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Characterization and Functional Analysis of the Oropharyngeal Microbiome in ICU staff via Metagenomic Sequencing

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Abstract

Background

ICU staff face an elevated risk of colonization by drug-resistant bacteria, potentially associated with persistent occupational exposure to hospital environments. The oropharynx, as a key ecological niche at the interface of the respiratory and digestive tracts, serves as an important reservoir for opportunistic pathogens. This study employed shotgun metagenomic sequencing to systematically characterize the composition and functional profiles of the oropharyngeal microbiota in ICU staff.

Methods

A cross-sectional study was conducted, enrolling 10 full-time ICU staff (with ≥ 5 years of clinical experience) and 10 healthy non-medical controls with no history of medical-related employment. Oropharyngeal swabs were collected for metagenomic sequencing. Multi-dimensional comparative analyses were conducted between the two groups, covering community structure, taxonomic composition, KEGG metabolic pathways, virulence factor profiles, and antibiotic resistance gene (ARG) profiles.

Results

Compared with the control group, ICU staff exhibited significantly reduced genus-level richness (Chao1 index) but elevated community evenness, resulting in higher Shannon and Simpson indices, with a clearly distinct overall microbial community structure. The abundances of potential pathogenic genera including Pseudomonas, Streptococcus, Escherichia, Mycobacteroides, and Helicobacter were significantly enriched, whereas core oral commensal genera such as Prevotella were depleted. Functionally, ICU staff exhibited significant enrichment in bacterial biofilm formation, drug metabolism–cytochrome P450, and ABC transporter pathways; marked upregulation of virulence factors associated with adhesion, immune evasion, and endotoxin biosynthesis; and elevated abundances of five resistance genes primarily derived from Streptococcus and Haemophilus species (mel, patB, mdtG, hmrM, and rlmA(II)).

Conclusion

ICU occupational exposure is associated with systematic remodeling of the oropharyngeal microbiota, characterized by depletion of core oral commensals and enrichment of opportunistic pathogens. Functionally, this remodeling supports the formation of a potential synergistic resistance system of "biofilm barrier–P450 metabolic inactivation–ABC active efflux," providing novel metagenomic evidence for microbiome-based surveillance and occupational health protection of healthcare workers.

Introduction

The intensive care unit (ICU) is a high-risk area for nosocomial infections and colonization with drug-resistant bacteria. Healthcare workers in this setting are chronically exposed to multidrug-resistant organisms and complex microbial aerosols, making them a key population in occupational health research[1, 2]. In recent years, with the widespread adoption of high-throughput sequencing technologies, the relationship between the human microbiome and occupational exposure has drawn increasing attention. Studies have confirmed that healthcare workers exhibit significant differences not only in gut microbiota composition compared with non-healthcare controls, but also notable deviations in their oral microbiome[3, 4].

The oropharynx, as a critical habitat bridging the oral cavity and the lower respiratory tract, harbors a rich commensal microbiota and also serves as a common reservoir for numerous clinically important opportunistic pathogens[5]. Using traditional bacterial culture methods to compare oropharyngeal swabs between healthcare workers and non-healthcare individuals at a tertiary hospital in India, Amritha et al. found that the colonization rate of Streptococcus pneumoniae and Haemophilus influenzae in healthcare workers (65%) was significantly higher than that in non-healthcare subjects (32%) [6]. These findings indicate that the hospital work environment can substantially increase oropharyngeal carriage of respiratory pathogens. Zhang et al. applied 16S rRNA amplicon sequencing to compare oropharyngeal swab samples from healthcare workers in four different hospital departments and found significant deviations in oral microbiota diversity, structure, and predicted functions across clinical settings; however, the lack of a non-healthcare healthy control group precluded the determination of the net effect of the hospital environment itself on microbiota shaping[3]. Furthermore, another 16S rRNA-based gut microbiota study, which included short-term and long-term healthcare workers as well as non-healthcare controls, revealed significant shifts in gut microbial diversity and structure in the healthcare worker cohort[4]. However, both of these studies relied on amplicon sequencing technology, which limits resolution to genus-level taxonomic composition and provides only rough functional predictions. This approach cannot accurately identify pathogenic bacteria at the species level, nor can it comprehensively characterize the virulence factor profiles and antibiotic resistance gene (ARG) repertoires carried by the microbial community.

Shotgun metagenomic sequencing, with its amplification-free, whole-genome coverage, enables precise species-level identification and direct functional gene annotation simultaneously, offering a more powerful tool for microbiome research[7, 8]. Recently, Xie B et al. employed a shotgun metagenomic strategy and found that the gut microbiome diversity of ICU staff was significantly reduced, accompanied by elevated abundances of virulence factors and carbohydrate-active enzymes[9]. A concurrent multicenter cross-sectional study further confirmed that the total abundance of gut ARGs in ICU staff was significantly higher than that in healthy controls[10]. These studies provided initial evidence regarding the gut microbiome that occupational exposure shapes the resistome and virulence factors; however, they were limited to fecal samples and did not address the upper respiratory tract, a microbial habitat more directly exposed to the hospital environment.

In summary, this study collected oropharyngeal swab samples from ICU staff and non-healthcare healthy controls outside the hospital and applied shotgun metagenomic sequencing. The aim was to systematically characterize differences in species composition and community structure of the upper respiratory tract microbiota between the two groups, and to compare their multi-dimensional features, including KEGG metabolic pathways, virulence factor profiles, and resistomes.

Methods

Participants and sample collection

A cross-sectional study was conducted to characterize and compare the oropharyngeal microbiota composition and functional profiles between ICU staff and healthy non-medical controls using shotgun metagenomic sequencing. We enrolled 10 full-time healthcare workers from the ICU of the Affiliated Hospital of Yunnan University. The inclusion criteria for ICU staff were: (1) age between 25 and 45 years, regardless of race or sex; (2) physicians or nurses directly involved in the treatment and care of ICU patients, with at least 5 years of ICU work experience and no continuous absence exceeding one month during this period. The non-medical controls consisted of 10 healthy individuals with the following inclusion criteria: (1) age between 25 and 45 years, regardless of race or sex; (2) no history of working in or visiting a hospital in the year prior to enrollment; and (3) no cohabitation with any healthcare worker employed in the ICU. Exclusion criteria for all participants comprised the presence of respiratory tract infections or other respiratory diseases, and the use of antibiotics within the three months prior to sampling. Participants were required to fast (no food or drink) for 3 hours before sampling. All fresh oropharyngeal swabs were collected by the same operator, and immediately stored at − 80°C within 2 hours after sampling until DNA extraction. Data regarding age, sex, Smoking, Alcohol consumption and Daily toothbrushing frequency were also collected using a questionnaire (Supplementary File S1). This study was approved by the Medical Ethics Committee of the Affiliated Hospital of Yunnan University (No. KJK#20250825-R1-041).

Metagenomic sequencing

Genomic DNA was assessed for purity (NanoDrop spectrophotometer), concentration (Qubit 4.0 fluorometer), and integrity (1% agarose gel electrophoresis, 120 V, 45 min) before library construction. After passing quality control, 0.5 μg of genomic DNA per sample was processed with the VAHTS Universal DNA Library Prep Kit for Illumina V3: DNA was fragmented by sonication (Bioruptor Pico), size-selected and purified with magnetic beads, subjected to end repair and adapter ligation, and enriched by PCR to generate double-stranded libraries. Qualified libraries were converted to single-stranded circular DNA, amplified via rolling circle amplification to form DNA nanoballs (DNBs), and sequenced on the DNBSEQ-T7 platform with a paired-end 150 bp (PE150) strategy.

Bioinformatic analysis (gene-based pipeline)

All analyses were performed under a unified gene-based framework. The core workflow was as follows: 1) Quality control: Raw reads were filtered with fastp (v0.21.0) to remove adapters, low-quality sequences (average quality score < Q20) and ambiguous bases; human-derived reads were depleted by alignment to the GRCh38 reference genome. 2) Assembly and gene catalog construction: Clean reads were assembled into contigs using MEGAHIT. Open reading frames were predicted with MetaGeneMark and clustered by CD-HIT (95% identity, 90% coverage) to build a non-redundant gene catalog. 3) Quantification and annotation: Gene abundance was normalized to reads per kilobase per million mapped reads (RPKM). Taxonomic assignment was performed by alignment to the NCBI nr database, and functional annotations were conducted against the KEGG, VFDB and CARD databases. Abundances were aggregated to corresponding taxonomic and functional levels for downstream analysis.

Microbial diversity and taxonomic differential analysis

Based on the genus-level abundance matrix, alpha diversity indices (Chao1, Shannon, Simpson) were calculated for each sample. Beta diversity was evaluated by PCA, PCoA and NMDS. Differentially enriched taxa between groups were identified by LEfSe.

Functional annotation and differential analysis

Functional abundance profiles for KEGG pathways, virulence factors and antibiotic resistance genes were derived from the gene catalog. LEfSe was used to detect differentially enriched functional features between the two groups.

Statistical analysis

All statistical analyses were performed using SPSS 26.0 and R software (version 4.2.1). A two-tailed P value < 0.05 was defined as statistically significant. For baseline characteristics, continuous variables were presented as mean ± standard deviation and compared by independent samples t-test; categorical variables were expressed as number (percentage) and compared by Fisher's exact test. For microbial community analysis, alpha diversity indices were compared by Wilcoxon rank-sum test. Beta diversity differences were tested by PERMANOVA (999 permutations) and verified by ANOSIM (999 permutations). For all differential analyses (taxa, KEGG pathways, virulence factors, ARGs), LEfSe was applied with uniform criteria: Wilcoxon rank-sum test P < 0.05 and LDA score (log10) > 3.0; the Benjamini–Hochberg method was used for false discovery rate correction. Significance levels in figures were denoted as: ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

Results

Population data

A total of 20 participants were enrolled in this study, including 10 ICU staff and 10 healthy non-medical controls. The baseline demographic characteristics of the two groups are summarized in Table 1. The mean age was 34.30 ± 3.02 years in the healthy non-medical control group and 35.90 ± 2.56 years in the ICU healthcare worker group. No statistically significant difference in age was observed between the two groups (P = 0.217). Males accounted for 40.0% (4/10) of participants in both groups, with identical sex distribution (P = 1.000). There were also no significant intergroup differences in the prevalence of smoking (P = 1.000) and alcohol consumption (P = 1.000). All participants in both groups reported brushing their teeth twice daily, with no variation between groups (Table 1).

Table 1
Baseline characteristics of study participants

baseline demographic characteristics	Healthy non-medical controls (n = 10)	ICU healthcare workers (n = 10)	P value
Age, years	34.30 ± 3.02	35.90 ± 2.56	0.217
Sex, n (%)			1.000
Male	4 (40.0)	4 (40.0)	
Female	6 (60.0)	6 (60.0)	
Smoking, n (%)			1.000
Yes	1 (10.0)	2 (20.0)	
No	9 (90.0)	8 (80.0)	
Alcohol consumption, n (%)			1.000
Yes	4 (40.0)	4 (40.0)	
No	6 (60.0)	6 (60.0)	
Daily toothbrushing frequency, times/day	2.00 ± 0.00	2.00 ± 0.00	—
Values are presented as mean ± SD or n (%).			

Distinct microbial diversity and community structure in ICU staff

We first assessed the genus-level diversity and structural differences in the oropharyngeal microbiota between ICU staff and the non-medical control group. For alpha diversity, significant differences were observed between the two groups, yet the direction of these differences varied by index. Specifically, the Chao1 index was significantly lower in ICU staff than in non-medical controls (P = 0.0039), indicating reduced microbial genus-level richness (Fig. 1A). In contrast, the Shannon index (P = 0.0021) and Simpson index (P = 0.0021) were significantly higher in ICU staff (Fig. 1B, C). As both indices integrate genus-level richness and community evenness, their elevation suggests that reduced dominance of high-

abundance taxa and increased community evenness offset the decline in richness, leading to higher overall community diversity.

Beta diversity analysis was further performed to assess differences in community structure across samples. Principal component analysis (PCA) (Fig. 2A), principal coordinate analysis (PCoA) (Fig. 2B), and non-metric multidimensional scaling (NMDS) (stress = 0.1032) (Fig. 2C) consistently demonstrated a distinct separation in microbial community structure between the two groups. PERMANOVA confirmed the statistical significance of this separation (Bray–Curtis distance: $R^2 = 0.2612$, $P = 0.001$; Jaccard distance: $R^2 = 0.1951$, $P = 0.001$) (Fig. 2D, E).

In addition, Taxonomic profiles were summarized from phylum to species level based on annotation results, and differential analysis was focused on the genus level using LEfSe. LEfSe analysis showed that genera significantly enriched in the non-medical control group were predominantly common oral commensals, such as Prevotella, Veillonella, and Alloprevotella. In contrast, genera significantly enriched in ICU staff included multiple well-recognized potential or opportunistic pathogens, such as Helicobacter, Streptococcus, Pseudomonas, Escherichia, and Mycobacteroides (Fig. 2F).

KEGG functional pathway enrichment of the oropharyngeal microbiome

ICU staff and the non-medical control group exhibited markedly distinct enrichment patterns in KEGG functional pathways (Fig. 3). The pathways significantly enriched in ICU staff were categorized into three functionally coherent classes: (i) infection- and pathogenesis-related pathways, represented by Staphylococcus aureus infection and bacterial biofilm formation pathways (including modules associated with Vibrio cholerae and Escherichia coli); (ii) detoxification and cofactor metabolism pathways, including drug metabolism–cytochrome P450, metabolism of xenobiotics by cytochrome P450, folate biosynthesis, and ABC transporters; (iii) stress adaptation and metabolic regulatory pathways, including the sulfur relay system, transcription factor-related pathways, and central carbon metabolism modules. Notably, several human disease-related pathways (e.g., diabetic cardiomyopathy, hepatocellular carcinoma, central carbon metabolism in cancer) were also mapped as differentially enriched. These annotations stem from sequence homology between microbial genes and eukaryotic pathway genes, and do not represent direct pathogenic functions of the oropharyngeal microbiota.

In contrast, pathways significantly enriched in the non-medical control group were primarily associated with cellular processes and basal metabolism, including lipopolysaccharide biosynthesis, glycosaminoglycan degradation, lysosomal pathways, carbohydrate metabolism, pathways involving protein phosphatases and associated proteins, exopolysaccharide biosynthesis, and beta-lactam resistance.

Virulence factor profiles of the oropharyngeal microbiome

Marked differences in virulence factor enrichment patterns were observed between the two groups (Fig. 4). A total of 21 virulence factors were significantly enriched in ICU staff, which could be

categorized into three functional classes: (i) adhesion, colonization, and invasion factors, represented by fibronectin-binding protein PfbA of Streptococcus pneumoniae, fibronectin-binding protein PavA, laminin-binding protein Lmb, lipoprotein SlrA, sortase SrtA, neuraminidase NanB of S. mitis, and outer membrane protein PomA of Haemophilus parahaemolyticus. The manganese/zinc transporter PsaA plays dual roles in both bacterial adhesion and metal ion acquisition. (ii) immune evasion and stress adaptation factors, including zinc metalloprotease RopA of S. pneumoniae, choline-binding protein Pce/CbpE, phosphatase CppA, and high-temperature requirement protease HtrA/DegP of S. mitis. These factors collectively mediate host immune evasion and intracellular stress adaptation. (iii) nutrient acquisition and endotoxin biosynthesis factors: iron/metal ion uptake systems, represented by the iron transport system HitABC of H. parahaemolyticus, iron uptake protein PiuA of S. mitis, and the aforementioned PsaA. Factors involved in lipopolysaccharide/endotoxin biosynthesis and modification include lipid A synthases LpxA and LpxH of H. haemolyticus, KDO kinase KdkA of H. influenzae, as well as KdsA and GmhA/LpcA of H. parahaemolyticus, which participate directly in the synthesis and modification of lipid A and core polysaccharides and enhance endotoxin activity. Notably, these enriched virulence factors were primarily derived from Streptococcus and Haemophilus species, consistent with the significant enrichment of these two genera in ICU staff. Virulence factors associated with other enriched pathogens such as Pseudomonas and Mycobacteroides did not reach statistical significance, likely due to their relatively low absolute abundance in the oropharyngeal niche. In contrast, no significantly enriched virulence factors were detected in the non-medical control group. Only a small number of genes, such as the hemoglobin receptor gene hmbR and pilus retraction protein gene pilT of Neisseria subflava, were detected at baseline abundance levels without statistical significance, reflecting an overall low-virulence homeostatic state of the commensal microbiota.

Antibiotic resistance gene profiles of the oropharyngeal microbiome

Resistome heatmap visualization revealed distinct overall distribution patterns of oral ARGs between the two groups: ICU staff was characterized by higher overall abundance of ARGs derived from Streptococcus species, whereas the non-medical control group was dominated by specific ARGs carried by Veillonella species (Fig. 5).

Compared with the control group, five resistance genes exhibited significantly higher abundances in ICU staff, which could be categorized into two mechanistic classes. Four were efflux pump-encoding genes: mel (a macrolide efflux pump mediating resistance to erythromycin and azithromycin) carried by Streptococcus pneumoniae, patB (a fluoroquinolone efflux pump conferring resistance to ciprofloxacin and levofloxacin) carried by S. mitis, mdtG (a multidrug efflux pump mediating cross-resistance to macrolides, β-lactams, and other agents) carried by S. mitis, and hmrM (a macrolide efflux pump) carried by Haemophilus influenzae. The remaining gene, rlmA(II) (carried by S. mitis), encodes a 23S rRNA methyltransferase that confers macrolide resistance via ribosomal target modification. The predominance of efflux pump–type ARGs is consistent with the significant enrichment of ABC

transporter pathways observed in the KEGG functional analysis. In contrast, only two ARGs—lnuC (a lincosamide nucleotidyltransferase gene mediating resistance to lincomycin and clindamycin) carried by Veillonella rogosae, and lsaC (an ABC-F family resistance gene mediating LSAP-type cross-resistance to lincosamides, streptogramin A and pleuromutilins) carried by Lachnoanaerobaculum umeaense—were significantly more abundant in the non-medical control group than in ICU staff. No statistically significant differences were observed in the abundances of the remaining ARGs between the two groups.

Notably, although beta-lactam resistance was identified as an enriched pathway in the control group at the KEGG functional level, no individual beta-lactamase gene reached statistical significance at single-gene resolution. This discrepancy is likely attributable to high inter-individual variability and the low abundance of individual resistance genes, which collectively drive pathway-level enrichment but fail to reach significance at the single-gene level.

Discussion

This study employed metagenomic sequencing to systematically characterize the oropharyngeal microbiota of ICU staff and healthy non-medical controls recruited from outside the hospital setting, comparing them across multiple dimensions including species composition, community structure, KEGG metabolic pathways, virulence factor profiles, and the resistome. The results revealed that the oropharyngeal microbiota of ICU staff exhibited profound remodeling patterns which may be associated with occupational exposure to the hospital environment.

First, the oropharyngeal microbial community richness of ICU staff decreased significantly, while evenness increased significantly relative to the control group. This is consistent with previous reports on the microecology of healthcare worker populations. Zhang et al. conducted 16S rRNA sequencing of oropharyngeal swabs from 65 healthcare workers across four different clinical departments and found that while oral microbial diversity was highest among the four departments, microbial richness was lower relative to other departments, albeit not statistically significant[3]. A possible explanation for this dysbiosis is that the persistent and taxonomically similar microbial sources in the ICU environment exert a homogenizing selective pressure via continuous environmental inoculation on healthcare workers, significantly reducing the variability in community structure between individuals and thus increasing evenness; concurrently, dominant commensal bacteria are suppressed, leading to decreased richness. The combination of these two effects establishes a pattern of "decreased richness but increased evenness," which may represent a distinctive signature of microbial community shift associated with occupational exposure.

The healthcare facility environment constitutes a unique background of microbial exposure for medical staff. A previous study analyzing water samples and airborne dust from different hospital departments found that Pseudomonas exhibited the highest prevalence, accounting for 7.95% of the total bacterial community, while Mycobacterium, Streptococcus, and Escherichia were also detected[11]. Furthermore, Baker et al. demonstrated that hospital tap water can serve as a reservoir for Mycobacterium

immunogenum[12]. Ussowicz et al. further identified potentially pathogenic bacteria including Pseudomonas and Enterobacteriaceae on cleaning sponges and faucet edges, with faucet samples enriched in human-associated genera such as Staphylococcus and Streptococcus[13]. In research on the gut microbiome of ICU staff, an enrichment of Pseudomonas, Streptococcus, and Enterobacteriaceae was similarly observed, along with a depletion of Prevotella[4]. In studies of the oropharyngeal microbiome, the abundance of Streptococcus in the upper respiratory tract of healthcare workers was significantly higher, while that of Prevotella was lower[3]. Consistent with these findings, our study demonstrated that, compared with non-medical controls, ICU staff had significantly higher abundances of potentially pathogenic bacteria such as Pseudomonas, Streptococcus, Escherichia, and Mycobacteroides in the upper respiratory tract, whereas commensal bacteria such as Prevotella were less abundant. These results collectively point to a noteworthy phenomenon: the enrichment profile of potentially pathogenic bacteria in two anatomically and functionally distinct niches, the oropharynx and the gut, closely mirrors the dominant bacterial genera detected in the hospital environment. This cross-niche consistency indicates that occupational exposure to the hospital environment is systematically linked to convergent microbial community remodeling at multiple body sites in healthcare workers, resulting in a detrimental pattern of microbial dysbiosis characterized by the expansion of potential pathogens and the depletion of commensals. This dysbiosis profile aligns with the occupational exposure-associated signature described above. Furthermore, our study revealed significant enrichment of the genus Helicobacter in the oropharynx of ICU staff, among which H. pylori is widely recognized as an occupational pathogen for certain healthcare workers, particularly for those in high-exposure settings such as gastroenterology endoscopy suites, ICUs, and dentistry departments[14, 15]. The enrichment of Helicobacter not only further corroborates the role of occupational exposure in reshaping the microbiota of healthcare workers but also suggests that colonization by specific pathogens from the hospital environment may pose additional health risks, warranting special attention in occupational protection.

The microbiota remodeling associated with ICU occupational exposure reflects not only shifts in species abundance but also a profound alteration in the community's functional potential. The significant enrichment of adhesion-, colonization-, and invasion-related virulence factors in ICU staff is consistent with the enrichment of bacterial biofilm formation pathways identified in the KEGG functional analysis. Key adhesins enriched in ICU staff—such as PfbA, PavA, and Lmb—are critical molecular mediators of early biofilm stages, including surface adhesion and microcolony aggregation[16–18]. Prior work has established PfbA as a surface adhesin and invasin that binds host fibronectin and plasminogen, serving as a core virulence factor. Furthermore, homologs of adhesion genes, including pavA, psaA, and lmb, are conserved across S. mitis and S. oralis clinical isolates[19].

Notably, KEGG analysis revealed co-enrichment of biofilm formation, drug metabolism–cytochrome P450, and ABC transporter pathways in ICU staff, highlighting three synergistic resistance mechanisms. Biofilm acts as a passive barrier: the extracellular polysaccharide matrix restricts antibiotic penetration, while nutrient and metabolite accumulation upregulates efflux pumps and quorum sensing, further enhancing resistance[20–22]. For example, a study using static and continuous-flow biofilm experiments has demonstrated that the matrix polysaccharide Psl of Pseudomonas aeruginosa acts as a physical

barrier, impeding a variety of biochemically distinct antibiotics[23]. The cytochrome P450-mediated drug metabolism pathway facilitates the metabolic inactivation of antibiotics via enzymatic modification or degradation[24, 25]. Notably, evidence for this pathway in the current study remains at the functional prediction level; direct experimental validation in oropharyngeal isolates is still lacking. Furthermore, studies have demonstrated that a bacterial CYP enzyme from Pseudomonas aeruginosa (CYP107S1) can directly metabolize fluoroquinolone antibiotics[26]. ABC transporter efflux pumps confer active resistance by extruding intracellular antibiotics and are also implicated in virulence, biofilm formation, and immune evasion[27, 28]. For example, Poudyal et al. demonstrated that activation of ABC transport systems by the MerR-like regulator BrlR in Pseudomonas aeruginosa biofilms confers high-level tobramycin tolerance, whereas inactivation of these systems restores drug susceptibility by increasing intracellular drug accumulation[29]. Such efflux-mediated resistance mechanisms often act in concert with virulence pathways, representing a widespread adaptive strategy across bacterial pathogens [30]. Collectively, these three mechanisms form a coordinated "passive barrier–metabolic inactivation–active efflux" system that yields resistance far exceeding that of any single mechanism alone. Consistent with these findings, four of the five significantly enriched resistance genes in ICU staff—mel, patB, mdtG, and hmrM—encode efflux pumps mediating resistance to macrolides, fluoroquinolones, and β-lactams; among them, the ABC-family transporter PatB directly aligns with the enriched ABC transporter pathway. The fifth gene, rlmA(II), mediates resistance via ribosomal target modification, representing an additional non-efflux resistance mechanism. This gene-level enrichment aligns with the pathway-level findings of biofilm formation, drug metabolism–P450, and ABC transporters, reinforcing that occupational exposure is associated with a functional shift in the oropharyngeal microbiota characterized by synergistic strengthening of passive defense, metabolic inactivation, and active efflux.

Several limitations of this study should be acknowledged. First, as a cross-sectional observational study, our findings only demonstrate associations between ICU occupational exposure and oropharyngeal microbial remodeling, and cannot establish a definitive causal relationship. Second, the relatively small sample size may limit the statistical power to detect subtle differences and affect the generalizability of the results. Third, all participants were recruited from a single center, which may further limit the generalizability of the findings to other regions or healthcare settings. Fourth, functional pathway and virulence factor annotations are based on bioinformatic predictions from metagenomic sequencing, and further in vitro functional experiments are required to verify the actual activity of the identified resistance and virulence traits. Finally, we did not fully adjust for potential confounding factors such as hand hygiene frequency, disinfectant exposure habits, and dietary patterns, which may also influence the oropharyngeal microbiota composition. These factors should be systematically collected and analyzed in future studies.

Conclusion

Our study revealed that ICU occupational exposure is associated with systematic remodeling of the oropharyngeal microbiota. This remodeling was characterized by an enrichment of potential pathogens and a depletion of commensals, functionally establishing a synergistic network of pathogenicity and

antimicrobial resistance. This microecological pattern—defined by pathogen enrichment, enhanced virulence, and intensified resistance—constitutes a distinctive signature of microbiota dysbiosis associated with occupational exposure, offering a novel scientific foundation for microbiome-based surveillance and the occupational health protection of healthcare workers.

Abbreviations

ICU
Intensive Care Unit
ARG
Antibiotic Resistance Gene
VFDB
Virulence Factor Database
CARD
Comprehensive Antibiotic Resistance Database
KEGG
Kyoto Encyclopedia of Genes and Genomes
PCA
Principal Component Analysis
PCoA
Principal Coordinate Analysis
NMDS
Non-metric Multidimensional Scaling
UPGMA
Unweighted Pair Group Method with Arithmetic Mean
LEfSe
Linear Discriminant Analysis Effect Size
PERMANOVA
Permutational Multivariate Analysis of Variance
ANOSIM
Analysis of Similarities
LDA
Linear Discriminant Analysis
RPKM
Reads Per Kilobase per Million mapped reads
DNB
DNA Nanoball
PE150
Paired-End 150 bp

Declarations

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of the Affiliated Hospital of Yunnan University (No. KJK#20250825-R1-041). All procedures involving human participants were performed in strict accordance with institutional and national ethical guidelines, including the Declaration of Helsinki (1964) and its later amendments. In addition, informed consent to participate was obtained from all participants in this study.

Consent for publication

Not applicable.

Availability of data and materials

The metagenomic sequencing datasets generated and/or analysed during the current study are available in the Genome Sequence Archive (GSA) at the National Genomics Data Center (NGDC), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences, under BioProject accession number PRJCA073801.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Conception and design:Guanghui Xiu and Zeqin Fan. Sample collection: Kailv Zhou and Junjie Li. Data analysis and interpretation: Kailv Zhou, Junjie Li and Bin Ling. Manuscript writing: Kailv Zhou. Manuscript revision: Zeqin Fan.

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Figures

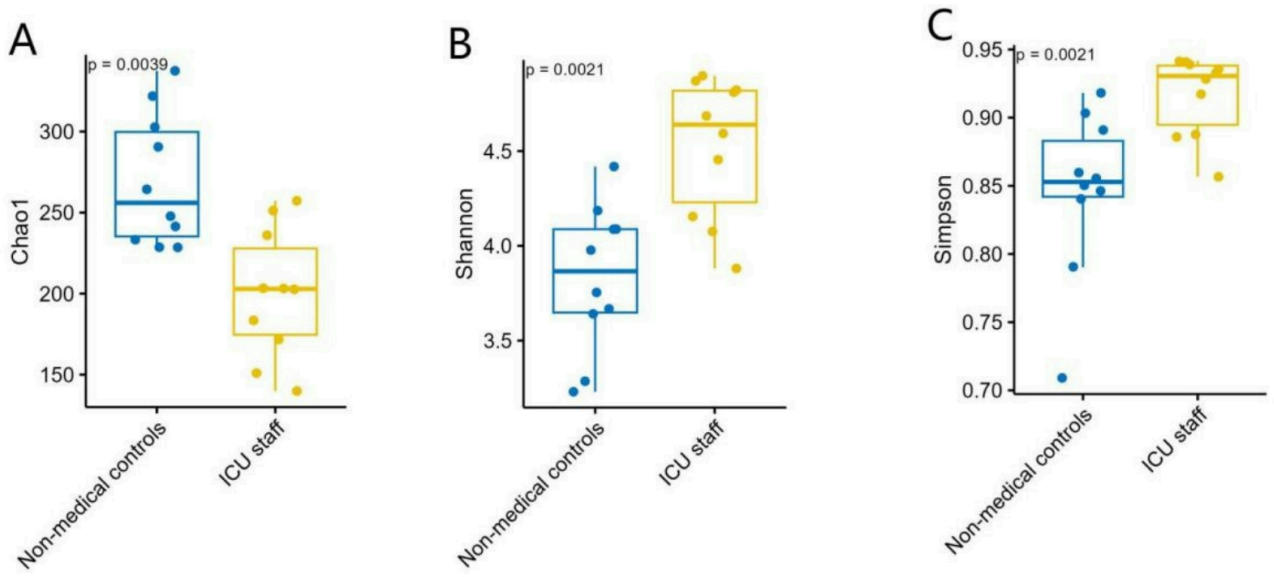


Figure 1

Alpha diversity of oropharyngeal microbiota. (A) Chao1 index reflecting microbial richness, significantly lower in ICU staff (P = 0.0039). (B) Shannon index integrating richness and community evenness, significantly higher in ICU staff (P = 0.0021). (C) Simpson index representing overall community diversity, significantly higher in ICU staff (P = 0.0021).

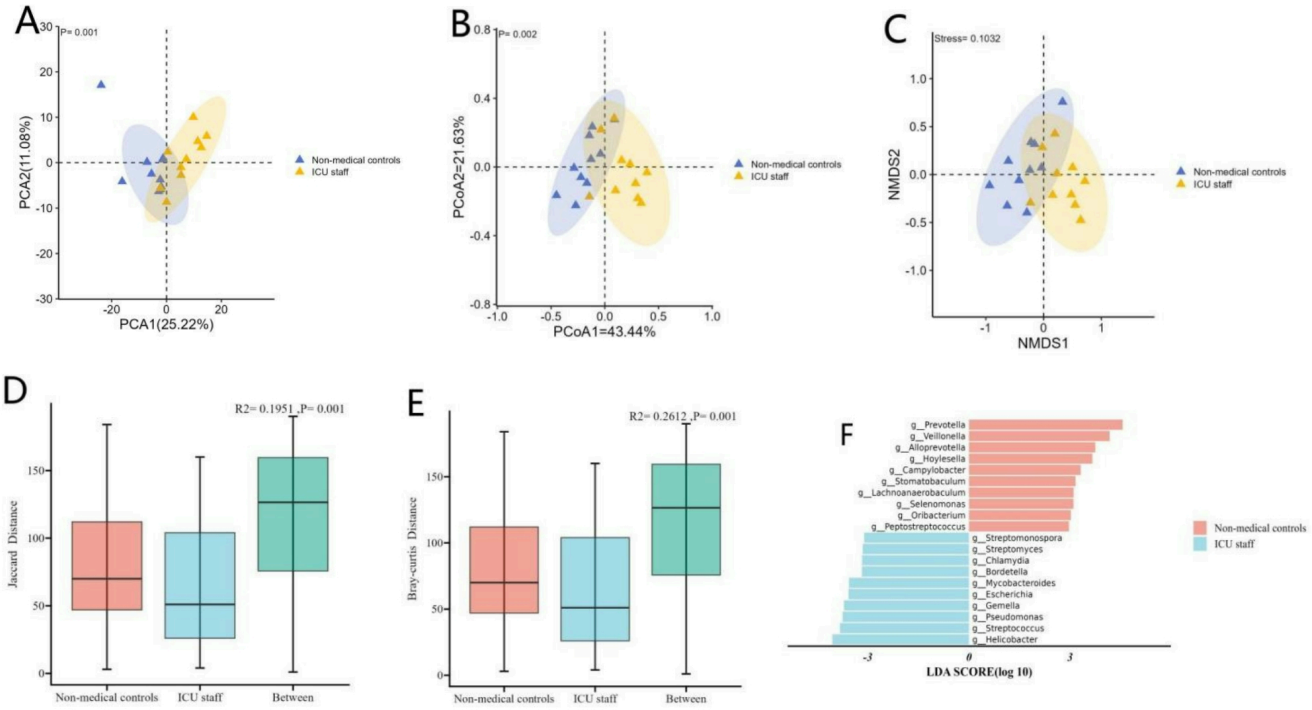


Figure 2

Beta diversity and differentially enriched genera. (A) Principal component analysis (PCA) showing distinct separation between groups. (B) Principal coordinate analysis (PCoA) showing distinct separation between groups. (C) Non-metric multidimensional scaling (NMDS) with stress = 0.1032, showing clear separation between groups. (D–E) PERMANOVA results for Jaccard distance (R^2 = 0.1951, P = 0.001) and Bray-Curtis distance (R^2 = 0.2612, P = 0.001) with 999 permutations. (F) Differentially enriched genera identified by LEfSe (LDA score > 3.0, P < 0.05), with oral commensal genera enriched in controls and potential pathogenic genera enriched in ICU staff.

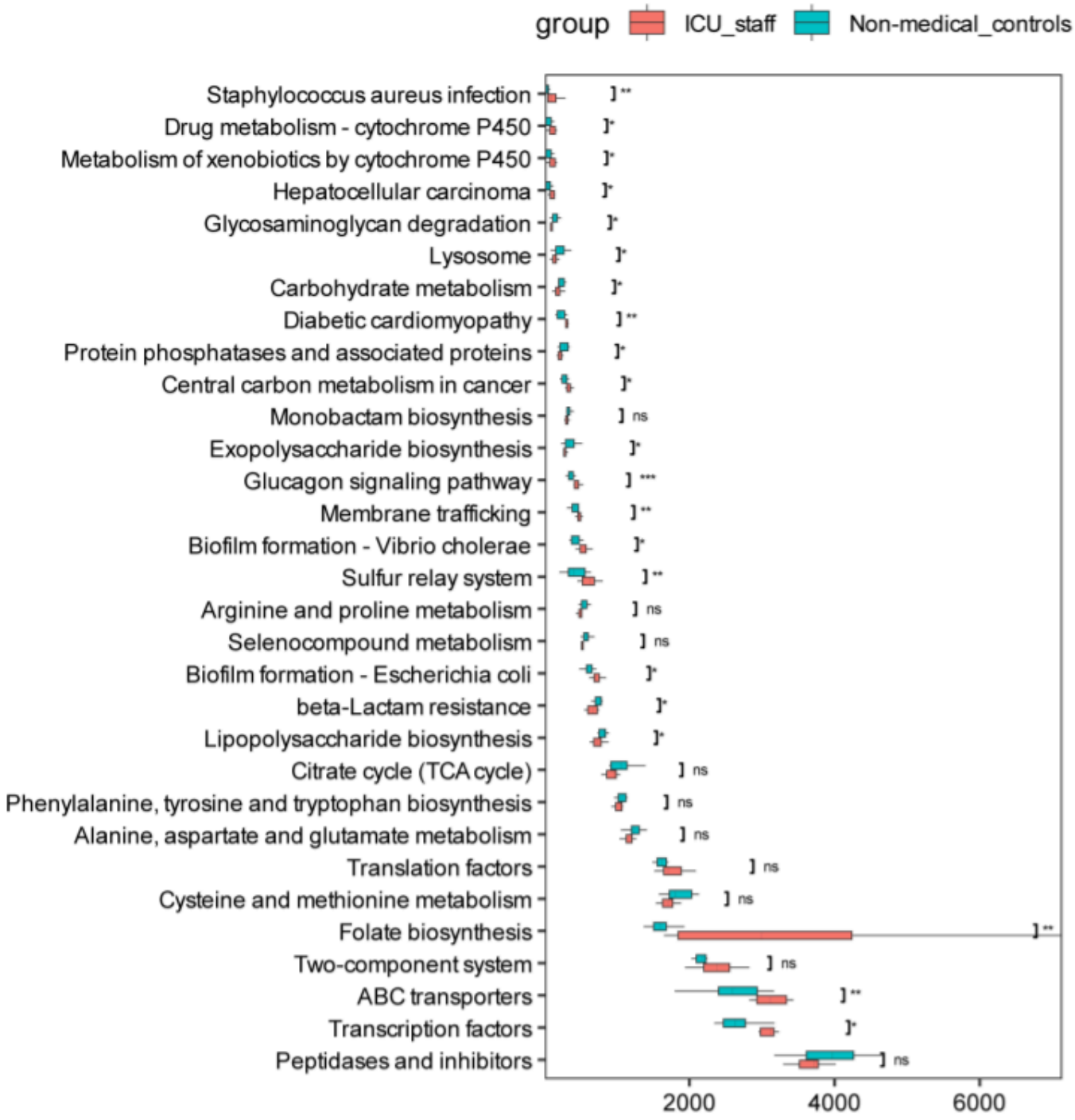


Figure 3

Functional enrichment patterns of the oropharyngeal microbiome between ICU staff and non-medical controls. ns: not significant, * P< 0.05, **P < 0.01, ***P< 0.001.

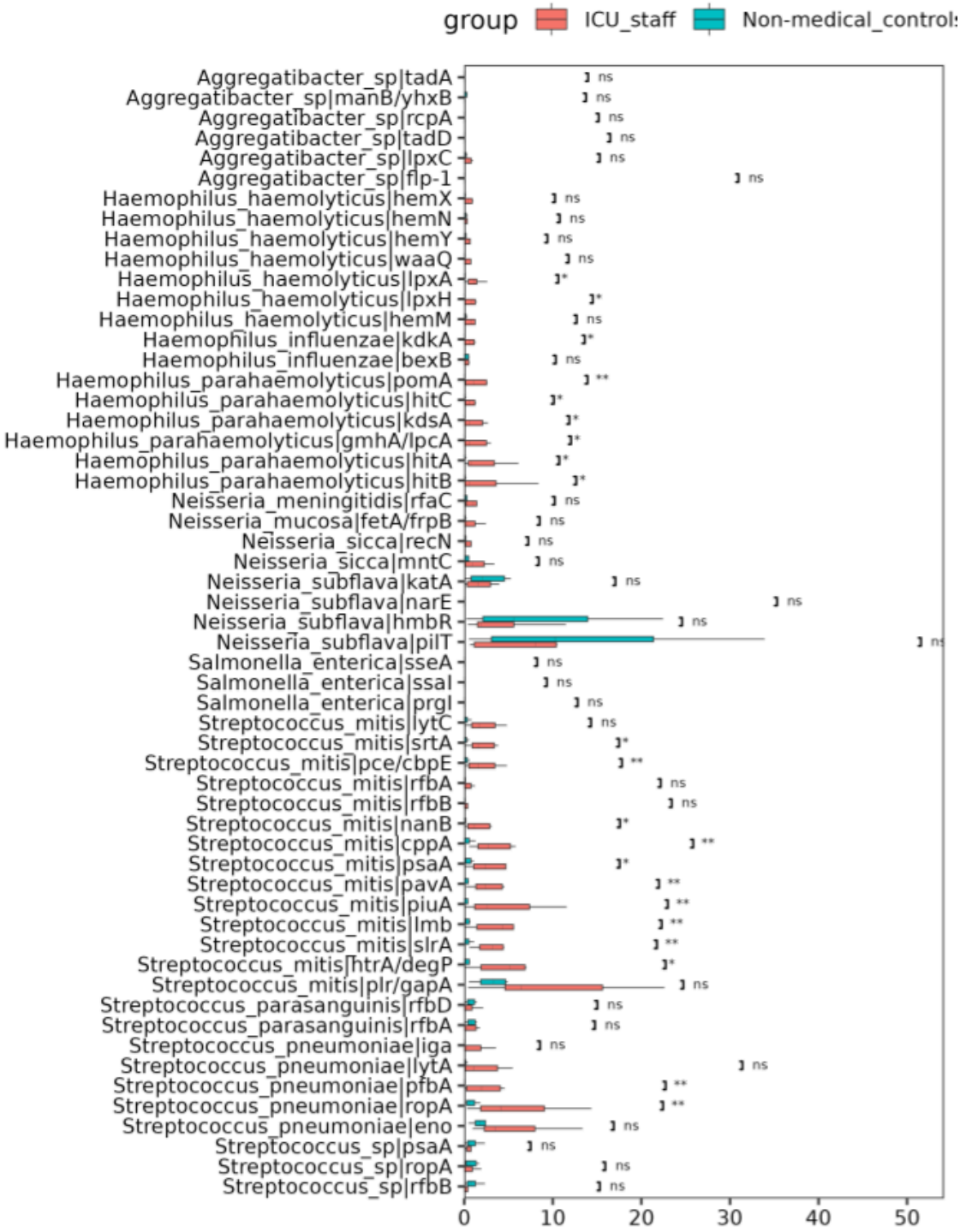


Figure 4

Differentially enriched virulence factors between the two groups. ns: not significant, * P< 0.05, **P < 0.01.

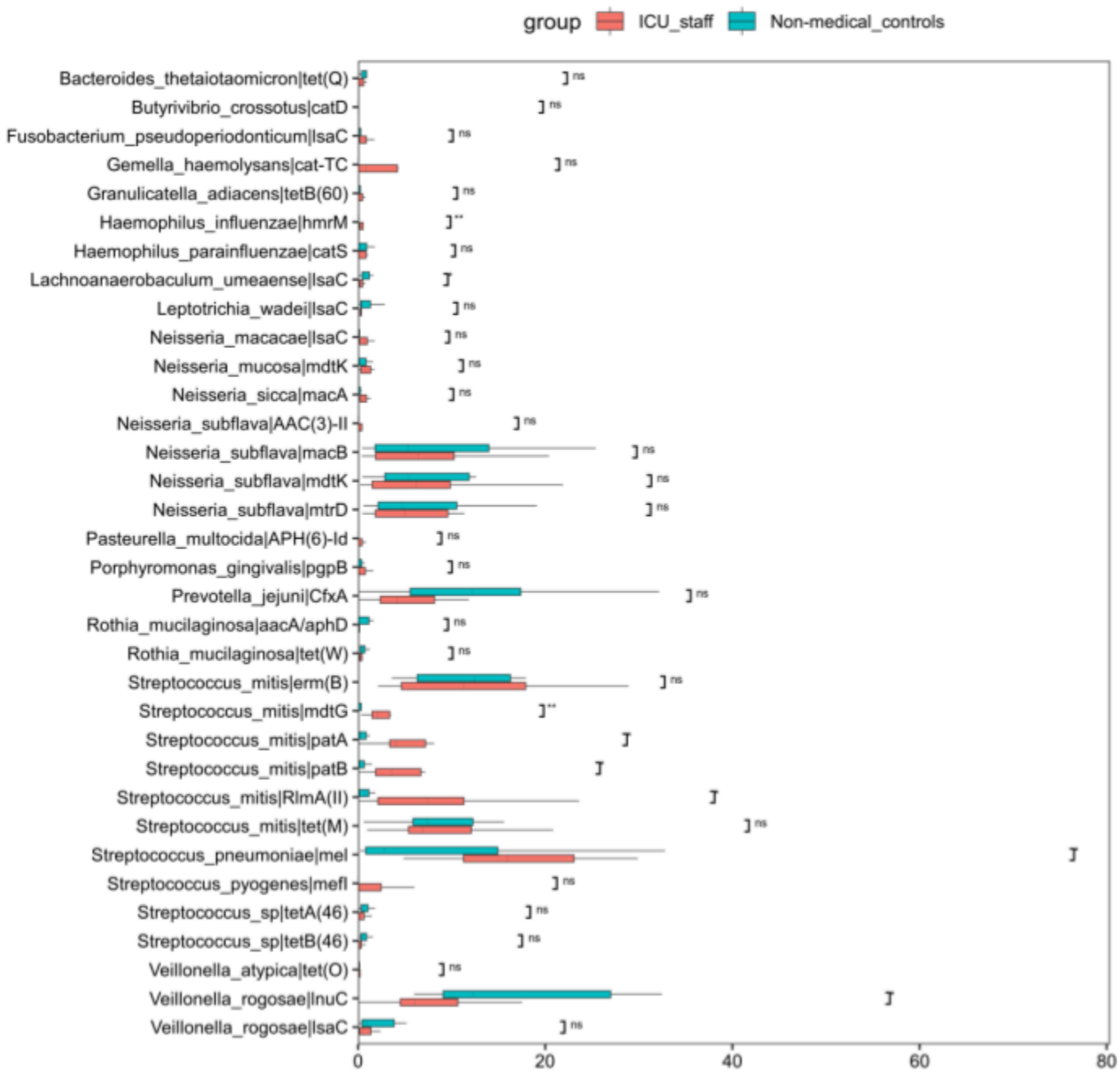


Figure 5

Abundance distribution of oropharyngeal antibiotic resistance genes between the two groups. ns: not significant, * P< 0.05, **P < 0.01.

Supplementary Files

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