30 seconds, 50 °C for I min, 72 °C for 90 seconds, and final extension of 72 °C for 10 minutes. The Next-Genertion Sequencing of PCR products was done from macrogen Korea.

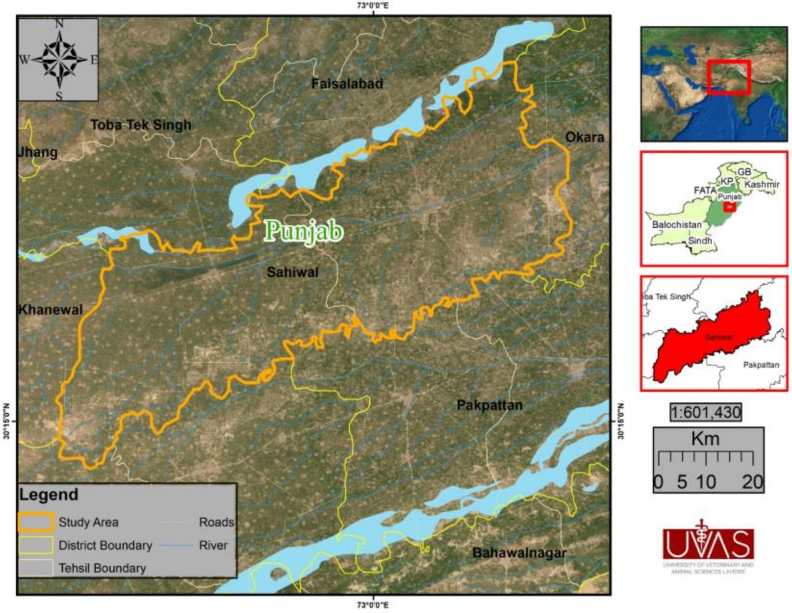

Fig. 1. GIS based map of the study area

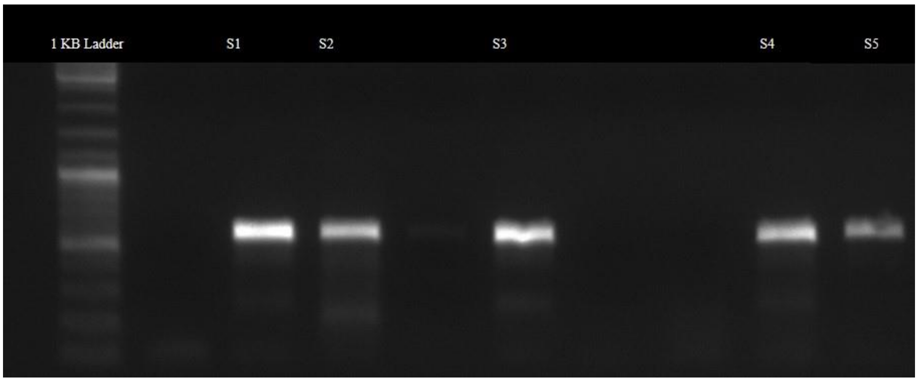

Fig. 2. The results of agarose gel electrophoresis for the PCR amplification of target DNA sequences: the leftmost lane represents the DNA marker/ladder used for estimating the size of the DNA fragments; the subsequent lanes display amplified DNA products with distinct bands indicating successful amplification

Table 1
Quantitative analysis of DNA using NanoDrop spectrophotometer showing nucleic acid concentration, purity ratios and absorbance values for various specimens

Specimens	Nucleic acid, ng/μL	A260/A280	A260/A230	A260	A280	Baseline correction, nm	Baseline absorbance
S1	428.450	1.530	0.742	9.024	4.430	382	0.512
S2	425.322	1.440	0.816	8.992	4.880	366	0.472
S3	417.290	1.560	0.820	8.760	5.026	318	0.508
S4	365.850	1.598	0.798	4.080	3.020	324	0.176
S5	338.900	1.602	0.804	3.780	2.808	308	0.168

Note: S – specimens.

The raw 16S rRNA sequence data was imported in PKSSU4.0 database of prokaryotic for initial analysis. The valid DNA sequences were used for the picking of the operational taxonomic unit (OUT). Then VSEARCH tool was used to arrange the OUT data. The relative abundance of bacterial communities were assessed using OTU data. The reference sequences data was saved as a FASTA file using QIIME 2. The Q2 feature classifier plugin and the Nave Bayes classifier were used to assign possible categories to each DNA read. The bacterial microbiota at the genus and species level were assembled. Finally valid DNA reads (FASTQ) from the original DNA fragments were imported in QIIME 2 v2021.4 software and the KRONA plot was constructed (Hussain et al., 2024).

Results

The KRONA plot (Fig. 3) provides a detailed visualization of the relative abundance and hierarchical distribution of the bacterial taxa.

The following phyla were identified and their percentages were as follow Proteobacteria 58.0% > Bacteriodetes 20.0% > Firmicutes 12.0% > Fusobacteria 7.0% > Patescibacteria 2.0%. These phyla represented the majority of the microbial community within the mongooses' oral cavity. At the class level, Gammaproteobacteria accounted for the largest proportion (57.0%), followed by Bacteroidia (20.0%), Bacilli (4.0%), and Alphaproteobacteria (0.8%). Similarly, at order level, Pseudomonadales, Bacteriodales, Flavobacteriales, Clostridiales, Fusobacteriales, Betaproteobacteriales, Lactobacillales, Absconditabacteriales, Saccharimonadales, Bacillales were identified. The order of relative abundance was as follows Pseudomonadales, Bacteriodales, Flavobacteriales, Clostridiales were 19.0% > 10.0% > 9.0% > 7.0% > respectively while Fusobacteriales and Betaproteobacteriales were at 5.0%, Lactobacillales, Absconditabacteriales, Saccharimonadales were at 4.0% > 1.0% > 0.1% respectively.

The order of identified families and their relative abundance was as follows Pasteurellaceae 29.0% > Weeksellaceae 5.0% > Neisseriaceae 4.0% > Peptostreptococcaceae 3.0% > Erysipelotrichaceae, Leptotrichiaceae and Enterobacteriaceae were 2.0% > Cardiobacteriaceae and Burkholderiaceae were at 1.0% > Xanthomonadaceae 0.8% > Carnobacteriaceae 0.5% > Streptococcaceae 0.2% > while genera including *Pasteurella*, *Paracoccus*, *Escherichia*, *Shigella*, *Moraxella*, *Stenotrophomonas*, *Neisseria*, *Conchiformibius*, *Bergeyella*, *Capnocytophaga*, *Fusobacterium*, *Oceanivirga*, *Streptococcus*, *Bacillus* and Defluviitaleaceae were identified. The percentage of *Moraxella* was 19.0% > *Fusobacterium* and *Bergeyella* both were 5.0% > *Capnocytophaga* 4.0% > *Escherichia*, *Shigella*, Defluviitaleaceae and *Conchiformibius* were 2.0% > *Pasteurella* and *Neisseria* were at 1.0% > *Paracoccus* 0.8% > *Oceanivirga* 0.6% > *Stenotrophomonas* 0.4 % > *Streptococcus* 0.2% and > *Bacillus* was 0.06%.

Discussion

The present study provides the first detailed insights into the oral microbiota of the small Indian mongoose through next-generation sequencing (NGS). The results revealed the dominance of the phyla Proteobacteria (58.0%), Bacteroidetes (20.0%), Firmicutes (12.0%), and Fusobacteria (7.0%), with a minor representation of Patescibacteria (2.0%). These findings align partially with prior studies on carnivorous mammals, where Proteobacteria and Firmicutes frequently dominate microbial communities (Kistler et al., 2018). The relative abundance of Gammaproteobacteria was 57.0% as the most abundant bacterial class is significant. Previous research has reported Proteobacteria and Gammaproteobacteria from oral microbiota of venomous snakes (Chen et al., 2020). This consistency is might be due to adaptation to the carnivorous diet and ecological niches of predatory animals.

Pasteurella (relative abundance, 19.0%), *Fusobacterium* (5.0%), and *Moraxella* (5.0%) were among the most abundant genera. *Pasteurella* are reported from the oral and respiratory tracts of various mammals but can also act as an opportunistic pathogen. Similarly, *Fusobacterium* has been observed in both healthy and diseased specimens, particularly in periodontal conditions in humans and animals (Wade, 2013). The presence of such genera raises questions about the potential zoonotic risks associated with the small Indian mongoose, as *Pasteurella* and *Fusobacterium* are known to harbour pathogenic strains capable of cross-species transmission (Everard et al., 1976).

Recent advancement in comparative genomics, biochemistry and developmental biology has enabled researchers to define species with remarkable precision. However, the complex relationships between hosts and the diverse microorganisms that live within and on them have introduced new challenges in determining what makes up an individual. The richness of bacterial families and orders observed, such as Pasteurellaceae (29.0%) and Weeksellaceae (5.0%), highlights the complex and diverse nature of the mongoose's oral microbiota. This diversity is comparable to findings in other wild carnivores, reflecting an adaptation to varied diets and environmental exposures (Zepeda et al., 2018). The identification of Pseudomonadales and Flavobacteriales as dominant orders further supports this notion, as these taxa are frequently observed in opportunistic feeders and predators due to their broad metabolic capabilities. The observed abundance of Fusobacteriales (7.0%) and Lactobacillales (4.0%) is consistent with studies on other wild carnivores, which have reported these orders as essential components of the oral microbiota due to their roles in health and disease (Aas et al., 2005). These findings suggest that the mongoose's microbiota is shaped by its carnivorous diet, environmental interactions, and possibly its invasive nature. Interestingly, the presence of genera like *Escherichia*, *Shigella* and *Fusobacterium*, suggests that this oral microbiome could harbor zoonotic risks. This is predominantly significant given the invasive nature of *H. auropunctatus*, which often found in human dominated habitats (Ulloa et al., 2019).

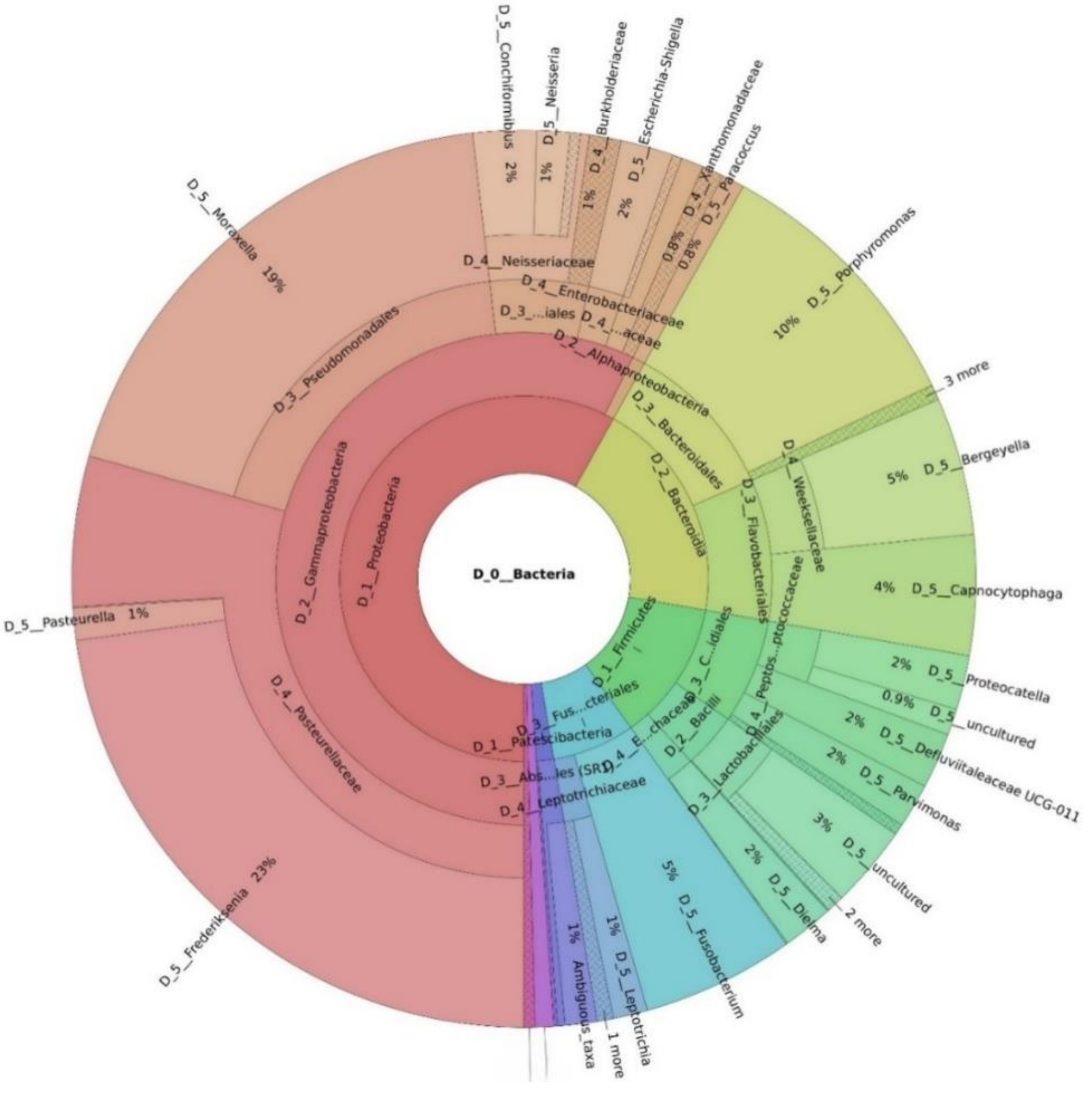

Fig. 3. KRONA plot of bacterial taxa identified during the present study

Pereira et al. (2020) reported that members of the phylum Firmicutes are found abundantly in the saliva of many small mammals. Our study revealed that within the Firmicutes, the Clostradiales are represented by only 2.0% of oral microbiota and the relative abundance of the phylum Proteobacteria was 58.0%. This result is in line with another study conducted by Maurice et al. (2015) indicating that Proteobacteria are common in various animals' microbiomes. During the present study, the most abundant genus was *Moraxella* (19.0%) of family Neisseriaceae. Many diseases are caused by *Morexella* such as infections in the sinus, middle ear, eye, blood and meningitis.

Remarkably, the phyla Proteobacteria and Fusobacteria each contributed up to 10.0% more to the fecal bacterial composition in small Indian mongooses than in Egyptian ones, thereby reducing the relative dominance of Firmicutes. Ruparell et al., 2020 hypothesize that dietary variation between Egyptian and small Indian mongooses is the primary cause of the observed differences in the microbial makeup of their gut, notwithstanding technical artefacts resulting from different experimental designs in the two investigations. According to a recent study conducted in Portugal, the diet of the Egyptian mongoose is primarily composed of mammals (63.1%), reptiles (16.3%), amphibians (7.9%), invertebrates (6.6%), and carrion (4.1%), with a minor inclusion of fish (1.0%), plant material (0.6%), eggs (0.4%), and birds (0.1%) (Bandeira et al., 2018).

A high ratio of Proteobaceria was reported in the oral saliva of small Indian mongoose. Members of Proteobacteria, predominantly Enterobacteriaceae including *Escherichia* spp. were identified in our study, which is in line with the study of Pereira et al., (2020) who reported the same species in the gut micobiota of Egyptian mongoose.

Similarly members of the orders Clostridiales, Lactobacillales, Fusobacteriales and *Escherichia* spp. were reported during the present study, which is also in line with the study of Pereira et al., (2020) which also showed the presence of *Clostridioides* spp. (25.0%), *Blautia* spp. (7.0%), *Collinsella* spp. (5.0%), *Lactobacillus* spp. (4.0%), *Escherichia* spp. and *Fusobacterium* spp. (2.0% each) in the gut micobiota of Egyptian mongoose. Our results revealed the presence of genera such as *Capnocytophaga* and *Bergeyella* and these findings are similar to the study of Dewhirst et al. (2010, 2012). Deng & Swanson (2015) also reported the existence of Bacteriodetes in the oral microbiome of small mammals including cats and dogs.

Our study showed the presence of genera such as *Paracoccus*, *Clostridium*, *Bergeyella* and *Capnocytophaga*, which is in contrast with the study of Bermingham et al. (2013), who reported genera *Blautia*, *Clostridium*, *Megamonas*, and *Peptostreptococcus* in Egyptian mongooses while they lack *Patescibacteria*. Rosalino et al., (2009) documented the presence of *Escherichia coli* and *Shigella* sp. in the intestinal tract of various small mammals. Our study also revealed the presence of enterococci which belongs to the family Enterobacteriaceae and order Enterobacteriales and bacteria *Shigella* sp. from saliva of the small Indian mongoose. The presence of pathogenic genera such as *Shigella* and *Neisseria* highlights the potential zoonotic risks associated with mongoose-human interactions. Interestingly, the study identified *Escherichia, Shigella* (2.0%), which is commonly associated with gut microbiota but occasionally found in oral microbiota under specific conditions (Lamont et al., 2018). This suggests possible cross-contamination or environmental factors influencing the microbial composition.

Given the mongoose's invasive status and its known role as a reservoir for zoonotic pathogens, the presence of potential pathogens in its oral microbiota has important public health implications. The identification of genera such as *Pasteurella* and *Neisseria* highlights the need for monitoring potential risks to humans and native wildlife populations. These findings also support the hypothesis that the mongoose's microbiota may play a role in its adaptability and ecological success in non-native regions (Barun et al., 2011).

Conclusion

The findings reveal a complex and diverse microbial community, dominated by Proteobacteria, Bacteroidetes, and Firmicutes, with potential implications for health, zoonotic risks, and ecological adaptability. While this study provides valuable insights, it is limited by its small sample size (n = 5) and geographical scope. Future studies should include larger sample sizes and comparative analyses with other mongoose populations across different habitats. The identification of genera such as *Pasteurella*, *Neisseria*, *Escherichia*, and *Shigella* warrants further investigation into their potential role as reservoirs of zoonotic pathogens, especially given the mongoose's invasive nature and close contact with human and animal populations.

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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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