

Serotyping Distribution of Invasive Pneumococcal Disease (IPD) in Iranian Patients

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The previous research conducted in abundance invasive pneumococcal infections has shown about 35 percent. This implementation of the vaccination program has been inevitable. Geographic distribution of serotypes, but infection is not known. The aim of this study was to determine the distribution of serotypes causative invasive *S.pneumoniae* infections. 135 Clinical isolates of *S.pneumoniae* were studied. Based on bacteriological and molecular methods they were identified. Then they were stereotyped with Quelling test (based on SSI protocol - Statens Serum Institut). The data were analyzed by SPSS version 17.0. The results showed that, the frequency distributions of age were: 27 patients were under 10 and 88 patients were over 11 years' olds. The finding of this study showed that the types of 6,19,6,11 are more prevalent in patients totally. This study also indicated that the most predominant types were in respiratory tract infections (group 7*), eye infection (group 19*), blood infection (type 6,5,3 and 4) and CFS (type20) respectively. Firstly, This study was showed which type of *Streptococcus pneumoniae* is more prevalent in the patients in Iran. It seems the infections could be prevented by Pnumococcal 23 valent vaccines.

Key words: Streptococcus pneumoniae, Serotyping, Invasive Pneumococcal Disease (IPD).

Streptococcus pneumoniae remains a leading cause of serious infectious disease in many countries¹. However, there is no epidemiological data regarding serotypes distribution of invasive pneumococcal disease (IPD) in I. R. IRAN. Investigation of the serotype distribution, antimicrobial resistance patterns and molecular epidemiology of *Streptococcus pneumoniae* (*S.*

pneumoniae) isolated from invasive pneumococcal diseases is crucial factors in order to provide rationales for antibiotics application and immunity control of *S. pneumonia* infections². Because of, Invasive Pneumococcal Disease (IPD) is a major health problem worldwide³. Due to ongoing serotype replacement and diversity occurrence, current efforts are focused in an attempt to identify the regional serotypes. In addition, the rapid increase in multiresistant serotypes causes of invasive and respiratory pneumococcal disease has been associated⁴. The research reports that, 92 capsular serotypes of *Streptococcus pneumoniae* differ greatly in nasopharyngeal carriage prevalence, invasiveness, and disease incidence⁵. Therefore, there has been some necessitate, though, regarding whether serotype independently affects

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the outcome of invasive pneumococcal disease (IPD). In a few countries, an active surveillance system was established to monitor Pneumococcal serotype prevalence⁶. Some investigators reported that, i.e the most prevalence of serotypes were 9V and 14 which significantly greater than 1 serotype in the Czech Republic⁷. The results research indicated that the emergence and rapid dissemination of antibiotic-resistant Pneumococci was observed in southern and Eastern Europe, North America, South America, Africa, and Asia⁸⁻¹¹. Great geographical variability, both in serotype distribution and in the prevalence of resistant Pneumococci, has been reported. However, the highest rates of resistance to penicillin and erythromycin worldwide were found in serotypes 6B, 6A, 9V, 14, 15A, 19F, 19A, and 23F¹². Our previous reports indicate that the frequency in *Streptococcus* infections is about 35%. However, the distribution of serotypes responsible for disease is not known. In addition, mass vaccination against Pneumococcal disease had not been implemented in Iran. The objectives of this study were to determine the serotypes and their distribution in patients and regional population.

MATERIAL AND METHODS

Clinical definitions

All IPD cases were obtained from six Tehran hospitals. We prospectively abstracted information about patient's such as clinical sign and syndromes and underlying conditions. Clinical presentations were categorized as meningitis or nonmeningitis IPD (for example; pneumonia, pneumococcal otitis media, sepsis, peritonitis, arthritis, and endophthalmitis). Meningitis was defined as CSF culture positive for *S. pneumoniae* and/or clinical diagnosis of meningitis in combination with a blood culture positive for *S. pneumoniae*. Invasive pneumonia was physician diagnosed and confirmed by Laboratories methods such as culture positive from Lavages, blood and other normally sterile body fluid for *S. pneumoniae*.

Bacterial Strain collection

During this cross-sectional study we have analyzed all invasive pneumococcal isolates collected at the Microbiology Laboratory of the University Baqiyatallah, Tehran, I R Iran, from

January 2006 to December 2010 (n= 135). Detailed description of our institution and the demographic data was reported elsewhere. The clinical syndrome was classified according to the International Classification of Disease, (ICD-9), specific for diseases caused by *S. pneumoniae*, including sepsis, bacteremia, meningitis, pneumonia, peritonitis, arthritis, and endophthalmitis.

Bacteriological and Molecular Methods: All pneumococci isolated from patients in six Tehran hospitalized are identified by standardized Bacteriological and molecular methods. The day of isolation *S. pneumoniae* identification was confirmed by conventional methods based on the colony morphology, direct smear observation, alpha hemolysis, Gram stain, bile solubility, and optochin susceptibility tests. *S. pneumoniae* isolates from non-sterile sites (nasopharynx, pharynx, tonsils, or sputum) were excluded. *S. pneumoniae* ATCC 49619 was used as a control strain.

PCR assays were performed to amplify the 16srRNA as described previously. The sequential universal and specific primer sets (Table 1) used in this study have been previously published (9 and 10). PCR were performed in 25 µl reaction volumes that contained 1µl PCR buffer, 2.4 mM MgCl₂, 240nM of each deoxynucleoside triphosphate, 2U of Taq polymerase (Cinagen), Each primer 1µl (35 nM), and 1 µl of DNA suspension. The PCR was performed on as follows: 95°C for 5 min for one cycle followed by 35 cycles at 95°C for 30 s, 61°C for 45s, and 72°C for 1 min, with the last cycle at 72°C for 5 min. The amplified products were analyzed by 1% agarose gel electrophoresis, then ethidium bromide stained, and by visualization under transeliminatore (Uvi Doc; Serial No MOZO312) and the equivalent band to the molecular weight of the corresponding gene.

Serotype Analysis

All isolates were serotyped by the Quellung reaction using antiserum (Statens Serum Institute, Copenhagen, Denmark). In addition, during the study all isolates identified were also tested in our laboratory with rapid specific PCR methods.

Statistical Analysis

Statistical analysis was performed by using SPSS software version 19.0 (SPSS, IBM

corporation, USA). Serotype proportion in each period was compared using the Fisher exact test, as appropriate.

RESULTS

The results demonstrated that, a total 135 patients infected samples suspected of invasive streptococcal disease (IPD) were subjected to PCR and bacteriological methods. Out of which, 115 strains of *S. pneumoniae* was isolated and confirmed by PCR method. In our study, the primers pair that was previously reported was applied (13, 14). As shown in Figure 1, using primer pairs U8 and U3 had amplified fragments of 1000 bp. In addition, the use of each primer

pairs with a specific primer of *Streptococcus pneumoniae* were amplified a 300 bp fragment (Fig 1).

The analysis of patient's sex frequency revealed 53 males and 62 were females and the frequency of isolated serotypes in patients according to sex was interesting. The frequency distributions of age were: 27 patients were under 10 and 88 patients were over 11 years' olds. The distributions of isolated strains were: 43 strains from sputum or lung lavage, 36 strains from blood, 17 strains from CSF, 13 strains from peritonitis and 6 strains from endophthalmitis.

The results of this study showed, some *S. pneumoniae* serotypes were found in both sexes and some only in one sex invasive infections. For

Table 1. Universal and specific primers used in this study

Length(nucleotides)	Sequence (5'-3')	position 16srRNA	PRIMER
25	AACTCCGTGCCAGCAGCCGCGGTAA	509-533	U3
18	GTACAACGAGTCGCAAGC	1246-1263	STREP
25	AAGGAGGTGATCCAGCCGCAGGTTC	1541-1517	U8

example, serotypes 10, 14, 18 and 22 were only in female patients with infections. While serotypes 20 were only isolated from men infected. The analysis of the results had suggested that serotypes 6 and 19 are the most abundant bacterial strains isolated from patients. Total 20 strains of serotype 6 was identified only six of them were isolated from male patients and 14 strains from patients female. Similarly, 14 strains were belong to serotype 19 which only 4 of them were isolated from men and 10 from women. In other words, the frequency of serotypes 6 and 19 were higher in women. While the frequency of serotypes 1, 2, 5 and 7 were higher in men. out of 11 strains belonging to serotypes 3, only three cases from women and 8 cases from men were isolated.

Another interesting finding of this study were the serotypes frequency distribution patterns in different ages. The members of group G serotypes and 5, 6, 14 and 19 serotypes were found at all age levels. While, the serotypes 1, 2, 3, 4, 7,

8, 10, 17, 18 and 20 were isolated only from the elderly and the serotypes 22 was isolated only from children the frequency analysis of strains isolated from patients showed that, the serotypes 5, 6, 14, 19, 22 and G groups were isolated from children

As shown in figure 1, the most abundant serotypes isolated in this study were: serotypes 20, 14, group G and also serotype 11 can be named respectively.

DISCUSSION

Streptococcus pneumoniae (pneumococcus) is a cause of life threatening bacterial infections, including septicemia, meningitis, community-acquired pneumonia and acute otitis media in childhood and adults, with more than 1 million children estimated to die annually from these diseases¹⁵. In a few countries based on serotyping study, the introduction of the pneumococcal vaccine into the childhood

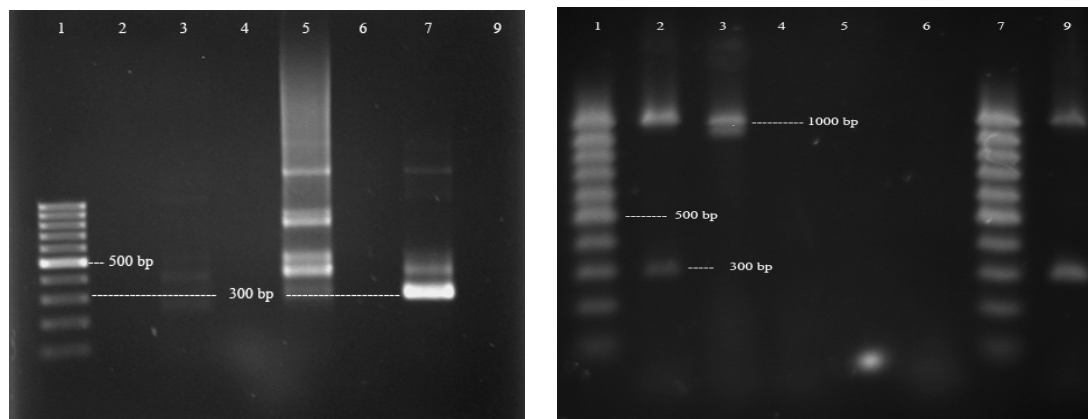


Fig. 1. In the left side, line 1 is a 100 bp ladder; the lines 3, 5 and 7 were the optimized the PCR conditions with reference strains of *Streptococcus pneumoniae* genome (ATCC 49619). The lines 2, 4, 6 and 8 were negative controls. The right side image was shown direct amplification of samples suspected. Lines 1 and 7 were a 100 bp Ladder. Lines 2 and 8 shows amplified a 300 bp and a 1000 bp fragments. Line 3 was shown only a 1000 bp fragment

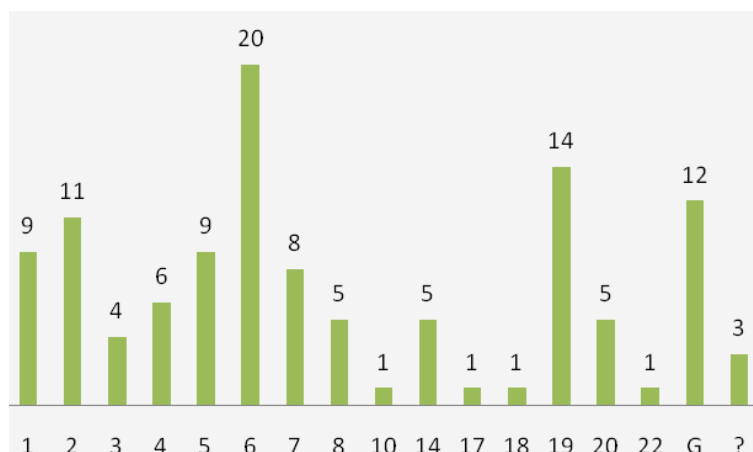


Fig. 3. The frequencies of isolated serotypes from invasive Punomoccal disease (IPD) are shown

vaccination calendar produced a dramatic reduction in the incidence of invasive pneumococcal disease (IPD) among children and adults¹⁶.

In our country, despite high prevalence of invasive pneumococcal infections exact circumstances of the type and distribution of serotypes responsible for disease does not exist. Therefore, this study was designed and implemented to determine the serotype

distribution of invasive *Streptococcus pneumoniae*. The 48 month prospective surveillance for IPD cases, which referred to four hospitals in Tehran, was carried out. This study is one of the longest reported for a very large population with wide ages range. Our results indicate that the serotypes 2 and 6 in adults and 6 and 19 in females infants have long fascinated epidemiolog and microbiolog. This is may be ability to cause outbreaks (in confined populations). However, we could not find that

Table 2. Comparison of vaccine serotypes with serotypes identified in this study

Name	Valent vaccine																						
	1	2	3	4	5	6B	7F	8	9N	9V	10A	11A	12F	14	15B	17F	18C	19A	19F	20	22F	23F	33F
PPV23	1																						
PCV13	1		3	4	5	6A	7F			9V				14			18C	19A	19F			23F	
PCV10	1			4	5	6B	7F*			9V				14			18C		19F			23F	
PCV7				4		6B				9V				14			18C		19F			23F	
Serotypes isolated in this study	1	2	3	4	5	6	7	8			10			14		17		19		20		22	

age and clinical syndrome influence the severity of illness more than serotype. Consider increasing occurrence IPD and propensity to cause disease our populations offered necessitate more investigates in feature. Our data is different from other area research. For example, some study were shown that, the serotypes 1 and 5 were commonly cause IPD¹⁷⁻²⁰

based on this study, may not be suitable introduced choice of vaccine alone. But compared to serotypes defined in this study with the serotypes include by seven-valent differences is large. In fact, the seven-valent vaccine could not covering serotypes 1, 2, 3, 5, 7, 8, 10, 17, 20 and 22. Similarly, compared with vaccine serotypes isolated from 13-valent in serotype 2, 10, 17, 20 and 22 are different. Hence, the only vaccine that is able coverage serotypes our country is the 23- valent vaccine (Table 2).

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