

HIGH PREVALENCE OF NON-VACCINE SEROTYPES
AMONG PEDIATRIC NON-INVASIVE PNEUMOCOCCAL
ISOLATES IN THE PCV10 VACCINE-ERA IN BULGARIA

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Abstract

Streptococcus pneumoniae is a vaccine-preventable agent, due to the available pneumococcal conjugate vaccines (PCVs), but still is a leading bacterial pathogen of non-invasive pneumococcal diseases (NIPD). We conducted a study on the serotype distribution and antibiotic nonsusceptibility in 202 *S. pneumoniae* isolates recovered from children with NIPD during the PCV10-era in Bulgaria (2015–2020). Serogrouping/serotyping were performed using latex agglutination and capsular swelling reaction. Serogroup 6 strains were subjected to serotype-specific PCR's. The antibiotic susceptibilities were assessed by broth microdilution. The PCV10-vaccinated children were 190 (94.1%). The antimicrobial non-susceptibility showed highest levels in oral penicillin (53.5%), erythromycin (52.0%), trimethoprim-sulfamethoxazole (48.5%), clindamycin (47.5%), tetracycline (45.0%), ceftriaxone (14.8%) and chloramphenicol (13.4%). More than half of the strains (53.5%) were MDR. We disclosed 80.2% non-vaccinal serotypes (NVTs) and 17.8% PCV10-serotypes. Except serotype 19F (19.4%), all emergent pediatric NIPD serotypes in our geographic area during the studied PCV10-period were NVTs: 19A (15.8%), 3 (14.8%), 6C (9.9%), 15A (6.0%) and 23A (4.0%). MDR-serotypes were 19A (22.2%), 19F (17.6%), 6C (15.7%), 15A (8.3%), and 23A (8.3%). Surveillance studies must be performed systematically to monitor the vaccine-induced changes and trends in antimicrobial resistance.

Key words: *S. pneumoniae*, vaccine, serotype

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Introduction. *S. pneumoniae* is an etiologic agent of invasive infections (IPD) such as meningitis and septicemia and non-invasive diseases (NIPD) like otitis media, non-bacteremic pneumonia, bronchitis, sinusitis, conjunctivitis, and rhinopharyngitis. Nearly every child has experienced NIPD by the age of five years [1].

The first pneumococcal conjugate vaccine introduced in the Bulgarian routine immunization programme in 2010 was PCV10. The vaccine reduced the incidence of IPD, but did not affect significantly the cases of NIPD.

PCV10 influenced the distribution of vaccine-serotypes (VT) and reflected the nasopharyngeal carriage isolates. There are 101 recognized capsular serotypes, associated with carriage and diseases, that differ by disease severity, antibiotic resistance profiles and case fatality ratio [2]. They form the primary target of conjugate vaccine strategies. Since the available vaccines provide protection against a limited number of serotypes, their distribution and characteristics warrant careful monitoring. Children are colonized most frequently, playing the key role in spreading and selecting multidrug-resistant strains. Many studies observed increasing antibiotic resistance in serotypes responsible for infections of the middle ear, nasal cavity and pharynx in children [3,4].

This study aimed to define the serotype distribution and antimicrobial susceptibility of non-invasive pediatric *S. pneumoniae* isolates during a 2016–2020 PCV10-period in Bulgaria.

Materials and methods. Patients and specimen collection. We collected 202 NIPD isolates from microbiological laboratories in Sofia, Plovdiv, Varna, and Pleven. The children's age was from one month to 16 years. Clinical and demographic data were collected (age, date of hospital admission, diagnosis, vaccine status).

The isolates were obtained from nasopharynx, throat, conjunctiva, and sputum. Children who had received 2 primary doses of PCV10 + 1 booster dose were defined as PCV10-vaccinated.

All isolates were identified according to susceptibility to optochin and bile-solubility.

Serotyping. Serogrouping/serotyping of *S. pneumoniae* isolates were performed by latex agglutination and capsular swelling reaction using serotype-specific factors antisera (SSI, Denmark). All serogroup 6 isolates were verified by PCR-serotyping [5]. The non-typeable (NT) strains were tested for specific amplification of *lytA* gene [6].

Antimicrobial susceptibility testing. The Minimum Inhibitory Concentrations (MICs) were determined by broth microdilution using Sensitre plate STP6F (TREK systems) according to the EUCAST breakpoints [7]. Multidrug resistance (MDR) was defined by non-susceptibility to at least three or more classes of antimicrobial agents.

Statistical analysis. Fisher's exact test was used for analysis of categorical

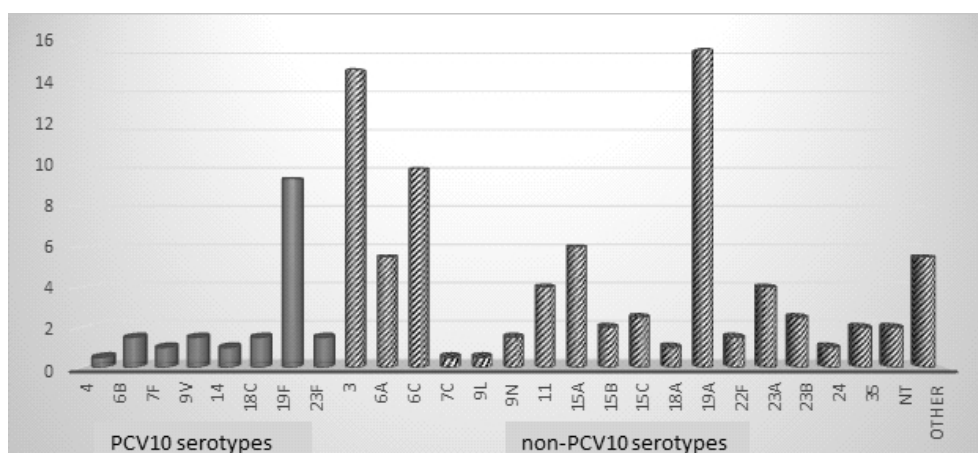


Fig. 1. Serotype distribution among 202 non-invasive pediatric *S. pneumoniae* isolates in PCV10-period (2015–2020) in Bulgaria

Notes:

NT – Non-typeable strains with Pneumotest kit-sera

OTHER – Expected to be serotypes/serogroups (strains were positive with one of the pooled sera only): 16,36,37 ($n = 3$, 1.9%); 25,38,43 – 46,48 ($n = 3$, 1.9%); 13,28 – ($n = 2$, 1.3%); 21,39 ($n = 1$, 0.6%); 29,34,35,42,47 ($n = 1$, 0.6%), 31,40 ($n = 1$, 0.6%)

data. Statistical significance was set at $p \leq 0.05$.

Results. We analysed 202 NIPD isolates recovered from children in the following age groups: 70 children (34.6%) from 1 month to 2 years, 87 children (50.5%) at age 2–7 years and 30 (14.9%) patients were above 7 years old. The PCV10-vaccinated children were 190 (94.1%), 12 children received two primary PCV10 doses and ten were without an applied vaccine.

The specimen collection included nasopharyngeal samples ($n=118$), middle ear fluids (48), throat samples ($n = 22$), eye secretions ($n = 10$) and sputum ($n = 4$). AOM was the most common NIPD ($n = 124$), followed by bronchitis ($n = 33$), and rhinopharyngitis ($n = 24$). Eleven patients manifested non-bacteremic pneumonia. Episodes of conjunctivitis were detected in ten cases.

Serotyping. We disclosed 35 different serotypes and four NT strains. Non-PCV10 serotypes were found in 162 isolates (80.2%). PCV10 serotypes were detected in 36 isolates (17.8%). Eleven strains were positive with only one serum and belonged to more than one serogroup or serotype.

The most common serotypes among the studied population were non-vaccinal serotypes (NVTs) 19A (15.8%), 3 (14.8%), and 6C (9.9%). The same serotypes were leading in the PCV10-vaccinated children. Other serotypes arousing interest were VT 19F (19.4%), NVTs 15A (6.0%), and 23A (4.0%). Among the ten non-vaccinated children we disclosed NVT serotypes 3 ($n = 4$), 6C ($n = 2$), 11 ($n = 1$), serogroup 13/28 ($n = 1$), and VT 18C ($n = 2$).

T a b l e 1

Antimicrobial non-susceptibility and resistance according to serotype in 202 non-invasive pediatric *S. pneumoniae* isolates recovered in PCV10 period 2016–2020

Antibiotics ^a	Total <i>n</i> (%) of isolates	Non-PCV10-Serotypes (<i>n</i>) isolates	Non-PCV10-Serotypes (%)	PCV10-Serotypes (<i>n</i>) isolates	PCV10-Serotypes (%)
PEN – I	84 (41.5)	6C(23), 15A(17), 23B(7), 19A(6), 6A(5), 3(4), 11(2), NT(2), 15B(1), 15C(2), 7B(1), 24(1), 35(1), 16/36/37(1)	73 (86.9)	9V(3), 19F(6), 23F(2)	11 (13.1)
PEN – R	30 (14.8)	19A(16), 15A(3), 6C(2), 15B(1), NT(1)	23 (76.7)	19F(6), 23F(1)	7 (23.3)
CRO – I	17 (8.4)	19A(9), 6C(2), 15A(1), 15B(1), NT(1)	14 (82.4)	19F(2), 23F(1)	3 (17.6)
CRO – R	13 (6.4)	19A(13)	13 (100)	–	–
ERY – NS	105 (52.0)	19A(23), 6C(16), 15A(9), 6A(9), 23A(7), 3(6), NT(4), 15C(3), 7B(1), 11(1), 14(2), 15B(1), 22F(1), 23A(1), 24(1)	84 (81.0)	19F(18), 6B(2)	20 (19.0)
CLI – NS	96 (47.5)	19A(24), 6C(15), 15A(9), 23A(8), 3(6), 6A(4), NT(2), 11(1), 14(1), 15B(1), 15C(2), 23B(1), 24(1)	75 (78.1)	19F(18), 6B(2), 23F(1)	21 (21.9)
TET – NS	91 (45.0)	19A(24), 6C(15), 15A(9), 3(6), 23A(4), NT(2), 15C(2), 6A(2), 14(1), 15B(2), 11(1), 18A(1), 23B(1), 24(1)	71 (78.0)	19F(17), 6B(2), 23F(1)	20 (22.0)
CHL – NS	27 (13.4)	6A(8), 3(6), 23A(5), 19A(3), 6C(1), 14(1)	24 (88.9)	6B(1), 19F(1), 23F(1)	3 (11.1)
SXT – NS	98 (48.5)	19A(25), 6A(10), 23A(8), 15A(4), 6C(4), 23B(4), NT(4), 14(2), 15B(3), 15C(3), 16/36/37(2), 3(1), 9N(1), 11(1)	72 (73.5)	19F(17), 23F(3), 9V(3), 6B(2), 18C(1)	26 (26.5)
MDR	108 (53.5)	19A(24), 6C(17), 15A(9), 23A(7), 6A(7), 3(6), NT(4), 6A(2), 14(2), 15B(2), 11(1), 15C(2), 23A(1), 24(1)	85 (81.0)	19F(19), 23F(2), 6B(2)	23 (19.0)

^aAccording to EUCAST breakpoints (2021), the criteria used for penicillin (benzylpenicillin) were ≤ 0.06 , 0.12–1 and > 2 , and for ceftriaxone were ≤ 0.5 , 1 and > 2 , for classification of susceptibility, intermediate resistance and resistance, respectively.

For the other antibiotics, intermediate and resistant isolates are combined (NS – Antimicrobial nonsusceptibility).

PEN – benzylpenicillin, CRO – ceftriaxone, ERY – erythromycin, CLI – clindamycin, TET – tetracycline, CHL – chloramphenicol, SXT – trimethoprim-sulfamethoxazole

MDR – Multidrug resistance. MDR was defined as resistance to three or more antimicrobials.

Antimicrobial non-susceptibility. Antimicrobial non-susceptibility to benzylpenicillin and ceftriaxone was revealed in 114 (56.3%) and 30(14.8%) isolates, respectively. The erythromycin- and clindamycin-resistant isolates were 52.0% and 47.5%. Resistant isolates to tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole were 45.0%, 13.4%, and 48.5%, respectively. More than half of the strains (53.5%) were MDR. The most common MDR combination among all resistant strains was: PEN, ERY, CLI, TET, SXT (38.8%).

The widespread serotypes among PEN-non-susceptible and resistant strains were 19A (20.4%), followed by 6C (15.7%) and 19F (14.8%). Serotypes 19A and 19F were responsible for high benzylpenicillin resistance ($\text{MIC} > 2 \text{ mg/L}$).

Serotypes 19A (22.9%), 19F (16.2%) and 6C (15.2%) prevailed also in the ERY-resistant strains. Serotypes 15A and 23A were frequent in MDR strains, as well.

Discussion. NIPD continues to emerge in the pediatric population [8,9]. We noted high prevalence of NVTs (80.2%). Except 19F (9.4%), all leading serotypes were NVTs: 19A (15.8%), 3 (14.8%), 6C (9.9%), 15A (6.0%), and 23A (4.0%). The serotype replacement is a consequence of new recombinant antigenic variants, which escape PCV10. Comparing with our earlier AOM study for pre-vaccine period 1994–2011, serotypes 3 and 19A showed remarkable increase among the same isolates ($p = 0.0001$ and $p = 0.03$, with a significant result at $p = 0.05$). Serotypes 6C and 15A were even not detected in the pre-vaccine era among these isolates. Serotypes 6A and 19F continue to expand in the post-vaccine era. ($p = 0.6647$ and $p = 0.6837$ at $p = 0.05$) [10].

More than half of the isolates were MDR. The spread of antimicrobial-resistant clones and antibiotic selection pressure resulted in MDR strains. High MDR prevalence was distinctive for serotypes 19A and 6C. Serotype 3 disclosed mainly susceptible isolates.

Conclusion. We observed a serious serotype replacement with NVTs in the vaccinated population. The levels of antibiotic resistance are high in our settings. MDR NVTs isolates expand in the post-vaccine era and lead to therapeutic implications in the treatment of the NIPD.

On-going surveillance is essential to monitor the shift of serotypes and the impact of the vaccine.

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