



Regional Variation in 16S rRNA Gene Profiles of Upper Respiratory Tract Microbiome in Kazakh Horses

ErmeK Anarkulov ¹, Zhanat Bimenova ^{1,*}, Assem Kalykova ^{2,*}, Vitali Strochkov ¹, Gulnur Kuzembekova ¹, Zhuldyzay Kenzhebekova ¹ and Shinara Kassymbekova ¹

¹Kazakh National Agrarian Research University, Almaty, Kazakhstan

²Al-Farabi Kazakh National University, Almaty, Republic of Kazakhstan

*Corresponding author: zhanat.bimenova@kaznaru.edu.kz (ZhB); a.kalykova@gmail.com (AK)

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ABSTRACT

The equine upper respiratory tract microbiome (URTM) plays a pivotal role in respiratory health, yet its regional variability in relation to environmental factors remains underexplored. This study characterized and compared the URTM of Kazakh horses from three ecologically distinct regions - Pavlodar, Zhetysu, and Mangystau-using high-throughput 16S rRNA gene sequencing and a multi-layered bioinformatic approach. Alpha diversity metrics indicated significantly greater richness and evenness in Mangystau samples ($P < 0.05$, Tukey's HSD test), suggestive of a more stable and resilient microbial community under arid climatic pressures. Beta diversity analyses revealed distinct clustering patterns aligned with regional origin, highlighting strong biogeographic structuring. Taxonomic profiling identified both core genera (e.g., *Nicoletella*, *Streptococcus*, *Moraxella*) and region-specific taxa, including environmental genera (*Planococcus*, *Psychrobacter*) in Mangystau and mucosa-associated genera (*Lactobacillus*, *Staphylococcus*) in Zhetysu. LEfSe analysis confirmed regionally enriched bacterial biomarkers, supporting functional adaptation to climate and ecology. Functional predictions derived from BugBase, PICRUST2, and FAPROTAX revealed pronounced differences in metabolic and ecological capacities: Mangystau horses were enriched in stress-tolerant phenotypes and xenobiotic degradation pathways, while Pavlodar and Zhetysu horses showed greater representation of host-associated functions such as nitrogen fixation and fermentation. These findings demonstrate that regional environmental conditions exert a measurable influence on both the composition and function of the equine URTM. This study provides a foundation for microbiome-informed respiratory health monitoring and regional veterinary strategies in horses raised under extensive management systems.

Keywords: Kazakh horses, 16S rRNA sequencing, Microbial diversity, Geographic variation, Equine respiratory health, Functional prediction

INTRODUCTION

The upper respiratory tract (URT) of horses serves as a dynamic interface between the environment and the host, providing both physical and immunological barrier against inhaled pathogens. Over the last decade, advances in next-generation sequencing (NGS) have revolutionized our understanding of the URT microbiome (URTM), revealing its intricate role in maintaining respiratory health and mediating host-pathogen interactions. While the gastrointestinal microbiota of equines has been widely characterized, the microbial ecology of the equine respiratory tract, particularly in relation to geographic and environmental variation, remains insufficiently explored (Bond et al. 2022).

Previous studies have demonstrated that the composition and diversity of URTM are influenced by several extrinsic and intrinsic factors, including stable management, ambient air quality, pasture access, breed, age, and even climatic seasonality (Richard et al. 2012; Knottenbelt 2019; Léguillette et al. 2019; Holcombe et al. 2020; Bond et al. 2022). Several bacterial genera commonly identified in the equine respiratory tract play distinct ecological roles; for instance, *Nicoletella* and *Streptococcus* are typically considered commensal organisms contributing to mucosal homeostasis, whereas *Moraxella* and *Actinobacillus* are more frequently implicated in respiratory infections and exhibit pathogenic potential (Orazymbetova et al. 2023; Zaitseva et al. 2023; Kabyzbekova et al. 2024; Kassymbekova et al. 2025).

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These microbial communities are not static; rather, they exhibit considerable plasticity in response to environmental stressors and geographical constraints (Morley et al. 2015; Cursons et al. 2018; Arroyo et al. 2019; Viitanen et al. 2019; Scheffler et al. 2020; Huber et al. 2021; Kennedy et al. 2021; Motoshima et al. 2022).

Kazakhstan, characterized by a wide spectrum of climatic zones from continental steppe to semi-desert, provides a valuable natural laboratory to examine environmental effects on equine microbiomes. Horses in this region are predominantly raised on open pastures under extensive management systems, thereby offering a unique opportunity to assess the impact of geography on the URTM under relatively standardized husbandry practices (Huber et al. 2021; Orazymbetova et al. 2023; Zaitseva et al. 2023; Kabyzbekova et al. 2024; Kassymbekova et al. 2025).

This study aims to characterize and compare the URTM of Kazakh horses from Pavlodar, Zhetysu, and Mangystau regions using high-throughput 16S rRNA amplicon sequencing. We hypothesize that microbial composition and diversity will reflect regional ecological variation, thereby contributing to the foundation of targeted strategies for respiratory health surveillance and intervention in equine populations.

MATERIALS AND METHODS

Sample collection

The research protocol was reviewed and approved by the local ethical committee of the Kazakh National Agrarian University (protocol dated March 11, 2024). The research was carried out in the autumn-winter period of 2023 in three different regions of Kazakhstan (Pavlodar, Zhetysu, Mangystau). The Kazakh-breed horses involved in this study were 3-8 years old and were located on natural grasslands of autumn grass, respectively, in the northern, western and southern regions of Kazakhstan. Swabs of the upper respiratory tract samples were taken from 15 horses. All samples were frozen in liquid nitrogen, delivered to the laboratory and stored in a freezer at -20°C until DNA extraction. They included Zhabe-type Kazakh horses from Pavlodar (n=5) and Zhetysu (n=5) regions, and Adai-type Kazakh horses (n=5) from Mangystau region. Nasopharyngeal swabs were collected from 15 clinically healthy Kazakh horses representing two phenotypic types and three geographic regions of Kazakhstan. Horses of the Zhabe type were sampled in Pavlodar (n=5) and Zhetysu (n=5) regions, while horses of the Adai type were sampled in the Mangystau region (n=5). Sterile swabs were used to obtain samples from the upper respiratory tract (nasopharyngeal area) under aseptic conditions. Immediately after collection, all samples were flash-frozen in liquid nitrogen and subsequently stored at -20°C until further processing for DNA extraction.

DNA extraction, library construction and sequencing

Microbiome DNA extraction was performed using the PureLink Microbiome DNA Purification Kit (Thermo Fisher, USA) according to the manufacturer's protocol. The DNA concentration and purity were quantified with a Nanodrop 2000® (Thermo Fisher, USA) and Qubit 3.0 (Thermo Fisher, USA), respectively. 1% agarose gel electrophoresis was used to examine DNA quality.

The amplification of hypervariable regions for NGS sequencing was conducted using the Ion 16S Metagenomics Kit (Thermo Fisher, USA) targeting the V2-4-8, V3-6, 7-9 region of the 16S rRNA gene, following the manufacturer's protocol. The Ion 530 chip was prepared for sequencing the obtained libraries with the Ion 510™&Ion 520™&Ion 530™ Kit – Chef. Metagenomic sequencing was performed on the IonTorrent S5 platform (Thermo Fisher, USA) at the Kazakh National Agrarian Research University (Almaty, Kazakhstan).

Bioinformatic and statistical analyses

Data analysis was performed using RStudio version 2024.04.1+748 and R-based EasyAmplicon pipeline (Liu et al. 2023). Raw sequences were quality-filtered and dereplicated using VSEARCH v.2.21.1 subcommand -fastx_filter and -derep_fulllength, respectively (Rognes et al. 2016). Then, the non-redundancy sequences were denoised into amplicon sequence variants (ASVs) with USEARCH v11.0.667 (Edgar 2010) (via -unnoise3). Chimeras were removed by VSEARCH -uchime_ref against the SILVA database (Quast et al. 2013). Feature tables were created by vsearch -usearch_global. The taxonomy of the features was classified by the USEARCH syntax algorithm in SILVA v123. Diversity analysis was carried out using the vegan v2.6-6.1 package (<https://cran.r-project.org/web/packages/vegan/>) and visualized by using the ggplot2 v3.5.1 (<https://cran.r-project.org/web/packages/ggplot2/>) package in R v4.4.0. LEfSe was conducted with the online platform ImageGP (<http://www.ehbio.com/ImageGP/index.php/Home/Index/LEfSe.html>). Functional profile prediction of microbial communities was conducted by PICRUSt (Langille et al. 2013) with the Greengenes as the reference database. The Greengenes database was used in accordance with PICRUSt v1 requirements, as it remains the compatible reference for functional prediction despite its older taxonomy.

RESULTS

Alpha diversity

Alpha Diversity Indicates Region-Specific Microbial Richness and Evenness (Fig. 1), and revealed statistically significant variation in microbial community across the three regional groups. Metrics including Chao1, ACE, Shannon, and Simpson indices were calculated to assess species richness and distribution.

Mangystau horses demonstrated the highest richness values, with a mean Chao1 index of 4295 and ACE index of 4482, indicating a broader spectrum of rare taxa compared to Pavlodar (Chao1=2434, ACE=2511). The Shannon index in Mangystau samples averaged 4.77 versus 3.65 in Pavlodar, highlighting greater taxonomic evenness and suggesting a more balanced microbial ecosystem. These trends were consistently observed across all metrics.

Post hoc analysis using Tukey's HSD (Table 1) test confirmed that the observed differences were statistically significant. For instance, the Chao1 index difference between Mangystau and Pavlodar reached 1861.1 (95% CI: 345.7–3376.4, P=0.017), while the Shannon index difference between the same groups was 0.97 (95% CI: 0.11–1.84, P=0.027). Zhetysu also showed significantly

Table 1: Summary of Statistically Significant Alpha Diversity Differences (Tukey HSD Post Hoc)

Comparison	Metric	Difference	95% CI Lower	95% CI Upper	Adjusted P-value
Mangystau vs Pavlodar	Chao1	1861.1	345.7	3376.4	0.017
Zhetysu vs Pavlodar	Chao1	1785.4	270.0	3300.7	0.021
Mangystau vs Pavlodar	Shannon	0.97	0.11	1.84	0.027
Zhetysu vs Pavlodar	Shannon	2.04	0.22	3.85	0.028
Mangystau vs Pavlodar	ACE	1970.4	158.7	3782.2	0.033

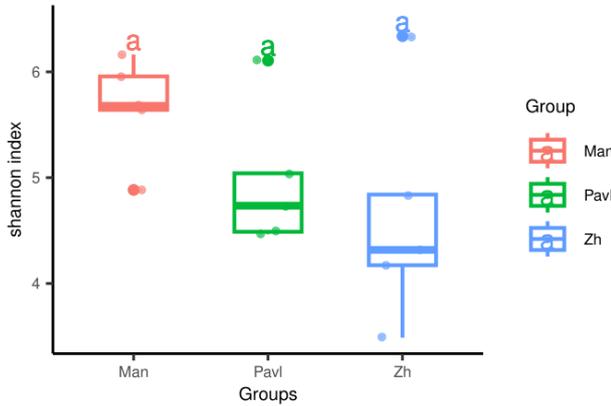


Fig. 1: Alpha diversity (Shannon index) across geographic regions. Mangystau horses exhibit significantly higher richness and evenness compared to Pavlodar and Zhetysu.

greater diversity compared to Pavlodar in both Chao1 (difference=1785.4, P=0.021) and Shannon (difference=2.04, P=0.028). The detailed statistical comparisons are summarized in Table 1.

These patterns strongly support the hypothesis that regional ecological pressures, such as forage diversity, dust exposure, and ambient climate, shape the richness and structure of the URTM. The data imply that horses in Mangystau and Zhetysu harbor more ecologically robust microbiomes than those in Pavlodar.

These results, visualized in Fig. 1, provide robust evidence for region-specific differences in microbial ecology within the upper respiratory tract of Kazakh horses.

Beta diversity

Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarities revealed distinct clustering of microbial communities according to geographic origin. While the first two axes of the PCoA plot together explained 24.7% of the total variance in the dataset (Axis 1=13.5%, Axis 2=11.2%), distinct grouping patterns were visible among samples from the three regions.

Samples from Mangystau tended to cluster separately, forming a relatively compact group, whereas samples from Pavlodar and Zhetysu exhibited greater dispersion, indicating higher within-group variability. This suggests that the URTM in Mangystau horses is more compositionally consistent, potentially reflecting strong environmental filtering.

These patterns were further supported by hierarchical clustering of Bray–Curtis dissimilarity distances, which grouped samples predominantly according to geographic origin (Fig. 2). The visualization highlights distinct microbial community structures, reinforcing the idea that geography contributes substantially to URT microbial differentiation in Kazakh horses.

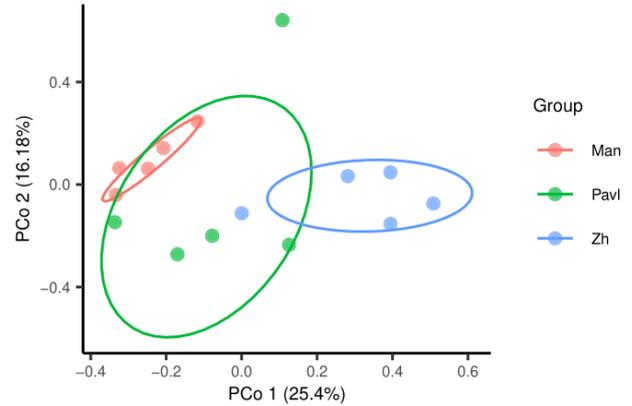


Fig. 2: Principal Coordinate Analysis (PCoA) based on Bray–Curtis dissimilarity reveals distinct clustering of URTM communities by region.

The regional clustering and lower dispersion of Mangystau samples imply that climatic and ecological pressures such as aridity and dust exposure may exert a stronger homogenizing influence on URT microbiota. This is consistent with findings from other animal studies that link environmental harshness to community convergence.

These patterns were further supported by hierarchical clustering of Bray–Curtis dissimilarity distances, which grouped samples predominantly according to geographic origin. The clustering heatmap (Fig. 3) showed that samples from the same region consistently grouped together, reinforcing the presence of structured microbial communities shaped by geography.

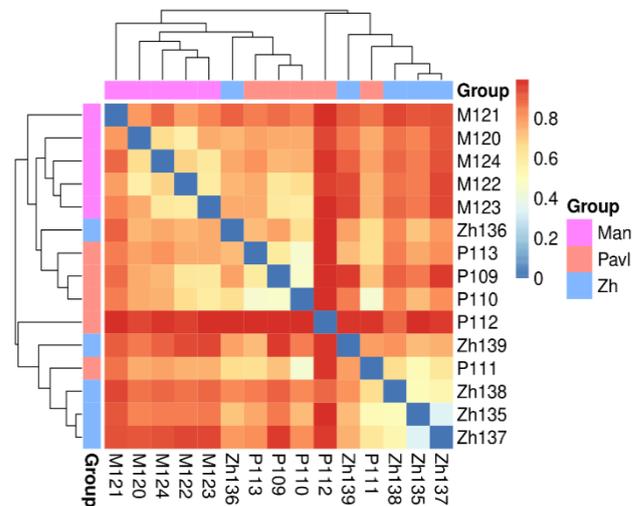


Fig. 3: Heatmap of Bray–Curtis dissimilarity distances showing hierarchical clustering of URTM samples by geographic region.

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pressures such as aridity and dust exposure may exert a stronger homogenizing influence on URT microbiota. This is consistent with findings from other animal studies that link environmental harshness to community convergence.

Together, alpha and beta diversity analyses confirm that region-specific environmental conditions shape distinct microbial ecosystems in the upper respiratory tract of horses raised under extensive pasture-based systems.

Taxonomic composition

Across all samples, the most abundant genera included *Nicoletella*, *Bacillus*, *Moraxella* and *Klebsiella*. Notably, *Nicoletella* was particularly prevalent in Pavlodar, a genus previously linked to both commensal roles and opportunistic infections (Huber et al. 2021). *Planococcus* and *Planomicrobium*, genera associated with soil and plant matter, were significantly enriched in Mangystau horses, likely reflecting regional dust exposure and pasture-specific microflora. Several potentially beneficial genera, including *Lactobacillus*, were uniformly distributed across all groups, suggesting a core respiratory microbiota.

Taxonomic classification at the genus level showed that while all horses shared a set of core bacterial taxa, several genera were differentially abundant depending on geographic origin. The most dominant genera overall included *Nicoletella*, *Moraxella*, *Streptococcus*, *Bacillus*, and *Corynebacterium*, but their relative abundances varied notably across regions.

In Pavlodar horses, *Nicoletella* and *Streptococcus* dominated the upper respiratory tract microbiome, together accounting for over 40% of the community. In contrast, Mangystau samples were characterized by a higher proportion of environmental genera such as *Planococcus*, *Psychrobacter*, and *Micrococcus*, which are often associated with dust and arid ecosystems. Zhetysu horses showed increased levels of *Staphylococcus* and *Lactobacillus*, which are generally linked to mucosal surfaces and may reflect regional differences in plant exposure and forage composition.

These trends are visualized in Fig. 3, which displays genus-level bar plots of relative abundance across the three groups. The taxonomic shifts support the hypothesis that distinct environmental exposures such as temperature, humidity, and soil microbiota influence URTM structure. Complementary heatmap clustering of Bray–Curtis distances (Fig. 3) reinforced these findings: Mangystau samples showed higher within-group similarity, with interregional distances clearly demarcated. This geographic differentiation supports the interpretation that environmental factors -such as aridity, plant and dust exposure, and pasture microbiota - exert a selective pressure on URTM composition.

In Pavlodar, *Nicoletella* was the most abundant genus (22.1%), aligning with previous studies on healthy equine URTM. Mangystau samples exhibited greater prevalence of environmental genera such as *Planococcus* (14.7%) and *Psychrobacter* (10.1%), potentially reflecting increased dust and soil contact in the arid landscape. Meanwhile, Zhetysu microbiota showed intermediate profiles but were enriched in *Staphylococcus* and *Lactobacillus*, indicating differences potentially driven by pasture flora and humidity.

Fig. 4 genus-level barplots that illustrate the regional dominance of key taxa and overall taxonomic structure across groups. These findings support the hypothesis that local ecological conditions act as environmental filters shaping respiratory microbial communities in horses.

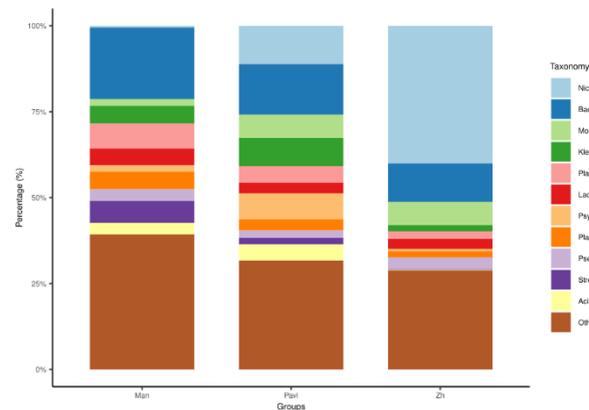


Fig. 4: Relative abundance of bacterial genera in the URT microbiome of Kazakh horses, highlighting region-specific taxonomic profiles.

LEfSe identifies region-enriched bacterial biomarkers

Linear discriminant analysis effect size (LEfSe) revealed bacterial taxa that significantly differed in abundance across geographic groups. Among the top discriminative genera, *Streptomyces* (LDA score: 4.6; $P=0.018$), *Planomicrobium* (LDA: 4.1; $P=0.024$), and *Micrococcus* (LDA:4.0; $P=0.031$) were strongly associated with horses from Mangystau. These genera are known for their environmental resilience and capacity to persist under desiccating, dusty, and UV-exposed conditions, making them plausible biomarkers of the semi-arid region.

In contrast, *Staphylococcus* (LDA: 3.9; $P=0.029$) and *Lactobacillus* (LDA:3.5; $P=0.034$) were enriched in Zhetysu horses, while *Nicoletella* (LDA:4.2; $P=0.022$) remained dominant in Pavlodar. These enrichments were statistically significant with $P<0.05$, as determined through Kruskal–Wallis and pairwise Wilcoxon rank-sum tests.

The cladogram generated by LEfSe (Fig. 5) illustrates the taxonomic hierarchy of differential taxa, highlighting family- and genus-level biomarkers across all three groups. These signatures not only reflect ecological adaptation but may serve as microbial indicators of environmental exposure relevant to respiratory health and surveillance programs.

Functional prediction

Functional prediction based on PICRUSt2 and BugBase analyses demonstrated significant regional differences in the metabolic and phenotypic potential of URTM microbial communities. These predictions provide insight into how local environmental pressures may select for microbial traits relevant to respiratory health and ecological adaptation.

BugBase analysis revealed that stress-tolerant bacterial phenotypes were most prevalent in Mangystau horses, with a mean relative abundance of 41.3%, compared to 30.1% in Zhetysu and 26.4% in Pavlodar. This phenotype is typically associated with taxa capable of withstanding oxidative stress and desiccation, consistent

with the arid climate and dusty conditions of Mangystau. Furthermore, anaerobic phenotypes were more prominent in Mangystau samples, suggesting a higher prevalence of facultative or obligate anaerobes, potentially introduced via soil and particulate matter. These phenotypic trends are visualized in Fig. 6.

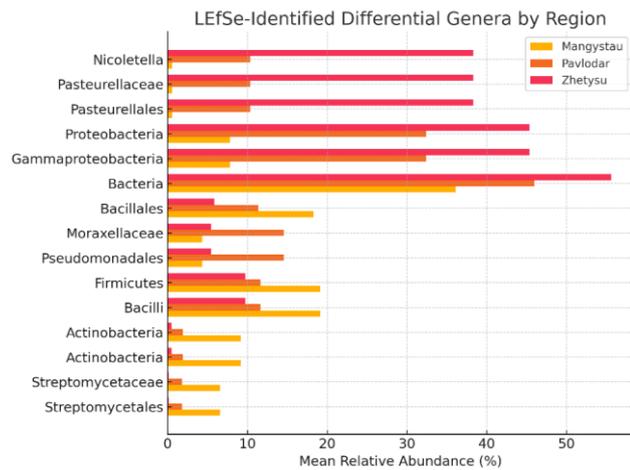


Fig. 5: LEfSe-based differential abundance of top bacterial genera across regions. Barplot shows average relative abundance in Mangystau, Pavlodar, and Zhetysu.



Fig. 6: PICRUSt2-predicted KEGG pathways enriched in Mangystau horses, particularly in xenobiotic degradation and carbohydrate metabolism.

PICRUSt2 analysis predicted an enrichment of several KEGG metabolic pathways in the Mangystau group. Notable among these were xenobiotic degradation pathways such as benzoate and caprolactam degradation, alongside carbohydrate metabolism pathways including fructose, mannose, and galactose metabolism. For instance,

the predicted abundance of aromatic compound degradation pathways was 1.8-fold higher in Mangystau compared to Pavlodar ($P < 0.05$). In contrast, Pavlodar samples exhibited higher representation of core energy metabolism pathways such as glycolysis and the TCA cycle. These differences are presented in Fig. 7.

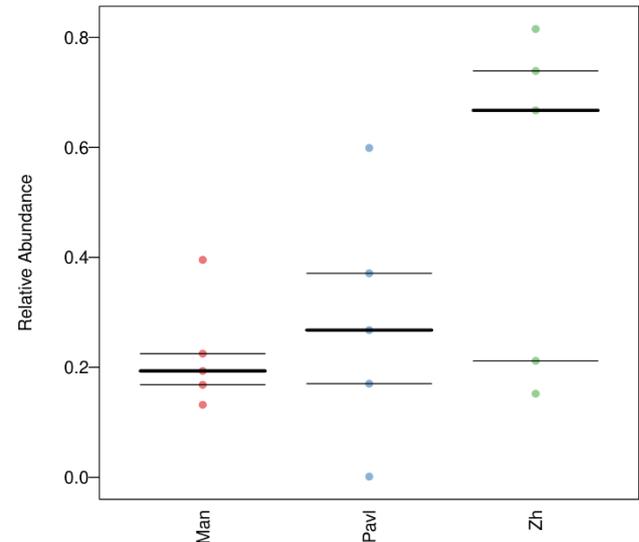


Fig. 7: BugBase prediction of stress-tolerant bacterial phenotype across geographic regions, indicating higher abundance in Mangystau samples.

Overall, the results support the hypothesis that URTM microbial communities are not only taxonomically distinct but also functionally adapted to region-specific environmental exposures. The functional profiles of Mangystau horses reflect an ecologically resilient microbiome, while those of Pavlodar indicate a more host-associated, metabolically conservative community.

FAPROTAX function-based classification

Functional annotation using FAPROTAX provided further insight into the ecological roles of the URTM microbiota in each region. Mangystau samples were enriched in microbial functions such as aerobic chemoheterotrophy, nitrate reduction, and hydrocarbon degradation—traits commonly associated with environmental bacteria from dust, arid soil, and low-nutrient niches. These predicted activities reflect the adaptation of the local microbial communities to the harsh ecological conditions of the Mangystau region.

In contrast, Zhetysu and Pavlodar horses harboured URTM microbiota enriched in functions related to fermentation, ureolysis, and nitrogen fixation. These functions suggest a microbial composition influenced more strongly by mucosal substrate metabolism and stable nutrient availability, possibly linked to local vegetation and host interactions.

Fig. 8 shows a FAPROTAX heatmap comparing Mangystau and Zhetysu, highlighting functional partitioning—e.g., enrichment of fermentation and methanogenesis in Zhetysu, versus hydrocarbon degradation in Mangystau. Fig. 9 complements this by presenting a second heatmap for Mangystau and Pavlodar, illustrating elevated nitrogen fixation and ureolysis in Pavlodar samples.

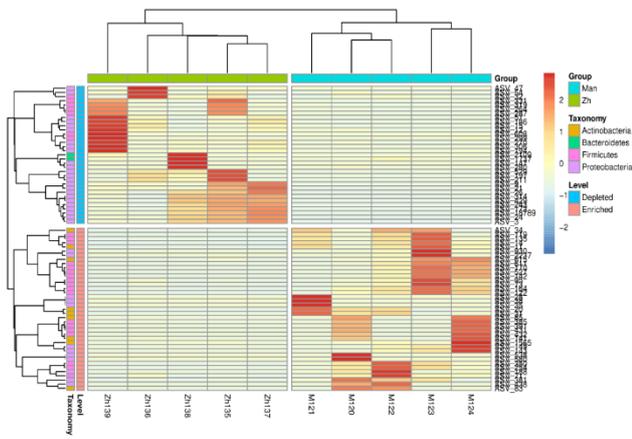


Fig. 8: FAPROTAX-based functional heatmap comparing microbial functions between Mangystau and Zhetysu horses, indicating region-specific ecological adaptations.

The enrichment of plant and xenobiotic degradation pathways in Mangystau horses reinforces the view that local environmental conditions—dust, sparse vegetation, and UV exposure—play a formative role in shaping both the taxonomic and functional landscape of the URTM microbiota. Collectively, functional predictions derived from BugBase, PICRUSt2, and FAPROTAX analyses confirm that geographic location not only shapes the microbial composition of the URT in Kazakh horses but also drives statistically supported functional divergence ($P < 0.05$) in key metabolic and ecological traits, as shown in Fig. 6–9.

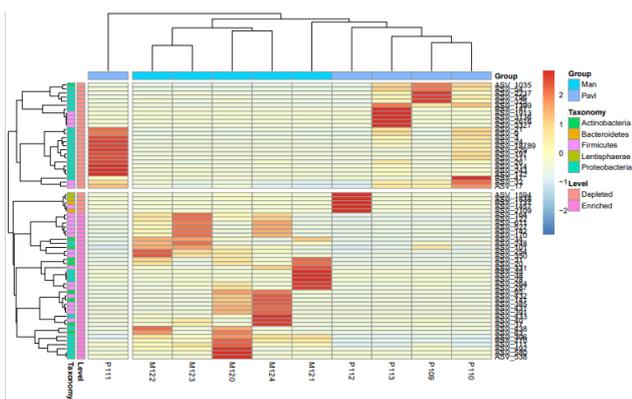


Fig. 9: FAPROTAX-based functional heatmap comparing microbial functions between Mangystau and Pavlodar horses, showing enrichment of host-associated functions in Pavlodar samples.

DISCUSSION

This study provides a comprehensive overview of the upper respiratory tract microbiome (URTM) in Kazakh horses across three climatically diverse regions: Pavlodar, Zhetysu, and Mangystau. By employing 16S rRNA gene sequencing and a suite of bioinformatic tools, we demonstrated that both microbial composition and predicted function exhibit clear regional structuring, likely shaped by environmental gradients and management conditions.

Alpha diversity analysis revealed significantly higher species richness and evenness in Mangystau samples, as indicated by elevated Chao1, ACE, Shannon, and Simpson

indices (Fig. 1, Table 1). These metrics suggest a more stable and resilient microbiome under arid environmental pressures. Similar regional influences on URTM diversity have been observed in horses across distinct climate zones, as reported Zhou et al. (2020) and Weese and Costa (2017). In contrast, Pavlodar horses exhibited the lowest diversity indices, reflecting potentially more restricted ecological niches in temperate steppe systems.

Beta diversity results corroborated these differences, with principal coordinate analysis (PCoA) and Bray–Curtis heatmap clustering revealing distinct regional separation (Fig. 2 and 3). Mangystau samples formed compact clusters, suggesting strong community cohesion potentially driven by dust-borne microbial influx and xerophilic selection pressures (Bond and Dechant 2021; Soto et al. 2021). The observed dispersion among Pavlodar and Zhetysu samples may be indicative of more heterogeneous environmental inputs and pasture ecology.

Taxonomic composition further reflected regional adaptation. While core genera such as *Nicoletella*, *Streptococcus*, and *Moraxella* were shared across all groups, Mangystau samples harbored higher abundances of *Planococcus* and *Psychrobacter*—genera commonly associated with desiccation-tolerant soil microbiota (Fig. 4). In Zhetysu, increased levels of *Staphylococcus* and *Lactobacillus* likely reflect mucosal adaptation and dietary influences, possibly mediated by forage type (Lange et al. 2022; Woodward et al. 2022). The relative dominance of *Nicoletella* in Pavlodar supports its previously established role in healthy equine URT communities (Holcombe et al. 2017).

LEfSe biomarker analysis (Fig. 5) confirmed region-specific enrichment patterns, identifying *Streptomyces* and *Planomicrobium* as characteristic taxa of Mangystau, and *Lactobacillus* and *Staphylococcus* as enriched in Zhetysu. These genera exhibit functional traits that support resilience to regional stressors such as high UV exposure and fluctuating humidity. The role of *Nicoletella* as a Pavlodar-specific biomarker strengthens its candidacy as a diagnostic indicator of URT homeostasis in temperate conditions.

Functional prediction analyses provided additional insight into ecological specialization. BugBase phenotypic profiles revealed a higher abundance of stress-tolerant and anaerobic bacteria in Mangystau horses, supporting the idea of selective pressures favoring survival-oriented microbial traits (Fig. 6). PICRUSt2-inferred KEGG pathways enriched in xenobiotic degradation and sugar metabolism in Mangystau further highlight adaptive potential to environmental pollutants and complex plant substrates (Fig. 7). Conversely, Pavlodar horses displayed enrichment in core energy metabolism, suggestive of a more host-adapted and metabolically stable microbiome.

Functional annotation using FAPROTAX provided further insight into the ecological roles of the URTM microbiota in each region. Mangystau samples were enriched in microbial functions such as aerobic chemoheterotrophy, nitrate reduction, and hydrocarbon degradation—traits commonly associated with environmental bacteria from dust, arid soil, and low-nutrient niches. These predicted activities reflect the adaptation of the local microbial communities to the harsh ecological conditions of the Mangystau region.

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In summary, this study demonstrates that geography, and by extension climate and ecological exposure, significantly influences the URT microbiome of Kazakh horses. The integration of taxonomic, diversity and functional profiling underscores the importance of environmental filtering in microbial community assembly. These findings provide a foundation for region-specific monitoring and potential microbiome-informed management strategies aimed at improving equine respiratory health.

Conclusion

This research establishes that the equine upper respiratory tract microbiome in Kazakhstan is shaped by strong geographic structuring, with clear differentiation in microbial richness, composition, and function among Pavlodar, Zhetysu and Mangystau regions. The use of high-throughput 16S rRNA gene sequencing enabled fine-scale resolution of these communities, supported by robust ecological and bioinformatic analysis and represents the first functional and taxonomic characterization of the equine URTM in Central Asia.

The observed enrichment of stress-tolerant, environmentally associated genera and functions in Mangystau horses suggests microbiome adaptation to arid and dusty conditions. In contrast, Pavlodar and Zhetysu horses showed signatures more consistent with mucosal and host-associated microbiota. These differences likely reflect local climate, vegetation, soil exposure, and management practices.

Our study uniquely demonstrates the region-specific microbial and functional signatures of Kazakh horses, highlighting the ecological plasticity of the equine URTM and its responsiveness to regional environments. Such

insights are foundational for future research into respiratory disease susceptibility, microbiome-based diagnostics, and targeted probiotic or environmental interventions in equine health management. As the first study of its kind across Central Asia, this work lays the groundwork for integrating microbiome surveillance into broader veterinary and ecological frameworks in the region, including tailored respiratory health strategies and potential development of region-specific probiotic interventions.

DECLARATIONS

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: All sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive under BioProject accession number PRJNA1096463.

Ethics Statement: All procedures involving animals were conducted in accordance with ethical standards and approved by the Local Ethical Committee of the Kazakh National Agrarian University.

Author’s Contribution: AE: conceptualization, data analysis, manuscript drafting. ZhB: supervision, study design. AK: literature review, manuscript writing and editing, supervision. VS, GK: statistical analysis and manuscript writing. ZhK, SK: data analysis, statistical work, funding acquisition, project administration, methodological development.

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