Original Article

Ten-year surveillance of invasive *Streptococcus pneumoniae* isolates in central Turkey prior to the introduction of a conjugate vaccine

Duygu Percin[1], Yasemin Ay Altintop[1], Bulent Sumerkan[1]

[1]Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Erciyes University, 38039-Kayseri/Turkey

Abstract

Introduction: The aim of this study was to characterize the serotypes and antimicrobial susceptibility patterns of invasive *Streptococcus pneumoniae* isolates in central Turkey.

Methodology: A total of 332 invasive *S. pneumoniae* isolates were identified, serotyped and tested for antimicrobial susceptibility by routine microbiological methods.

Results: The most common serogroups/serotypes were 1, 19, 3, 18, 6, 14, and 7 in rank order. Serogroup/serotype coverage of the 23-valent polysaccharide vaccine, and the 7-, 10-, and 13-valent conjugate vaccines were 96%, 44%, 78.6%, 96.4%, respectively. Overall, 20 (6%) of the isolates were resistant to penicillin, 1 (0.3%) to cefotaxime, 20 (6%) to erythromycin, 13 (4%) to cloramphenicol, and 120 (36%) to trimethoprim-sulfamethoxasole. Among cerebrospinal fluid (CSF) isolates, 20 (18.5%) were resistant to penicillin (26.3% and 11.5%, respectively, of child and adult meningitis cases; $p \geq 0.05$).

Conclusions: Although the seven-valent conjugate vaccine is expected to protect less than half of children younger than three years of age, of the incorporation of this vaccine into the routine immunization program of Turkey is advised to continue. However, the 13-valent conjugate vaccine, including serotypes 1, 3, 5, and 7, has the most potential prevent the highest burden of invasive pneumococcal diseases in this age group.

Key words: *Streptococcus pneumoniae*, vaccine, serotyping, antimicrobial susceptibility

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Introduction

Streptococcus pneumoniae is one of the most important causes of invasive bacterial infections, including sepsis and meningitis. Despite the availability of potent antimicrobial therapies and vaccines for pneumococci, infections result in a high mortality rate among several different age groups [1]. In the central region of Turkey, *S. pneumoniae* is the most frequently isolated bacteria in community-acquired bloodstream infections and the rate of death attributable to pneumococcal sepsis approaches 50% in adults [2]. The majority of invasive pneumococcal diseases are linked to a limited number of serotypes, the prevalence of which varies from country to country. In Turkey, serogroups/serotypes 1, 6, 19, and 23 cause the majority of cases of invasive infections [3]. Similarly, among the penicillin-resistant *S. pneumoniae* isolates in Turkey (rates of intermediate resistance and resistance to penicillin are 29% and 3%, respectively) the most prevalent serogroups/serotypes are 6, 14, 19, 23 [3-6].

A 7-valent conjugate vaccine including serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, which was licensed in Europe in 2001, was introduced to Turkey in 2007 and added to the National Immunization Program by the Ministry of Health of Turkey in 2008. The aim of this study was to identify serotypes and to determine the antimicrobial susceptibilities of invasive *S. pneumoniae* isolates over a time period prior to the introduction of the 7-valent conjugate pneumococcal vaccine. The data allowed us to assess the potential impact of different conjugate vaccines in preventing invasive pneumococcal infections among different age groups in central Turkey.

Methodology

This prospective study was conducted between January 1998 and July 2007 in Erciyes University Hospital, a tertiary-care hospital with 1,350 beds, four intensive care units, a severe burns unit, and an active program of renal transplantation and bone marrow transplantation. It has a large rural patient

base, serving more than 3,000,000 people. The study used 332 pneumococci isolated from invasive infection. The study timeframe occurred prior to the introduction of a 7-valent conjugate pneumococcal vaccine. All isolates were unique isolates from separate patients. Bile-solubility and opthochin susceptibility tests were conducted to identify the strains. Serotyping was performed by Quellung reaction using Pneumotest (Statens Serum Institute, Copenhagen, Denmark). Because antisera was not available, subtypes of the isolates could not be determined. The minimal inhibitory concentration (MIC) of each isolate to penicillin G and cefotaxime was determined using E-test (AB Biodisk, Solna, Sweden) and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints [7]. Susceptibility to erythromycin, chloramphenicol, and trimethoprim-sulfamethoxasole was determined using the disk-diffusion method and was similarly interpreted following CLSI criteria [7]. *S. pneumoniae* ATCC 49619 was used as the quality control strain for antimicrobial susceptibility testing. Statistical analyses were done using the Chi-square test. A p value ≥ 0.05 was deemed significant.

Results

Of the 332 strains identified, 131 (39.5%) were isolated from children under 16 years of age and 201 (60.5%) were from adults. A total of 169 (51%) isolates were from cerebrospinal fluid, 109 (33%) from blood, 37 (11%) from peritoneal fluid, 15 (4.5%) from pleural fluid, and 2 (0.5%) from joint fluid specimens. Serogroups/serotypes 1, 19, 3, 18, 6, 14, and 7 were found to be the most common amongst the patient isolates (Figure 1). The majority (n = 95; 28.6%) belonged to serotype 1, followed by 62 (18.7%) for serogroup 19. At $\geq$ 15 strains each, 30.4% were comprised of serogroups/serotypes 3, 18, 6, 14, and 7. Coverage rates of four different pneumococcal vaccines are given in Figure 2. Coverage rates were different between age groups, but all ranked coverage of the 23-valent polysaccharide vaccine better than the 13-valent conjugate vaccine, which was better than the 10-valent conjugate vaccine, and which was better than the 7-valent conjugate vaccine. However, the overall mean coverage rates of the 23-valent polysaccharide vaccine and the 7-, 10-, and 13-valent conjugate vaccines were 96%, 44%, 78.6%, 96.4%, respectively, that is, the 13-valent conjugate vaccine provided the most coverage. Serogroups 15 and 20, which were not present in any of conjugate vaccines, caused 10% of invasive infections among children younger than three years of age.

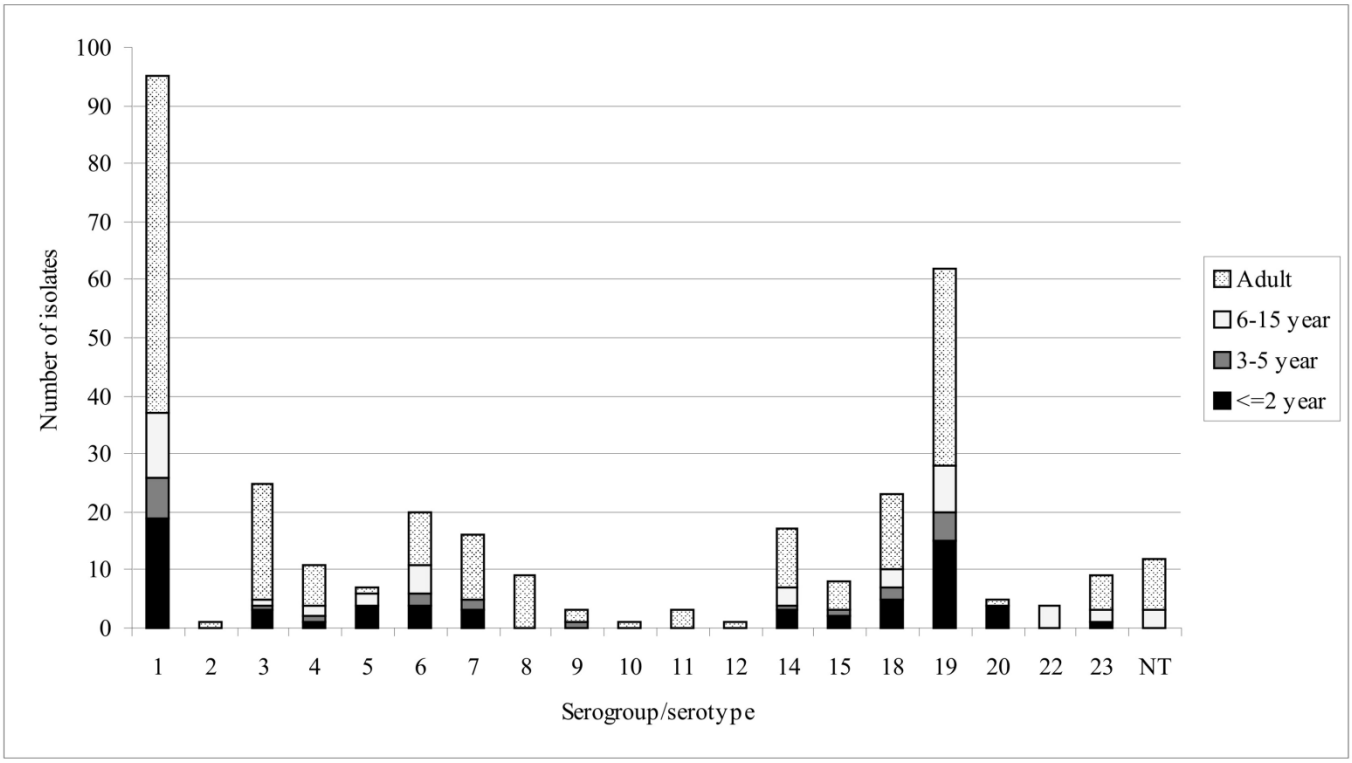
Overall resistance rates for penicillin, cefotaxime, erythromycin, chloramphenicol, and trimethoprim-sulfamethoxazole were 6% (*n* = 20), 0.3% (*n* = 1), 6% (*n* = 20), 4% (*n* = 13), and 36% (*n* = 120), respectively, among all isolates. The penicillin resistance rate was 26.3% (*n* = 15) among the strains isolated from cerebrospinal fluids of children with meningitis, whereas the rate was 11.5% (*n* = ?) in adults. The difference in penicillin resistance rates between children and adults was statistically significant for CSF isolates ($p < 0.05$), whereas there were no significant difference for blood, pleural or peritoneal fluid isolates. Serogroups/serotypes 19, 1, 6, and 14 were the most common serogroups/serotypes among the penicillin-resistant *S. pneumoniae* isolates (n = 17, 11, 5, 3, respectively). There was only one cefotaxime resistant isolate which was also resistant to penicillin. This strain was isolated from the cerebrospinal fluid of a four-year-old child and belonged to serogroup 14. The distribution of multidrug resistant *S. pneumoniae* invasive isolates amongst different age groups is shown in Table 1. Overall, 18 (5.4%) isolates were resistant to two or more antimicrobials. Of these multidrug resistant strains, 17 (94.4%) were resistant to at least penicillin and trimethoprim/sulfamethoxasole, while 6 (35.3%) belonged to serogroup/serotype 19.

Table 1. Distribution of multidrug resistant *S. pneumoniae* invasive isolates amongst different age groups

Antimicrobial agent	children < 3 years old (*n* = 64)		children + adults ≥ 3 years old (*n* = 268)	
	No	**%**	**No**	**%**
Pen G + T/S	2	3.1	10	3.7
Pen G + T/S + E	0	0	3	1.1
Pen G + T/S + E + C	1	1.6	0	0
Pen G + T/S + C	1	1.6	0	0
Pen G + CTX	0	0	1	0.4

Pen G: Penicillin G, T/S: Trimethoprim/sulfamethoxazole, E: Erythromycin, C: Chloramphenicol, CTX: Cefotaxime.

Figure 1. Age distrubution of serogroups/serotypes (NT: Non-typable)

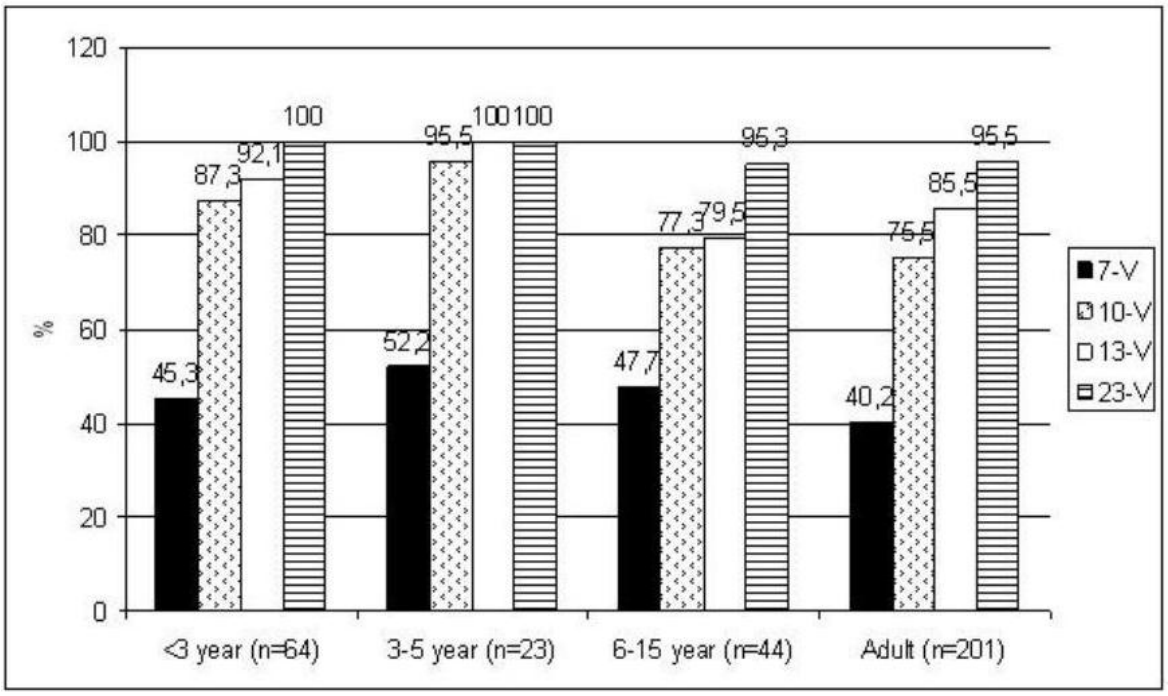


Discussion

Pneumococcal conjugate vaccines have been reported to be effective in children aged two years and under [8]. Serotypes causing invasive diseases differ from country to country; therefore, it is of utmost importance to identify the most common serotypes causing invasive diseases and to determine which of these are resistant to antimicrobial agents. Serogroup coverage rate for the 7-valent conjugate vaccine for children has been reported as 78% in the United States of America [9], 75.3% in Greece [10], 71% in England [11], 56% in Gambia [12], and 48.4% in Scotland [13]. Serotypes 1 and 5, which are not found in the 7-valent conjugate vaccine, have been reported to be common serotypes, especially in developing countries. Mokaddas *et al*. [14] has reported the coverage of the 7-valent conjugate vaccine against invasive serotypes in children 2 years old or younger at 55%. They concluded that there is a need for the formulation of an improved vaccine for better coverage in Kuwait. The coverage rate of this vaccine has been reported to be 49% to 83% in the Arabian peninsula and Egypt [15].

Recent data have shown that the use of the 7-valent conjugate vaccine is very effective in reducing not only invasive infections but also carriage of vaccine serotypes in the nasopharynx of vaccinated children [17-18]. Although vaccine use causes an

Figure 2. Coverage rates of pneumococcal vaccines in different age groups (As subtyping of the serogroups could not be determined, it may influence the coverage data of vaccines for all serogroups having subtypes like 6, 7, 9, 18, 19 and 23).



increase in herd immunity and a reduction in the number of vaccine-related diseases, the greatest concern is a possible increase in invasive pneumococcal diseases caused by non-vaccine serotypes resulting from the selective pressure of the vaccine [18-20]. In this regard, Martens *et al.* [16] have reported that capsular serotypes influenced the outcome of invasive pneumococcal diseases and that infection with serotype 3 resulted in an increased relative risk of death, whereas infection with serotype 1 was associated with a decreased risk of death.

Of the strains, 4% were non-typeable, which means the optimum coverage of the 23-valent polysaccharide vaccine was 96% in this region. However, it is generally accepted that the efficacy of this vaccine is not much higher than 70% and the level of antibody decreases at the end of first year after vaccination [21]. It should also be noted that children younger than two years of age and certain groups of patients with conditions associated with immunosuppression have a poor antibody response to the polysaccharide vaccine [22]. There are some conflicting data about the efficacy of pneumococcal vaccines in adults. Whereas Moberley *et al*. [23] recommend the pneumococcal polysaccharide vaccine to prevent invasive pneumococcal diseases in adults, Huss *et al*. [24] found very little evidence of protection among elderly people or adults with chronic respiratory illness, for whom the pneumococcal vaccine is recommended.

It has been reported that when strains not susceptible to penicillin cause meningitis, the outcome is fatal unless an antimicrobial therapy active against penicillin-resistant strains is provided [25]. The result of the present study suggests a higher virulence of these penicillin resistant strains as indicated by their significantly higher occurrence in children. In light of the availability of antibiotics without prescription in Turkey, the low prevalence of penicillin resistance is surprising. The reason for this low consumption of antibiotics could be due to economic reasons, but this is hard to prove. Third-generation cephalosporins are the first choice of treatment of penicillin resistant pneumococci [26]. However, some studies have indicated that pneumococci have become resistant to the third-generation cephalosporins [6, 27].

In conclusion, although this study indicates that the 7-valent conjugate vaccine can protect fewer than half of the children younger than three years of age, the introduction of this vaccine into the routine immunisation program in Turkey is advisable. However, the 13-valent conjugate vaccine including serotypes 1, 3, 5, and 7 will prevent a higher burden

of invasive pneumococcal diseases among children under 3 years of age.

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Corresponding author
Duygu Percin
Department of Microbiology
Faculty of Medicine, Erciyes University
38039 Kayseri, Turkey
Mobile Phone: (+90) 532 2053860
Fax: (+90) 352 4379335
Email: duygu.percin@hotmail.com

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