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Molecular Characterization of *Streptococcus Pneumoniae* from Patients Diagnosed with Pneumonia: Recommendation for Vaccination Program

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Abstract

Background: Infections caused by *Streptococcus pneumoniae* (*S. pneumoniae*) have remained a significant public health concern worldwide. In developed countries, the highest prevalence of *S. pneumonia* has been reported among the elderly. The aim of this study was to evaluate the coverage of genotypes in the 13-valent pneumococcal conjugate vaccine (PCV-13) in the Iranian elderly population.

Methods: A total of 41 isolates of *S. pneumoniae* were collected in the current retrospective cross-sectional study. The samples comprised 33 inpatients hospitalized for pneumococcal pneumonia and 8 outpatients. Multiplex polymerase chain reaction assay was performed to categorize the bacteria isolated into specific genotypes. Statistical analyses were performed using SPSS software, and the chi-square test was used to assess the statistical significance in percentages.

Results: A total of 68 genotypes were identified in this study, in which 39 isolates (57.3%) were associated with invasive infections. The most common genotypes were 6A/B [8 (19.5%)], 1 [7 (17.5%)], 14 [5 (12.2%)], and 19A [4 (9.75%)], respectively. The coverage rates of PCV-7, PCV-10, and PCV-13 vaccines were 51.17%, 70.7%, and 99.9%, respectively. According to our results, the pneumococcal coverage rate of PCV-7, PCV-10, and PCV-13 vaccine types is estimated to be 51.2%, 70.7%, and 99.9%, respectively. Furthermore, the trend of pneumococcal serotypes included in the PCV-13 was steadily increasing during the study period.

Conclusion: It can be concluded that the most circulating pneumococcal serotypes were in accordance with specific serotypes included in the PCV-13 vaccine types. Therefore, including PCV-13 vaccines in immunization programs against pneumococcus in the elderly can effectively reduce the rate of infections.

Keywords: Pneumococcal Vaccine, Immunization, Elderly, Genotype

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Introduction

Infections caused by *Streptococcus pneumoniae* (*S. pneumoniae*) have remained a significant public health

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↑What is "already known" in this topic:
PCV-13 could cover the majority of the invasive pneumococcal isolates. To decrease the prevalence of pneumococcal illness and pneumococcal-resistant strains in Tehran, it is advised that all babies receive the PCV-13 vaccine.

→What this article adds:
According to this study, the serotypes of PCV-13 were the most common circulating serotypes in the elderly population of Tehran. Therefore, the use of this vaccine can be highly effective in immunization programs for the elderly and adults.

concern worldwide. According to the World Health Organization, these infections claim more than 1.6 million lives annually worldwide (1). Susceptibility to *S. pneumoniae* is age-dependent, with the highest prevalence, mortality, and complication rates reported among children and the elderly (>65 years) (2). In developed countries, the highest prevalence of *S. pneumonia* has been reported among the elderly (3). Invasive pneumococcal diseases (IPDs), such as septicemia, meningitis, and pneumonia are known to have high morbidity and mortality rates worldwide. They can be categorized into at least 100 serotypes based on their capsular polysaccharides (2, 3). Protection from severe and perhaps deadly pneumococcal infections is provided by the pneumococcal vaccination. The PCV-13 contains strains 1, 3, 4, 5,6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, and the PCV-23 includes 1, 2, 3, 4, 5, 6B, 7F, 8, 9V, 9N, 10A, 11A, 12F, 14A, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F strains (4).

The elderly account for an increasingly larger percentage of the human population in Iran, which is in accordance with the increasing trend of the world's aging population. Given the increased risk of pneumococcal disease with advancing age, besides the increased cost of treatment and increased antibiotic resistance, pneumococcal vaccination has become a public health priority. Therefore, it is essential to identify common strains in individuals over the age of 65 and implement appropriate vaccination strategies. This study was carried out among our country's older population for the first time. We demonstrated that this population can be vaccinated with the PCV-13 vaccine.

Methods

Study Design

This retrospective cross-sectional study was conducted among inpatient and outpatient individuals hospitalized with pneumonia in 2 tertiary hospitals (Rasoul Akram and Milad hospitals) during 2017 and 2019. The diagnosis of *pneumonia* was often clinical and based on symptoms and signs combined with laboratory findings. The patient's demographic data, including age and underlying disease, were collected from medical records. Informed consent form was obtained from all participants.

The isolated bacteria that met the inclusion criteria of the present study were outsourced for further laboratory analysis to the Pediatric Infectious Diseases Research Center of Iran University of Medical Sciences. A total of 80 *S. pneumonia* isolates were collected from 2 medical centers and stored at -80°C. Out of 80 isolates, 39 were not revived due to fluctuation of freezer temperature, whereas 41 samples could be subcultured and cryostored for the study. For the molecular characterization of the pneumococcal isolates, the selected samples were revived on chocolate agar. A multiplex polymerase chain reaction (PCR) assay was implemented to differentiate the isolates into genotypes (4). The Ethics Committee of Iran University of Medical Sciences gave its approval to this project (IR.NIMAD.REC.1395.077).

DNA Extraction and Multiplex PCR Assay

Genomic DNA was extracted from the samples using a SinaClon kit (SinaClon). Next, a multiplex PCR assay was performed, as described earlier (4).

The serotypes were categorized into PCV-13 and non-PCV-13 serotypes. Using SPSS Version 18, the *t*-test was used to analyze the data. $P < 0.05$ was regarded as statistically significant.

Culture

The *S. pneumoniae* strains were subcultured on enriched chocolate agar plates. The plates were incubated overnight at 37 °C with 5% CO_2 (4).

DNA Extraction

PCR and a culture test were used to determine if *S. pneumoniae* was present in the samples.

Statistical Analyses

Statistical analyses were performed using SPSS software, Version 18.0 for Windows (SPSS Inc), and $P < 0.05$ were considered statistically significant. The chi-square test was used to assess the statistical significance of percentages.

Results

A total of 41 isolates were analyzed in the present study. The following samples were collected from the patients: blood samples (46.3%; n = 19), bronchoalveolar lavage (BAL) samples (19.5%; n = 8), trachea tube samples (19.5%; n = 8), pleural fluid samples (4.8%; n = 2), ascites fluid samples (2.4%; n = 1), brain abscess samples (2.4%; n = 1), cerebrospinal fluid (CSF) (2.4%; n = 1), and urine samples (2.4%; n = 1).

Pneumococcal strains isolated from the blood, CSF, pleural fluid, ascites fluid, and cerebral abscess samples were considered IPD, while the rest were considered non-invasive. The youngest and oldest patients were 16 and 92 years old, respectively. The patients were classified into 7 age groups, with an age difference of 10 years (Table 1); the majority of the participants were 71 to 81 years old ([n = 13] 32%).

Most of the identified genotypes were detected in the age group of 49-59 years; this age group accounted for 19.5% of pneumococcal cases. A total of 68 genotypes were identified by multiplex PCR; however, 4 could not be identified, and 57.3% (n = 39) of the genotypes belonged to the invasive category (Table 2). Based on our findings, 51.17%, 70.7%, and 99.95% of isolates were compatible with genotypes included in the PCV-7, PCV-10, and PCV-13 vaccines, respectively. The most common genotypes were 6A/B (n = 8 [19.5%]), 1 (n = 7 [17.5%]), 14 (n = 5 [12.2%]), and 19A (n-4 [9.75%]), respectively (Fig. 1). It can be deduced that PCV-13 could be considered the best choice for a vaccine program (Fig. 2).

In this study, 57.3% (39/68) of genotypes isolated from the blood (n = 31), CSF (n = 4), and peritoneal (n = 4) were considered to be invasive. IPD, such as septicemia 87.5% (35/40), meningitis 10% (4/40), and 39.7% (27/68) genotypes from pneumonia were identified. Pneumonia

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Table 1. Information of patients with different types of genotypes identified among isolated pneumococcal strains

Age (Year)	Prevalence N=41 (%)	Different types of genotypes	Genotypes identified n=64
16-26	3 (7.3%)	1 ,6A (2*),6B (2*), 23F, 31, 35B	8
27-37	2 (4.8%)	12F,15B,19F, 35B,	4
38-48	4 (9.7%)	6A,6B, 19F,19A, Cps	5
49-59	8 (19.5%)	1(3*), 6A,6B, 9V,12F, ,23F, ,34(2*),35F, 15B (2*), cps,17F	15
60-70	7 (17%)	1, 6A (3*),6B (3*),14(2), 19A 3*), 35B (2*)	14
71-81	13 (32%)	1, 14(3*), 19F, 7F,9V, ,23F, cps (4*),12F	13
82-92	4 (9.7%)	1,6A,6B,10A, cps	5

* Numbers in parentheses indicate the relative frequency of the genotype.

Table 2. Coverage of 13-valent vaccine genotypes identified in IPD

Types of vaccines	IPD (n=39)	Non-IPD (n=29)	*P* value
PCV-13 (n=41)	24 (61.5%)	17 (58.6%)	0.253
Non- PCV-13 (n=27)	15 (38.5%)	12 (41.4%)	

specimens included BAL 44.4% (12/27) and trachea 55.6% (15/27). The distribution of genotypes is shown separately in Figure 1.

Genotype 1 was observed in all age groups, with the highest prevalence reported in the age group of >50 years, particularly the age group of 49-59 years. The next most common genotype was 6A/B, which was the most common genotype in the age group of >50 years, more resembling genotype 1. Genotype 14 was solely detected in the age group of >50 years. Genotype 10A was only found in the age group of 82-92 years. In contrast, genotype 31 was only observed in young adults (16-26 years).

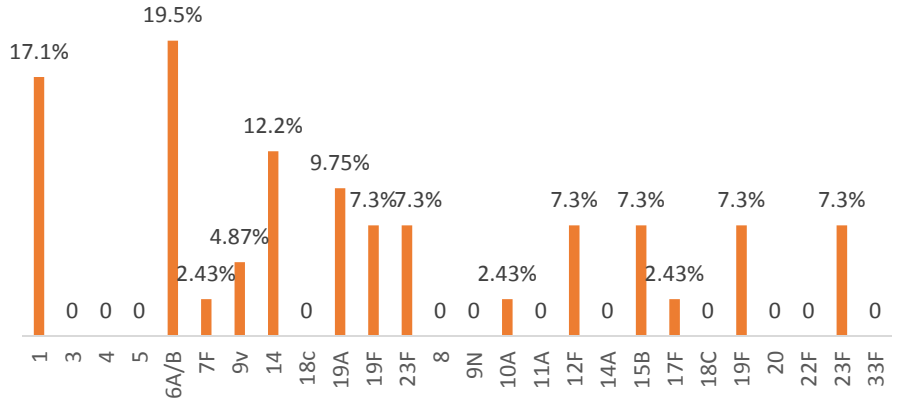

Fig. 1. Prevalence of genotypes among Streptococcus pneumoniae strains isolated from patients in this study

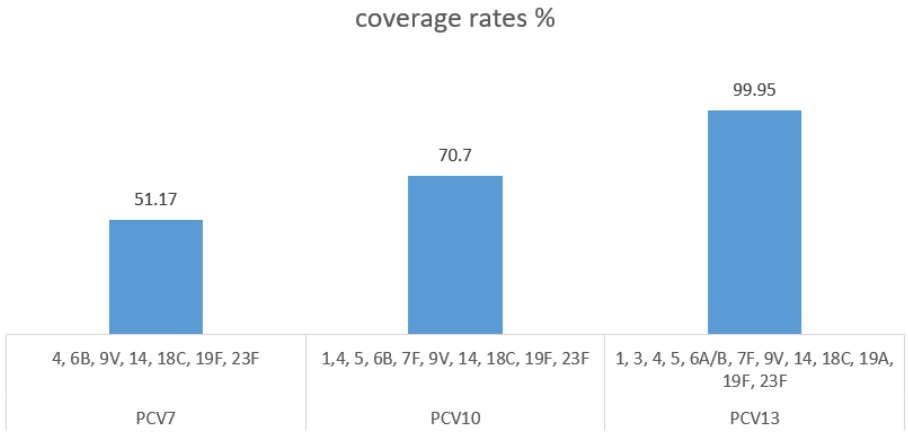

Fig. 2. Vaccination coverage of PCV-7, PCV-10 and PCV-13 in the individuals in this study

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Discussion

In the present study, the mean age of the patients with community-acquired pneumonia was 61.39 years, similar to a survey by Feizabadi et al (5) in Tehran, Iran. On the other hand, in a study by Vila-Corcol et al (6) in Turkey, the mean age of the patients was 13.53 years. Almost all patients studied in Turkey were at risk of infections (96.9%). Pneumonia is the most common presentation of pneumococcal infections in adults, and pneumonia and septicemia account for approximately 80% to 90% of all IPDs (7). According to our findings, 78% (n = 32) of the patients above 50 years were exposed to a high risk of IPDs. Pneumonia and septicemia were considerably more prevalent among older patients than their younger counterparts (22% in the age group of 16-49 years).

According to research by Habibi et al (8), the most prevalent serotypes were 23F (17.5%), 14 (16.3%), 19F (16.3%), 3 (12.5%), and 19A (12.5%), while PCV-7, PCV-10, and PCV-13 vaccination coverage rates were 52.6%, 52.6%, and 83.7%, respectively. On the other hand, in the present study, the coverage rates of these vaccines were 51.17%, 70.7%, and 99.95%, respectively. Generally, genotype 19A is recognized as a drug-resistant and virulent genotype, the prevalence of which increased following PCV-7 vaccination in Massachusetts, USA (9). This genotype was also observed in the present study, and its prevalence was 3 times higher in the age group of >50 years compared with younger age groups. Habibi et al (8) reported the 19A genotype as a specific genotype in IPD patients (19%). The prevalence of this genotype was similar to the rates reported in several other countries before the introduction of pneumococcal vaccination. In the present study, a prevalence of 9.75% was reported for this genotype.

In a study by Feizabadi et al, similar to other studies conducted in Asia (10, 11), most isolates were identified by the multiplex PCR method, where 6A/B, 19A, 19F, and 23F were the dominant capsule types, respectively. In the present study, 6A/B, 1, 14, and 19A genotypes were predominant, respectively. Genotypes 6A/B and 19A were detected in both studies using PCV-13. Moreover, Vladimir Tatochenko et al (12) found 19F, 14, 6B, and 23F to be the most common genotypes in Russia. Serotype 19A was common in community-acquired pneumonia, middle ear infection, nasopharyngeal infection, and antibiotic-resistant isolates in all age groups.

In this study, 6A/B, 1, 14, and 19A serotypes were the most common pneumococcal serotypes in Iran, respectively. The widespread use of PCVs can significantly reduce the incidence of pneumococcal diseases. It is well-established that 19F and 14 serotypes are the most common (7). High antibiotic resistance has been reported in some serotypes, such as 23F, 19A, 19F, and 14 (13). These serotypes, which can be found in PCV-13, were observed in the present study. The distribution of serotypes, clones, and antimicrobial resistance patterns of *S. pneumonia* varies depending on age and geographic region; therefore, vaccine modifications based on the prevalent serotypes are essential. There were some limitations to the study. Freezer failure during the study caused us to lose a large number of strains. Incomplete information on hospitalized patients led us to exclude some strains from the study.

Conclusion

According to the present findings, the serotypes included in the PCV-13 vaccine were the most common circulating serotypes in the study population. Therefore, this vaccine type could be highly effective in immunization programs for the elderly and adults. Besides, the identification of the most common pneumococcal serotypes circulating in the community, analytical evaluation of the effectiveness of pneumococcal vaccines, and finally, assessment of the efficacy of PCV-10, PCV-7, and PCV-13 vaccines can help us examine the protective effects of PCV-13 accurately. The immunization of the elderly is strongly advised due to the high cost of vaccination against IPDs compared with antibiotic therapy options and the growing incidence of IPDs worldwide.

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Authors' Contributions

Project administration: A.E.; Conceptualization: A.E. and S.S.; Formal analysis: A.N. and A.B.; Writing-original draft: A.B. and A.T.; Methodology and investigation: A.B., Z.F.A., S.S., K.K., A.T., M.F., M.N., and M.R.; Data interpretation: A.E.

Ethical Approval

This study was approved by the Ethics Committee of Iran University of Medical Sciences (IR.NIMAD.REC.1395.077).

Conflict of Interests

The authors declare that they have no competing interests.

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