

Assessment of differences in bacterial and fungal microbiota of the respiratory tract in correlation with selected molecular biomarkers - study in patients with chronic obstructive pulmonary disease (COPD).

Authors

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Introduction

Chronic obstructive pulmonary disease (COPD) is a respiratory disease characterized by the permanent restriction of airflow through the airways. According to World Health Organization COPD is **the third leading cause of death worldwide, causing 3.2 million deaths in 2021.** Bacterial and fungal infections and chronic inflammation lead to acute worsening of respiratory symptoms requiring a change in treatment. Exacerbations of COPD along with poorly controlled disease course, are most often associated with increased mortality, especially in the elderly group.



Hypothesis

Research hypothesis: Colonisation with *Haemophilus influenzae*, *Streptococcus spp.*, *Enterobacteriaceae*, *Candida spp.* are associated with the development and course of COPD, molecular regulators and can affect the airway obstruction and lung function parameters.



Materials and Methods

Samples were collected **from 35 patients form research group** during exacerbations of COPD according to GOLD grade 3 and 4 and from **23 patients form control group** (Tab. 1.).

	Research group	Control group	p value
Age (mean)	69.79 (95% CI 67.53-72.04)	65.42 (95% CI 63.63-67.21)	0.006*
Sex	17M, 18F	10M, 9F	0.949
GOLD 3 grade	7(20%)	-	-
GOLD 4 grade	28(80%)	-	-
FEV1 (L)	1.20 (95% CI 0.79-1.61)	2.74 (95% CI 2.36-3.11)	0.0002*
FEV1%/FVC ex(%)	44.91 (95% CI 38.45-51.38)	82.0 (95% CI 71.59-92.42)	0.000002*
FEV1 predicted (%)	43.68 (95% CI 32.46-54.91)	93.77 (95% CI 83.28-104.27)	0.000005*
Smokers	23(65.71%)	13(68.42%)	0.986
Former smokers	6(17.14%)	3(15.79%)	0.897
Never smokers	6(17.14%)	3(15.79%)	0.897

Tab. 1. Characteristic of the research group and the control group

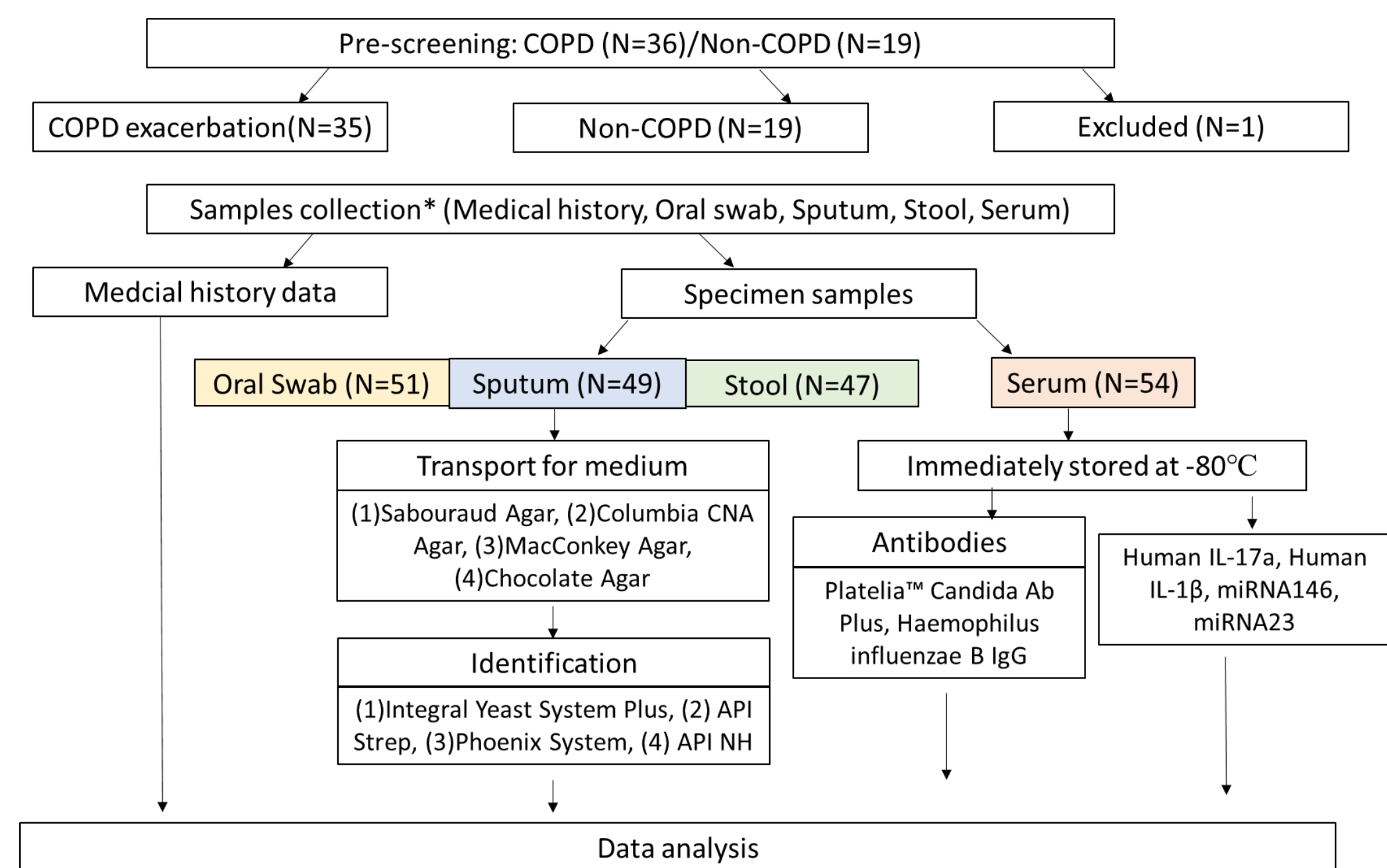


Fig. 1 Materials and methods used used in the identification of selected microorganisms

Quantitative and qualitative assessment of *Haemophilus influenzae*, *Streptococcus spp.*, *Enterobacteriaceae*, *Candida spp.* in large intestine, lower respiratory tract and oral cavity. Serum antibody levels to selected microorganisms were performed by ELISA. Molecular analysis of miRNA146a, miRNA 23 and IL-1β, IL-17 were performed by qPCR.

Results

Yeast (*Candida spp.*, *Cryptococcus spp.*) were isolated from 34 patients from AECOPD group and in 4 people from control group, **7 species have been identified**, the **dominant was C. albicans**, antibiotic resistance was assessed for yeast. **Haemophilus spp. were isolated from 8 patients from AECOPD group and 4 people from control group.** Two species have been identified: **H. influenzae and H. parainfluenzae**. **Streptococci were isolated from 13 AECOPD patients and 2 people from control group.** The **dominant S. milleri**. *Enterobacteriaceae* were not identified. Analysed bacteria species were connected with coinfection with *C. albicans* or *C. tropicalis*.

Localisation	<i>Streptococcus spp.</i>		<i>Haemophilus spp.</i>	
	Isolated species	Number of bacteria (mean)	Isolated species	Number of bacteria (mean)
Oral cavity	<i>S. milleri</i>	500 cfu/swab	-	
	<i>S. sanguis</i>	3300 cfu/swab		
	<i>S. salivarius</i>	1320 cfu/swab		
	<i>E. faecalis</i>	120 cfu/swab		
Lower airways	<i>S. milleri</i>	7100 cfu/1ml	<i>Haemophilus spp.</i>	177 778 cfu/1ml
	<i>S. salivarius</i>	53050 cfu/1ml		
Large intestine			<i>H. influenzae</i>	2 117 901 cfu/1g
	<i>E. faecalis</i>	321 822 cfu/1g	<i>H. parainfluenzae</i>	452 381 cfu/1g
			<i>Haemophilus spp.</i>	159 090 cfu/1g

Tab. 2. Number of isolated bacteria in oral cavity, lower airways and large intestine.

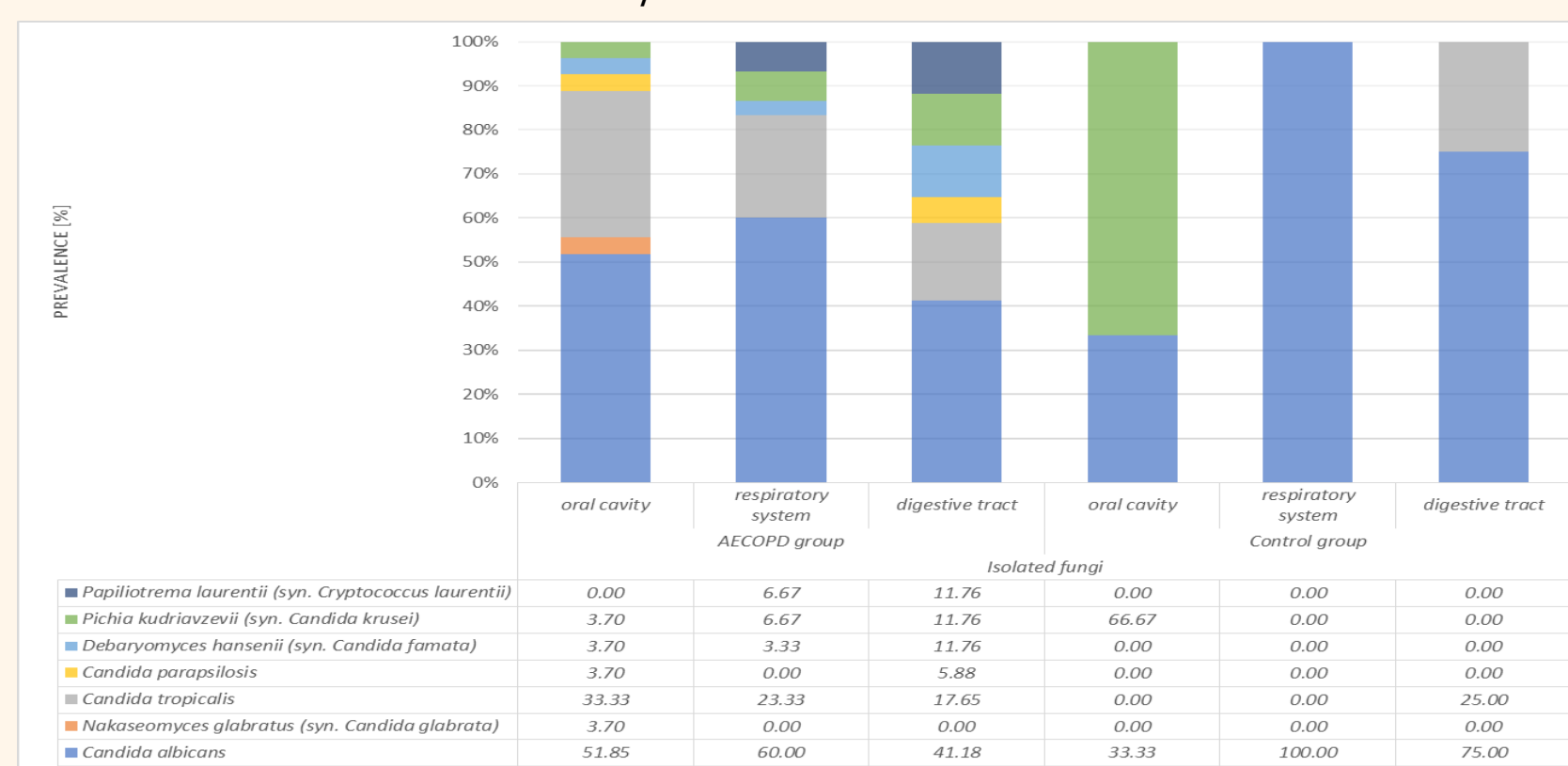


Fig. 2. Prevalence of individual species of fungi isolated from A. AECOPD group and B. control group in biological material collected from the oral cavity, respiratory tract and large intestine.

Conclusions

- Fungi were isolated significantly more often from the AECOPD group than the controls ($Z=5.632$, $p=0.00000018$; Mann-Whitney U-test). The number of yeasts in the oral cavity presented as CFU per swab was statistically significant in the AECOPD group ($Z=4.613$, $p=0.00004$). A significantly higher number of fungal cells was found at the AECOPD group when comparing material from the respiratory tract, in CFU/ml of sputum ($Z=5.027$, $p=0.000005$), and from the digestive tract, expressed in CFU/g of feces ($Z=2.891$, $p=0.0038$).
- Due to differences in the isolation of identified bacteria, especially in the respiratory system; in Streptococci, the species diversity between the research group and the control group was statistically significant ($Z=2.69820$, $p=0.006972$), while the species diversity and isolation of *Haemophilus spp.* is not statistically significant in the comparison of the research and control groups.
- The presence of *Pseudomonas aeruginosa* was not identified in the tested isolates. *Streptococcus milleri* was isolated in the highest amount (cfu/swab) compared to other selected species from the oral cavity. *Haemophilus spp.* was isolated in the highest amount (cfu/ml) compared to other selected species from the respiratory tract. *H. influenzae* was isolated in the highest amount (cfu/1g of faeces) compared to other selected species from the gastrointestinal tract

Affiliations

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