Molecular characterization, antibiotic resistance pattern and capsular types of invasive *Streptococcus pneumonia* isolated from clinical samples in Tehran, Iran

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Abstract

Background: *Streptococcus pneumoniae* causes serious infections worldwide. The aim of this study was to determine the molecular characteristic, antibiotic resistance pattern and capsular types of invasive *S. pneumonia* in Tehran, Iran.

Results: Of the 44 pneumococcal invasive isolates, 39 (89%) were isolated from children and 5 (11%) from adults. The results show that all pneumococcal isolates were susceptible to linezolid but had varying resistance to trimethoprim-sulfamethoxazole (86%), erythromycin (73%), tetracycline (66%), clindamycin (43%), penicillin (16%), chloramphenicol (14%) and levofloxacin (2%). The range of erythromycin, tetracycline and penicillin MICs were 2 - ≥ 256 μg/mL, 4 - ≥ 48 μg/mL, and 0.047 - ≥ 256 respectively. All of the penicillin resistant isolates were multidrug resistant (MDR) and in addition to penicillin were resistant to tetracycline, erythromycin and trimethoprim-sulfamethoxazole. The most common capsular types detected in 64% of the pneumococcal isolates was 6A/B, 19A, 15A, 23F. The multilocus sequence typing (MLST) of 10 pneumococcal isolates revealed 9 different sequence types (STs), including ST 15139 (capsular type 19A) and ST 15140 (capsular type 23F), which have not previously been reported.

Conclusions: The study revealed that the *S. pneumonia* isolates belonged to diverse capsular types and clones with high rate of resistance to erythromycin, tetracycline, and penicillin.

Background

Streptococcus pneumoniae is the leading cause of invasive disease in young children, older adults and individuals with impaired immune systems [1, 2]. The invasive pneumococcal disease (IPD) is an important cause of morbidity and mortality worldwide [1]. IPD is described by isolation of S. pneumonia from a normally sterile site, such as blood; cerebrospinal fluid (CSF), and pleural or ascitic fluid [1]. The polysaccharide capsule is a main virulence factor in IPD, providing protection the bacterium from the host's immune system. To date 98 capsular serotypes have been identified based on the antigenic capsular polysaccharide [2–4]. Although universally, the majority of IPD (80%) have caused by a small number of capsular types including 1 6A/B, 23F, 7F 9V 14 [1, 2, 4].

The incredible capacity of S. pneumoniae to uptake genes has facilitated the spread of resistance in the pneumococcal population to penicillin and other antibiotics such as macrolides that used routinely to treat the disease [4–7]. The resistance mechanism to penicillin is structural modification in the penicillin binding proteins (PBPs) which have a major role in the synthesis of cell wall. Six PBPs have been identified in S. pneumoniae of which three PBPs (PBP2b, PBP2x and PBP1a) are the most often associated with penicillin resistance [7, 8]. While, macrolide resistance mechanisms in S. pneumonia is conferred by two mechanisms. The major resistant determinant is acquisition of the ermB gene that encoding a methylase [7, 9, 10]. The second mechanism is acquisition of mefA/E genes that encoding an active efflux pump [7, 9, 10]. Noticeably, the majority of isolates that encode ermB exhibit the MLS_B

(Macrolide, lincosamide and streptogramin B) phenotype. While, the majority of isolates that carries mef reveal the M phenotype [7, 9–11]. Also the most common mechanism of resistance to tetracycline in S. pneumoniae is acquisition one of the two genes, tetM and less frequently the tetO genes [10, 12, 13] both of which located in mobile genetic elements such as transposons and encode ribosomal protection proteins [12, 13]. Interestingly, resistance to erythromycin and tetracycline is generally related to the insertion of the ermB gene into the transposons that contains tetM gene, raising worry about the role of tetracycline-resistant strains in the spread of macrolides -resistant strains. The high prevalence of tetracycline resistance among macrolide resistant S. pneumoniae has reported [7, 10]. As well, the transposons of the Tn916 or Tn917 family such as Tn6002, Tn3872, Tn6003 and Tn1545 have been described in pneumococci [7, 10]. Also, the main source of the tetM gene is Tn916 family [12].

Many molecular methods have been used to determine the genotypic background of S. pneumonia. One of these methods is multilocus sequence typing (MLST), which relies on polymerase chain reaction (PCR) and sequencing of house-keeping genes [2, 5, 14, 15]. The most common sequence types (STs) were ST320 that is a frequently multidrug resistant (MDR) type and ST695, associated with susceptibility to all antibiotics except for clarithromycin [2]. The most prevalent STs reported in some Asian countries are ST81, ST283 and ST236 [16, 17].

The purpose of the current study was to analyze the molecular characteristic, antibiotic resistance pattern and capsular types of invasive S. pneumonia in Tehran, Iran.

Results

The pneumococcal isolates were obtained from blood cultures. Of the 44 pneumococcal invasive isolates, 39 (89%) were isolated from children and 5 (11%) from adults.

The antibiotic susceptibility pattern and molecular characteristics of pneumococcal isolates are summarized in Table 3. The results show that all isolates were susceptible to linezolid but had varying resistance to trimethoprim-sulfamethoxazole (86%), erythromycin (73%), tetracycline (66%), clindamycin (43%), penicillin (16%), chloramphenicol (14%) and levofloxacin (2%). The range of erythromycin, tetracycline and penicillin MICs were 2 - ≥ 256 μg/mL, 4 - ≥ 48 μg/mL, and 0.047 - ≥ 256, respectively.

The co resistance to erythromycin and clindamycin (the constitutive phenotype) was observed in 43% (19/44) of the isolates and resistance to erythromycin, but not to clindamycin (the M phenotype) was observed in 29.5% (13/44) of the isolates. As to macrolide resistant genes, the most prevalent gene was ermB found in 52% (23/44) of the isolates, followed by mefA/E found in 50% (22/44) of the isolates. The rate of coexistence of ermB and mefA/E was 23% (10/44). Analysis of resistance genes illustrated the significantly higher prevalence of ermB and tetM genes in MDR isolates (P = 0.0001).

The co resistance to erythromycin and tetracycline was found in 54.5% (24/44) of the isolates. The rate of tetM gene and coexistence of ermB and tetM were 68% (30/44) and 50% (22/44) respectively. Our

result also showed that all of the penicillin resistant isolates were MDR and in addition to penicillin were resistant to tetracycline, erythromycin and trimethoprim-sulfamethoxazole.

In the present study, of 44 isolates 36% (16/44) were positive for transposon genes. The most prevalent transposon genes were Tn6002 14% (6/44) and Tn2009 7% (3/44) followed by Tn1545/6003 4.5% (2/44).

The pneumococcal isolates belonged to the following capsular types: 6A/B (18%), 19A (16%), 15A (16%), 23F (14%), 11A (9%), 14 (4.5%), 15B/C (4.5%), 19F (4.5%), 9V (4.5%), 1 (2%) and noticeable (7%).

PCR analysis of virulence genes revealed that 100% (44/44), 100% (44/44), 100% (44/44), 73% (32/44), and 41% (18/44) of isolates harboured the cbpA, cpsA, lytA, ply and pspA genes, respectively.

The MLST typing of 10 pneumococcal isolates revealed 9 different ST types, including ST 15139 (capsular type 19A) and ST 15140 (capsular type 23F), which have not previously been reported (Fig. 1). The two ST 9533 isolates belonged to capsular type 11A. Both isolates were resistant to penicillin, erythromycin, clindamycin and tetracycline, but differed in the MIC levels. The MIC of isolate No.1 to penicillin, and tetracycline was > 256 μg/ml and 24 μg/ml, respectively, whilst isolate No.5 was 48 μg/ml and 12 μg/ml, respectively.

Discussion

This study investigated molecular characteristics, antimicrobial resistance patterns and capsular types of S. pneumoniae isolated from blood samples in Tehran, Iran. Of the 44 isolates, 73%, 68% and 16% were resistant to erythromycin, tetracycline and penicillin, respectively which were similar to levels reported for these antibiotics among pneumococci isolated in Iran (71%, 67%, 17%) and the rate of resistance in penicillin (16–28%) and erythromycin (67%) in some Asian countries such as Malaysia and Thailand [18, 19].

In our study, the major mechanism conferring resistance to macrolide antibiotics, was the constitutive phenotype that 43% of isolates showed this resistance and 52% of the isolates harboring ermB gene. In agreement with this finding, other studies in the Turkey, China and Taiwan showed that the ermB gene was the most frequent macrolide resistance determinant. while the most common resistance gene in the Canada and United States was mefA/E gene [20, 21]. In this study the rate of co resistance related to ermB and mefA/E gens was found in 23% of isolates which accordance with a report from Turkey (20%) [20]. As for frequency of double resistance to erythromycin and clindamycin has been spread globally. The high prevalence of dual resistance in S. pneumoniae was found in South Korea and South Africa [5, 21, 22].

The majority of macrolide-resistant strains 75% (24/32) were also resistant to tetracycline. This association is due to the insertion of ermB into composite transposons of the Tn916 family that contain tetM gene [7, 20, 23]. While, the existence of unexpressed tetM genes in tetracycline sensitive isolates

showed that transposons of the Tn916 family may be more widespread in S. pneumonia than expected to firmly associated with resistant tetracycline [23, 24].

This study showed that the most common capsular types detected in 64% of the pneumococcal isolates was 6A/B, 19A, 15A, 23F. These results are partly similar to those from most Asian countries such as Japan (6B, 23F, and 19F), China (14, 19A, 23F) and Turkey (1, 19A, 19F, 6A/6B, 14) [25–27]. Generally, a small number of capsular types (1 6A/B, 23F, 19A, 19F, 7F 9V and 14) are the most prevalent capsular types in IPD isolates [1, 2, 4].

Capsular type 19A is frequent among MDR isolates which has already been described in many non-vaccinated regions such as Korea [28]. Researchers have formerly exhibited that the spread of MDR capsular types 19A isolates is due to antibiotic misuse in developing countries [29]. In our country, irrational use of antibiotics has contributed to the emergence of MDR isolates

In current study, more than 70% (5/7) of capsular type 19A isolates were MDR and showed resistance to erythromycin (majority MIC ≥ 256 μg/ mL), tetracycline (MIC ≥ 8 μg/ mL), clindamycin, and trimethoprim-sulfamethoxazole. One isolate of capsular type 19A in addition of mention antibiotics showed relatively high resistance to penicillin which carried Tn2009. As for, all (6/6) of capsular type 23F isolates and 50% (2/4) of capsular type 11A, 15B/C (1/2) isolates were MDR (high level MIC for erythromycin and tetracycline) as capsular type 19A isolates but 50% of 11A, 15B/C and 23F were resistance penicillin (MIC ≥ 32–48 μg/ mL).

Despite the importance of capsular type as an invasive determinant, other virulence determinants were also associated with invasive isolates [30]. As our results, the majority of the isolates contained the virulence determinants that probably indicate the essential of virulence determinants in the ability of an isolate to cause invasive disease [30]. Interestingly, pspA gene was encoded by the most pneumococcal isolates which only detected in 41% of our isolates. This is in accordance with the study suggesting that probable limitation of detection by conventional PCR and confirmed this hypothesis by a quantitative PCR assay at high level detection [31].

As for capsular type is assumed to be more important than genotype in the ability of an isolate led to invasive disease but also underline the role of genetic background in invasion [30, 32, 33]. Since pneumococcal isolates with diverse MLST profiles have showed various pathogenicity potential [32]. According to the other studies suggesting high capsular type and genetic diversities in IPD isolates [10, 25, 33, 34], there was important diversity among our isolates base on capsular types and different MLST profiles. In pneumococcal isolates, one of the important factor to selective pressure is use of antibiotics [34, 35], so the antibiotic selection pressure may be led to different genetic diversity of IPD isolates that observed in this study [34, 35]. However, causing agents associated with genetic diversity should be further studied.

Reliable and comprehensive data regarding antimicrobial resistance and genetic characteristics, S. pneumoniae are scarce, in Iran. This prompted our research. The main limitations of this study are rather

small number of invasive isolates and the pneumococcal isolates were collected from a few hospitals.

Conclusion

The study revealed that the *S. pneumonia* isolates belonged to diverse capsular types and clones with high rate of resistance to erythromycin, tetracycline, and penicillin.

Methods

Bacterial isolates

A total of 44 invasive pneumococcal isolates were involved in the present study. The isolates were collected from October 2016 to September 2017. Only one isolate was investigated per patient. The organisms were identified to the species level using standard biochemical methods based on typical colony morphology, Gram staining, catalase, hemolysis, and optochin sensitivity testing (Difco, USA). To confirm the identification of the isolate as *S. pneumonia* the *lytA* and *ply* genes were amplified by a PCR, using primers: *LytA-F*, 5'-CGGACTACCGCCTTTATATCG-3'; *lytA*-R, 5'-GTTTCAATCGTCAAGCCGTT-3' [36] and *ply*-F, 5'-ATTTCTGTAACAGCTACCAACGA-3'; *ply*-R, 5'- GAATTCCCTGTCTTTTCAAAGTC-3' [37].

Antibiotic susceptibility determination

Antimicrobial susceptibility testing was performed according to the Clinical Laboratory and Standards Institute (CLSI (guidelines. Disk agar diffusion (DAD) method was performed on Mueller-Hinton agar with 5% defibrinated sheep blood, incubated at 35°C and 5% CO2 for 20–24 hours, and zones of inhibition measured after incubation. All isolates were tested against Oxacillin (1 µg), tetracycline (30 µg), erythromycin (15 µg), levofloxacin (5 µg), chloramphenicol (30 µg), linezolid (30 µg), clindamycin (2 µg), trimethoprim/ sulfamethoxazole (1.25/23.75 µg). All of the antibiotic discs were purchased from Mast Diagnostics Ltd (Merseyside, UK). Minimum inhibitory concentration (MIC) for erythromycin, tetracycline and penicillin were determined with E-test (0.016-256 µg/ml-Liofilchem, Via Scozia, Italy). The MIC was interpreted according to the CLSI breakpoints [38]. MDR was considered as resistance to three or more different classes of antimicrobial. *S. pneumonia* ATCC 49619 was used for quality control strain to ensure the reliability of the results.

Capsular typing

The PCR were performed with capsular specific primers as described by Ahn *et al*. as Table 1[39]. First of all the confirmed pneumococcal isolates were examined for amplification of *cpsA gene* (Table 1) [40].Then, the capsular primers used to detect most common capsular types that were outlined in Table 2 [39].

Detection of Resistance genes, Virulence genes and Transposon profiles

The PCR assays were used to detect the macrolide resistance genes (*ermB* and *mef A/E*), tetracycline resistance genes (*tetM*, *tetO*, *tetL* and *tetK*), virulence genes including autolysin A (*lytA*), pneumolysin (*ply*), pneumococcal surface protein A (*pspA*), and Choline binding protein A (*cbpA)* with primers specific for each gene.

The transposons were detected using PCR assay for Tn916 and Tn917 transposon-related genes including *xis, int, tndX, tnpR* and *tnpA*. The resistance genes related to the different transposons were Tn2009 (*tetM, int, xis, mef*), Tn6002 (*ermB, tetM, int, xis*), Tn3872 (*ermB, tetM, tnpA, tnpR*), Tn2010 (*ermB, tetM, int, xis, mef*), Tn6003/Tn1545 (*ermB, tetM, int, xis, aph3'-III*), Tn6002+ MEGA (macrolide efflux genetic assembly) [10]. All primers are listed in Table 2.

MLST analysis

MLST was performed with selected isolates using the internal fragments of seven housekeeping gene including *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl* as specified by Enright *et al.* [15].

The sequences types (STs) were determined by the comparison with those of corresponding allelic profiles at MLST database (http://pubmlst.org/spneumoniae/). Minimum spanning trees were produced using PHYLOViZ 2.0 software [41].

Statistical analysis

The statistical analysis of the difference in the frequency of the pneumococcal genes was evaluated by using the chi-square and Fisher's as appropriate. The differences less than 0.05 were considered significant statistically.

Abbreviations

Invasive pneumococcal disease (IPD)

Cerebrospinal fluid (CSF)

Macrolide, lincosamide and streptogramin B (MLS_B)

Multilocus sequence typing (MLST)

Multidrug resistant (MDR)

Minimum Inhibitory Concentration (MIC)

Penicillin binding proteins (PBPs)

Polymerase chain reaction (PCR)

Autolysin A (*lytA*)

Pneumolysin (*ply*)

Pneumococcal surface protein A (*pspA*)

Choline binding protein A (*cbpA)*

Macrolide efflux genetic assembly (MEGA)

Sequences type (ST)

Clinical Laboratory and Standards Institute (CLSI)

Disk agar diffusion (DAD)

Clindamycin (CD)

Chloramphenicol (CLR)

Erythromycin (E)

Levofloxacin (Lvo)

Oxacillin (Oxa)

Penicillin (P)

Tetracycline (T)

Trimethoprim/sulfamethoxazole (TS)

Non-determined (ND)

Transposon (Tn)

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Declarations

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Availability of data and materials

Please contact author for data requests.

Authors' contributions

ME, FJ, MMF and FBH designed the study and experiments. MB conducted the experiments, RB drafted the manuscript. ME and MB revised the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Tehran University of Medical Sciences. Consent to participate is not applicable for this study because the isolates included in the study were obtained from existing clinical collections routinely assembled as part of laboratory practices of university hospitals.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest

Tables

Figures

Table 1. Sequences of capsular primers.

Primer	Sequence	Amplicon size (bp)	Reference
23F	5'-GTAACAGTTGCTGTAGAGGGAATTGGCTTTTC-3' 5'-CACAACACCTAACACACGATGGCTATATGATTC-3'	384	[39]
19F	5'-GTTAAGATTGCTGATCGATTAATTGATATCC-3' 5'-GTAATATGTCTTTAGGGCGTTTATGGCGATAG-3'	304	
4	5'-CTGTTACTTGTTCTGGACTCTCGATAATTGG-3' 5'-GCCCACTCCTGTTAAAATCCTACCCGCATTG-3'	430	
6A/B	5'-AATTTGTATTTTATTCATGCCTATATCTGG-3' 5'-TTAGCGGAGATAATTTAAAATGATGACTA-3'	250	
14	5'-CTTGGCGCAGGTGTCAGAATTCCCTCTAC-3' 5'-GCCAAAATACTGACAAAGCTAGAATATAGCC-3'	208	
19A	5'-GTTAGTCCTGTTTTAGATTTATTTGGTGATGT-3' 5'-GAGCAGTCAATAAGATGAGACGATAGTTAG-3'	478	
3	5'-ATGGTGTGATTTCTCCTAGATTGGAAAGTAG-3' 5'-CTTCTCCAATTGCTTACCAAGTGCAATAACG-3'	371	
15A	5'-ATTAGTACAGCTGCTGGAATATCTCTTC-3' 5'-GATCTAGTGAACGTACTATTCCAAAC-3'	434	
15B/C	5'-TTGGAATTTTTTAATTAGTGGCTTACCTA-3' 5'-CATCCGCTTATTAATTGAAGTAATCTGAACC-3'	496	
1	5'-CTCTATAGAATGGAGTATATAAACTATGGTTA-3' 5'-CCAAAGAAAATACTAACATTATCACAATATTGGC-3'	280	
11A	5'-GGACATGTTCAGGTGATTTCCCAATATAGTG-3' 5'-GATTATGAGTGTAATTTATTCCAACTTCTCCC-3'	463	
9V	5'-CTTCGTTAGTTAAAATTCTAAATTTTTCTAAG-3' 5'-GTCCCAATACCAGTCCTTGCAACACAAG-3'	753	
7F	5'-CCTACGGGAGGATATAAAATTATTTTTGAG-3' 5'-CAAATACACCACTATAGGCTGTTGAGACTAAC-3'	826	

Table 2. Sequences of antibiotic resistance and virulence factor genes oligonucleotide primers

Primer	Sequence	Amplicon size (bp)	Reference
lytA	5'-CGGACTACCGCCTTTATATCG-3' 5'-GTTTCAATCGTCAAGCCGTT-3'	229	[36]
ply	5'-ATTTCTGTAACAGCTACCAACGA-3' 5'-GAATTCCCTGTCTTTTCAAAGTC-3'	347	[37]
cpsA	5'-GCAGTACAGCAGTTTGTTGGACTGACC-3' 5'-GAATATTTTCATTATCAGTCCCAGTC-3'	160	[40]
erm(B)	5'-TGGTATTCCAAATGCGTAATG-3' 5'-CTGTGGTATGGCGGGTAAGT-3'	745	[42]
mef(A/E)	5'-AGTATCATTAATCACTAGTGC-3' 5'-TTCTTCTGGTACTAAAAGTGG-3'	346	[11]
tetO	5'-AACTTAGGCATTCTGGCTCAC-3' 5'-TCCCACTGTTCCATATCGTCA-3'	515	[11]
tetL	5'-ATAAATTGTTTCGGGTCGGTAAT-3' 5'-AACCAGCCAACTAATGACAATGAT-3'	1077	[13]
tetK	5'-GTAGCGACAATAGGTAATAGT-3' 5'-GTAGTGACAATAAACCTCCTA-3'	361	[13]
tetM	5'-AGTGGAGCGATTACAGAA-3' 5'-CATATGTCCTGGCGTGTCTA-3'	159	[13]
aphA3	5'-GCCGATGTGGATTGCGAAAA-3' 5'-GCTTGATCCCCAGTAAGTCA-3'	292	[24]
int	5'-GCGTGATTGTATCTCACT-3' 5'-GACGCTCCTGTTGCTTCT-3'	1046	[13]
xis	5'-AAGCAGACTGAGATTCCTA-3' 5'-GCGTCCAATGTATCTATAA-3'	194	[13]
tnpR	5'-CCAAGGAGCTAAAGAGGTCCC-3' 5'-GTCCCGAGTCCCATGGAAGC-3'	1548	[13]
tnpA	5'-GCTTCCATGGGACTCGGGAC-3' 5'-GCTCCCAATTAATAGGAGA-3'	2134	[13]
tndX	5'-ATGATGGGTTGGACAAAGA-3' 5'-CTTTGCTCGATAGGCTCTA-3'	611	[13]
pspA	5'-CATAGACTAGAACAAGAGCTCAAA-3' 5'-CTA CAT TAT TGT TTT CTT CAG CAG-3'	214	[31]
cbpA	5'-GCTAATGTAGCGACTTCAGATCAA-3' 5'-AGCTTGGAAGAGTTTCTTCACCTA-3'	142	[31]

Table 3. Antimicrobial resistance pattern, antibiotic resistance genes, virulence genes, capsular type and sequence type of isolates

Isolate	Resistance		MIC (µg/ml)			Tn	Virulence Factor	Capsular	ST
	Phenotype	genes	P	E	T		Genes	type	
1	CD, E, Oxa, T, TS	*ermB, mefA/E, tetM*	≥256	≥256	24	-	*cbpA, cpsA, lytA*	*11A*	9533
2	Oxa, TS	*tetM*	0.75	ND	ND	-	*cbpA, cpsA, lytA, ply, pspA*	-	-
3	CD, E, Oxa, T, TS	*ermB, tetM*	1.5	≥256	16	-	*cbpA, cpsA, lytA, ply, pspA*	*19A*	15139
4	Oxa, TS	*mefA/E*	0.094	ND	ND	-	*cbpA, cpsA, lytA, ply, pspA*	-	12224
5	CD, E, Oxa, T, TS	*ermB, mefA/E, tetM*	48	≥256	12	-	*cbpA, cpsA, lytA*	*11A*	9533
6	CD, E, Oxa, T, TS	*ermB, mefA/E, tetM*	48	≥256	16	-	*cbpA, cpsA, lytA, ply*	*15B/C*	-
7	CD, E, Oxa, T, TS	*ermB, mefA/E, tetM*	0.19	≥256	8	-	*cbpA, cpsA, lytA, ply, pspA*	*19A*	-
8	CD, CLR, E, Oxa, T, TS	*ermB, mefA/E, tetM*	48	≥256	16	-	*cbpA, cpsA, lytA, ply*	*23F*	15140
9	CD, CLR, E, Oxa, T, TS	*ermB, tetM*	48	≥256	16	-	*cbpA, cpsA, lytA, ply, pspA*	*23F*	-
10	CD, CLR, E, Oxa, T, TS	*ermB, tetM*	1.5	≥256	8	-	*cbpA, cpsA, lytA, ply, pspA*	*23F*	-
11	Oxa, T, TS	*tetM*	1	ND	4	-	*cbpA, cpsA, lytA, ply, pspA*	*15A*	
12	E, Oxa, TS	*mefA/E*	0.125	8	ND	-	*cbpA, cpsA, lytA, ply, pspA*	*14*	-
13	CD, CLR, E, Oxa, T, TS	*ermB, mefA/E, tetM*	32	≥256	24	-	*cbpA, cpsA, lytA, ply, pspA*	*23F*	-
14	E, Oxa, TS	*mefA/E*	1	12	ND	-	*cbpA, cpsA, lytA, ply*	*6A/B*	-
15	Oxa, TS	-	0.38	ND	ND	-	*cbpA, cpsA, lytA, ply*	*6A/B*	-
16	CD, E, Lvo, Oxa, T, TS	*ermB, tetM*	0.5	≥256	8	-	*cbpA, cpsA, lytA, ply*	*19A*	-
17	TS	-	1	ND	ND	-	*cbpA, cpsA, lytA, ply*	*15A*	-
18	CD, CLR, E, Oxa, T, TS	*ermB, tetM*	2	≥256	12	6002	*cbpA, cpsA, lytA, ply*	*23F*	-
19	E, TS, Oxa	*mefA/E*	0.047	2	ND	-	*cbpA, cpsA, lytA, ply, pspA*	*6A/B*	-
20	E, CD, T, Oxa, TS	*ermB, tetM*	0.5	≥256	12	6002	*cbpA, cpsA, lytA*	*15A*	-
21	E, Oxa, TS	*ermB, mefA/E*	0.19	3	ND	-	*cbpA, cpsA, lytA*	*15B/C*	1888
22	CD, E, Oxa, T, TS	*ermB, tetM*	0.5	≥256	8	6002	*cbpA, cpsA, lytA*	*15A*	-
23	Oxa, TS	*tetM*	1.5	ND	ND	-	*cbpA, cpsA, lytA, pspA*	*15A*	-
24	E, Oxa, T, TS	*mefA/E, tetM*	0.5	12	12	2009	*cbpA, cpsA, lytA, ply*	*9V*	-
25	Oxa, T	*tetM*	1.5	ND	8	916	*cbpA, cpsA, lytA, pspA*	*1*	-
26	E, Oxa, T, TS,	*mefA/E, tetM*	16	2	24	2009	*cbpA, cpsA, lytA*	*19A*	1339
27	CD, E, Oxa, T, TS	*ermB, tetM*	0.38	≥256	16	-	*cbpA, cpsA, lytA, ply, pspA*	*14*	-
28	Oxa, TS	-	1	ND	ND	-	*cbpA, cpsA, lytA, ply*	*6A/B*	-
29	CD, CLR, E, Oxa, T, TS	*ermB, tetM*	2	≥256	24	6002	*cbpA, cpsA, lytA, ply*	*23F*	-

30	CD, E, Oxa, T, TS	*ermB, tetM*	1	24	16	-	*cbpA, cpsA, lytA, ply*	*19F*	-
31	E, Oxa, TS	*mefA/E*	0.75	3	ND	-	*cbpA, cpsA, lytA, ply, pspA*	*6A/B*	-
32	CD, E, T, TS	*ermB, tetM*	0.094	4	8	3872	*cbpA, cpsA, lytA, ply*	*19A*	12888
33	CD, E, T, TS	*ermB, tetM*	0.38	12	24	6002	*cbpA, cpsA, lytA, ply*	*6A/B*	-
34	E, Oxa, TS	*mefA/E*	0.75	4	ND	-	*cbpA, cpsA, lytA, ply*	*9V*	-
35	E, Oxa, TS	*mefA/E*	0.094	4	ND	-	*cbpA, cpsA, lytA, ply*	*6A/B*	-
36	Oxa	*mefA/E*	0.75	ND	ND	-	*cbpA, cpsA, lytA*	*19A*	-
37	Oxa, T, TS	*tetM*	0.75	ND	8	916	*cbpA, cpsA, lytA, ply*	-	-
38	Oxa, T, TS	*ermB, tetM*	0.5	ND	4	6002	*cbpA, cpsA, lytA, ply*	*15A*	-
39	Oxa, T, TS	*tetM*	4	ND	8	916	*cbpA, cpsA, lytA, ply*	*19A*	-
40	E, Oxa, T	*ermB, mefA/E, tetM*	1	3	16	1545/6003+MEGA	*cbpA, cpsA, lytA, pspA*	*11A*	-
41	E, Oxa, T	*ermB, mefA/E, tetM*	0.75	2	16	1545/6003+MEGA	*cbpA, cpsA, lytA, pspA*	*11A*	-
42	E, Oxa, T	*mefA/E, tetM*	0.094	3	48	2009	*cbpA, cpsA, lytA, pspA*	*15A*	-
43	CD, E, Oxa, T, TS	*ermB, mefA/E, tetM*	0.75	≥256	6	2010	*cbpA, cpsA, lytA, ply*	*19F*	2533
44	E, Oxa	*mefA/E*	0.19	4	ND	-	*cbpA, cpsA, lytA, ply, pspA*	*6A/B*	1876

CD: clindamycin, CLR: chloramphenicol, E: erythromycin, Lvo: levofloxacin ,Oxa: oxacillin, P: penicillin, T: tetracycline, TS: trimethoprim/sulfamethoxazole, ND: non-determined, Tn: transposon, ST: sequence type

eakpoints: Penicillin: S ≤ 2; I= 4; R ≥ 8, Tetracycline: S ≤ 1; I= 2; R ≥ 4, Erythromycin: S ≤ 0.25; I= 0.5; R ≥ 1

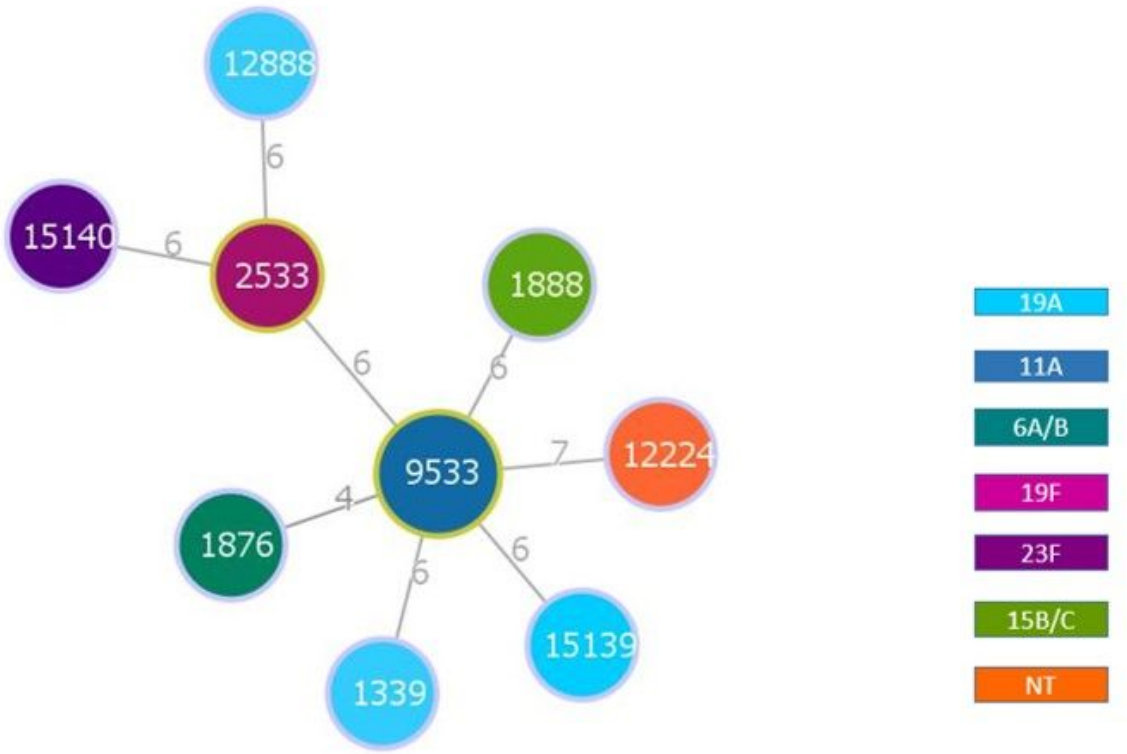

Figure 1

Minimum spanning tree of STs of S. pneumoniae constructed by PHYLOViZ 2.0. Green outlines indicate a group founder; light blue outlines indicate relatedness to founder; the STs are displayed as circles; Numbers indicate the number of differences between the ST profiles of the two connected circles; capsular types are characterized by circles different colors.