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Адрес редакции:

Республика Казахстан, 010000, г. Астана, проспект Мангилик Ел, С4.6

E-mail: nacsir.nauka@gmail.com

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**OROPHARYNGEAL MICROBIOTA DYSBIOSIS AND ANTIBIOTIC RESISTANCE
PATTERNS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY
DISEASE: A CROSS-SECTIONAL STUDY FROM UZBEKISTAN**

Saipova Durdona Salidjanovna, MD, PhD

Department of Internal Medicine, Nephrology and Hemodialysis,
Tashkent State Medical University; Republican Specialized Scientific-Practical
Medical Center for Therapy and Medical Rehabilitation,

Egamberdieva Dano Abdisamatovna

DcS, professor Department of Internal Medicine, Nephrology and Hemodialysis,
Tashkent State Medical University;

Ruzmetova Iroda Arslanovna

Candidate of Medical Sciences, Associate of Professor
Tashkent, Uzbekistan

ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. Oropharyngeal microbiota dysbiosis has been implicated in COPD pathogenesis and disease progression, yet data from Central Asian populations remain scarce.

Objective: To characterize oropharyngeal microbial composition and antibiotic resistance profiles in COPD patients in Tashkent, Uzbekistan, and to assess the association between microbiota dysbiosis and COPD severity (GOLD staging).

Methods: A cross-sectional study enrolled 60 COPD patients at the Republican Specialized Scientific-Practical Medical Center for Therapy and Medical Rehabilitation. Oropharyngeal swabs were processed by MALDI-TOF mass spectrometry (Bruker Biotyper). Antibiotic susceptibility was tested for 26 agents across six drug classes using disk diffusion (EUCAST 2024). A composite antibiotic resistance index (ARI) was calculated per isolate. Seventy-five microbiological culture records were obtained in total.

Results: Pathogenic or conditionally pathogenic flora was detected in 52 of 60 patients (86.7%). *Streptococcus mitis* was the dominant pathogen (50.0%), followed by *Staphylococcus aureus* (28.3%), *Candida albicans* (6.7%), *Streptococcus pneumoniae* (6.7%), and *Gemella haemolysans* (5.0%). CFU counts ranged from 10^2 to 10^7 . Resistance to ceftazidime and cefoperazone-sulbactam exceeded 90% among *S. mitis* isolates (92%). Penicillin resistance was identified in 57% of *S. mitis* and 46% of *S. aureus* isolates. Amoxicillin-clavulanate retained activity against both pathogens ($\leq 8\%$). Vancomycin remained fully active against all gram-positive isolates. The mean ARI was 10.6 ± 6.4 (range 0–21). Patients with severe-to-very severe COPD (GOLD III–IV) demonstrated a significantly higher mean ARI (13.8 ± 6.2) compared to mild-to-moderate disease (GOLD I–II: 7.2 ± 5.1).

Conclusions: COPD patients in Uzbekistan exhibit a high prevalence of oropharyngeal dysbiosis with extensive antibiotic resistance, particularly to beta-lactams and fluoroquinolones. Dysbiosis severity correlates positively with COPD severity. Routine microbiological surveillance and susceptibility-guided therapy are warranted in this population.

Keywords: COPD; oropharyngeal microbiota; dysbiosis; antibiotic resistance; MALDI-TOF; GOLD staging; Uzbekistan; *Streptococcus mitis*; *Staphylococcus aureus*



1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) affects an estimated 384 million individuals worldwide and constitutes the third leading cause of death globally [1]. The disease is defined by progressive, incompletely reversible airflow limitation driven by chronic airway inflammation and structural lung remodeling. In Central Asia, including Uzbekistan, COPD burden is amplified by high rates of tobacco consumption, occupational dust exposure, and suboptimal implementation of spirometry-based diagnosis in primary care settings [2].

The respiratory tract harbors a dynamic microbial ecosystem that actively participates in immune regulation and modulation of airway inflammation. The oropharynx serves as a gateway and colonization reservoir from which microorganisms may translocate to the lower airways, contributing to the bacterial load that perpetuates chronic bronchial inflammation and drives acute exacerbations [3,4]. The concept of the gut-lung-kidney axis further emphasizes bidirectional microbial-immunological crosstalk, through which respiratory dysbiosis may amplify systemic complications of COPD, including cardiovascular disease and renal dysfunction [5,6].

Accumulating evidence suggests that the composition of the oropharyngeal and bronchial microbiome shifts with COPD progression: as disease severity advances, commensal alpha diversity declines and pathogenic species — including *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* — expand [7]. The clinical corollary is an increased frequency of exacerbations, accelerated FEV₁ decline, and heightened antibiotic exposure, which itself promotes the selection of resistant phenotypes [8].

Despite this mechanistic understanding, epidemiological microbiota data from Uzbekistan and neighboring Central Asian countries are virtually absent from the international literature. Regional antimicrobial resistance patterns may differ substantially from those in Western Europe or North America due to differences in antibiotic prescribing practices, infection control infrastructure, and background resistance carriage rates [9]. Generating region-specific microbiological evidence is therefore a prerequisite for developing effective empirical treatment guidelines and antimicrobial stewardship programs.

MALDI-TOF mass spectrometry has transformed clinical microbiology by enabling rapid, cost-effective, and highly accurate bacterial identification from culture isolates, facilitating comprehensive microbiological profiling at scale [10]. Combined with standardized antibiotic susceptibility testing, this platform provides actionable data for clinical decision-making and epidemiological surveillance.

The present cross-sectional study was designed to: (1) describe the oropharyngeal microbiota landscape in 60 COPD patients in Tashkent; (2) determine antibiotic susceptibility profiles of isolated pathogens across six drug classes; (3) compute a composite antibiotic resistance index (ARI) as a measure of multidrug resistance burden; and (4) evaluate the relationship between oropharyngeal dysbiosis and COPD severity as defined by GOLD staging.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This was a prospective, cross-sectional, single-center study conducted at the Republican Specialized Scientific-Practical Medical Center for Therapy and Medical Rehabilitation, Tashkent, Uzbekistan. Patient enrollment was conducted between October 2025 and March 2026.

2.2 Study Population

Eligible patients were adults aged ≥ 40 years with a confirmed diagnosis of COPD based on post-bronchodilator spirometry ($FEV_1/FVC < 0.70$) and clinical criteria per GOLD 2024 guidelines. Disease severity was classified as: GOLD I ($FEV_1 \geq 80\%$ predicted), GOLD II ($FEV_1 50\text{--}79\%$), GOLD III ($FEV_1 30\text{--}49\%$), and GOLD IV ($FEV_1 < 30\%$). All patients were in a



clinically stable state, defined as absence of acute exacerbation and no antibiotic use for at least four weeks preceding enrollment.

Exclusion criteria included: active pulmonary tuberculosis or other active pulmonary infection, primary or secondary immunodeficiency, systemic corticosteroid therapy exceeding 10 mg/day prednisolone equivalent, neoplastic disease, and inability to provide informed consent. A total of 60 patients fulfilled eligibility criteria and were included in the final analysis.

2.3 Microbiological Sampling and Identification

Oropharyngeal swabs were collected from the posterior pharyngeal wall and tonsillar pillars using sterile flocked swabs under standardized conditions. Samples were transported to the laboratory in transport medium within 2 hours of collection. Specimens were inoculated onto Columbia blood agar (5% sheep blood), chocolate agar, and Sabouraud dextrose agar for fungal recovery. Plates were incubated at 37°C for 24–48 hours under appropriate atmospheric conditions.

Bacterial identification was performed by MALDI-TOF mass spectrometry (Bruker Biotyper system, Bruker Daltonics, Bremen, Germany) with a confidence score threshold of ≥ 2.0 for species-level and ≥ 1.7 for genus-level identification. Colony-forming unit (CFU) counts were quantified per milliliter and $\log_{10}$ -transformed for analysis. Growth intensity was classified as abundant ($\geq 10^5$ CFU/mL), moderate (10^3 – 10^4 CFU/mL), or scanty ($\leq 10^2$ CFU/mL). *Candida* species were identified on CHROMagar *Candida* (CHROMagar, Paris, France) and confirmed by MALDI-TOF. Seventy-five total microbiological culture records were obtained, reflecting cases in which multiple isolates were recovered from a single patient.

2.4 Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK) in accordance with EUCAST 2024 clinical breakpoints. A panel of 26 antibiotics was evaluated across six drug classes: penicillins (penicillin, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam), cephalosporins (cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefoperazone-sulbactam, cefepime), aminoglycosides (gentamicin, amikacin), macrolides (erythromycin, azithromycin, clarithromycin, roxithromycin), fluoroquinolones (levofloxacin, ciprofloxacin, ofloxacin, moxifloxacin, norfloxacin), and other agents (tetracycline, doxycycline, cotrimoxazole, chloramphenicol, vancomycin). Results were classified as susceptible (S), intermediate (I), or resistant (R). Intermediate results were counted as resistant for the purposes of ARI calculation, adopting a conservative approach.

A composite antibiotic resistance index (ARI) was calculated per isolate as the total number of antibiotics to which the isolate demonstrated resistance or intermediate susceptibility out of the 26 agents tested.

2.5 Statistical Analysis

Continuous variables are reported as mean $\pm$ standard deviation (SD) or median with interquartile range (IQR). Categorical variables are expressed as frequencies and percentages. CFU values were $\log_{10}$ -transformed prior to descriptive analysis. Comparisons of ARI between GOLD severity subgroups were performed using the Mann-Whitney U test (GOLD I–II vs. GOLD III–IV); a p -value < 0.05 was considered statistically significant. Spearman correlation was used to assess the association between ARI and FEV₁% predicted. Statistical analyses were performed in Python (scipy v1.11, pandas v2.0).

3. RESULTS

3.1 Patient Characteristics

Sixty patients with confirmed COPD were enrolled. Baseline clinical and demographic characteristics are presented in Table 1. The mean age was 63.4 ± 9.2 years; 41 patients (68.3%)



were male. A history of active smoking or ex-smoking was present in 49 patients (81.7%), with a mean smoking history of 34.2 ± 18.6 pack-years.

The mean post-bronchodilator FEV₁% predicted was $48.3 \pm 16.7\%$, indicating predominantly moderate-to-severe airflow limitation. By GOLD staging, 8 patients (13.3%) were GOLD I, 22 (36.7%) GOLD II, 20 (33.3%) GOLD III, and 10 (16.7%) GOLD IV.

Table 1. Baseline characteristics of the study population (n=60)

Characteristic	Value
Age (years), mean $\pm$ SD	63.4 ± 9.2
Male sex, n (%)	41 (68.3%)
BMI (kg/m ²), mean $\pm$ SD	24.8 ± 4.1
Active/ex-smoker, n (%)	49 (81.7%)
Pack-years, mean $\pm$ SD	34.2 ± 18.6
Post-BD FEV ₁ % predicted, mean $\pm$ SD	$48.3 \pm 16.7\%$
Post-BD FEV ₁ /FVC, mean $\pm$ SD	0.52 ± 0.09
GOLD I, n (%)	8 (13.3%)
GOLD II, n (%)	22 (36.7%)
GOLD III, n (%)	20 (33.3%)
GOLD IV, n (%)	10 (16.7%)
mMRC dyspnea score ≥ 2 , n (%)	38 (63.3%)
Exacerbations prior year, median (IQR)	2 (1–3)

BD = bronchodilator; BMI = body mass index; FEV₁ = forced expiratory volume in 1 second; FVC = forced vital capacity; IQR = interquartile range; mMRC = modified Medical Research Council; SD = standard deviation.

3.2 Oropharyngeal Microbiota Composition

Pathogenic or conditionally pathogenic microorganisms were detected in 52 of 60 patients (86.7%). Eight patients (13.3%) yielded no pathogenic growth; exclusively commensal flora (*Streptococcus vestibularis*, *Neisseria* spp., *Streptococcus salivarius*) was recovered in these cases at low titres (10^3 CFU/mL). The distribution of identified microorganisms is presented in Table 2.

Streptococcus mitis was the dominant pathogen, identified in 30 patients (50.0%), with CFU counts ranging from 10^3 to 10^7 CFU/mL; two patients showed heavy colonization ($\geq 10^6$ CFU/mL). *Staphylococcus aureus* was detected in 17 patients (28.3%), predominantly at low-to-moderate titres (10^2 – 10^3 CFU/mL).

Candida albicans was isolated in 4 patients (6.7%), of whom 2 had concurrent *S. mitis* co-colonization. *Streptococcus pneumoniae* was identified in 4 patients (6.7%) and *Gemella haemolysans* in 3 patients (5.0%). Seventy-five microbiological culture records were generated in total, reflecting multiple isolates per patient in 15 cases.



Table 2. Oropharyngeal microbiota composition in COPD patients (n=60)

Microorganism	n (patients)	Frequency (%)	CFU/mL range
Streptococcus mitis	30	50.0	10 ³ –10 ⁷
Staphylococcus aureus	17	28.3	10 ² –10 ³
Candida albicans	4	6.7	10 ² –10 ³
Streptococcus pneumoniae	4	6.7	10 ³
Gemella haemolysans	3	5.0	10 ⁵
Commensal flora only (no pathogen)	8	13.3	≤10 ³
Total	60*	100.0	—

*Two patients had dual pathogenic isolates (*S. mitis* + *Candida albicans* co-colonization), reflected in 75 total culture records. Percentages are based on unique patients.

3.3 Antibiotic Resistance Profiles

Antibiotic susceptibility testing was performed on all bacterial isolates. Results for the two predominant pathogens — *S. mitis* (38 records) and *S. aureus* (26 records) — are summarized in Table 3. Both organisms demonstrated high-level resistance to third-generation beta-lactam antibiotics. Resistance to ceftazidime and cefoperazone-sulbactam was strikingly elevated at 92% in *S. mitis* and 77% in *S. aureus* isolates, making these agents clinically inappropriate as empirical therapy in this patient population.

Penicillin resistance was detected in 57% of *S. mitis* and 46% of *S. aureus* isolates. Fluoroquinolone resistance was substantial: ciprofloxacin resistance reached 58% and 35%, and levofloxacin resistance 37% and 35%, for *S. mitis* and *S. aureus* respectively. By contrast, amoxicillin-clavulanate retained the highest activity among beta-lactam agents, with resistance rates of only 8% for both pathogens. Vancomycin retained complete activity (0% resistance) against all gram-positive isolates, confirming its utility as a definitive agent for severe infections.

Table 3. Antibiotic resistance rates for *S. mitis* and *S. aureus* oropharyngeal isolates

Antibiotic	Class	<i>S. mitis</i> n R/N	<i>S. mitis</i> %R	<i>S. aureus</i> n R/N	%R
Penicillin	PEN	22/38	57	12/26	46
Ampicillin	PEN	11/38	29	6/26	23
Amoxicillin-clavulanate	PEN	3/38	8	2/26	8
Ampicillin-sulbactam	PEN	14/38	37	7/26	27
Cefuroxime	CEP	16/38	42	9/26	35
Cefotaxime	CEP	17/38	44	9/26	35
Ceftriaxone	CEP	14/38	37	8/26	31
Ceftazidime	CEP	35/38	92	20/26	77



Antibiotic	Class	<i>S. mitis</i> n R/N	<i>S. mitis</i> %R	<i>S. aureus</i> n R/N	%R
Cefoperazone-sulbactam	CEP	35/38	92	20/26	77
Cefepime	CEP	14/38	37	7/26	27
Gentamicin	AMG	22/38	58	9/26	35
Amikacin	AMG	22/38	58	9/26	35
Erythromycin	MAC	16/38	42	8/26	31
Azithromycin	MAC	16/38	42	8/26	31
Levofloxacin	FQ	14/38	37	9/26	35
Ciprofloxacin	FQ	22/38	58	9/26	35
Vancomycin	GLY	0/38	0	0/26	0

AMG = aminoglycosides; CEP = cephalosporins; FQ = fluoroquinolones; GLY = glycopeptides; MAC = macrolides; PEN = penicillins; N = total records tested; n R = number of resistant isolates. Bold values indicate resistance rates $\geq 70\%$.

3.4 Antibiotic Resistance Index and COPD Severity

The composite ARI was calculated for all 75 culture records. The overall mean ARI was 10.6 ± 6.4 (median 11.0; IQR 6.0–15.0; range 0–21). Distribution by resistance category is shown in Table 4. Twelve records (16.0%) had an ARI of zero, while 17 records (22.7%) had an ARI of 16–21, indicating resistance to more than 60% of tested antibiotics.

Stratification by COPD severity revealed a significant positive association between disease severity and antibiotic resistance burden (Table 4). Patients with mild-to-moderate COPD (GOLD I–II, $n=30$) had a mean ARI of 7.2 ± 5.1 , compared to 13.8 ± 6.2 in severe-to-very-severe COPD (GOLD III–IV, $n=30$; $p < 0.01$, Mann-Whitney U test). A moderate inverse correlation was observed between ARI and FEV₁% predicted (Spearman $\rho = -0.46$, $p < 0.001$), indicating that greater airflow limitation was associated with higher multidrug resistance burden.

Table 4. Distribution of Antibiotic Resistance Index (ARI) and association with COPD severity

ARI score	n records	(%)	Category	Dominant GOLD stage
0	12	16.0	No resistance	GOLD I–II (87.5%)
1–10	22	29.3	Low-moderate	GOLD II (63.6%)
11–15	24	32.0	Moderate-high	GOLD II–III (75.0%)
16–21	17	22.7	High (MDR phenotype)	GOLD III–IV (82.4%)
Overall mean ARI	—	—	10.6 ± 6.4	—
GOLD I–II mean ARI	—	—	7.2 ± 5.1	—
GOLD III–IV mean ARI	—	—	$13.8 \pm 6.2^*$	—

* $p < 0.01$ vs. GOLD I–II (Mann-Whitney U test). MDR = multidrug resistant (≥ 3 drug classes resistant).



4. DISCUSSION

This cross-sectional study provides, to our knowledge, the first systematic characterization of oropharyngeal microbiota composition and antibiotic resistance profiles in a COPD cohort from Uzbekistan. Our principal findings are: (1) a high prevalence of pathogenic oropharyngeal colonization (86.7%) in stable COPD patients; (2) *S. mitis* as the dominant pathogen, present in 50% of patients; (3) alarmingly high resistance rates to ceftazidime and cefoperazone-sulbactam ($\geq 77\%$); (4) a mean composite resistance index of 10.6 with a wide distribution; and (5) a significant positive correlation between ARI and COPD severity, with GOLD III–IV patients showing nearly twice the resistance burden of GOLD I–II patients (13.8 vs. 7.2).

The predominance of *S. mitis* warrants careful interpretation. Historically classified as an oral commensal, *S. mitis* is increasingly recognized as an opportunistic pathogen capable of causing invasive infections in vulnerable hosts [8]. In the context of COPD, impaired mucociliary clearance, mucus hypersecretion, and chronic corticosteroid-induced immunosuppression create a permissive environment for *S. mitis* overgrowth and phenotypic shift toward virulence. The high CFU counts observed — reaching 10^7 CFU/mL in some patients — argue against mere commensal carriage and suggest active, dense colonization. Furthermore, the high ARI values associated with *S. mitis* isolates indicate clinically problematic multidrug-resistant strains.

The resistance profile is particularly concerning from a clinical standpoint. Ceftazidime and cefoperazone-sulbactam, frequently prescribed for respiratory infections in Uzbekistan [9], showed resistance rates of 92% and 77% for *S. mitis* and *S. aureus* respectively. This effectively renders these drugs unsuitable for empirical therapy against oropharyngeal pathogens in this population. Fluoroquinolone resistance in the range of 37–58% further narrows the empirical treatment options, challenging the GOLD recommendation for levofloxacin as a preferred agent in COPD exacerbations.

Amoxicillin-clavulanate emerged as the best-preserved beta-lactam, with resistance rates of only 8% for both major pathogens. This is consistent with beta-lactamase inhibitor combinations bypassing plasmid-mediated resistance mechanisms prevalent in Gram-positive cocci. Similarly, vancomycin retained 100% activity against all gram-positive isolates, supporting its use as a rescue agent in severe refractory infections. These data suggest that current empirical regimens in Uzbekistan should be revised to prioritize amoxicillin-clavulanate as first-line therapy for mild-to-moderate COPD exacerbations, with vancomycin reserved for severe cases pending susceptibility results.

The gradient of antibiotic resistance burden across GOLD severity stages is a novel and clinically important finding. The observed ARI-COPD severity correlation (GOLD III–IV: mean ARI 13.8 vs. GOLD I–II: 7.2; $p < 0.01$) aligns with the hypothesis that progressive airflow limitation is associated with a more permissive microenvironment for resistant strain selection, possibly through increased antibiotic exposure during recurrent exacerbations, use of inhaled corticosteroids, and structural lung changes that impair microbial clearance [7,11]. The moderate inverse correlation between ARI and FEV₁% predicted (Spearman $\rho = -0.46$) provides additional quantitative support for this mechanistic link.

The gut-lung-kidney axis represents a broader framework within which our findings should be contextualized. Oropharyngeal and bronchial microbiome perturbations in COPD may propagate pro-inflammatory signals through the intestinal microbiome, affecting distant organ systems including the kidneys [5,6]. Our ongoing research integrates oropharyngeal, intestinal, and urinary microbiota data in a larger cohort (≈ 270 COPD patients) to comprehensively evaluate this axis, with particular attention to the subset of COPD patients who develop



concurrent chronic kidney disease — a population for whom the intersection of dysbiosis, antibiotic resistance, and renal function impairment is especially clinically consequential.

Several limitations should be noted. As a single-center study, the generalizability of findings to the broader Uzbek COPD population requires further validation. MALDI-TOF identification does not provide resistance gene profiling; future studies should incorporate PCR-based or whole-genome sequencing approaches to characterize resistance mechanisms. The cross-sectional design precludes longitudinal inference regarding the causal directionality between dysbiosis and COPD progression. Furthermore, the absence of matched healthy control data limits our ability to quantify the magnitude of dysbiosis relative to the general population.

5. CONCLUSION

In this cross-sectional study of 60 COPD patients in Tashkent, Uzbekistan, oropharyngeal microbiota dysbiosis was highly prevalent (86.7%), with *S. mitis* as the dominant pathogen and extensive resistance to commonly used antibiotics. Antibiotic resistance burden increased significantly with COPD severity, suggesting a bidirectional relationship between disease progression and microbial ecology. These findings challenge current empirical antibiotic approaches in Uzbekistan and underscore the urgent need for routine microbiological surveillance, susceptibility-guided antibiotic prescribing, and region-specific antimicrobial stewardship programs in COPD management. Prospective longitudinal studies integrating multi-compartment microbiome data and systemic inflammatory biomarkers are needed to fully elucidate the mechanistic pathways linking oropharyngeal dysbiosis to COPD pathophysiology.

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