Serotype distribution and antibiotic resistance of *Streptococcus pneumoniae* isolates collected from unvaccinated children with pneumonia at a province in central Vietnam

Bui Anh Son1,*, Tang Xuan Hai1†, Tran Van Cuong1, Duong Dinh Chinh2, Thi-Hong-Hanh Le3, Nguyen Manh Dung4, Vu Nhat Dinh5*, Do Ngoc Anh6†

1Department of Pediatrics, Nghe An Obstetrics and Pediatrics Hospital, Nghe An, Vietnam
2Department of Neurology, Nghe An Friendship General Hospital, Nghe An, Vietnam
3Department of Respiratory Diseases, National Pediatrics Hospital, Hanoi, Vietnam
4Department of Scientific Management, 108 Military Centre Hospital, Hanoi, Vietnam
5Department of Trauma and Orthopedic Surgery, 103 Military Hospital, Hanoi, Vietnam
6Department of Laboratory Medicine, 103 Military Hospital, Hanoi, Vietnam
7Department of Medical Parasitology, Vietnam Military Medical University, Hanoi, Vietnam

Received: May 2022, Accepted: July 2022

**ABSTRACT**

**Background and Objectives:** Identification of pneumococcal serotypes and antimicrobial resistance provides helpful information for the use of suitable vaccines and antibiotics; however, very limited data is available on these issues in Vietnam. The present study aimed to find the serotype distribution and drug resistance patterns of *Streptococcus pneumoniae* isolated from unvaccinated children less than 5 years of age with pneumonia at a province in central Vietnam.

**Materials and Methods:** A total of 126 clinical pneumococcal strains isolated from unvaccinated children less than 5 years of age with pneumonia at the Nghe An province, Vietnam between Nov 2019 and Mar 2021. All strains were identified using conventional microbiological method, VITEK® 2 Compact system, specific PCR and sequencing. The serotypes and antimicrobial resistance patterns of pneumococcal strains were determined using the multiplex PCR assays and VITEK® 2 Compact system.

**Results:** The results showed that, eight different pneumococcal serotypes were identified. The most common serotypes were 19F (67.46%), followed by 23F (10.32%), 19A (9.52%), 6A/23B (3.17%), 15A (2.38%), 9V (3.17%), 11A (1.59%) and 14 (0.80%), respectively. More than half of the pneumococcal strains were non-susceptible to penicillin. The resistance rate to ceftriaxone and cefotaxime were 41.3% and 50.8%. The percentage of pneumococci strains resistant to clarithromycin, azithromycin, erythromycin, cotrimoxazole, tetracyclin, and clindamycin were more than 93% of all strains. All pneumococcal serotypes were highly resistant to clarithromycin, azithromycin, erythromycin, cotrimoxazole, and clindamycin.

**Conclusion:** Our findings showed high antibiotic resistance rates of the strains causing pneumococcal pneumonia, mostly macrolide resistance, among unvaccinated children.

**Keywords:** *Streptococcus pneumoniae*; Serotypes; Antibiotic resistance; Children; Pneumonia

1*These authors contributed equally to this work.

2Corresponding author: Do Ngoc Anh, Ph.D, MD, Department of Laboratory Medicine, 103 Military Hospital, Hanoi, Vietnam; Department of Medical Parasitology, Vietnam Military Medical University, Hanoi, Vietnam. Tel: +84-989255773 Fax: +84-36883994 Email: dranhk61@gmail.com; anhdn_vmmu1@vmmu.edu.vn

Copyright © 2022 The Authors. Published by Tehran University of Medical Sciences. This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International license (https://creativecommons.org/licenses/by-nc/4.0). Noncommercial uses of the work are permitted, provided the original work is properly cited.
INTRODUCTION

The bacterium *Streptococcus pneumoniae* (S. *pneumoniae*, pneumococcus) causes pneumococcal disease. This is a Gram-positive, facultative anaerobic bacterium that is an important pathogen causing community pneumonia, sinusitis, otitis media, and invasive infections such as bacteremia and bacterial meningitis, which are the leading causes of morbidity and mortality among children less than 5 years of age (1-3). The occurrence of diseases caused by this etiological agent are a prominent global public health issues (4, 5). According to the study reported by Wahl et al. (2018), diseases caused by *S. pneumoniae* kill 317,300 children under five years of age every year, mostly in lower income countries (6). *S. pneumoniae* also occurs as a cause of invasive infections in elderly persons (7).

Pneumococcus is currently divided into more than ninety serotypes based on the antigenic capsular polysaccharide (CPS) (8). The CPS is an important virulence factor of *S. pneumoniae* and of the pneumococcal serotypes, serotypes 6, containing four confirmed serotypes (6A-6D), are reported to account for the most common serotypes of pneumococcal disease worldwide (5, 9, 10). Epidemiological studies around the world have showed that distribution of pneumococcal serotypes varies by age and geographical area (11-13). According to previous studies, although there are many pneumococcal serotypes, only certain types lead to invasive diseases worldwide such as serotypes 1, 4, 6A/6B, 7F, 9V, 14, 15B/15C, 18C, 19F, 19A, and 23F, which were found to account for about 80-90% of invasive pneumococcal disease (IPD) in children, especially in unvaccinated areas (2, 14, 15). Therefore, the type of pneumococcal vaccines should be selected in accordance with the circulating serotypes in each country (5, 11).

The antibiotic treatment for pneumococcal infections seems to be the primary choice. However, increasing antibiotic drug-resistance of pneumococcus, mainly to β-lactams, has been noted worldwide, especially in Asia, that has made the role of antibiotics is limited (11, 13). This showed that the importance of disease prevention (11). Vaccines have demonstrated to be an effective means of preventing pneumococcal disease worldwide (13). In the US, after the introduction of seven-valent pneumococcal conjugate vaccines (PCV7) to prevent pneumococcal diseases, the rate of IPD has significantly reduced from >200 cases/100 000 persons to >50 cases/100 000 persons (7). To date, 146 member countries out of WHO members have added pneumococcal conjugate vaccine (PCV) into their National Immunization Program (16). Nevertheless, only about 55% (approximately 74 million) of the global infant population are receiving PCV (7).

In addition, antibiotic resistance in *S. pneumoniae* strains is rising in all parts of the world, including Vietnam (17). Thus, an understanding of the serotype distribution and antibiotic resistance patterns of pneumococcus is necessary to guidance for the use of suitable vaccines and antibiotics (3). The aim of this study was to determine the serotypes and patterns of antibiotic resistance of *S. pneumoniae* isolated from unvaccinated children less than 5 years of age with pneumonia at a province in centre Vietnam.

MATERIALS AND METHODS

Bacterial isolates and identification of *S. pneumoniae*. In current study, a total of 126 *S. pneumoniae* clinical isolates were isolated from sputum samples of pneumonia children, aged between 2 and 59 months, at the Nghe An Obstetrics and Pediatrics Hospital (500 beds), Nghe An province, Vietnam, during the period between November 2019 and March 2021. Sputum specimens for each patient were taken by trained nurses using a clean suction and were then transported to the clinical microbiology laboratory within 2 h for isolation of *S. pneumoniae*. All samples were inoculated onto agar plates containing 5% sheep blood (Himedia, India) at 37°C in 5% CO₂ atmosphere for 18-24 h. The samples that no growth on the agar after 24 h were followed up for a further 24 h before being pronounced as negative. Colonies of suspected isolates was taken to identify as *S. pneumoniae* using conventional microbiological method (Gram staining, the alpha hemolysis test, the optochin sensitivity test) in combination with the VITEK® 2 Compact system (bioMérieux, North Carolina 27712, USA) according to the manufacturer’s instructions and PCR analyses using species-specific primers as described previously (1). All isolates were stored at -80°C in cryotubes containing trypicase soy broth (Merck, Germany), 20% glycerol (Merck, Germany) and 10% horse serum for further analysis.

Determination of pneumococcal serotypes. Ge-
nomic DNA of *S. pneumoniae* was extracted from the bacterial cultures using G-spin™ Genomic DNA Extraction Kit (iNtRON Biotechnology, Korea), following the manufacturer’s protocol. First of all, the pneumococcal isolates were confirmed by molecular method using the specific primer pair of *cpsA*-F (5’-GCA GTA CAG CAG TTT GTT GGA CTG ACC-3’) and *cpsA*-R (5’-GAA TAT TTT CAT CAT CAG TCC CAG TC-3’) (Integrated DNA Technologies, USA) for amplification of *cpsA* gene (8). And then, the most common serotypes of *S. pneumoniae* isolates were identified by multiplex PCR (mPCR) using twenty-one capsular specific primer pairs (Table 1) as described in previous reports (1, 8). The capsular types were collected on five groups as follows: Types 14, 19A, 19F and 23F, 6A/B, 9V, 15A and 15B/C; 1, 3, 10A and 11A; 4, 5, 7C and 17F; 7F, 8, 12A, 10 and 23B (Table 1). The mPCR reactions were carried out in 25 μl volumes containing 2 μl of DNA solution, 12.5 μl 2X Master mix (Cat.# M7505, Promega, USA), 0.5 μl of each primer (0.2 μM) and distilled water up to 25 μl. Thermal cycling was performed in Thermo Mastercycler Gradient system (Thermo Fisher Scientific, USA) under the following conditions: 94°C for 5 minutes; followed by 35 cycles at 94°C for 45 seconds, 54°C for 45 seconds and 65°C for 150 seconds; and a final extension of 72°C for 10 minutes. The mPCR products were analyzed on a 2% agarose gel containing 0.5 μg/ml ethidium bromide at 100V for 60 minutes and visualized with UV transillumination (UVP, Canada). The sizes of the mPCR products were determined by 100bp DNA Ladders (Cleaver, UK). Pneumococcal isolates that could not be serotyped by mPCR were classified as non-typeable. Total DNA isolated from *S. pneumoniae* strain ATCC 49619 were used for quality control.

**16S rRNA gene sequencing.** Two PCR primers, namely 27F (5’-AGA GTT TGA TCC TGG CTC AG-3’) and 1492R (5’-GTT TAC CTT GTA AGC ACT T-3’), were chosen to amplify the 16S rDNA gene (18). PCR products of 16S ribosomal RNA genes from twenty-two isolates were randomly selected and were sent to Apical Scientific Sdn. Bhd (Selangor, Malaysia) for purification and automatic DNA sequencing, using the same primer pair. Species confirmed *S. pneumoniae* isolates were accurately examined by two-directional sequencing. The 16S rRNA gene sequences of these strains were deposited in the DDBJ/EMBL/GenBank databases under accession number MW672550-MW672562 and MZ007491-MZ007499, respectively.

**Antimicrobial susceptibility testing.** The antimicrobial susceptibility tests of each isolate with penicillin (PEN, 0.0625-8.0 μg/mL), cefotaxim (CXM, 0.125-8.0 μg/mL), ceftiraxone (CEF, 0.125-8 μg/mL), chloramphenicol (CLP, 1.0-16.0 μg/mL), azithromycin (AZM, 0.125-8.0 μg/mL), clarithromycin (CLA, 0.25-16.0 μg/mL), erythromycin (ERY, 0.125-8.0 μg/mL), clindamycin (CLI, 0.25-1.0 μg/mL), levofloxacin (LEV, 0.25-16.0 μg/mL), linezolid (LIN, 2.0-8.0 μg/mL), moxifloxacin (MXF, 0.0625-4.0 μg/mL), rifampicin (RIF, 0.0625-4.0 μg/mL), tetracyclin (TET, 0.25-16.0 μg/mL), vancomycin (VAN, 0.125-8.0 μg/mL) and trimethoprim-sulfamethoxazole (SXT, 10.0-320.0 μg/mL) were performed for each strain using VITEK® 2 Compact system according the manufacturer’s instructions. The breakpoints used for *S. pneumoniae* were classified in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2020 criteria. For quality control of the susceptibility tests, *S. pneumoniae* ATCC 49619 was chosen as the reference strain.

**Statistical analysis.** The statistical analysis was carried out by IBM SPSS Statistics software, version 20.0 developed by IBM Corp. (Armonk, NY, USA). Chi-squared and Fisher’s exact tests were performed to check the significance of the data. P values less than 0.05 were considered significant statistically. The 16S gene sequences of pneumococcal isolates were compared to the publicly available DNA sequences in the Genbank databases, using BLAST programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Ethics approval and consent to participate.** The purpose and benefits of the current study were informed to parents/legal guardians of each participant who also signed a written informed consent before the study procedure was performed. The study protocols was approved by the Ethical Committee of the National Institute of Malariaiology, Parasitology and Entomology (Ha Noi, Vietnam) in March 2018 (ethics code: 225/QD-VSR). Furthermore, this study is based on the Declaration of Helsinki Principles.

**RESULTS**

All of the 126 individual isolates which were *S.
| Reaction | Serotype/Primer | Primer sequence (5'-3') | Product size (bp) |
|----------|----------------|-------------------------|------------------|
| 1        | 14-F           | CTT GGC GCA GTG GTC AGA ATT CCC TCT AC | 208              |
|          | 14-R           | GCC AAA ATA CTG ACA AAG CTA GAA TAT AGC C |                |
|          | 19A-F          | GTT AGT CCT GTT TTA GAT TTA TTT GTT GAT GT | 478              |
|          | 19A-R          | GAG CAG TCA ATA AGA TGA GAC GAT AGT TAG |                |
|          | 19F-F          | GTT AAG ATT GCT GAT CTA TTA ATT GAT ATC C | 304              |
|          | 19F-R          | GTA ATA TGT CTT TAG GCC GTT TAT GCC GAT AG |                |
|          | 23F-F          | GTA ACA GTT GCT GTA GAG GGA ATT GGC TTT TC | 384              |
|          | 23F-R          | CAC AAC ACC TAA CAC ACC ATG GCT ATA TGA TTC |                |
| 2        | 6A/B-R         | AAT TTG TAT TTT ATT CAT GCC TAT ATC TGG | 250              |
|          | 9V-F           | CTT CGT TAG TTA AAA TTC TCA ATT TTT CTA A | 753              |
|          | 9V-R           | GTC CCA GTA CCA GTT CCT GTA GCA ACA CAA G |                |
|          | 15A-F          | ATT AGT ACA GCT GCT GGA AAT TCT TTT C | 436              |
|          | 15A-R          | GAT CTA GTG AAC GTA TTA TTC CAA AC |                |
|          | 15B/C-F        | TTG GAA TTG TTT ATT TAG TGG CTT ACC TA | 496              |
|          | 15B/C-R        | CAT CCG CCT ATT AAT TGA AGT AAT CTG AAC C |                |
| 3        | 1-F            | CTC TAT AGA ATG GAG TAT ATA AAC TAT GGT TA | 280              |
|          | 1-R            | CCAAGAAGAAATACATACAGTTACAACATGGAATTTGCG |                |
|          | 3-F            | ATG GTG TGA TTT CTC CTA GAT TGG AAA GTA G | 371              |
|          | 3-R            | CTT CTC TAA CTT TCT ACC AAC AGG TGG AAC AAT G |                |
|          | 10A-F          | GGT GTA GAT TTA CCA TTA GTG TCG GCA GAC | 628              |
|          | 10A-R          | GAA TTT CCT CTT TAA GAT TCG GAT ATT TTC C |                |
|          | 11A-F          | GGA CAT GTT CAG GTG ATT TTC CAA TAT AGT G | 463              |
|          | 11A-R          | GAT TAT GAG TGG AAT TTA TTC CAA CTT CTC CC |                |
| 4        | 4-F            | CTT TTA CTT GTT GTG GAC TCT CGA TAA TTG G | 430              |
|          | 4-R            | GCC CAC TCC TGT TAA AAT CCT ACC CGC ATT G |                |
|          | 7F-R           | CAA TTA CCA CAC ATG TTG TTG AGA CTA AC | 826              |
|          | 7F-R           | CAA ATA CAC CAC TAT AGG CTG TTG AGA CTA AC |                |
|          | 8-F            | GAT GCC ATG AAT CAA GCA GTG GTC ATA AAT C | 294              |
|          | 8-R            | ATC TCT GTG TAT AAT TTG AGG TAT GGC ACC |                |
|          | 12A-F          | ACT CTG TTA AAT TGT TAT GCT TTT ATT GAT TC | 656              |
|          | 12A-R          | ATG AAT GAG AAA AGG AAC TTA AAA TCT ATC GCA |                |
|          | 20-F           | GAG CAA GAG TTT TTC ACC TAG CAG CGA GAA G | 514              |
|          | 20-R           | CTA AAT TCC TGT AAT TTA GCT AAA ACT CTT ATC |                |
|          | 23B-F          | TTG TTA GTG GTA TTA AAT TGG GGA CTA CTA GAG | 216              |
|          | 23B-R          | ATA CCT ATC TCA AGT GTT ATT AAC CCA CCA AC |                |
|          | cpsA-F         | GCA GTA CAG CAG TTT GTT GGA CTG ACC | 160              |
|          | cpsA-R         | GAA TAT TTT CAT TAT CAG TCC CAG TC |                |

Table 1. Primers used to confirm and identify serotypes of *S. pneumoniae*
pneumoniae culture-positive also indicated a positive PCR result for the cpsA gene (Fig. 1). 22 sequences of the 16S rDNA regions of different pneumococcal isolates were also deposited in the NCBI database under accession number MW672550-MW672562 and MZ007491-MZ007499, respectively.

Fig. 1. Gel electrophoresis of S. pneumoniae-specific PCR products targeting the 160 bp cpsA gene
Lane 1: DNA Ladder 100 bp Standard; lane 2: negative control; lanes 3-7 (strain Sp8107, Sp8279, Sp8281, Sp8294, and Sp8298): clinical samples; lane 8: positive control

By multiplex PCR assays, of the 126 S. pneumoniae isolates analyzed, 124 strains (98.41%) could be serotyped, of which the eight different pneumococcal serotypes were classified. Only one serotype per patient was detected. The remaining 2 isolates (1.59%) could not be serotyped. The serotype distribution is shown in Figs 2 and 3.

Pneumococcal serotype distribution varied between age groups (Fig. 4), but the difference was not statistically significant between the two groups (p > 0.05).

Table 2 showed the trends of antimicrobial resistance patterns of S. pneumoniae strains. Accordingly, the observed resistance rates of 126 S. pneumoniae isolates to CLA, AZM, SXT, TET, CLI, ERY, CEF, and CXM were high, i.e. 100% (126), 100% (126), 93.7% (118), 96% (121), 96% (121), 99.2% (125), 41.3% (52), and 50.8 (64), respectively. This bacteria showed 100% susceptibility to the RIF, CLP, VAN, LIN and MZX. The susceptibility rates of pneumococcus to levofloxacin were 97.6%.

Antimicrobial resistance of pneumococcus among different serotypes is shown in the Table 2. These results indicated that the observed resistance rates of serotype 19F were highest. All of 8 serotypes exhibited high rates of resistance to macrolides, tetracyclin and clindamycin.

DISCUSSION

Invasive pneumococcal disease is known to be a major cause of morbidity and mortality among children under five years of age, especially those under 2 years of age, although preventive actions have been implemented in many countries (1, 19). According to the previous studies, the distribution of pneumococcal serotypes, which plays an important role in

http://ijm.tums.ac.ir
IRAN. J. MICROBIOL. Volume 14 Number 5 (October 2022) 653-661
the cause of invasive infections, varies in different geographical area (11-13). Thus, routine screening for local serotype distribution of *S. pneumoniae* was necessary to inform the developing a safe and effective vaccine and guidance for the use of appropriate antibiotics (3). According to the WHO, universal vaccination is the best way against pneumococcal disease (2).

The epidemiological data from around the world have indicated that serotypes 1, 4, 6A/B, 7F, 9V, 14, 15B/C, 18C, 19F, 19A, and 23F represent around 80–90% of IPD in children, especially in unvaccinated areas (2, 14, 15). The current study detected pneumococcal serotypes 6A/B, 9V, 11A, 14, 15A, 19F, 19A, and 23F among unvaccinated children under five years of age in Nghệ An province. The three major
serotypes 19F, 19A, and 23, were found to account for about 90% of S. pneumoniae isolates, while serotypes 6A/B, 9V, 11A, 14, and 15A were represented in lower percentages, ranging from 0.80 to 3.17%. Notably, serotype 19A has a rather high prevalence. The serotype distribution in this study was quite similar to those found previous studies in southern Vietnam, ASEAN countries, and Taiwan (20-24). Serotypes 6B, 23F and 19F are the most prevalent among children in Japan, while serotypes 1, 5, 6ABC, and 19F predominate in Egypt (25, 26). Serotype 14 was most common among strains from Paulo, Brazil and Casablance, Morocco (27, 28). Serotypes 23F, 14 and 3 are the most common in Tehran, Iran (11). In China, several studies have demonstrated the distribution of serotypes of S. pneumoniae varies between cities and different years (3, 29, 30). The results from different studies indicate that the prevalence of pneumococcal serotypes varies different depending on the population, region, and change over time (29, 31, 32). Thus, additional investigations should be carried out to identify the pneumococcal serotypes in different regions of Vietnam to provide information for the development of appropriate vaccines.

The emergence of resistance to antibiotics in pneumococci is increasing and it is becoming increasingly predictive factor since it is directly related to persistent disease or disease mortality (3, 33, 34). The results of this study indicated that antimicrobial resistance patterns of S. pneumoniae is a matter of great concern. The resistance rates of S. pneumoniae isolates to AZM, CLA, CLI, ERY, SXT, and TET were higher than 96%. The previous studies in China, Taiwan and other Asian countries, including Vietnam also indicated bad in vitro activity of macrolides (ERY, CLA and AZM), lincosamide (CLI), tetracyclines (TET), and SXT against S. pneumoniae isolates (3, 23, 24). Our results suggested that these antibiotics are not appropriate for the treatment of pneumococcal disease in Vietnam. Besides, the rates of decreased susceptibility to penicillins and cephalosporins showed a rising trend. This result is in agreement with previous studies conducted in China, Taiwan and Vietnam, where more than 50% of patients infected with non-susceptible to penicillins and cephalosporins (3, 24, 29, 30, 35). In our study, all the S. pneumoniae strains were susceptible to RIF, CLP, VAN, MXF and LIN. Our findings also indicated the prevalence of LEV resistance in S. pneumoniae isolates were low (2.4%). The results of current study have shown that RIF, CLP, VAN, MXF, LIN and LEV may provide an opportunity for treating β-lactam, macrolides, lincosamide, tetracyclines, and cotrimoxazole-resistant pneumococcal disease in Vietnam.

In the present study, the prevalence of multidrug resistance of all eight serotypes were 100%. This rate was higher in the current research than previously findings in southern Vietnam (20-22). Notably, the high rates of antimicrobial resistance of serotype 19A were observed. This serotype is not covered by the PCV-7, thus the use of these vaccines may not be effective in preventing pneumococcal disease (3, 11). Therefore, PCV-13 should be recommended for future vaccination in Nghe An because of its broader serotype coverage.

CONCLUSION

In the current study, eight different pneumococcal serotypes were identified in Nghe An, Vietnam. Among that, 19F, 23F and 19A were the most prevalent serotypes. The high frequency of serotype 19A was a notable characteristic. In addition, the rate of antibiotic resistance of S. pneumoniae is considerable. Cautious use of antibiotics is extremely important and necessary to prevent the appearance of resistant pneumococci.

ACKNOWLEDGEMENTS

This work was partially supported by the Department of Science and Technology of Nghe An province, Vietnam, (grant no. 901/HD-SKHCN).

REFERENCES

1. Ahn JG, Choi SY, Kim DS, Kim KH. Enhanced detection and serotyping of Streptococcus pneumoniae using multiplex polymerase chain reaction. Korean J Pediatr 2012; 55: 424-429.
2. Houri H, Tabatabaei SR, Saee Y, Fallah F, Rahbar M, Karimi A. Distribution of capsular types and drug resistance patterns of invasive pediatric Streptococcus pneumoniae isolates in Teheran, Iran. Int J Infect Dis 2017; 57: 21-26.
3. Liu C, Xiong X, Xu W, Sun J, Wang L, Li J. Serotypes
and patterns of antibiotic resistance in strains causing invasive pneumococcal disease in children less than 5 years of age. PLoS One 2013; 8(1): e54254.
4. Van de Vooren K, Duranti S, Curto A, Garattini L. Cost effectiveness of the new Pneumococcal vaccines: A systematic review of European studies. Pharmacoeconomics 2014; 52: 29-45.
5. Shi W, Zhou K, Yuan L, Meng Q, Dong F, Gao W, et al. Serotype distribution, antibiotic resistance patterns and molecular characteristics of serogroup 6 Streptococcus pneumoniae isolates collected from Chinese children before the introduction of PCV13. J Glob Antimicrob Resist 2018; 14: 23-28.
6. Wahl B, O’Brien KL, Greenbaum A, Majumder A, Liu L, Chu Y, et al. Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. Lancet Glob Health 2018; 6(7): e744-e757.
7. International Vaccine Access Center. Johns Hopkins Bloomberg School of Public Health. VIEW-hub report: Global vaccine introduction and implementation, March 2019.
8. Beheshti M, Jabalameli F, Feizabadi MM, Hahsemi FB, Beigverdi R, Emaneini M. Molecular characterization, antibiotic resistance pattern and capsular types of invasive Streptococcus pneumoniae isolated from clinical samples in Tehran, Iran. BMC Microbiol 2020; 20: 167.
9. Wang J, Liu F, Ao P, Li X, Zheng H, Wu D, et al. Detection of serotype distribution and drug resistance of Streptococcus Pneumoniae isolated from pediatric patients. Lab Med 2017; 48: 39-45.
10. Huang S, Liu X, Lao W, Zeng S, Liang H, Zhong R, et al. Serotype distribution and antibiotic resistance of Streptococcus pneumoniae isolates collected at a Chinese hospital from 2011 to 2013. BMC Infect Dis 2015; 15: 312.
11. Habibi Gahfarokhi S, Mosadegh M, Ahmadi A, Pourmand MR, Azarsa M, Rahbar M, et al. Serotype distribution and antibiotic susceptibility of Streptococcus pneumoniae isolates in Tehran, Iran: A surveillance study. Infect Drug Resist 2020; 13: 333-340.
12. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. Lancet Infect Dis 2005; 5: 83-93.
13. Zhao C, Li Z, Zhang F, Zhang X, Li P, Zeng J, et al. Serotype distribution and antibiotic resistance of Streptococcus pneumoniae isolates from 17 Chinese cities from 2011 to 2016. BMC Infect Dis 2017; 17: 804.
14. Song JY, Nahm MH, Moseley MA. Clinical implications of pneumococcal serotypes: invasive disease potential, clinical presentations, and antibiotic resistance. J Korean Med Sci 2013; 28: 4-15.
15. Nuorti JP, Whitney CG. Prevention of pneumococcal disease among infants and children - use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine - recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2010; 59: 1-18.
16. International Vaccine Access Center. Johns Hopkins Bloomberg School of Public Health. VIEW-hub Report: Global Vaccine Introduction and Implementation, March 2020.
17. Reinert RR. The antimicrobial resistance profile of Streptococcus pneumoniae. Clin Microbiol Infect 2009; 15 Suppl 3: 7-11.
18. Miller CS, Handley KM, Wrighton KC, Frischkorn KR, Thomas BC, Banfield JF. Short-read assembly of full-length 16S amplicons reveals bacterial diversity in subsurface sediments. PLoS One 2013; 8(2): e56018.
19. Tan TQ. Pediatric invasive pneumococcal disease in the United States in the era of pneumococcal conjugate vaccines. Clin Microbiol Rev 2012; 25: 409-419.
20. Vo TT, Phan T, Ngo HTM, Pham H, Ho T. Antibiotic susceptibility of invasive Streptococcus pneumoniae isolates in southern Vietnam. Int J Infect Dis 2020; 101: 53-54.
21. Parry CM, Diep TS, Wain J, Hoa NT, Gainsborough M, Nga D, et al. Nasal carriage in Vietnamese children of Streptococcus pneumoniae resistant to multiple antimicrobial agents. Antimicrob Agents Chemother 2000; 44: 484-488.
22. Parry CM, Duong NM, Zhou J, Mai NTH, Diep TS, Thinh LQ, et al. Emergence in Vietnam of Streptococcus pneumoniae resistant to multiple antimicrobial agents as a result of dissemination of the multiresistant Spain(23F)-1 clone. Antimicrob Agents Chemother 2002; 46: 3512-3517.
23. Jaunenkaite E, Jefferies JM, Hibberd ML, Clarke SC. Prevalence of Streptococcus pneumoniae serotypes causing invasive and non-invasive disease in South East Asia: A review. Vaccine 2012; 30: 3503-3514.
24. Wu CJ, Lai JF, Huang IW, Shiau YR, Wang HY, Laiderdale TL. Serotype distribution and antimicrobial susceptibility of Streptococcus pneumoniae in pre- and post- PCV7/13 Eras, Taiwan, 2002–2018. Front Microbiol 2020; 11: 557404.
25. Sakata H. Invasive pneumococcal diseases in children in Hokkaido, Japan from April 2000, to March 2015. J Infect Chemother 2016; 2: 22-26.
26. El-Kholy A, Badawy M, Gad M, Soliman M. Serotypes and antimicrobial susceptibility of nasopharyngeal isolates of Streptococcus pneumoniae from children less than 5 years old in Egypt. Infect Drug Resist 2020; 13: 3669-3677.
27. Medeiros MIC, Almeida SCG, Guerra MLS, Da Silva P, Carneiro AMM, De Andrade D. Distribution of
Streptococcus pneumoniae serotypes in the northeast macro-region of São Paulo state/Brazil after the introduction of conjugate vaccine. *BMC Infect Dis* 2017; 17: 590.

28. Diawara I, Zerouali K, Katfy K, Zaki B, Belabbes H, Najib J, et al. Invasive pneumococcal disease among children younger than 5 years of age before and after introduction of pneumococcal conjugate vaccine in Casablanca, Morocco. *Int J Infect Dis* 2015; 40: 95-101.

29. Pan F, Han L, Huang W, Tang J, Xiao S, Wang C, et al. Serotype distribution, antimicrobial susceptibility, and molecular epidemiology of *Streptococcus pneumoniae* isolated from children in Shanghai, China. *PLoS One* 2015; 10(11): e0142892.

30. Yu Y-Y, Xie X-H, Ren L, Deng Y, Gao Y, Zhang Y, et al. Epidemiological characteristics of nasopharyngeal *Streptococcus pneumoniae* strains among children with pneumonia in Chongqing, China. *Sci Rep* 2019; 9: 3324.

31. Johnson HL, Deloria-Knoll M, Levine OS, Stoszek SK, Freimanis Hance L, Reithinger R, et al. Systematic evaluation of serotypes causing invasive Pneumococcal disease among children under five: the Pneumococcal global serotype project. *PLoS Med* 2010; 7(10): e1000348.

32. Ktari S, Jmal I, Mroua M, Maalej S, Ben Ayed NE, Mnif B, et al. Serotype distribution and antibiotic susceptibility of *Streptococcus pneumoniae* strains in the south of Tunisia: A five-year study (2012–2016) of pediatric and adult populations. *Int J Infect Dis* 2017; 65: 110-115.

33. McGee L, McDougal L, Zhou J, Spratt BG, Tenover FC, George R, et al. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. *J Clin Microbiol* 2001; 39: 2565-2571.

34. Lonks JR, Garau J, Gomez L, Xercavins M, De Echagüen AO, Gareen IF, et al. Failure of macrolide antibiotic treatment in patients with bacteremia due to erythromycin-resistant *Streptococcus pneumoniae*. *Clin Infect Dis* 2002; 35: 556-564.

35. Kim SH, Song J-H, Chung DR, Thamlikitkul V, Yang Y, Wang H, et al. Changing trends in antimicrobial resistance and serotypes of *Streptococcus pneumoniae* isolates in Asian countries: an Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Antimicrob Agents Chemother* 2012; 56: 1418-1426.