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Serotypes distribution and antibiotic susceptibility of Streptococcus pneumoniae strains: five-year surveillance results of post-PCV-13

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Abstract

Background Approximately 100 capsular serotypes of *S. pneumonia* have been identified according to the composition of their capsular polysaccharides, currently available vaccines do not cover many of these. Pneumococcal vaccination serotype coverage is essential for preventing noninvasive and invasive illnesses as well as asymptomatic carriage. We aimed to determine the serotype distribution and antimicrobial susceptibility pattern of pneumococcal clinical isolates in this study. We also analyzed the serotype coverage rates of PCV13, which is applied in the NIP, and PCV-15 and PCV20, which have been introduced recently.

Methods This study is a retrospective surveillance of pneumococcal infections including invasive pneumococcal isolates (IPIs) and non-invasive pneumococcal isolates (non-IPIs).

Results A total of 420 isolates from 356 different patients aged 0–89 years were enrolled in the study. A total of 420 pneumococcal isolates were serotyped and 26 different serotypes were detected. Serotype 19 F was the most prevalent serotype ($n=96$, 22.8%), followed by 6 A/B ($n=55$, 13.1%), 23 F ($n=49$, 11.6%), 3 ($n=22$, 5.2%) and 19 A ($n=16$, 3.8%).

Conclusions Surveillance studies of pneumococcal diseases are critical to investigating current serotype distributions, antibiotic resistance status, and frequency of IPD cases. Considering the increasing antibiotic resistance rates of *S. pneumoniae*, it is necessary to provide protective immunization by switching to more comprehensive PCV vaccines rather than treatment.

Clinical trial number Not applicable.

Keywords *Streptococcus pneumoniae*, Serotypes distribution, Antibiotic susceptibility

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Background

Streptococcus pneumoniae can cause noninvasive pneumococcal diseases (IPDs), such as otitis media, sinusitis and pneumonia, as well as more severe invasive pneumococcal diseases (IPDs), such as bacteremia, meningitis and empyema [1]. Approximately 100 capsular serotypes of *S. pneumoniae* have been identified according to the composition of their capsular polysaccharides; currently available vaccines do not cover many of these serotypes [2]. As estimated by the World Health Organization (WHO), approximately 80% of all IPDs are caused by 20 serotypes [3]. Currently, there is a 23-valent pneumococcal polysaccharide vaccine (PPV23) and a 13-valent pneumococcal conjugate vaccine (PCV13), and in the last few months, the 20-valent pneumococcal conjugate vaccine (PCV20) has become available in Turkey. Pneumococcal vaccination serotype coverage is essential for preventing noninvasive and invasive illnesses as well as asymptomatic carriage. In this respect, efforts to increase the number of serotypes contained in pneumococcal vaccines aim to reduce pneumococcal diseases caused by nonvaccine serotypes. All kinds of clinical and laboratory studies analyzing the coverage of current 15- and 20-valent pneumococcal vaccines together with previous pneumococcal vaccines are important and will help in vaccine application policies.

There are limited data about the distribution of pneumococcal serotypes and antibiotic susceptibility patterns for the current postvaccination period in the literature, which is a public health concern. We aimed to determine the serotype distributions and antimicrobial susceptibility patterns of pneumococcal clinical isolates in this study. We also analyzed the serotype coverage rates of PCV13, which is applied in the NIP (National Immunization Program), and PCV-15 and PCV20, which were introduced recently.

Methods

This study was performed at Marmara University Pendik Training and Research Hospital. The hospital opened in 2011 and started accepting patients in January 2011. This study is a retrospective surveillance of pneumococcal infections, including invasive pneumococcal isolates (IPIs) and noninvasive pneumococcal isolates (non-IPIs). This report presents data from 5 years after opening the hospital. Microbiology department records and microbiology laboratory stocks were used to identify the isolates. The clinical and demographic data of the patients were retrieved from the hospital's electronic database. This retrospective study design was reported to the Ethics Committee of Marmara University School of Medicine and was conducted with the approval of Marmara University Microbiology Department.

Isolation of pneumococcal strains

S. pneumoniae strains isolated via routine microbiological methods in the clinical microbiology laboratory between January 2011 and December 2015 were used. Unviable isolates and patients whose clinical information was insufficient were excluded. Pneumococcal infections can be classified into invasive and noninvasive, depending on whether the *S. pneumoniae* is detected in sterile or nonsterile body areas. Pneumococcal isolates (IPI and non-IPI) were evaluated in the study, and nasopharyngeal isolates for asymptomatic carriage were not included. IPI was defined as the detection of *S. pneumoniae* from a sterile sample such as blood, cerebrospinal fluid, pleural fluid, urine and joint fluid for this study. Non-IPI was defined as the isolation of the bacteria from areas other than sterile sites.

Multiplex PCR reactions for serotyping

Multiplex PCR procedures were performed according to the appropriate protocol, and the *S. pneumoniae* isolates were serotyped according to the Centers for Disease Control and Prevention (CDC) methods [4]. For the eight different multiplex PCR reactions, 40 primer pairs were used to detect the serotypes, as follows: 1, 2, 3, 4, 5, 6 A/B, 6 C, 7 C/7B/40, 7 F/7A, 8, 9 N/9L, 9 V/9A, 10 A, 10 F/10 C/33 C, 11 A/11D, 12 F, 13, 14, 15 A/15F, 15 C, 16 F, 17 F, 18 C, 19 A, 19 F, 20, 21, 22 F/22A, 23 A, 23B, 23 F, 24 F, 31, 33 F/33A/37, 34, 35 A/35 C/42, 35B, 35 F/47, 25 F/38 and 39. Another primer pair amplifying the cpsA locus found in all *S. pneumoniae* strains was used as an internal control for the PCR reactions [4].

Antibiotic susceptibility

The data were assessed via breakpoint standards, and the Clinical and Laboratory Standards Institute (CLSI) standards were used to interpret the MICs [5]. Susceptibility to non-β-lactam antibiotics was evaluated via the disk diffusion method, and the results were analyzed according to the CLSI criteria [5]. The following antimicrobial agents were used: penicillin ($n = 420$), ceftriaxone ($n = 420$), erythromycin ($n = 420$), trimethoprim-sulfamethoxazole (TMP-SMX) ($n = 420$), vancomycin ($n = 420$), tetracycline ($n = 398$), chloramphenicol ($n = 384$), rifampin ($n = 380$), clindamycin ($n = 374$), levofloxacin ($n = 368$) and moxifloxacin ($n = 263$).

Statistical analysis

The data were entered into Microsoft Office Excel (Microsoft Corp., USA) and analyzed via the SPSS for Windows (SPSS, Chicago, IL, USA) program.

Results

Patients and pneumococcal isolates

A total of 420 isolates from 356 different patients aged 0–89 years were included in the study. Among these isolates, 43 (10.2%) IPIs were obtained from 40 (11.2%) patients, and 377 (89.8%) non-IPIs were obtained from 316 (88.8%) patients (Table 1). The age range for the pediatric group was 0–18 years ($n = 200$, mean age = 8.72 ± 5.3 years), whereas the ages in the adult group ranged from 19 to 89 years ($n = 156$, mean age 53.86 ± 18.05 years). The overall mean age was 26.02 ± 24.9 years. The age distribution of the patients was 11.6% for 0–24 months, 5.9% for 25–59 months, 38.7% for 5–18 years, 30.9% for 19–65 years and 23% for >65 years. Among the isolates, 251 (59.8%) were collected from males, and 169 (40.2%) were collected from females. The number of isolates obtained from pediatric patients aged 0–18 years was 259 (61.7%), and the remaining 161 (38.3%) isolates were obtained from adult patients over 18 years of age. Among the total 259 isolates obtained from pediatric patients aged 0–18 years, 19 were IPIs, and 240 were non-IPIs. The isolation rates were 11.7% ($n = 49$), 5.5% ($n = 23$), 44.5% ($n = 187$), 26.9% ($n = 113$), and 11.4% ($n = 48$) in the following age groups: 0–24 months, 25–59 months, 5–18 years, 19–65 years and >65 years, respectively (Table 1).

The distribution of the IPIs by source was as follows: 20 (46.5%) isolates from blood, 12 (27.9%) isolates from cerebrospinal fluid, 5 (11.6%) isolates from pleural fluid, 4 (9.3%) isolates from sterile urine samples, and 2 (4.7%) isolates from articular fluid. For the non-IPIs, the distribution by source was as follows: 224 (59.4%) isolates from sputum, 60 (15.9%) isolates from endotracheal aspirates, 43 (11.4%) isolates from nasopharyngeal exudates, 41 (10.9%) isolates from bronchoalveolar lavage fluid, and 9 (2.4%) isolates from wound and abscess samples. There was no comorbidity in 110 (30.9%) patients. The most common comorbidity was asthma (17.7%), followed by bronchiectasis (12.9%), malignancy (7.9%), cystic fibrosis (7.6%), and immunodeficiency (6.8%) (Table 1).

Serotype distribution

A total of 420 pneumococcal isolates were serotyped, and 26 different serotypes were detected. Serotype 19 F was the most prevalent serotype ($n = 96$, 22.8%), followed by 6 A/B ($n = 55$, 13.1%), 23 F ($n = 49$, 11.6%), 3 ($n = 22$, 5.2%) and 19 A ($n = 16$, 3.8%). We also compared the serotypes by dividing them into IPIs and non-IPIs. For IPIs, the most common serotypes were 19 F ($n = 11$, 25.5%), 6 A/B ($n = 4$, 9.3%), 23 F ($n = 4$, 9.3%), 3 ($n = 2$, 4.6%) and 19 A ($n = 2$, 4.6%), accounting for more than 50.0% of all IPIs. Serotype 19 F was more common among IPIs and non-IPIs. The five most common serotypes were the same in the IPI and non-IPI groups. Eighty-two (19.5%) isolates were nontypable (Fig. 1). We analyzed the serotype distribution by year from 2011 to 2015. Serotype 19 F was more common in all years, and the rates of serotype 19 F were 72.2%, 32.7%, 20%, 25.3%, and 16.3% annually from 2011 to 2015. Among all serotypes, only serotype 19 A significantly decreased over time ($p = 0.001$). The rate changes by year for the other serotypes were not statistically significant ($p > 0.05$) (Table 2).

Coverage of pneumococcal vaccines

The first available 7-valent pneumococcal conjugate vaccine (PCV7) included serotypes 4, 6B, 9 V, 14, 18 C, 19 F, and 23 F. PCV10 included serotypes 1, 5, and 7 F in addition to all serotypes covered by PCV7, and PCV13 included serotypes 3, 6 A, and 19 A in addition to all serotypes covered by PCV10. PCV15 covers all serotypes covered by PCV13, as do serotypes 22 F and 33 F. PCV20 covered all serotypes covered by PCV13, as did serotypes 8, 10 A, 11 A, 12 F, 15B, 22 F, and 33 F. The 23-valent polysaccharide vaccine (PPV23) did not include 6 A but also included serotypes 2, 8, 9 N, 10 A, 11 A, 12 F, 15B, 17 F, 20, 22 F, and 33 F, which were added to PCV13. The serotype coverage rates of the PCV13, PCV15, PCV20, and PPV23 vaccines for the pediatric age group (≤18 years) were 60.2%, 62.2%, 66.4%, and 69.1%, respectively. In the entire study group, the rates were 59.3%, 61.2%, 66.4%, and 69.5%, respectively (Table 3).

Antibiotic susceptibility

The mean MIC values of penicillin and ceftriaxone for the *S. pneumoniae* strains were 1.28 ± 2.47 and 0.89 ± 2.22 µg/mL, respectively. The antibiotic susceptibilities of the isolates were determined on the basis of the CLSI criteria and are presented in Table 4. Among the IPIs, the nonsusceptible (NS = intermediate + resistance) rates were as follows: 67.4% to oral penicillin, 11.6% to parenteral penicillin (for nonmeningitis), 55.8% to parenteral penicillin (for meningitis), 20.9% to ceftriaxone (for nonmeningitis), 23.3% to ceftriaxone (for meningitis), 44.2% to erythromycin, 49.2% to TMP-SMX, 0% to vancomycin, 24.3% to clindamycin and 0% to moxifloxacin. The NS rates for non-IPIS patients were as follows: 70.8% for oral penicillin, 14.9% for parenteral penicillin (for nonmeningitis), 67.4% for parenteral penicillin (for meningitis), 17.5% for ceftriaxone (for nonmeningitis), 32.9% for ceftriaxone (for meningitis), 60.3% for erythromycin, 40.6% for TMP-SMX, 0.5% for vancomycin, 49.9% for clindamycin, 1.2% for levofloxacin and 2.2% for moxifloxacin (Table 4). The NS rates were similar for both groups.

The most common oral penicillin nonsusceptible (PNS) serotypes were 23 F, 19 F, and 6 A/B, and the rates of PNS for these serotypes were 96%, 88.5%, and 87.2%, respectively. The most common ceftriaxone nonsusceptible serotypes were 19 A (31.2%), 6 A/B (25.5%), and 19 F (25%).

Table 1 Characteristics of patients, and distribution of Pneumococcal isolates during the study period

	Invasive *n* (%)						**Non-invasive *n* (%)**					
	2011	**2012**	**2013**	**2014**	**2015**	**Total**	**2011**	**2012**	**2013**	**2014**	**2015**	**Total**
No of isolates	1 (0.2)	10 (2.4)	9 (2.1)	4 (1)	19 (4.5)	**43 (10.2)**	17 (4)	48 (11.4)	31 (7.4)	67 (16)	214 (51)	**377 (89.8)**
No of patients	1 (0.3)	10 (2.8)	8 (2.2)	4 (1.1)	17 (4.8)	**40 (11.2)**	14 (3.9)	40 (11.2)	26 (7.3)	57 (16)	179 (50.3)	**316 (88.8)**
Age groups of patient												
0–24 months	0 (0)	1 (0.3)	1 (0.3)	1 (0.3)	4 (1.1)	**7** (**2**)	1 (0.3)	1 (0.3)	1 (0.3)	7 (2)	24 (6.7)	**34 (9.6)**
25–59 months	0 (0)	0 (0)	1 (0.3)	0 (0)	1 (0.3)	**2 (0.6)**	1 (0.3)	1 (0.3)	3 (0.8)	0 (0)	14 (3.9)	**19 (5.3)**
5–18 years	1 (0.3)	2 (0.6)	1 (0.3)	2 (0.6)	3 (0.8)	**9 (2.5)**	9 (2.5)	18 (5)	12 (3.4)	23 (6.5)	67 (18.8)	**129 (36.2)**
19–65 years	0 (0)	4 (1.1)	5 (1.4)	1 (0.3)	4 (1.1)	**14 (3.9)**	2 (0.6)	16 (4.5)	8 (2.2)	23 (6.5)	47 (13.2)	**96 (27)**
>65 years	0 (0)	3 (0.8)	0 (0)	0 (0)	5 (1.4)	**8 (2.2)**	1 (0.3)	4 (1.1)	2 (0.6)	4 (1.1)	27 (7.6)	**38 (10.7)**
Comorbidities												
No comorbidity	0 (0)	2 (0.6)	5 (1.4)	2 (0.6)	7 (2)	**16 (4.5)**	4 (1.1)	5 (1.4)	5 (1.4)	20 (5.6)	60 (16.9)	**94 (26.4)**
Asthma	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**0** (**0**)	2 (0.6)	7 (2)	6 (1.7)	10 (2.8)	38 (10.6)	**63 (17.7)**
Bronchiectasis	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	**1 (0.3)**	2 (0.6)	8 (2.2)	6 (1.7)	5 (1.4)	24 (6.7)	**45 (12.6)**
Malignancy	0 (0)	3 (0.8)	1 (0.3)	1 (0.3)	2 (0.6)	**7** (**2**)	0 (0)	6 (1.7)	2 (0.6)	3 (0.8)	10 (2.8)	**21 (5.9)**
Cystic fibrosis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**0** (**0**)	1 (0.3)	3 (0.8)	2 (0.6)	3 (0.8)	18 (5.1)	**27 (7.6)**
Immunodeficiencies	0 (0)	2 (0.6)	0 (0)	0 (0)	0 (0)	**2 (0.6)**	0 (0)	3 (0.8)	4 (1.1)	8 (2.2)	7 (2)	**22 (6.2)**
Others	1 (0.3)	2 (0.6)	2 (0.6)	1 (0.3)	8 (2.2)	**14 (3.9)**	5 (1.4)	8 (2.2)	1 (0.3)	8 (2.2)	22 (6.3)	**44 (12.4)**

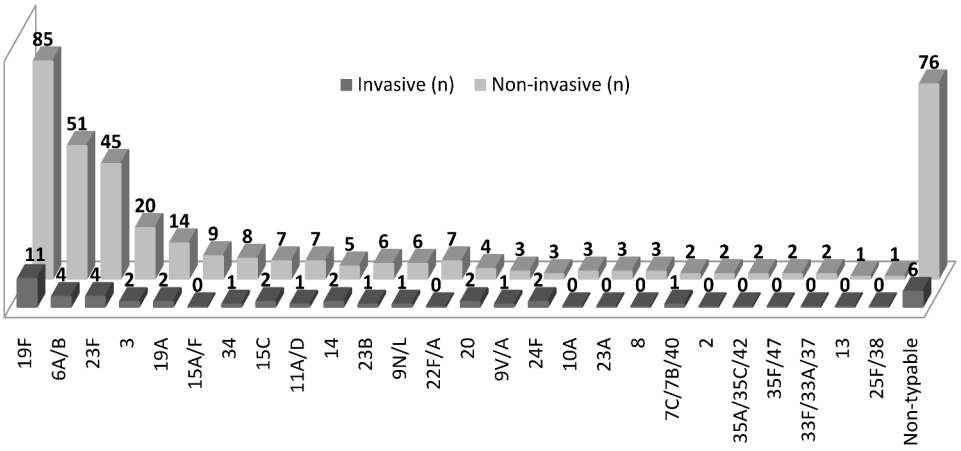

Fig. 1 The number of invasive pneumococcal isolates (IPIs), and non-invasive pneumococcal isolates (non-IPIs)

Table 2 Serotype distribution of *Streptococcus pneumoniae* isolates during the study period

	2011 *n* (%)	2012 *n* (%)	2013 *n* (%)	2014 *n* (%)	2015 *n* (%)	*p* value
Total number of isolates	18 (4.3)	58 (13.8)	40 (9.5)	71 (16.9)	233 (55.5)	
Serotype 19 F	13 (72.2)	19 (32.7)	8 (20)	18 (25.3)	38 (16.3)	< 0.001
Serotype 6 A/B	3 (16.6)	10 (17.2)	7 (17.5)	7 (9.9)	28 (12)	0.618
Serotype 23 F	1 (5.5)	9 (15.5)	3 (7.5)	12 (16.9)	24 (10.3)	0.346
Serotype 3	0 (0)	0 (0)	1 (2.5)	6 (8.4)	15 (6.4)	0.138
Serotype 19 A	0 (0)	1 (1.7)	1 (2.5)	3 (4.2)	11 (4.7)	0.708
Non-typable	1 (5.5)	10 (17.2)	7 (17.5)	10 (14)	54 (23.1)	0.211

Table 3 Coverage of Pneumococcal vaccines according to children and adult age groups

	≤ 18 years *n* (%)	> 18 years *n* (%)	Total *n* (%)
Invasive pneumococcal isolates (n19 + 24 = 43)			
PCV13	12 (63.2)	14 (58.3)	26 (60.5)
PCV15	12 (63.2)	14 (58.3)	26 (60.5)
PCV20	14 (73.7)	15 (62.5)	29 (67.4)
PPV23	15 (78.9)	17 (70.8)	32 (74.4)
Non-invasive pneumococcal isolates (n 240 + 137 = 377)			
PCV13	144 (60)	79 (57.7)	223 (59.1)
PCV15	149 (62.1)	82 (59.9)	231 (61.3)
PCV20	158 (65.8)	92 (67.2)	250 (66.3)
PPV23	164 (68.3)	96 (70.1)	260 (68.9)
All pneumococcal isolates (n 259 + 161 = 420)			
PCV13	156 (60.2)	93 (57.8)	249 (59.3)
PCV15	161 (62.2)	96 (59.6)	257 (61.2)
PCV20	172 (66.4)	107 (66.5)	279 (66.4)
PPV23	179 (69.1)	113 (70.2)	292 (69.5)

Discussion

In developing nations such as Turkey, insufficient data are available concerning the serotype distribution, antibiotic resistance, and vaccine coverage rates. In the case of pneumococcal illness, serotype surveillance is crucial. Identifying the most common disease-causing serotypes provides important data to health policymakers to select the best vaccine for local immunization campaigns and guidelines. Variations in the distribution of serotypes may differ depending on the nation, region, PCV being implemented, and vaccination policies. We found that the most common vaccine type (VT) serotypes in the IPD and non-IPD groups were 19 F (22.8%), 6 A/B (13%), 23 F (11.6%), 3 (5.2%), and 19 A (3.8%). The most common nonvaccine type (NVT) serotypes were 15 A/F (2.1%), 34 (2.1%), 15 C (2.1%), 23B (1.6%), and 24 F (1.1%). The rates of other less common serotypes were similar in the IPD and non-IPD groups. Soysal et al. reported that the most commonly isolated VT serotypes were 6 A/B/C (16.5%), 19 F (12.9%) and 23 F (10%); the nonvaccine serotypes accounted for 45% of the isolated strains; and the most common NVT serotypes were serotypes 15 A/F (5%), 22 A/F (4.3%), 12 F (3.5%) and 35B (3.5%) [6]. Ozdemir et al. reported that 19 F (11.6%), 23 F (7.8%) and 6B (7%) were the most common isolated VT serotypes; the most common NVT serotypes were 15/A/B/C/F (8.2%), 23 A (1.6%), 10 A/B (1.6%), 35 F/A/B/C (1.6%) and 6 C (1.6%)

Table 4 The antibiotic susceptibilities of the *Streptococcus pneumoniae* isolates

		Invasive *n* (%)				Non-invasive *n* (%)			
Antimicrobial Agents		**S**	**I**	***R***	**Total**	**S**	**I**	***R***	**Total**
Penicillin	Oral	14 (32.6)	22 (51.2)	7 (16.2)	43 (100)	110 (29.2)	183 (48.5)	84 (22.3)	377 (100)
	Parenteral (for non-meningitis)	38 (88.4)	5 (11.6)	0 (0)	43 (100)	321 (85.1)	41 (10.9)	15 (4)	377 (100)
	Parenteral (for meningitis)	19 (44.2)	0 (0)	24 (55.8)	43 (100)	123 (32.6)	0 (0)	254 (67.4)	377 (100)
Ceftriaxone	Parenteral (for non-meningitis)	34 (79.1)	7 (16.2)	2 (4.7)	43 (100)	311 (82.5)	47 (12.5)	19 (5)	377 (100)
	Parenteral (for meningitis)	33 (76.7)	4 (9.3)	6 (14)	43 (100)	253 (67.1)	79 (21)	45 (11.9)	377 (100)
Erythromycin		24 (55.8)	1 (2.3)	18 (41.9)	43 (100)	149 (39.5)	12 (3.2)	216 (57.3)	377 (100)
TMP-SMX		24 (55.8)	4 (9.3)	15 (34.9)	43 (100)	224 (59.4)	29 (7.7)	124 (32.9)	377 (100)
Vancomycin		43 (100)	0 (0)	0 (0)	43 (100)	375 (99.5)	0 (0)	2 (0.5)	377 (100)
Tetracycline		20 (48.8)	7 (17.1)	14 (34.1)	41 (100)	113 (31.7)	44 (12.3)	200 (56)	357 (100)
Chloramphenicol		38 (90.5)	0 (0)	4 (9.5)	42 (100)	308 (90.1)	0 (0)	34 (9.9)	342 (100)
Rifampin		41 (97.6)	0 (0)	1 (2.4)	42 (100)	335 (99.1)	0 (0)	3 (0.9)	338 (100)
Clindamycin		28 (75.7)	0 (0)	9 (24.3)	37 (100)	196 (58.2)	3 (0.9)	138 (40.9)	337 (100)
Levofloxacin		43 (100)	0 (0)	0 (0)	43 (100)	321 (98.8)	1 (0.3)	3 (0.9)	325 (100)
Moxifloxacin		31 (100)	0 (0)	0 (0)	31 (100)	227 (97.8)	0 (0)	5 (2.2)	232 (100)

S: sensitive, I: intermediate; R: resistance; TMP-SMX: Trimethoprim-sulfamethoxazole

[7]. Although the VT serotypes were similar, there were differences in NVT serotype distributions between these studies. In particular, in the manuscript by Liyanapathirana et al. and other studies, serotype 15 was highlighted, and it was reported that it might become the dominant NVT serotype in the near future. Consistent with these estimates, in our study, the most common NVT serotype was found to be serotype 15 [7–9].

This study may be important for our country and other developing countries, as it provides information on the changing patterns of pneumococcal rates, serotype distributions, risk factors, antimicrobial susceptibilities, and resistance statuses according to the most common serotypes 13 years after the implementation of PCV13 in the NIP and this year when the PCV20 vaccine was available. This study, which includes 5-year pneumococcal serotype data collected immediately after the routine implementation of PCV13 within the scope of the NIP in Turkey in 2011, will also contribute to the evaluation of the NIP. In four studies conducted during the prevaccination period in Turkey, the PCV7 coverage rates were determined to be 30%, 16%, 55%, and 51% [10–13]. In two studies conducted during the postvaccination period, the PCV-7 coverage rates were found to be 46.2% and 51.4% [6, 7]. In our study, the PCV7 coverage rate was 50%, which was consistent with the rates reported in studies conducted during the postvaccination period.

We detected PCV13 serotypes in 63.2% of sterile samples from children with IPD younger than 18 years and in 58.3% of sterile samples from adult patients older than 18 years. When PCV13 coverage was assessed in nonsterile samples from non-IPD patients, 60% of samples from pediatric patients under 18 years of age and 58.3% of samples from adult patients aged 18 years and over contained PCV13 serotypes. Among all the isolates, the PCV13 coverage in the IPD and non-IPD groups was 60.5 and 59.1, respectively, and there was no significant difference in PCV13 coverage between the two groups.

PPV23 contains all serotypes except 6 A, which is covered by PCVs; however, it is unable to produce immunological memory or reduce asymptomatic carriage in children. For these reasons, it is not preferred or ineffective in children under 2 years of age [14]. Recently, introduced novel PCVs have wider serotype coverage than PCV13 does and provide more promising data with improved results. In early 2022, two novel conjugate pneumococcal vaccines, 20-valent PCV (PCV20, Apexxnar) and 15-valent PCV (PCV15, Vaxneuvance), were approved for adult use. In March 2024, PCV20 (Prevnar 20, previously Apexxnar) was licensed for infants, children, and adolescents from 6 weeks to less than 18 years of age [15]. PCV15 includes serotypes 22 F and 33 F, and PCV20 includes 8, 10 A, 11 A, 12 F, 15B, 22 F and 33 F, in addition to the PCV13 vaccine serotypes [16, 17]. Our results revealed that in invasive pneumococcal isolates, PCV13 and PCV15 coverage was the same across all age groups. In the noninvasive group, PCV15 coverage was found to be approximately 2% greater. The coverage of PCV20 was found to be 4.2–10.5% greater than that of PCV13 in all the study groups. Notably, the highest coverage difference (10.5%) was more pronounced in the invasive pneumococcal isolate group in children under 18 years of age. In a recent large-scale study conducted with 410 pneumococcal isolates collected from 21

centers, the coverage of PCV20 was reported to be significantly greater than that of PCV13 in all study groups, which is consistent with our results [18]. It has also been reported in some studies conducted in different countries that PCV20 has greater coverage than PCV13 and PCV15 [19, 20]. This study revealed that PCV13 covered 59.3%, PCV15 covered 61.2%, and PCV20 covered 66.4% of all isolates identified from the specimens in the entire study population. The coverage for PPV23 was similar for the child and adult age groups and was approximately 69.5%. PCV20 and PPSV23 covered approximately two-thirds of the isolated serotypes.

The European Regional Office of the World Health Organization reported that the total antibiotic usage rate in Turkey was 42.3% according to the defined daily dose, and this incidence was the highest among European countries [21]. The effects of the PCV vaccination program on antibiotic resistance are controversial. While some clinical studies have not detected an effect of PCV vaccine administration on resistance, other studies have reported a decrease in antibiotic resistance [22, 23]. There are few studies on the antibiotic susceptibility of pneumococci before PCV was introduced into the vaccination schedule. In studies conducted during the prevaccination period, the percentage of penicillin-nonsusceptible isolates (PNS, intermediate, and resistant) was found to be 10.5% by Bayraktar et al., 12.9% by Aslan et al., and 39.3% by Uzuner et al. [10, 12, 24]. Özdemir et al. reported that the percentage of PNS isolates was 73% (intermediate, 50.6%, and resistant, 22.4%) in the early post-PCV7 period; Soysal et al. reported that the percentage of PNS isolates was 60% (intermediate, 42%, and resistant, 18%) in the early post-PCV13 period [6, 7]. In our study, the percentage of NSP isolates in the IPI serotypes was 67.4% (intermediate, 51.2%, and resistant, 16.2%), and the percentage of NSP isolates in the non-IPI serotypes was 70.8% (intermediate, 48.5%, and resistant, 22.3%). We reported the percentage of NSP isolates as 70.4% (intermediate, 48.8%, and resistant, 21.6%) among all serotypes in the study group. The PNS rates reported in this study were similar to the rates reported in studies conducted post-PCV and were significantly higher than the rates reported in studies conducted pre-PCV. The third most important observation of our study was the significant increase in macrolide and clindamycin resistance rates. Four studies reported macrolide resistance in the pre-PCV period. Uzuner et al. reported clindamycin and erythromycin resistance rates of 9.8% and 16.1%, respectively [24]. Torun et al. reported clindamycin and erythromycin resistance rates of 45% and 42%, respectively, and reported relatively high resistance rates [25]. Bayer et al. reported that the degree of macrolide resistance was 4% [26]. During the post-PCV period, Soysal et al. reported that the rates of erythromycin and clindamycin resistance were 43% and 31%, respectively [6]. The erythromycin and clindamycin resistance rates in the present study were 55.7% (41.9% in the IPI group and 57.3% in the non-IPI group) and 39.3% (24.3% in the IPI group and 40.9% in the non-IPI group), respectively.

This study had several limitations. The number of isolates obtained from clinical samples at a single center was limited and may not reveal the actual serotype distribution in the population. We compared our results with those of pre-PCV period studies conducted at various geographic locations within our nation because we lacked a pre-PCV period cohort to compare our study population with. Therefore, we cannot completely rule out the possibility that changes in particular geographic areas, rather than vaccine usage, were the source of the observed changes in the serotype distributions. The study was passive surveillance; more detailed epidemiological data can be obtained with prospective active surveillance. Finally, only 5 years of data were available for analysis following the implementation of PCV13; our study also included children vaccinated with both PCV7 and PCV13; therefore, we cannot exclude effects of the PCV vaccines and changes unrelated to the vaccines. Despite these limitations, this study provides the most up-to-date and extensive data revealing serotype distribution and antibiotic resistance in Turkey for academics, vaccine companies, and policymakers.

Conclusion

This surveillance study, which included up-to-date data on *S. pneumoniae* isolates after PCV13 immunization, analyzed the coverage of PCV13, PCV15 and PCV20. It was observed that PCV20 covered approximately two-thirds of the pneumococcal serotypes isolated. At the same time, antibiotic resistance rates were also found to be high in the study. Considering the increasing antibiotic resistance rates of *S. pneumoniae*, it is necessary to provide protective immunization by switching to more comprehensive PCV vaccines rather than treatment. More comprehensive studies with serotype analyzes and cost-effectiveness about *S. pneumoniae* and pneumococcal vaccines are needed to update immunization schedules.

Abbreviations

IPIs	Invasive pneumococcal isolates
non-IPIs	Noninvasive pneumococcal isolates
PCV13	13-Valent pneumococcal conjugate vaccine
PPV23	23-Valent pneumococcal polysaccharide vaccine
VT	Vaccine type
NVT	Nonvaccine type

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Author contributions
All authors have approved the submitted manuscript. SA, DG, EK, GS, AS contributed to the study conception and design. All programming and analyses were conducted by SA and DG. SA, EK, AS contributed to the acquisition and interpretation of the study results. SA and AS drafted the manuscript, with revisions made by SA, DG, EK, GS, and AS.

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Data availability
The datasets analyzed in this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This retrospective study design was conducted with the approval of Marmara University Microbiology Department. Since the study included laboratory data and had a retrospective design, we were unable to obtain informed consent from study participants. However, a letter of support was obtained from the microbiology medical director's office, and full anonymity was granted for the patient profile and patient data. Because this anonymized record-based data was used, the need for informed consent was waived. The study was conducted according to the ethical principles stated in the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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