



Metagenomic characterization of the bacterial microbiota in domestic cat (*Felis catus*) saliva

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Abstract

Purpose: *Felis catus* is a domestic animal that interacts with people frequently, both directly and indirectly. These interactions ultimately result in changes to each organism's microbiome. Studying the microbiota of organisms which are in contact with humans enables new data on potential health risks and provides new understanding for microbial changes and compositions. This study aimed to enabled a detailed profile of the cat oral microbiota, contributing to a more comprehensive understanding of animal microbiomes and their potential impact on human health, particularly in the context of zoonoses and bacterial infections.

Method: In this study, metagenomic analysis was used to understand *Felis catus* saliva's bacterial composition at various taxonomic levels. The saliva sample was sequenced and the V3-V4 region of bacterial 16S rRNA gene was targeted for this analysis.

Findings: At the phylum level, Bacterioidota (38%), Pseudomonadota (21%), Fusobacteriota (19%), Bacillota (11%), Spirochaetota (5%), and Mycoplasmatota (3%) and at the genus level *Porphyromonas* (30%), *Fusobacterium* (14%), *Treponema* (5%), *Peptostreptococcus* (5%), *Campylobacter* (5%) and *Oceanivirga* (4%) were dominant.

Conclusion: This study provides a foundation for future research on microbial transmission between animals and humans. In the further studies, modern sequencing techniques can be used to better understand the links between bacterial communities in domestic animals and their potential impact on human health.

Keywords: *Felis catus*, metagenomics, saliva, microbiota

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Evcil kedi (*Felis catus*) salivasının bakteriyal mikrobiyotasının metagenomik karakterizasyonu

Özet

Amaç: *Felis catus* insanlar ile doğrudan ve dolaylı olarak sıklıkla etkileşime giren bir evcil hayvandır. Bu etkileşimlerin sonucunda her organizmanın mikrobiyomunda değişiklikler meydana gelmektedir. İnsanlarla temas halinde olan organizmaların mikrobiyotasının incelenmesi potansiyel sağlık riskleri hakkında yeni veriler; mikrobiyal değişiklikler ve kompozisyonlar hakkında yeni anlayışlar sağlamaktadır. Bu çalışmanın amacı kedi oral mikrobiyotasının ayrıntılı bir profilini elde edilmesini sağlayarak hayvan mikrobiyomlarının ve bunların özellikle zoonoz ve bakteriyel enfeksiyonlar bağlamında insan sağlığı üzerindeki potansiyel etkilerinin daha kapsamlı anlaşılmasına katkıda bulunmaktadır.

Metod: Bu çalışmada, *Felis catus* salivasının çeşitli taksonomik düzeylerdeki bakteriyel bileşimini anlamak için metagenom analizi kullanılmıştır. Saliva örneğinin sekanslanması ve analizi için bakteriyel 16S rRNA geninin V3-V4 bölgesi hedeflenmiştir.

Bulgular: Filum seviyesinde baskın olarak Bacterioidota (%38), Pseudomonadota (%21), Fusobacteriota (%19), Bacillota (%11), Spirochaetota (%5) ve Mycoplasmatota (%3); cins seviyesinde ise *Porphyromonas* (%30), *Fusobacterium* (%14), *Treponema* (%5), *Peptostreptococcus* (%5), *Campylobacter* (%5) ve *Oceanivirga* (%4) tespit edilmiştir.

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Sonuç: Bu çalışma, hayvanlar ile insanlar arasındaki mikrobiyal bulaşmaya ilişkin gelecekteki araştırmalar için bir temel sağlamaktadır. Modern dizileme teknikleri, ilerleyen çalışmalarda evcil hayvanlardaki bakteri toplulukları arasındaki bağlantıları ve bunların insan sağlığı üzerindeki potansiyel etkilerini daha iyi anlamak için kullanılabilir.

Anahtar kelimeler: *Felis catus*, metagenom, saliva, mikrobiyot

1. Introduction

Humans interact on a regular basis with a variety of living groups, including plants and animals. These interactions occur at both the macroscopic and microscopic levels, affecting the microbiota of the creatures involved. The term microbiota refers to all microorganisms that live within a living organism. This includes the collective presence of viruses, fungi, archaea, and especially bacteria, primarily in the gastrointestinal, skin, respiratory, and urogenital systems. Since the early 2000s, microbiota research has attracted increasing attention, particularly studies on the gut microbiota, which has led to the intestine being regarded as the "second brain" [1].

Compared to other research areas, microbiota studies are not frequently carried out because they are expensive, time-consuming, labor-intensive, and require bioinformatics expertise, despite the fact that they are crucial for extensive and comprehensive projects. The microbiota has an important impact on individuals and society. It is estimated that it contains 10 times more cells than the host organism [2;3]. Individual microbiota differs more from species-specific microbiota. Depending on a number of variables, this dynamic and variable structure may show taxonomic differences between individuals at the species, phylum, and other levels. Every organism has a distinct microbiota, and changes in these microbial communities can result from interactions between organisms. Diet, age and genetic factors can affect microbiota [4].

Human-animal interactions can pose a variety of health risks. Zoonotic infections transmitted from animals to humans are known to cause epidemics and deaths. Especially EZDs "emerging zoonotic diseases" are very dangerous for global public health [5]. This circumstance requires a closer look at all animals that interact with humans, especially pets, as well as a study of their microbiome and other characteristics yet to be found. Recently, approximately 75% of infectious diseases spreading among humans have been of zoonotic origin [6].

The United Nations Sustainable Development Goal 3: Good Health and Well-Being focuses mostly on promoting healthy persons and encourages more efforts in this regard. A healthy society begins with healthy individuals. Microbiota research has demonstrated a correlation between individual health and a well-balanced microbiome. Changes in the microbiota's bacterial makeup, whether quantitative or qualitative, can affect health. Dybiosis, for example, can cause a variety of diseases and put people's immune systems at risk. As a result, both direct and indirect research of human microbiome is necessary to protect the individuals. Furthermore, understanding the microbiota of other species that regularly interact with humans is essential for enhancing and preserving public health. Factors such as nutrition, air quality, water sources, vegetation, and the microbiomes of various species all influence the human microbiota. A survey conducted in 2012 estimated the domestic cat population in the Ankara Metropolitan Region to be between 15,000 and 20,000 [7]. This statistic emphasizes the significant interaction between domestic cats and humans. Understanding the effects of animal interactions on the human microbiome is vital for understanding how animal microbiota, particularly that of cats, impacts human health.

Among the negative consequences of human-animal interactions are zoonoses and parasitic infections. The bacterial composition of the microbiota varies depending on the biological location within the host organism. Especially, oral microbial populations represent a community of microorganisms that are important for health. Research on the composition of cat saliva is also of great importance for understanding the risks associated with injuries such as cat bites, which can involve bacterial contamination [8]. Cats are the second most common animals that cause animal bites, which are one of the most frequent reasons people globally seek medical help. Many infections can originate from cat bites [9;10]. Cats can carry the protozoan infection caused by the parasite *Toxoplasma gondii*, which is known to transmit the disease toxoplasmosis to the environment. Although *Toxoplasma gondii* is a parasite, its seroprevalence is estimated to be 30-40 % [11]. New studies should be carried out in this area to reveal bacterial, viral and fungal interactions in the cat oral ecosystem[12].

Research on microorganisms has advanced significantly due to the development of biotechnological methods and techniques. In order to identify the types of microorganisms in a culture, it was previously necessary to isolate the target species from the sample as a single colony. However, metagenomic approaches have made it possible to directly study bacterial samples. But metagenomic technologies can now be used to study bacterial samples without need for cultivation. The goal of metagenomic omics is to identify and evaluate each molecules found in a sample. Metagenomic studies beneficial to understand compositions of bacteria without requiring a culture, and the genetic material of each bacterium is gathered for next generation sequencing. When analyzing bacterial populations, the V3-V4 variable regions are frequently used. Bacterial study use the 16S rRNA gene, which comprises nine variable regions ranging from V1 to V9 [13].

Saliva sample was collected to examine the bacterial composition of the cat and the V3-V4 regions of the 16S rRNA gene was targeted, advanced sequencing method, next generation sequencing technology was employed to assess

the composition of the oral microbiota in cats. This method allowed us to acquire an extensive knowledge of the bacterial variety in the cat's oral cavity, which contributed to a deeper comprehension of animal microbial communities and their potential impact on human health, including the spread of zoonotic and bacterial illnesses.

2. Materials and methods

2.1. Sample Collection and DNA Isolation

Saliva sample was collected from the mouth of a domestic cat in Niğde province, Türkiye by using dry swab and after sampling the tube was preserved at +4°C to protect the sample and DNA. The Invitrogen DNA Isolation Kit was used to isolate DNA following the instructions supplied. DNA concentration and quality were examined to assure compatibility with future steps. DNA concentration and purity were determined using Jenway Genova Nano spectrophotometer, by measuring the A260/A280 ratio and then the DNA's integrity was confirmed via agarose gel electrophoresis on a 1% gel.

2.2. PCR Amplification and Library Preparation

The PCR amplification consisted of the following components and volumes: dH₂O (13.5 µL), dNTP (1.25 µL), buffer (2.25 µL), forward and reverse primers (0.5 µL each), MgCl₂ (2 µL), DMSO (1.5 µL), Taq polymerase (0.5 µL) and DNA (5 µL) in a total reaction volume of 27 µL. For the amplification of the V3-V4 regions of the 16S rRNA gene, specific primers were used:

the forward primer (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and the reverse primer (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') [14]. The PCR amplification conditions were as follows: an initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 90 seconds, and a final extension at 72°C for 5 minutes (Table 1). The PCR was performed using the ABI Veriti 96 Well Thermal Cycler.

Table 1. The PCR amplification conditions

Step	Time	Temperature
Initial denaturation	3 min	94°C
Denaturation	45 sec	94°C
Annealing	45 sec	55°C
Extension	90 sec	72°C
Final extension	5 min	72°C

2.3. Next-Generation Sequencing and Data Analysis

The NGS process was outsourced to BM Biotechnology Company, Türkiye. The data obtained from the sample was converted to FASTA format, and metagenomic analysis was performed using QIIME 2. FastQC, QIIME 2, and DADA 2 were used to carry out the analysis steps. Quality assesment of reads was performed using FastQC and low-quality sequences were filtered with DADA2. QIIME 2 was used for read quality control and taxonomic identification. Additionally, primers, barcodes and chimeric reads were filtered during analysis. After reading quality assessment, bioinformatics analyses were performed.

3. Results

Using NGS technology, the composition of cat mouth microorganisms was analyzed. The phylogenetically informative regions of 16S rRNA genes of 460 bp were amplified from saliva sample used in the study. The results of the analysis of saliva composition are shown in Table 2 and Table 3.

Table 2. Bacterial composition of the saliva sample at the phylum level

Phylum	Composition (~%)
Bacteroidota	38%
Pseudomonadota	21%
Fusobacteriota	19%
Bacillota	11%
Spirochaetota	5%
Mycoplasmata	3%
Other	1%

Table 3. Bacterial composition of the saliva sample at the genus level

Genus	Composition (~%)
<i>Porphyromonas</i>	30%
<i>Fusobacterium</i>	14%
<i>Treponema</i>	5%
<i>Peptostreptococcus</i>	5%
<i>Campylobacter</i>	5%
<i>Oceanivirga</i>	4%
<i>Filifactor</i>	4%
<i>Frederiksenia</i>	4%
<i>Flavobacterium</i>	3%
<i>Pseudomonas</i>	3%
<i>Moraxella</i>	2%
<i>Mycoplasma</i>	2%
Other	19%

As a result of the analysis, the bacterial composition of the saliva was determined. The distribution of bacterial content at the phylum level is presented in the Krona chart in Figure 1 (a). As shown in Figure 1 (a), the relative abundance of these phyla is as follows: Bacteroidota accounts for 38%, Pseudomonadota 21%, Fusobacteriota 19%, Bacillota 11%, Spirochaetota 5%, and Mycoplasmatota 3%. The remaining 1% is attributed to other, unidentified strains.

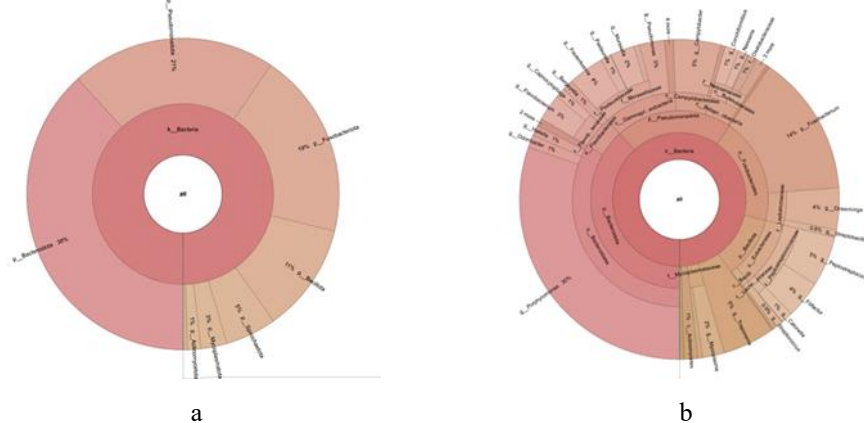


Figure 1. Bacterial composition of the saliva sample at the phylum (a) and genus (b) levels

The Krona chart in Figure 1 (b) shows the bacterial composition of the saliva sample at the genus level. The distribution of bacterial genera is as follows: *Porphyromonas* accounts for 30%, *Fusobacterium* 14%, *Treponema* 5%, *Peptostreptococcus* 5%, *Campylobacter* 5%, *Oceanivirga* 4%, *Filifactor* 4%, *Frederiksenia* 4%, *Flavobacterium* 3%, *Pseudomonas* 3%, *Moraxella* 2%, *Mycoplasma* 2%, with other genera constituting the remaining 19%.

4. Conclusions and discussion

This study focused on characterizing the microbial composition of a healthy cat's saliva and used NGS to perform a complete analysis of bacterial communities at both phylum and genus levels. According to the findings, there were various bacterial phyla in the cat saliva including Bacteroidota, Pseudomonadota, and Fusobacteriota. Furthermore, the findings are similar to past research in some aspects while also differing in others. The current study shows the microbial diversity in domestic cats and this information may help us to understand the roles of oral bacteria in feline health and its potential risks of zoonotic disease transmission through bites or scratches. On the other hand, until today various studies were conducted as well as dominant bacterial phyla were identified. As an example, Adler et al. (2016) studied on the feline oral microbiota by amplifying the V1-V3 region of 16S rRNA gene from supragingival plaque [15]. They used the next generation sequencing technology to discover the phyla in the samples. Three phyla were found as dominant: Bacteroidetes (31%), Firmicutes (24%) and Proteobacteria (21%). *Porphyromonas* was likewise found to be the most common genus, accounting for 14.9% of the total bacterial population, followed by *Treponema* (5.1%) and *Fusobacterium* (4.5%). In this study, the same molecular methods were used to identify the microbiota composition. At the genus level,

Porphyromonas was identified in the saliva sample at a significantly higher frequency (30%) compared to their study, where it accounted for 14.9%. This represents a notable difference in the dominance of *Porphyromonas* between the two studies, suggesting that *Porphyromonas* plays a more central role in the oral microbiome of the sample. At the phylum level, it was found that the majority of bacterial sequences that were derived from the phyla Bacteroidota (38%), Pseudomonadota (21%), Fusobacteriota (19%), Bacillota (11%), Spirochaetota (5%) and Mycoplasmatota (3%). This differs somewhat from the results of Adler et al. 2016, in which Bacteroidetes, Firmicutes, and Proteobacteria were the dominant phyla. However, the overall phylum distribution in this study also includes Pseudomonadota and Fusobacteriota to a significant extent.

On the other hand, a study analyzed various plaque samples from 92 cats with varying gingival health. They found *Porphyromonas* as the most dominant genus, especially in healthy gingiva, followed by *Moraxella* and *Fusobacteria*. The study found that Firmicutes (29.96%), Bacteroidetes (21.78%), and Proteobacteria (16.67%) were the most abundant phyla. They determined *Porphyromonas* as a dominant genus, but its abundance was higher in this study (30%) compared to their study (14.9%). Furthermore, they found Bacteroidetes as one of the dominant phyla (21.78%). This result is similar to this study in terms of Bacteroidetes [16].

In different research, the oral microbiota was shown to be mostly consisting of Proteobacteria (75.2%), Bacteroidetes (9.3%) and Firmicutes (6.7%), with minor proportions of other taxa such as Spirochaetes and Fusobacteria. Bacteroidota (a synonym for Firmicutes) was the most common phylum in this study (38%), whereas Firmicutes was found at a greater percentage (11%) than in their study (6.7%). Both investigations detected Spirochaetota, albeit this study had a somewhat lower abundance (5%) than the other (7.36%) (Sturgeon et al., 2014). Both this study and Thomas et al. (2021) discovered *Porphyromonas* and *Treponema* as prominent bacterial genera in the oral microbiota, which is consistent with the findings [17]. The identified genera were also discovered to be connected to oral disease.

In a recent study that used next generation sequencing technology, the most common phyla were Proteobacteria and Bacteroides, with the most abundant genera being *Porphyromonas* (22.48%), *Moraxella* (7.06%) and *Fusobacterium* (4.32%). In comparison, this study found that *Fusobacterium* (now *Fusobacteriota*) was the most abundant genus, accounting for 19% of the total. Both investigations identified *Porphyromonas* and *Moraxella* as significant genera, with *Porphyromonas* being similarly prevalent. However, this analysis revealed a higher fraction of *Fusobacteriota*, indicating a shift in the microbial community between the two studies [18].

Finally, the findings shed fresh light on the nature of the feline oral microbiota by highlighting significant bacterial communities and explaining their functions. While the findings support earlier studies, the different abundance patterns discovered in this study highlight the feline oral microbiome's diversity and variety. These findings add to the expanding body of information about feline oral health and highlight the need for more research to completely understand microbial dynamics.

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