SIGNIFICANCE AND GENO-/SEROTYPIC CHARACTERISTICS OF STREPTOCOCCUS PNEUMONIAE IN THE PATHOGENESIS OF EXACERBATIONS OF CHRONIC OBSTRUCTIVE RESPIRATORY DISEASES

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Abstract. *Pneumococcal infection caused by Streptococcus pneumoniae remains one of the leading causes of infectious morbidity and mortality worldwide, particularly among individuals with chronic respiratory diseases. According to the World Health Organization (WHO), more than 1.6 million deaths are recorded annually due to invasive forms of pneumococcal disease, with the highest vulnerability observed in elderly patients and individuals with comorbid conditions. Chronic obstructive pulmonary diseases (COPD) significantly increase the risk of bacterial colonization and the development of severe complications associated with S. pneumoniae. The present study aims to investigate the frequency of pneumococcal detection and to analyze its serotypic and phenotypic distribution among patients with COPD, in order to assess the local serotype landscape and to inform potential adjustments in vaccination strategies.*

Keywords*: Streptococcus pneumoniae, pneumococcal infection, serotypes, chronic obstructive pulmonary diseases (COPD), bronchial asthma, bronchiectasis, vaccination, phage therapy, multiplex PCR.*

Introduction. Pneumococcal infection remains one of the most significant challenges in clinical microbiology and respiratory medicine.*Streptococcus pneumoniae* is the leading etiological agent of community-acquired pneumonia, purulent sinusitis, otitis media, as well as invasive forms of disease including meningitis and bacteremia. According to estimates from the World Health Organization (WHO), the annual global mortality from pneumococcal infection exceeds 1.6 million people, with the highest vulnerability observed among young children, the elderly, and patients with chronic somatic comorbidities [9,3].Patients with chronic obstructive pulmonary diseases (COPD) are at high risk for primary colonization by *S. pneumoniae* as well as for exacerbations of their underlying disease triggered by pneumococcal infection.In the context of increasing antibiotic resistance of *S. pneumoniae* and the limited effectiveness of empirical antibacterial therapy, the importance of regional serotype surveillance is growing. This has direct clinical implications for the optimization of both vaccine-based prevention strategies and therapeutic interventions.

The aim of the present study was to determine the etiological detection rate of *Streptococcus pneumoniae* in patients with exacerbations of chronic obstructive respiratory diseases and to identify the genotypic and serotypic characteristics of the isolates using multiplex PCR diagnostics.

Material and methods of research. The study cohort included patients with chronic obstructive respiratory diseases who had laboratory-confirmed pneumococcal infection, identified using molecular genetic analysis by multiplex PCR. Patients were stratified according to nosological forms: 34 patients (35.1%) had chronic obstructive pulmonary disease (COPD) (GOLD, 2023), 54 patients (55.7%) had bronchial asthma (BA) (GINA, 2024), and 9 patients (9.3%) had bronchiectasis. The mean age of the patients was 60.04 ± 1.2 years. In terms of gender distribution, males accounted for 47 (48.4%) and females for 50 (51.6%). Among patients with COPD, 62.9% (61 individuals) had

comorbid cardiovascular diseases (CVD), and 10.3% (10 individuals) had type 2 diabetes mellitus (DM).

Research results. During the molecular genetic analysis of 202 nasopharyngeal swab samples using multiplex PCR, positive results for *Streptococcus pneumoniae* were detected in 97 patients with exacerbation of chronic bronchopulmonary pathology. The predominant genotype identified (see Table 1) was *LytA*, detected in 44.3% of cases, followed by *CpsA* in 35.1%, and the combined *LytA+CpsA* genotype in 20.6% of patients.

The *S. pneumoniae LytA* genotype was primarily observed in patients with bronchial asthma (BA) — 26 cases (60.5%) — and in 16 cases (32.2%) of chronic obstructive pulmonary disease (COPD). The *CpsA* genotype was identified in 58.8% of BA patients (20 out of 34), 29.4% (10 out of 34) with COPD, and 11.8% (4 out of 34) with bronchiectasis (BE). The combined *LytA+CpsA* genotype was found with equal frequency in both COPD and BA patients — 8 out of 20 (40%) in each group — and in 4 out of 20 (20%) of BE cases.

These findings highlight that the *LytA* and *CpsA* genotypes are predominantly associated with patients suffering from bronchial asthma, while the combined *LytA+CpsA* genotype is observed at a comparable frequency among patients with COPD and those with BA.

Table 1

Frequency of *S. pneumoniae* Genotypes in the Structure of Respiratory Pathology

Genotype	COPD (n = 34)	BA (n = 54)	BE (n = 9)	Total (n = 97)
LytA	16 (37,2%)	26 (60,5%)	1 (2,3%)	43 (44,3%)
CpsA	10 (29,4%)	20 (58,8%)	4 (11,7%)	34 (35,1%)
LytA + CpsA	8 (40,0%)	8 (40,0%)	4 (20,0%)	20 (20,6%)

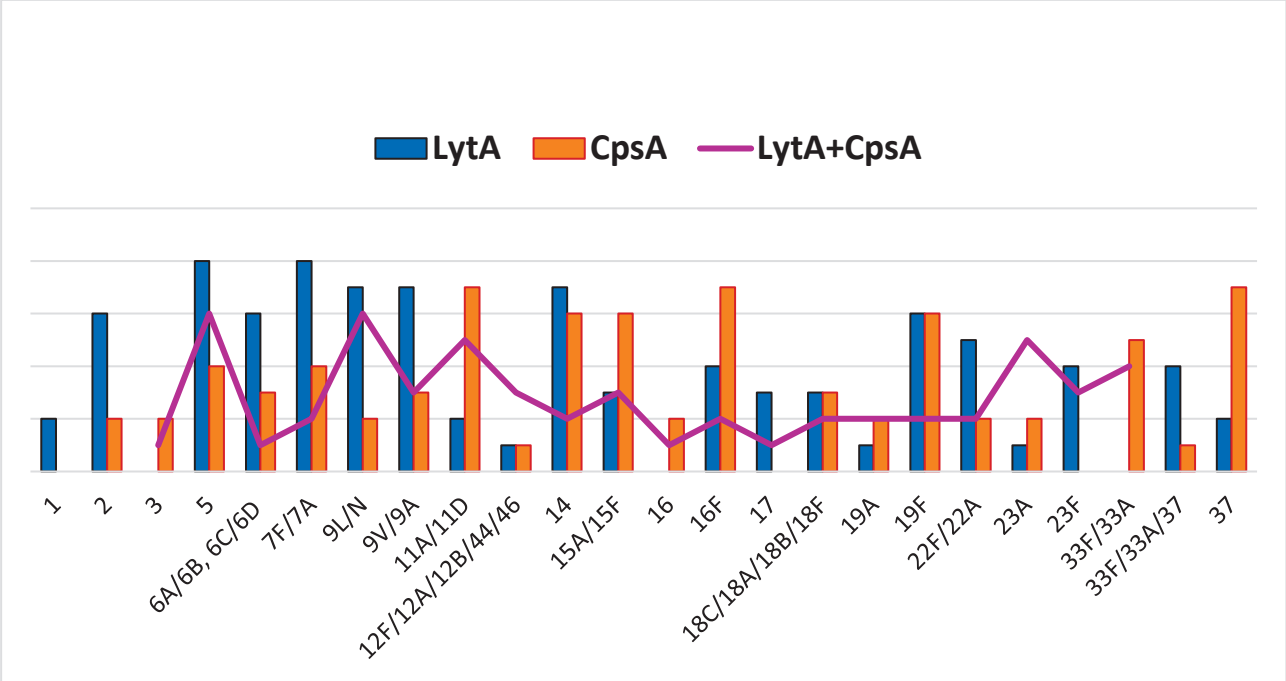

Fig.1. Distribution of Serotypes and Serogroups According to *S. pneumoniae* Genotype.

According to the results of the study (Fig. 1), the Streptococcus pneumoniae LytA genotype was associated with a specific spectrum of serotypes and serogroups, including 1 (2%), 2 (6.1%), 5 (8.2%), 6A/6B, 6C/6D (6.1%), 7F/7A (8.2%), 9L/N (7.2%), 9V/9A (7.2%), 11A/11D (2%), 12F/12A/12B/44/46 (1%), 14 (7.2%), 15A/15F (3%), 16F (4.1%), 17 (3%), 18C/18A/18B/18F (3%),

19A (1%), 19F (6.1%), 22F/22A (5.1%), 23A (1%), 23F (4.1%), 33F/33A/37 (4.1%), and 37 (2%). Serotypes 3, 16, and serogroup 33F/33A were not detected in this group.

For the S. pneumoniae CpsA genotype, associated with exacerbations of respiratory pathology in adults, the serotype and serogroup distribution included: 2 (2%), 3 (2%), 5 (4.1%), 6A/6B, 6C/6D (3%), 7F/7A (4.1%), 9L/N (2%), 9V/9A (3%), 11A/11D (7.2%), 12F/12A/12B/44/46 (1%), 14 (6.1%), 15A/15F (6.1%), 16 (2%), 16F (7.2%), 18C/18A/18B/18F (3%), 19A (2%), 19F (2%), 22F/22A (2%), 23A (5.1%), 33F/33A (5.1%), 33F/33A/37 (1%), and 37 (7.2%). Serotypes 1, 17, and 23F were absent.

The LytA+CpsA genotype was characterized by the following serotypes and serogroups: 1 (1%), 3 (1%), 5 (6.1%), 6A/6B, 6C/6D (1%), 7F/7A (2%), 9L/N (6.1%), 9V/9A (3%), 11A/11D (5.1%), 12F/12A/12B/44/46 (3%), 14 (2%), 15A/15F (3%), 16 (1%), 16F (2%), 17 (1%), 18C/18A/18B/18F (2%), 19A (2%), 19F (2%), 22F/22A (2%), 23A (5.1%), 23F (3%), 33F/33A (4.1%), and 37 (4.1%). Serotype 2 and serogroup 33F/33A/37 were not identified in this group.

The analysis of serotype distribution by S. pneumoniae genotypes (n = 97) revealed substantial serotype heterogeneity (Fig. 1). The most frequently identified serotypes across all genotypes were 5 (18 out of 97); 9L/N and 14 (15 out of 97); 19F, 7F/7A, and 11A/11D (14 out of 97); 9V/9A, 16F, and 37 (13 out of 97); 15A/15F (12 out of 97); and 6A/6B, 6C/6D (10 out of 97). These serotypes occurred in various combinations and were differently distributed among the genotypic groups.

A total of 24 distinct S. pneumoniae serotypes were identified in the sample. Among them, half (12 serotypes, 50%) are vaccine serotypes included in the 13-valent pneumococcal conjugate vaccine (PCV13), namely serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. In this study, 12 of the 13 PCV13 target serotypes were identified (92.3%), accounting for 50% of all isolated serotypes. Serotype 4, although included in the vaccine, was not found among the isolates, while all other vaccine serotypes were present.

The remaining 12 serotypes (50%) are classified as non-vaccine types, meaning they are not included in the PCV13 formulation. Thus, based on the number of unique serotypes identified in the sample, the proportions of vaccine and non-vaccine serotypes were equal, each representing 50% of the total serotype diversity.

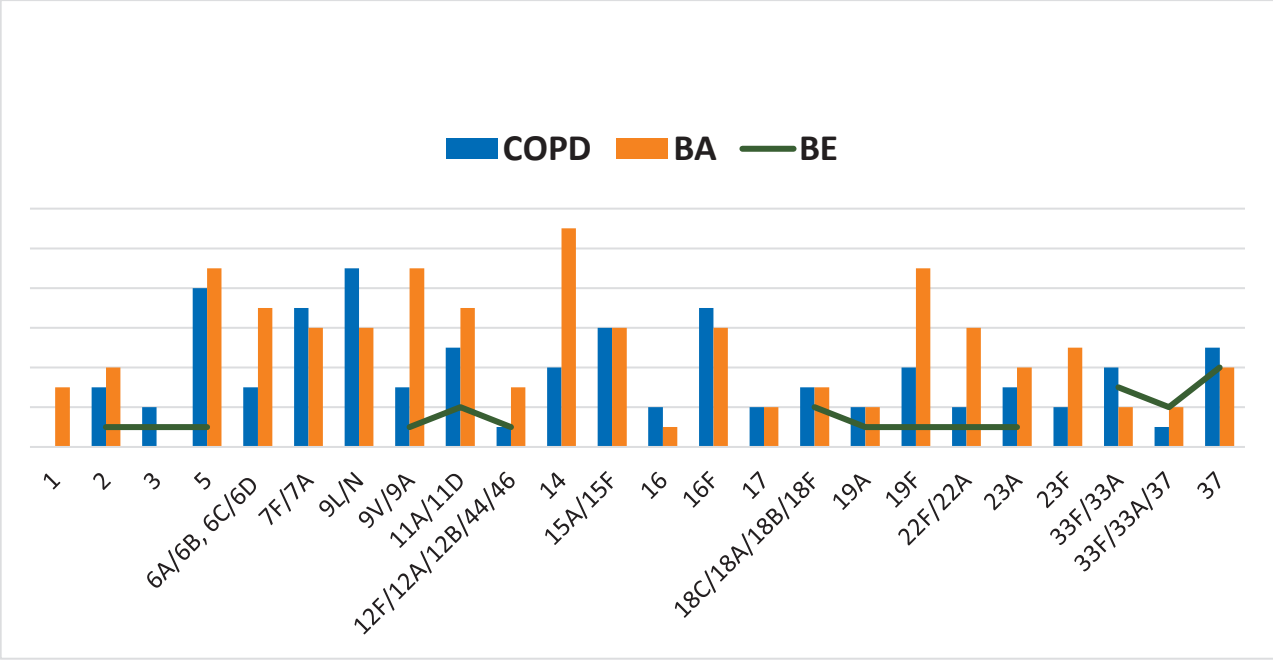

Fig. 2. Distribution of Streptococcus pneumoniae serotypes and serogroups by nosological groups.

According to the serotype analysis by nosological group (Fig. 2):

In patients with chronic obstructive pulmonary disease (COPD) (n = 34), the most frequently detected serotypes were: 5 (n = 8), 9L/N (n = 9), 7F/7A (n = 7), 16F (n = 7), 37 (n = 5), 15A/15F (n = 6), and 11A/11D (n = 5). Serotype 1 was not identified in this group. The study found that PCV13 vaccine-covered serotypes were observed in 41.6% of COPD cases.

In the cohort of patients with bronchial asthma (BA) (n = 54), the most prevalent serotypes included: 5 (n = 9), 9V/9A (n = 9), 9L/N (n = 6), 6A/6B, 6C/6D (n = 7), 11A/11D (n = 7), 14 (n = 11), 19F (n = 9), 23F (n = 5), 7F/7A (n = 6), 15A/15F (n = 6), 16F (n = 6), 22F/22A (n = 6). Serotype 3 was not detected in this group. The proportion of PCV13 vaccine-covered serotypes was also 41.6%.

Among patients with bronchiectasis (BE), there was a limited but distinct dominance of the following serotypes: 11A/11D (n = 2), 33F/33A (n = 2), 33F/33A/37 (n = 3), 37 (n = 4), 18C/18A/18B/18F (n = 2). The following serotypes and serogroups were not identified: 1, 6A/6B, 6C/6D, 9L/N, 14, 15A/15F, 16, 16F, 17, and 23F. The frequency of vaccine-covered serotypes in this group was 29%.

Discussions. The obtained data demonstrate a high etiological frequency of *Streptococcus pneumoniae* among patients experiencing exacerbations of chronic obstructive respiratory diseases (COPD, bronchial asthma, and bronchiectasis), thereby confirming the role of pneumococcal infection as a significant contributing factor to disease exacerbations in adults. Notably, among the serotypes identified in the cohort of patients with PCR-confirmed pneumococcal infection (n = 97), both vaccine-covered and non-vaccine serotypes were prevalent. This underscores the pressing need for enhanced regional seroepidemiological surveillance, especially in the context of increasing antibiotic resistance.

This study revealed statistically significant differences in the distribution of genotypes between disease groups: genotype *LytA* predominated among patients with COPD and bronchial asthma, whereas *LytA+CpsA* was more frequently found in individuals with bronchiectasis. This pattern may be attributed to pronounced mucosal damage, impaired mucociliary clearance, and a tendency for chronic colonization by multidrug-resistant strains in this patient population. These findings are consistent with the work of Gadsby et al. [6], who reported that pneumococcus was mainly detected as a monoinfection during asthma exacerbations, while in patients with bronchiectasis, mixed microbial associations and higher antimicrobial resistance were observed.

According to the current study, the most commonly identified serotypes during exacerbations of COPD and asthma included serotypes 5, 9L/N, 9V/9A, 11A/11D, 7F/7A, 23F, 14, 16F, 19F, and 37, with approximately 50% of isolates belonging to serotypes included in the PCV13 vaccine. These results align with data from similar studies conducted in Europe and Asia. For example, a multicenter study in the UK (Torres A. et al., 2023) [9], showed that serotypes 19A, 3, 6A, and 14 continued to circulate significantly among adult COPD patients even after the introduction of PCV13. Cillóniz C. et al. (2016) [3] also reported a high prevalence of *S. pneumoniae* in community-acquired pneumonia (30–50%), with dominance of the same serotypes identified in our study. Serotypes 5, 14, 19A, 7F, and 23F are classified as highly invasive and associated with severe clinical outcomes according to CDC (2023) [1] and Hausdorff et al. [5], reinforcing the need to include these serotypes in priority targets for vaccination.

In the study by Ben Fredj M. et al. (2020) [4], hospitalizations for acute respiratory infections (ARIs) accounted for 17.6% of all infectious disease admissions, with lower respiratory tract infections, including pneumonia, making up 77.5% of these cases. These figures correspond with our findings, indicating a substantial burden of pneumococcal serotypes associated with severe disease. Particularly noteworthy is the significant proportion of patients with comorbid conditions: in the present study, cardiovascular disease was observed in 62.9% of cases, and type 2 diabetes mellitus in 10.3%, which aligns with international criteria for high-risk complicated pneumococcal infections [8]. This also supports global data highlighting diabetes mellitus as a major risk factor for invasive

pneumococcal disease [2]. The combination of COPD and cardiovascular disease represents a particularly concerning clinical scenario, as both conditions potentiate systemic inflammation and suppress innate immunity.

In conclusion, the findings of this study emphasize the necessity of a comprehensive approach to immunization and prevention of pneumococcal infection in patients with chronic respiratory diseases. The presence of a substantial proportion of non-vaccine serotypes suggests the potential need for broader coverage using PCV15 or PCV20, as well as the prospective utility of adjunctive phage therapy in patients with frequent exacerbations and insufficient vaccine protection.

CONCLUSION**:**

1. The etiological role of *Streptococcus pneumoniae* in exacerbations of chronic obstructive respiratory diseases was identified in 48% of cases: 55.7% among patients with bronchial asthma (BA), 35.1% in those with chronic obstructive pulmonary disease (COPD), and 9.2% in patients with bronchiectasis (BE). These findings highlight the critical role of pneumococcal infection in the pathogenesis of exacerbations and the worsening of the clinical course of respiratory diseases.
2. Genotypic characterization revealed a predominance of *Streptococcus pneumoniae* strains harboring the **LytA** gene (44.3%) and **CpsA** gene (35.1%).
3. A high degree of heterogeneity was observed in pneumococcal serotypes and serogroups, with only 12 out of 24 identified variants covered by the **Prevenar-13** vaccine. The prevalence of vaccine-covered serotypes among patients with BA and COPD was 41.6%, while in the bronchiectasis group it was only 29%.

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