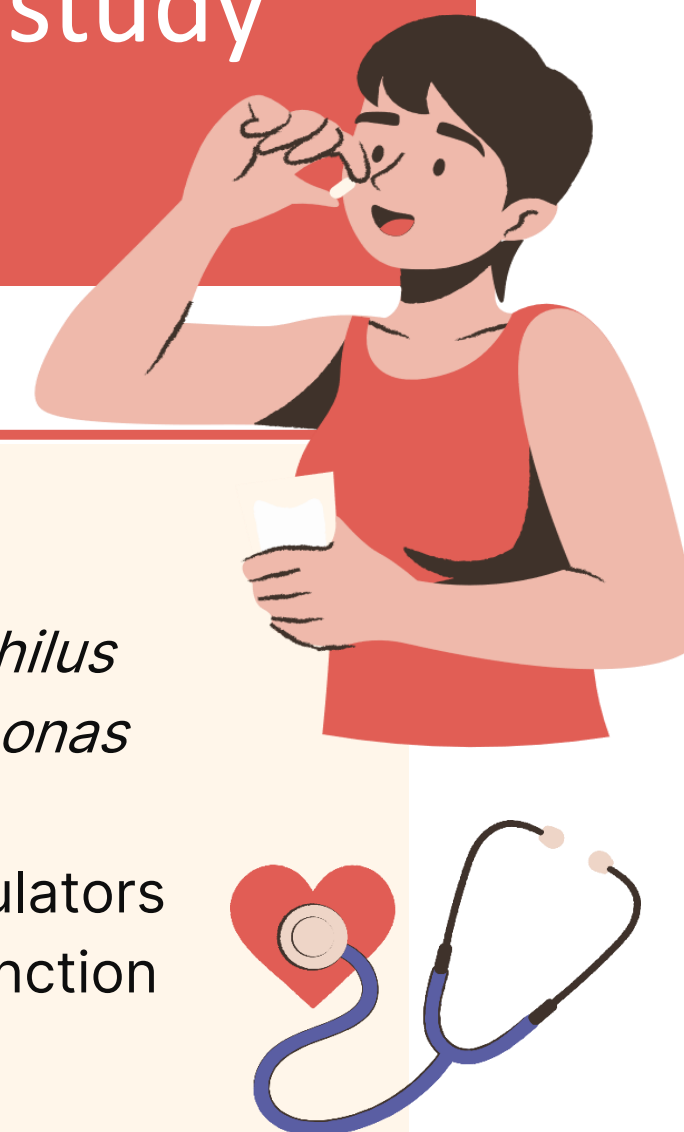


# Assessment of differences in bacterial and fungal microbiota of the respiratory tract in correlation with selected molecular biomarkers - study in patients with chronic obstructive pulmonary disease (COPD).

## 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 pneumoniae*, *Pseudomonas aeruginosa*, *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 **16 patients form control group** (Tab. 1).

	Research group (mean ±SD)	Control group (mean ±SD)
Sex	17 M 18F	9 M 7F
Age	67,18 ±6,01	65,875 ±4,58
Pack years of smoking	33,75 ±12,87	35 ±16,16
Exacerbations per year	2,9 ±1,97	-
CRP (mg/L)	41,34 ±81,19	0,7 ±0,81
EOS (g/L)	0,22 ±0,15	processing of results
FEV1/FVC (%)	14,39 ±29,97	processing of results
Baseline FEV1 (% predicted)	4,19 ±7,15	processing of results
GOLD 3 grade	2M 5F	-
GOLD 4 grade	15M 13F	-

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

Quantitative and qualitative assessment of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Candida spp.* in large intestine, lower respiratory tract and oral cavity. Serum antibody levels to selected microorganisms will be performed by ELISA. Molecular analysis of miRNA146a, miRNA 20a, miRNA 132, miRNA 145 and IL-1β, IL-17, IL-6, IL -5, TNFα will be performed by qPCR.

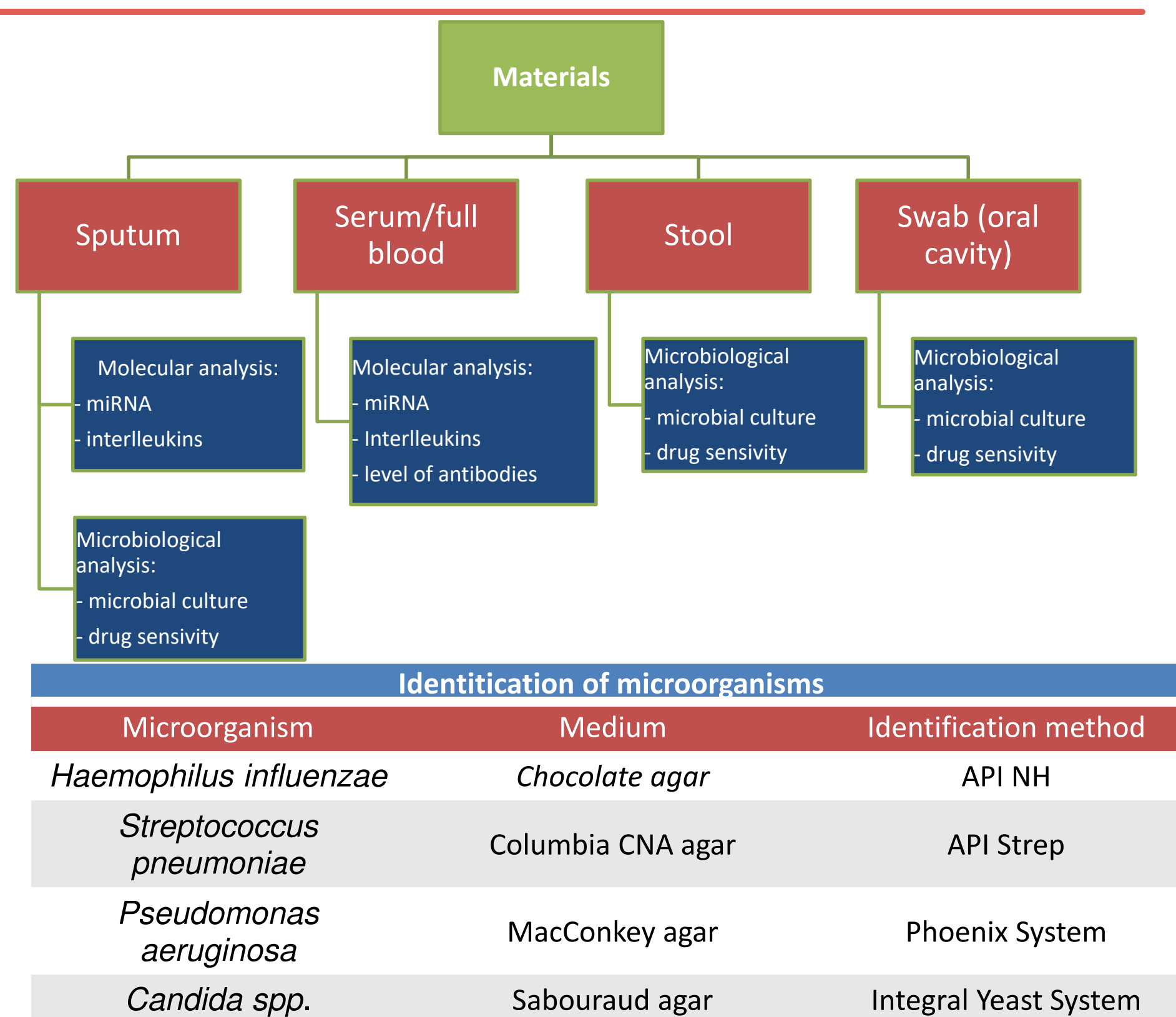


Fig. 1 Materials and methods used in the identification of selected microorganisms

## Results

Yeast (*Candida spp.*, *Cryptococcus spp.*) were isolated from 33 patients, **8 species have been identified**, the **dominant one being *C. albicans***, antibiotic resistance was assessed for yeast. ***Haemophilus spp.* were isolated from 5 patients.** Two species have been identified: ***H. influenzae* and *H. parainfluenzae***. **Streptococci were isolated from 5 patients**, 3 species have been identified, the **dominant *S. milleri***. *Pseudomonas spp.* were not identified in 16 patients. Analysed bacteria species were connected with coinfection with *C. albicans* or *C. tropicalis*, while multifocal colonisation with diverse mycobiota was connected with absence of analysed bacteria.

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>E. faecalis</i>	321 822 cfu/1g	<i>H. influenzae</i>	2 117 901 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.

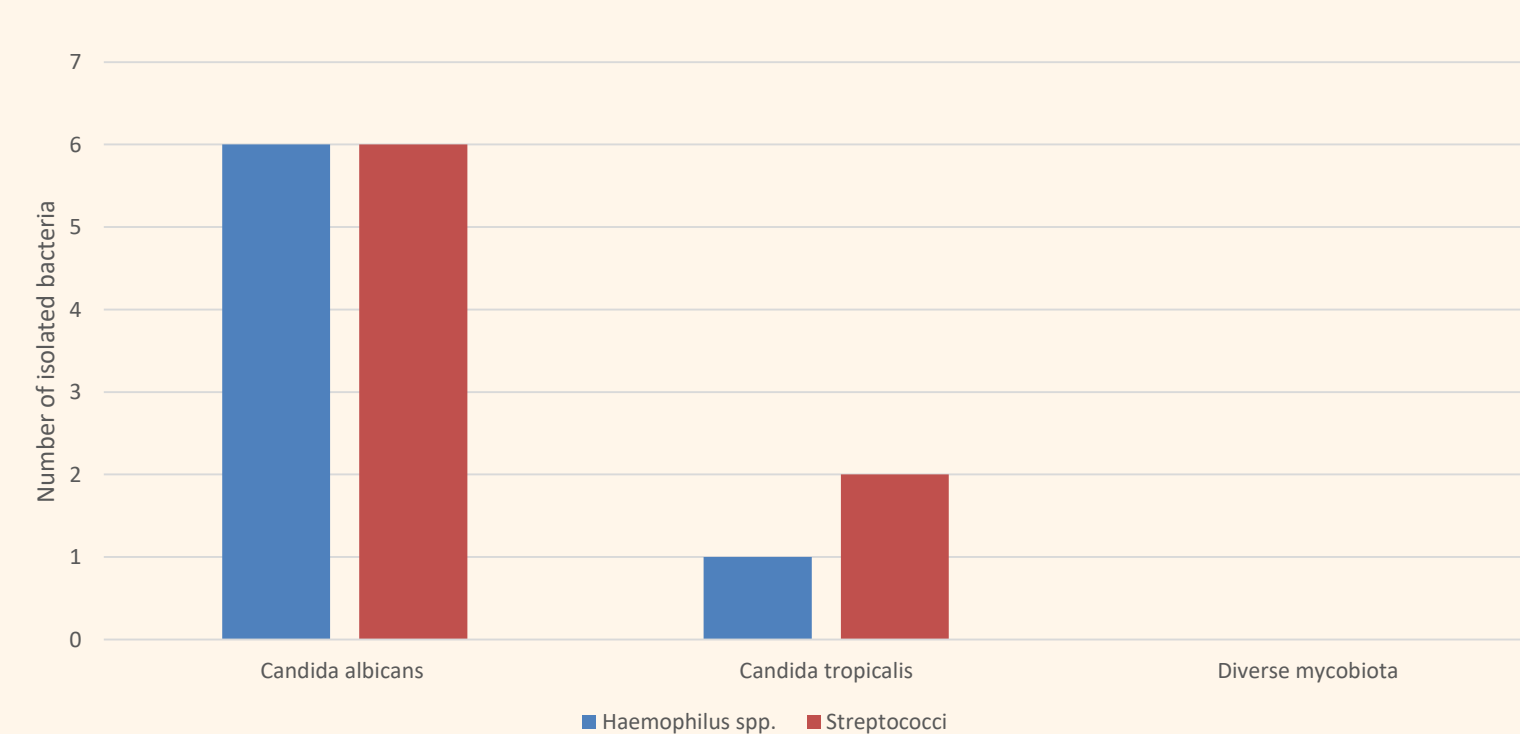
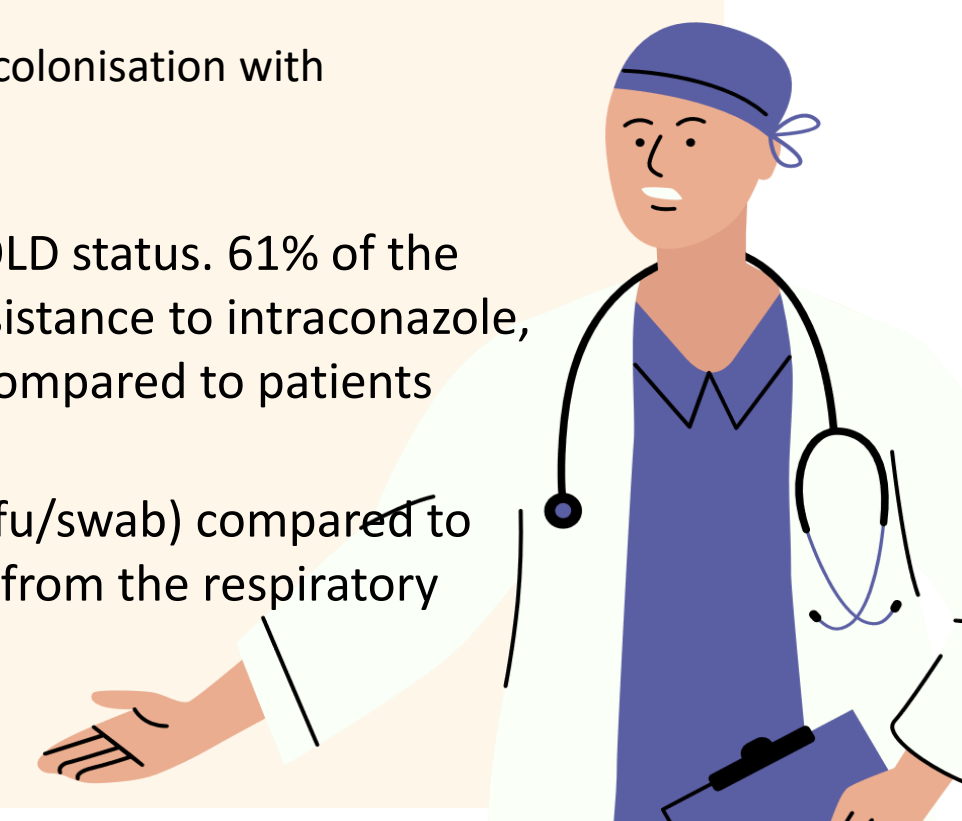


Fig. 2. Presence of bacteria from patients with multifocal colonisation with *C. albicans/C. tropicalis*.

## Conclusions

- The dominant species of fungi in the isolated materials was *Candida albicans*. There was no correlation between isolated yeast species and GOLD status. 61% of the isolated yeasts were sensitive to all antifungals tested, the remaining strains showed intermediate susceptibility and the highest amount of resistance to intraconazole, nystatin, fluconazole and amphotericin. The presence of *Candida albicans* or *Candida tropicalis* was associated with the presence of bacteria compared to patients with diverse mycobiota or different types of yeast in whom bacteria were not isolated.
- 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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