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Original Article

Molecular Detection and Serotyping of *Streptococcus Pneumoniae* in Children with Suspected Meningitis in Northeast Iran

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What's Known

- *Streptococcus pneumoniae* is one of the major causative agents of meningitis in children. Pneumococcal conjugate vaccine is one of the best methods to prevent pneumococcal disease.
- Pneumococcal capsular serotypes can vary in different geographical areas.

What's New

- The first attempt to evaluate the most common capsular serotypes in northeast Iran.
- Considering common capsular serotypes in Bojnurd (Iran), PCV-13 vaccine is the suitable choice for a vaccination program.

Abstract

Background: To date, more than 90 *Streptococcus pneumoniae* (*S. pneumoniae*) capsular serotypes are known. The prevalence of these serotypes varies according to the geographical area and the regional vaccination program. Due to the lack of regular vaccination programs for *S. pneumoniae* in developing countries, serotyping of the prevalent isolates is useful in selecting the correct vaccine. The present study aimed to evaluate common serotypes of pneumococcal meningitis in Bojnurd, Iran.

Methods: All cerebrospinal fluid (CFS) samples suspected for bacterial meningitis were analyzed. The samples were collected during 2014-2018 in the Laboratory of Imam Reza Hospital (Bojnurd, Iran). Due to the high rate of false-negative cultures, polymerase chain reaction (PCR) was used for the detection of *lytA* and *psaA* genes of *S. pneumoniae*. In addition, the modified Marimon's PCR method was used for serotyping the bacteria. The data were analyzed using Pearson's Chi-square test.

Results: Out of the 901 CSF samples, 106 cases tested positive for *S. pneumoniae* using the PCR method, while only 92 cases tested positive using the conventional methods. Based on the Marimon's PCR method, serotypes 23F, 19F, 19A, 1, 14, and serogroup 6A/B were the most common types. Serogroups 18C, 15A/F, 15B/C, 9A/V, 7A/F, 11A/D/F, and 22A/F were also detected in isolates. Note that 2.8% of the samples were non-typable (NT).

Conclusion: The results showed that only 13 serotypes were responsible for all meningitis cases. Pneumococcal capsular vaccine-13 (PCV-13) is the preferred choice against common serotypes of *S. pneumoniae* in northeast Iran.

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Keywords ● *Streptococcus pneumoniae* ● Serogroup ● Vaccines ● Meningitis

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) is a Gram-positive coccus that can colonize the nasopharyngeal area without major clinical symptoms. Clinical manifestation of the bacteria ranges from otitis media and sinusitis to bacteremia, invasive pneumonia, and meningitis.[1, 2] *S. pneumoniae* causes more than 14 million

cases of invasive infections and approximately one million deaths annually among children in developing and underdeveloped countries. During 1999-2010, in the Asian-Pacific region, the incidence of *pneumococcal* disease among children aged under two years was about 150 cases per 100,000.[3]

Meningitis is defined as the inflammation of meninges surrounding the brain and spinal cord. Infectious agents that cause bacterial meningitis are *S. pneumoniae*, *Neisseria meningitidis*, and *Hemophilus influenzae*. Of these, *S. pneumoniae* is the most common agent. It has various virulence factors such as streptolysin-O, IgA protease, and polysaccharide capsule. The bacterium has more than 90 capsular serotypes, but only a limited number is related to acute *pneumococcal* meningitis.[4]

The mortality rate due to meningitis among children and adults is 25-73% and 20-30%, respectively, and the survivors often suffer from neurological complications and sequelae.[4] The adverse effect depends on the patient's medical condition such as the underlying chronic disease, immune system, nutrition, etc.[5, 6] Considering the high morbidity and mortality rates associated with *pneumococcal* infections, it is important to implement life-saving programs for the prevention, timely diagnosis, and treatment of *pneumococcal* diseases. As part of a national vaccination program, two types of vaccines are currently used in developed countries, namely pneumococcal capsular vaccine-10 (PCV-10) and pneumococcal capsular vaccine-13 (PCV-13). These vaccines contain serotypes [1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F] and [1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F], respectively.[7] The production of PCV-7, the previous type of PCV vaccine, has already been stopped.

In some developing countries, such as Iran, *pneumococcal* vaccination is not included in the national vaccination program. Therefore, data on serotypes causing invasive *pneumococcal* infections are not readily available since vaccination is carried out locally in Iranian public and private hospitals.[4, 8] Patients with suspected meningitis are typically viewed as emergency cases and physicians immediately proceed with empirical anti-microbial therapy and obtain cerebrospinal fluid (CSF) for laboratory evaluations. In some cases, the CSF culture could be negative while other tests (e.g. serological and molecular diagnostic) could be positive. Consequently, methods such as polymerase chain reaction (PCR) and multiplex PCR are also used.[9]

Since the prevalence of serotypes causing invasive *pneumococcal* infections varies in different geographical areas, we focused our study on patients suspected of meningitis in Bojnurd, Iran. Using the molecular method, we evaluated CSF samples for *pneumococcal* serotypes to recommend a regional vaccination program.

Materials and Methods

An observational and descriptive study of CSF samples from patients with suspected meningitis was conducted in the Laboratory of Imam Reza Hospital, Bojnurd, Iran. The samples were collected during 2014-2018 from children aged <5 years with an increased level of C-reactive protein (CRP) or with fever and CSF leukocytosis. The samples were evaluated for *S. pneumoniae* using conventional methods, including CSF sediment gram staining, microscopic evaluation, and culture. The PCR assay was performed using *lytA* (N-acetylmuramoyl-L-alanine amidase) and *psaA* (pneumococcal surface adhesin A) as marker genes. In addition, capsular serotypes were determined using the modified Marimon's multiplex PCR method for wzy, wchE, and wciN beta genes.

The inclusion criteria were different physical examinations for children <12 months and for those between 12 months and 5 years old, such as bulging fontanel, nuchal rigidity, and positive Brudziński signs. The general inclusion criteria were increased levels of CRP, CSF leukocytosis, CSF protein; reduced level of CSF glucose; and physical signs of fever, neck stiffness, vomiting, headache, etc. The exclusion criterion was a previous PCV vaccination. The data were analyzed with Pearson's Chi-square test using SPSS software (version 22.0). $P<0.05$ was considered statistically significant.

The study was approved by the Ethics Committee of North Khorasan University of Medical Sciences, Bojnurd, Iran (code: 95/P/970). Written informed consent was obtained from the parents of the children.

Genomic DNA Extraction

Total genomic DNA of the CSF samples was extracted using QIAamp® DNA Blood mini kit (QIAGEN, Germany). In accordance with the manufacturer's instruction, lysozyme at the concentration of 20 units/ml (SinaClon, Iran) was added to the lysis buffer. To obtain better results, based on a previous study,[10] the CSF samples were centrifuged at 8,000 rpm for 3 minutes and then used as the precipitants for DNA extraction.[10]

PCR Assay

The PCR assay was performed using the

TAKARA PCR thermal cycler Gradient TP600 (TAKARA, Japan). For all PCR reactions, EmeraldAmp® MAX PCR Master Mix 2X (Takara, Japan) in a volume of 50 µl was used.

PCR Assay for Detection of S. pneumoniae

The *lytA* and *PsaA* genes were selected as marker genes for the detection of *S. pneumoniae*. PCR was performed with the following sequences: primary denaturation at 94 °C for 5 minutes, 35 cycles of amplification (denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, extension at 72 °C for 30 seconds), and a final extension at 72 °C for 7 minutes. ATCC® 33400 reference strain was used for PCR optimization and to determine the limit of detection (LOD) of the triplex PCR method. The third set of primers was added to the reaction as the internal control for preventing false-negative results. Specific primers were used as the control for the recognition of the human beta-globin gene. The list of primer sequences is described in table 1.

To assess the LOD, 10-fold serial dilutions (equivalent to 10^7-10^1 copies) of a *pneumococcal* reference strain ATCC® 33400 were prepared using tested sterile CSF as a solvent (due to the presence of human cells containing the beta-globin gene as the internal control) and the extracted DNA was tested by triplex PCR protocol. In positive reactions, the bands 268 (internal control), 187 (*lytA*), and 114 bp (*psaA*) were observed (figure 1). In negative reactions, only the internal control band (268 bp) was observed (table 2). The primers were evaluated using the software packages AlleleID 6.0 (Biosoft International, Palo Alto, CA, USA) and Oligo 6.0 (Life Science Software Resource, Minnesota, USA). The reactions without any PCR product bands were false-negative and the results were not reliable (table 2). As shown in figure 1 and table 3, the mean LOD of the reaction after triplicate testing was between 10^1-10^2 colony-forming unit (CFU).

The PCR was also performed on 51 clinical isolates of *S. pneumoniae* and all reactions tested positive for *lytA* and *psaA* genes; indicating 100% sensitivity. For the specificity evaluation, PCR was performed using the extracted DNA from *Haemophilus influenzae* (ATCC51907), *Neisseria meningitidis* (ATCC13077), *Staphylococcus aureus* (ATCC19323), *Streptococcus*

Table 1: Primer sequences for detection of *S. pneumoniae*

Primer	Sequence	size	Reference
lytA[1] forward	5'-CCATTATCAACAGGTCCTACC-3'	187 bp[3]	Present study
lytA reverse	5'-TAAGAACAGATTTGCCTCAAG-3'		
PsaA[2] forward	5'-GCCCTAATAAATTGGAGGATCTAATGA-3'	114 bp	Jourdain et al.[12]
PsaA reverse	5'-GACCAGAAGTTGTATCTTTTTTTCCG-3'		
Beta-globin forward	5'-GAAGAGCCAAGGACAGGTAC-3'	268 bp	Present study
Beta-globin reverse	5'-CAACTTCATCCACGTTCACC-3'		

[1]N-acetylmuramoyl-L-alanine amidase, [2]*S. pneumoniae* surface adhesin A precursor, [3]Base pair

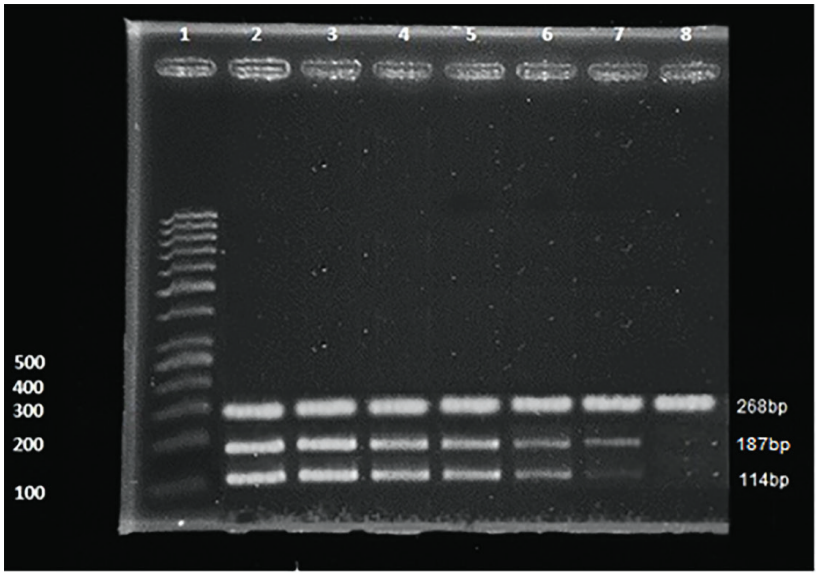

Figure 1: Agarose gel electrophoresis of triplex PCR on standard samples. The 268 bp, 187 bp, and 114 bp bands belong to beta-globin, *lytA*, *psaA* genes, respectively. Lanes, 1: 100 bp ladder; 2: 10^7 copy; 3: 10^6 copy; 4: 10^5 copy; 5: 10^4 copy; 6: 10^3 copy; 7: 10^2 copy; 8: 10^1 copy of *S. pneumoniae* genome.

Table 2: Interpretation of *S. pneumoniae* detection PCR results

Interpretation	Beta-globin: 268 bp	*lytA*: 187 bp	*psaA*: 114 bp
Unacceptable	-	-	-
S. pneumoniae -	+	-	-
S. pneumoniae +	+	+	+

Table 3: Determination of LOD using the PCR method

Number	Standard	CFU	Result
1	S1	10^7	+
2	S2	10^6	+
3	S3	10^5	+
4	S4	10^4	+
5	S5	10^3	+
6	S6	10^2	+
7	S7	10^1	-

CFU: Colony-forming unit (mL)

viridance (ATCC6249), *Enterococcus fecalis* (ATCC29212), *Enterococcus faecium* (ATCC700221), *Escherichia coli* (ATCC25922), *Streptococcus agalactiae* (ATCC13813), *Listeria monocytogenes* (ATCC19115), *Pseudomonas aeruginosa* (ATCC10145), and *Mycobacterium tuberculosis* (ATCC25177). All reactions were negative.

Multiplex PCR for Determination of Capsular Serotypes

The primers used for the amplification of various capsular types (*wzy*, *wchE,* and *wciN* genes) are listed in table 4. Marimon's method was modified and adapted to the conventional PCR and agarose gel electrophoresis settings.[9] Originally, Marimon designed 55 sets of primers for evaluation with a capillary electrophoresis system. However, in the absence of such a system, due to the similar size of the PCR products of different primer pairs in the Marimon's method, we performed the evaluation with the conventional PCR and electrophoresis. The 55 pairs of primers were grouped into nine separate multiplex reactions based on the difference in their PCR product size. The optimum difference in size for detection in agarose gel electrophoresis was about 100 bp. In some cases, when grouping based on product size was not possible, the PCR products were evaluated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The PCR thermal cycling condition was 15 minutes at 95 ºC; 35 cycles of 60 seconds at 95 ºC, 60 seconds at 58 ºC, and 60 seconds at 72 ºC; and a final extension step of 10 minutes at 72 ºC.

Results

Out of the 901 CSF sample, 92 cases tested positive for *S. pneumoniae* in culture and 106 cases tested positive for *lytA* and *psaA* genes using the molecular detection method. It should be noted that all the 92 culture-positive samples also tested positive in PCR. Of the 106 (11.8%) positive samples, 50 (47.2%) and 56 (52.8%) isolates were from female and male patients, respectively. There was no significant correlation between sex and *S. pneumoniae* isolation (P=0.92). Based on the primer pair combinations in table 1, all positive samples were evaluated for capsular serotypes using the modified Marimon's multiplex PCR method. As shown in figure 2, the types 23F, 19F, 19A, 1, 14, and group six were the most common serotypes and groups in our samples. It should be noted that 2.8% of the samples were Non-typable (NT). The results for capsular serotypes and their distribution are presented in figure 2 and table 5.

Discussion

Serotypes 23F, 19F, 1, 14, 19A, and serogroup 6 were the most common types in Bojnurd, Iran. PCV13 had the optimal coverage for children aged ≤5 years in this area. The Quellung reaction (Neufeld test) is the accepted gold standard method for *S. pneumoniae* serotyping. However, a number of factors (e.g., costs, the need for operational expertise, potential false-negative cultures especially in meningitis cases) hindered its use in clinical laboratory settings.[11] Molecular assays have recently been developed to identify the invasive pneumococcal disease (IPD) associated with serotype distribution.[9, 12] Consequently, we used the modified Marimon's molecular method for serotyping *S. pneumoniae*. A limited number of capsular serotypes were related to most of the IPDs.[13] In the present study, we found 13 serotypes, which were responsible

Table 4: Primer sequences for serotyping of *S. pneumoniae*

Reaction	Serotype	Size (bp)	Forward	Reverse
1	14	84	CCGTCTTTTTGTATGGTGCTATG	TGAACAGCCAATCCATCAATCAG
	22A, 22F	181	ACGTATAGGACGTTTCTCAATCC	ATCCCGAAACCAAATTGCTATCC
	33C	278	CGGCAGGTATAAGTATTATCGG	CCTACACCTCTTATAAACGTTGG
	35F, 47F	375	TTGCTACAGTTTTGATGTATCTCC	AGAATCCGTTTCATCATACAGCC
	6C, 6D	473	GTCGTGTAAAGTAGTATACAATCC	ATAATCCTCTGGATTATCCACCC
	24A, 24B, 24F	569	TCATGCTTATGTTATGTGTTACGG	GTGAGAGCTATATTTAGAACATGG
	23B	650	TTGCATATGGATTTAATGGTGGG	ACCATTGCTGATAGAAGTAGAGG
2	8	94	ATTAGCTGCATACGCAAGAACC	ATTAGCTGCATACGCAAGAACC
	3	191	AGAAATGCTATCCGCGTTGGG	TTGTCACGAGATTACGCTCAGG
	20	292	TATTGTTCCGAAAAAAGAGTGGG	TACTCAAAGATTGTGTGGTACGG
	11B, 11C	392	TAGAAATCGCAAGATAGCCTTCC	CTGATTATGAGCATAGTTGATCC
	7A, 7F	491	TTGACTGCAAGTGTTTCAATGGG	AAAGCACAAAATATTGGAACGAGG
	28A, 28F	578	AGGTAGACTACCAATTTCAATTCC	TACACCTGCTAATATCAATGTTCC
3	7B, 7C, 40	101	TTATTTTTTAGAAACATTAAAACTC	AACAATCATCTCTATTCGACC
	15B, 15C	201	CGGATGATTGTAGCGTTTTATCC	ACTGTAGATTGTGTTCTGATTCC
	31	301	TTTCAAGGATATGATAGTGGTGG	TAGCATTACAGATGTCACTAAGG
	17F	402	TCTTGTCAAATACATACTTACCCC	GTAGTCTCGCATTTCTATCATCC
	16A	501	GTTATGATAATGGTAACGCCTCC	CAGCCAATAAGTCATATACGCC
	15A, 15F	598	ATGAGAGGAAGATATATACTGGG	AAATAAGTTGTCCCATAGGAAGG
4	9L, 9N	111	CACTGTTGGCTATGTTAGCCTC	CTCTCCACGTGGCCAATATAC
	6A, 6B, 6C, 6D	211	GAAGTAGAAAATCGTGTAAGTGG	TCCAACAACTAACCTTATAAGGG
	23A	314	CTAGGTTCGTATCTCTTTGCGG	TACCAAATGGGTAATGGAGGGG
	41A, 41F	413	AGTTACTGGCCCTTTCTTATTCC	AGGTAAAAAGTCATATCCATTCCC
	19F	523	TGTTCTTAGTAATGGATATACGGG	AAAACTTCACCAGGATCTAATGG
	17A	615	TTTACCCAAGAATGGTTTCTAGG	AGCATAACAGTTTGCGCTATTGG
5	21	122	TTATGCTGGTTTAAATATCGCTCC	TAACAAATATGCCAAAACGTAGCC
	34	130	TGTAAGAGGAGATTATTTTCACCC	GTCACAATAAAAACTGTACCTCC
	25A, 25F, 38	140	CAACACAATGTCTTATTGCAGCC	AACGCACCCCAAAATAACTTTCC
	27	240	AATGCCGACGATTAATGCAGCC	CTAGCCATGCTGGATATTTCCC
	19B, 19C	338	TCAGTACGAATAGATGGAACACC	CCCAGTATCTAAATCCTAATCCC
	4	433	ATTCAGAGGCAGCTAGTTCAGG	CAGAAGCTACTGTTAGGCTGG
	48	532	CCCTTTGATAGCTATAGTATCGG	CCCTGGAATAGAAGTTTTCTAGG
	12A, 12B, 12F, 44, 46	627	CCTTTCTGATTCGTCCAGTTCC	AGTTGAACCAACTCCCCATCC
6	10A, 10B, 0C, 10F	151	CRATGAGGCTATATGTTGGAATAG	GTATTGAACYCATAGATAACAGAG
	29	251	TGTGGCAAAAATTTCTTTAGCGG	ATATACCCAGTAAACAGACAAGG
	11A, 11D, 11F	348	GGACATGTTCAGGTGATTTCCC	TGCGCCAAATTTGGTATCGACC
	5	443	TTATCTATTTTATCGCAGACTCCC	CTGCCGATAAAAAGATAGATGCC
	39	544	TATGAGGTATCATTTAGCAGGGG	ATCATCGAAATGGCAACTAAAGG
	33B, 33D	640	ACAACAGCAATGTTGTTGTTACTC	GAGAAGTAAGAGTTTTGTCATCC
7	43	160	GGAATAGTTTAGGATTTGTACACC	TAGAGTCTGCTAACTGTAATATCC
	23F	260	TTCACAAGTGATAGTGAACTTGG	TATTAGCTTTATCGGTAAGGTGG
	32A, 32F	357	CTTACAATGAGACGCTATTTTCC	GTTATTACCATTGAATTCGTTCCC
	33A, 33F, 37	453	ATGTTAGATTAGATGGTTTGCTGG	ATTCAACACATAAACCGTTGGGG
	1	555	TTTGCTAGATGGTGAGTTTGTATC	TTTAGAAGCTGCATTGTACTACTC
	36	230	TTCCGGATCTATTCAATTTCCCC	CAATAACAGCCTCCGTTTTACC
	2	383	GTTCAATATTTCTCCACTACACC	CTAAGAGTTCCAATACGTTGACC
	35B	483	CATTAGTGTTGCTATGTTGTTCC	GATTAGATAAATAAATACGCCCCC
	16F	606	ACTGCTTGCATATTAGCTTTATGG	TGATAGAGTGACAGAACAATTGG
8	9A, 9V	172	GATCAATGGCAACTATATTTACCC	GATTCACTGTCTGACTTTGAACC
	19A	269	ATTGGAGTAGCTGAGGTTTTTGG	TATCCAATTTAAAACCAGCACGG
	35A, 35C, 42	367	TCGTTCACCTACTTTATTAATGCC	AATAATTCCTAATACCATCTGCCC
	45	463	CAGATTGGTTTTCACATCACTCC	ACATAACACGACTTTTAGTGACC
9	18A, 18B, 18C, 18F	220	AGTCTTACTAGACGTAATGAACC	AAGATAAATTGACTAAGTCCTCCC
	13	328	TTAACAGGTAGATTACGACTTGG	ATATCCCAAAAACAAAATCGCTGG
	47A	423	AATACATTGTACGTCTTTAACCCC	CGAAGAATTAAACCCACATAACC

bp: Base pair

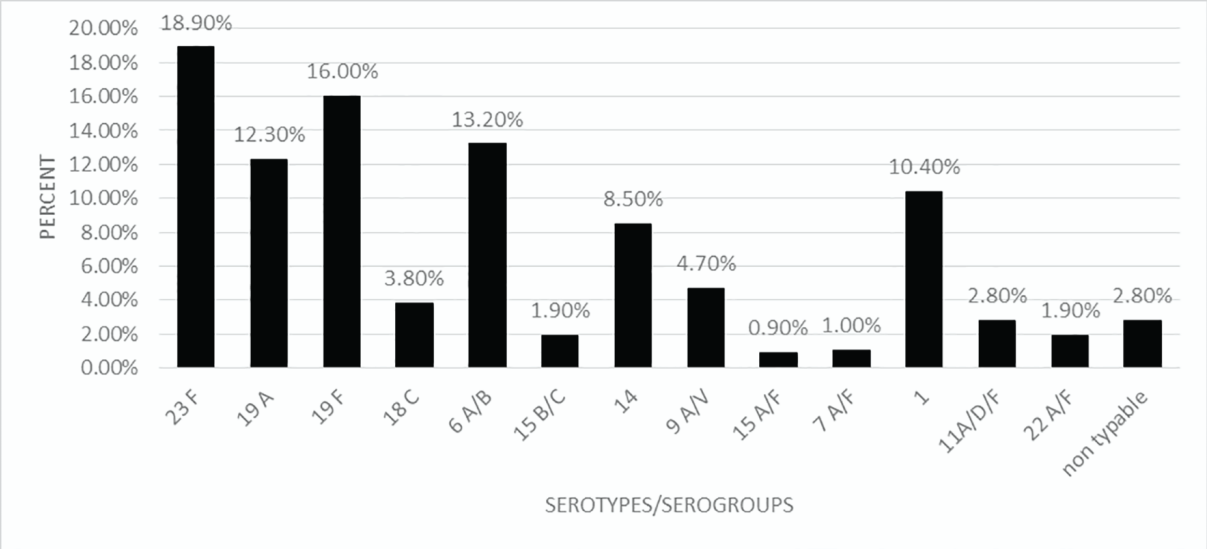

Figure 2: Serotype distribution among *S. pneumoniae* strains.

Table 5: Serotype distribution among *S. pneumoniae* strains in different years

		2014-2015 n (%)	2015-2016 n (%)	2016-2017 n (%)	2017-2018 n (%)	Total n (%)
Number of *S. pneumoniae* n [+, %]		160 [16, 10]	163 [19, 11.7]	278 [29, 10.4]	300 [42, 14]	901
S. Pneumoniae +[a]		16 (15.1)	19 (17.9)	29 (27.4)	42 (39.6)	106 (11.8)
Women[b]		10 (62.5)	8 (42.1)	16 (55.2)	16 (38.1)	50 (47.2)
Men[b]		6 (37.5)	11 (57.9)	13 (44.8)	26 (61.9)	56 (52.8)
Serotype[c]	23F	3 (15)	5 (25)	4 (20)	8 (40)	20 (18.9)
	19A	2 (15.4)	3 (23.1)	4 (30.8)	4 (30.8)	13 (12.3)
	19F	4 (23.5)	3 (17.6)	5 (29.4)	5 (29.4)	17 (16)
	18C	0	1 (25)	1 (25)	2 (50)	4 (3.8)
	6A/B	3 (21.4)	2 (14.3)	4 (28.6)	5 (35.7)	14 (13.2)
	15B/C	0	0	1 (50)	1 (50)	2 (1.9)
	14	1 (11.1)	2 (22.2)	2 (22.2)	4 (44.5)	9 (8.5)
	9A/V	1 (20)	1 (20)	1 (20)	2 (40)	5 (4.7)
	15A/F	0	0	0	1 (100)	1 (0.9)
	7A/F	0	0	0	2 (100)	2 (1.0)
	1	2 (18.2)	1(9.1)	4 (36.4)	4 (36.4)	11 (10.4)
	11A/D/F	0	0	2 (66.6)	1 (33.3)	3 (2.8)
	22A/F	0	0	0	2 (100)	2 (1.9)
	NT	0	1 (33.3)	1 (33.3)	1 (33.3)	3 (2.8)

[a]Number of positive cases; [b]Number of positive cases in female or male patients; [c]Number of positive cases of each serogroup or serotype; NT: Non-typable

for all meningitis cases. Note that 2.8% of the isolates were NT.

During the last two years, the prevalence of some serotypes (9A/V, 15A/F, 7A/F, 1, 11A/D/F and 22A/F) has increased in Bojnurd, Iran. Studies in the USA, UK, and Brazil have reported that only a few *pneumococcal* serotypes were related to the colonization and invasive diseases,[14-16] whereas a wide variety of such serotypes were identified in Asia.[17, 18] The serotypes 23F, 14, 19F, and 6B were predominant in East and Southeast Asia prior to the PCV vaccination.[3] Several studies have reported that serotypes 1, 4, 6A/B, 7F, 9V, 14, 15B/C, 18C, 19F, 19A, and 23F account for more than 80% of the IPD in children, especially in developing countries without any routine *pneumococcal* vaccination programs.[19-22] In the absence of such programs in Iran, we managed to identify the presence of serotypes 23F, 19A, 19F, 18C, 14, 1, and serogroups 6A/B, 15B/C, 9A/V, 15A/F, 7A/F, 11A/D/F, and 22A/F. In line with other studies,[1, 4, 23-26] we found that the most prevalent serotypes in meningitis cases were 23F, 19A, 19F and 6A/B, which could be controlled with the PCV-13 vaccine. In contrast,

Talebi and colleagues reported that the most common serotypes in pneumococcal isolates were 14 and 3, followed by 23F and 19F.[27]

Some researchers believe that the common serotypes in infants are different from those found in adults. A previous study reported that the serotypes 6 and 19 were common in IPD related isolates and the 23-valent vaccine was best for adults.[28] Moreover, in another study, they reported serotype 6 was most prevalent in adults, but serotype 19 in infants.[29] Dashti and colleagues reported that the most prevalent serotypes were 19, 6, 14, 17, 20, 23, and 21 in nasopharyngeal carriers under 10 years old in Tehran (Iran).[30] Another study reported that four serotypes of 19A, 6, 3, and 23F were common in both the carrier and clinical samples at a rate of at least 91%.[13] Gharailoo and colleagues reported that the serotypes 6A/B, 19A, 19F, and 23F were the common types of nasopharyngeal carriers,[31] whereas another study reported that the most common serotypes in nasopharyngeal carrier children were 19F, 6A/B, 15A, 11, 23F, 1, 19A, and 35B.[32] Based on the above-mentioned studies, we deduced that the source of meningitis related to *pneumococcal* serotypes was the nasopharyngeal area. Overall, the data showed that serotypes 6A/B, 14, 18C, 19F, 19A, and 23F were the most prevalent serotypes in Asia, especially in non-immunized areas.

We found that PCV-13 vaccine is the best candidate against common serotypes of meningitis-related *S. pneumoniae* strains. This vaccine is readily available in Iran and covers 77.13% of the most common serotypes in our region, especially 23F, 19A, 19F, and 6A/B. On the other hand, PCV-10 vaccine covers 65.06% of the serotypes but excludes the two major serotypes 19A and 6A. None of these vaccines could cover serotypes 22, 7, 15A/F, 15B/C, and 11. The present study was the first attempt to identify the distribution of serotypes in Bojnurd. Serotyping on more specimens is recommended to determine the extent of their coverage and evaluate the effectiveness of the vaccine.

Conclusion

The high pathogenicity of *S. pneumoniae* in different age groups and the increasing antimicrobial resistance are alarming. The management of this pathogen is essential to protect lives and to reduce therapeutic treatment costs. PCV-13 vaccine is the preferred choice in northeast Iran. More research on serotypes in different geographical regions allows the design of a nationwide vaccination program. Further studies on capsular serotyping, antimicrobial susceptibility testing, and molecular epidemiology should provide a better understanding of common pneumococcal types and allow choosing optimal therapeutic strategies in Iran.

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Conflict of Interest: None declared.

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