# Serotype prevalence and antibiotic susceptibility patterns of pneumococcal isolates in Zunyi city, China

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## ABSTRACT

**الأهداف**: لتقييم توزيع النمط المصلي و قابلية المضادة الميكروبات للمكورات الرئوية المعزولة من المرضى الداخليين من جميع الأعمار المشتبه وجود التهابات بكتيرية.

**الطريقة**: أجريت هذه الدراسة بأثر رجعي، وجمعت عزلات المكورات الرئوية على التوالي من المستشفى الثالث التابع لجامعة زونيي الطبية، في مدينة زوني، الصين، خلال الفترة من يناير 2014م وديسمبر 2016م. تم تحديد المكورات الرئوية وذلك باستخدام المقايسات الميكروبيولوجية الروتينية. أجرينا تحليلات الحساسية المضادة للميكروبات باستخدام البكتيريا تحديد / قابلية نظام VITEK2 والاختبارات الإلكترونية. تم تحديد أنواع المحليات من جميع العزلات بواسطة تفاعل متعدد البلمرة المتسلسلة.

**النتائج**: حددنا 778 عزلات المكورات الرئوية. كانت السلالات 19F، 6A / 6B، 19A، 23F، 15B / 15C السلالات الأكثر انتشارا، وهو ما يمثل 71.5% (556/778) من جميع العزلات. وتشير البيانات إلى أن 409 (70.4%) من العزلات يمكن تغطيتها من لقاح PCV13 لدى الأطفال الذين تقل أعمارهم عن سنتين. وبصرف النظر عن الأنماط المصلية، كانت العزلات 747 (96%) حساسة للبنسلين، في حين أن 720 إلى 778 (90% إلى 100%) عزلات لم تكن عرضة للإريثروميسين، التتراسيكلين، والميثوبريم / سلفاميثوكسازول. بالنسبة للعزلات المقاومة للبنسلين، سيفترياكسون، سيفوتاكسيم، و ميروبينيم، 22 إلى 39 (70% إلى 81.25%) سلالات تنتمي إلى الأنماط المصلية PCV13.

**الخاتمة**: لقد وجدنا زيادة كبيرة في العدد السنوي لعزل المكورات الرئوية منذ عام 2014م. وكان الأثر النظري لل PCV13 عالية في الأطفال أقل من سنتين، والبنسلين قد تكون فعالة ضد الالتهابات الرئوية في هذه المنطقة.

**Objectives:** To assess the serotype distribution and antimicrobial susceptibility of pneumococci isolated from inpatients of all ages suspected of having bacterial infections.

**Methods:** In this retrospective study, pneumococcal isolates were consecutively collected from the Third Affiliated Hospital of Zunyi Medical University, in Zunyi city, China, between January 2014 and December 2016. Pneumococci were identified using routine microbiological assays. We performed antimicrobial susceptibility analyses using the bacteria identification/ susceptibility system VITEK2 and E-tests. Capsular types of all isolates were determined by multiplex polymerase chain reaction.

**Results:** We identified 778 pneumococcal isolates. Serotypes 19F, 6A/6B, 19A, 23F, and 15B/15C were the most prevalent strains, accounting for 71.5% (556/778) of all isolates. Data show that 409 (70.4%) isolates could be covered by the PCV13 vaccine in children less than 2 years old. Irrespective of serotypes, 747 (96%) isolates were sensitive to penicillin, while 720 to 778 (90% to 100%) isolates were not susceptible to erythromycin, tetracycline, and trimethoprim/sulfamethoxazole. For isolates resistant to penicillin, ceftriaxone, cefotaxime, and meropenem, 22 to 39 (70% to 81.25%) strains belonged to PCV13 serotypes.

**Conclusion**: We found a substantial increase in the annual number of pneumococcal isolates since 2014. The theoretical impact of PCV13 was high in children less than 2 years old, and penicillin might be effective against pneumococcal infections in this region.

***Saudi Med J 2017; Vol. 38 (12): 1243-1249***
***doi: 10.15537/smj.2017.12.21090***

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*Received 27th August 2017. Accepted 8th November 2017.*

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*Streptococcus pneumoniae (S. pneumoniae)* remains an important gram-positive pathogen in children <5 years and adults >65 years of age worldwide. This bacterium commonly causes pneumococcal-related diseases such as pneumonia, meningitis, and bacteremia. An epidemiological report in 2012 documented a death rate of at least 400,000/year for children less than 6 years old who die from invasive pneumococcal disease worldwide.[1] Capsular polysaccharide (CPS) is a protective substance for pneumococci and constitutes the outermost layer of the bacterial body.[2] The CPS itself is nontoxic and non-inflammatory; however, by mainly interfering with leukocyte phagocytosis, it helps pneumococci survive inside the host.[2,3] So far, more than 90 immunologically distinct serotypes have been reported.[4] However, it was observed that only a small number of serotypes were related to most pneumococcal infections,[5,6] and the prevalence may vary geographically and temporally. China is a country with a high prevalence of pneumococcal infections.[5] However, epidemiologic studies of pneumococcal infections have mainly focused on some big and well-developed cities including Shanghai,[7] Beijing,[8] and HongKong.[9] There is limited serotype surveillance data for *S. pneumoniae* in rural or less-developed regions.

Zunyi is an important city in Guizhou province located in the southwest of China.[10] Zunyi city is comprised of 15 districts, with a total land area of 30,762 square kilometers and having a population of approximately 8 million people. Compared to other developed regions in China, the people here have a relatively lower socioeconomic status. They were seldom vaccinated with pneumococcal vaccines, and there is still no local epidemiology and antimicrobial susceptibility information for pneumococci present in this region. The 13-valent pneumococcal polysaccharide conjugate vaccine (PCV13) is currently the only conjugate vaccine approved by the China Food and Drug Administration for use in children in 2016, which has so far not been used in this region. This vaccine is an updated version of the PCV7 (which covers serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) and includes 6 additional capsular polysaccharide antigens (serotypes 1, 3, 5, 6A, 7F, and 19A). Thus, we used clinical pneumococcal isolates, which were isolated from several clinical specimens, mostly sputum, of inpatients suspected of having a pneumococcal infection at the Third Affiliated Hospital of Zunyi Medical University to evaluate the serotype prevalence and to estimate the impact of the PCV13 vaccine. In addition, we characterized the antimicrobial susceptibility patterns of the identified *S. pneumoniae* strains.

**Disclosure**. Authors have no conflict of interests, and the work was not supported or funded by any drug company. This project was supported by the Scientific Project of Health and Family Planning Commission of Guizhou Province (#gzwjkj2014-2-153), "15851 Talents Elite Project" of Zunyi city, Innovation Talent Team of Science And Technology Jointly provided by Zunyi city and the First People's Hospital # 17, 2015) and the Talent Team Project provided by the Guizhou Province and Zunyi city, China (Grant #4018, 2015).

## Methods.

***Ethics statement.*** This study was approved by the Ethics Committee of the Third Affiliated Hospital of Zunyi Medical University (also named the First People's Hospital of Zunyi; Reference number: 2014-20). The testing results were extracted from the laboratory information system. All patients or their guardians (for children) signed a written consent regarding the use of their samples and basic information when they were hospitalized. Our medical practices fully complied with the Declaration of Helsinki.

***Population and bacterial isolates.*** In this retrospective study, pneumococcal isolates were consecutively identified from inpatients suspected of having a bacterial infection that attended the third affiliated hospital of Zunyi Medical University, China, between January 2014 and December 2016. Sputum, blood, fiber bronchoscope lavage (only obtained from adult patients), and secretions (including wound and ear canal secretions) were the main specimen sources. Only qualified sputum specimens were acceptable, which was defined as a specimen having greater than 25 white blood cells, and the number of epithelial cells being less than 10 or greater than 25 for a sputum smear observed under low magnification.

Pneumococci were isolated and identified by routine microbiological methods. Blood specimens were inoculated directly into blood culture bottles after blood drawing and cultured in BAcT/ALERT 3D; other specimens were collected in a sterile container, sent to the laboratory within 2 hours, and plated onto agar plates supplemented with 5% sheep red blood cells. These plates were cultured at 37°C in a 5% $CO_2$ atmosphere for 18-24 hours. Suspected pneumococcal colonies were preliminarily identified according to the presence of α-hemolysis and gram-positive diplococci, and further confirmed by the optochin sensitivity test (Oxoid, Basingstok, UK), the bile solubility test (Shenggong, Shanghai), and an automatic bacterial

identification system (VITEK 2). Several isolates of the same type from the same patient were included only once. All isolates were stored at -80°C in 10% sterilized glycerol for capsular type determination. Capsular type determination and vaccine coverage. Capsular types were determined by multiplex polymerase chain reaction (MP-PCR), which has been well-established in several reports.[11-14] Capsular types that could not be determined by MP-PCR were defined as un-typeable (UT). The theoretical impact of 2 protein conjugate vaccines PCV7 and PCV13 were calculated in children less than 2 years old.

*Antimicrobial susceptibility.* Antimicrobial susceptibility tests were performed on the automatic bacteria identification/susceptibility system VITEK 2 (BioMérieux). Judgment of the pneumococcal susceptibility to antibiotics was based on guidelines provided by the Clinical and Laboratory Standards Institute (CLSI).[15] For the penicillin susceptibility breakpoint, we used a minimum inhibitory concentration (MIC) range of 2.0-4.0 μg/mL to define penicillin-intermediate *S. pneumoniae* (PISP), and >8.0 μg/mL to define penicillin-resistant *S. pneumoniae* (PRSP). In this study, the quality control strain was the *S. pneumoniae* ATCC49619.

*Statistical analysis.* Numeration data were reported by positive rate and analyzed using the $\chi^2$ test or Fisher's exact test. We did the statistical analysis using the Statistical Package for Social Sciences software for Windows version 19 (SPSS Inc., Chicago, IL, USA). A $p<0.05$ was regarded as statistically significant.

## Results.

*General properties of pneumococcal isolates.* Between 2014-2016, 778 pneumococcal strains were identified. Of them, 137 strains were identified in 2014, 319 strains were identified in 2015, and 322 strains were identified in 2016. Among the 778 strains, 540 strains (69.4%) were isolated from males. The specimen sources were sputum (756, 97.2%), blood (12, 1.5%), and fiber bronchoscope lavage (5, 0.64%). The remaining 5 samples were from ear canal secretions (2, 0.3%), wound secretions (2, 0.3%), and arthroedema (1, 0.1%).

*Serotype frequency.* Of the 778 isolates, 664 (85.3%) strains were successfully typed, and the remaining 114 (14.7%) strains were defined as UT by the method used. Capsular types 19F, 6A/6B, 19A, 23F, and 15B/15C were the most commonly isolated pneumococcal types, accounting for 71.5% (556) of all isolates (**Figure 1**). Serotype 19F was the most common serotype, which

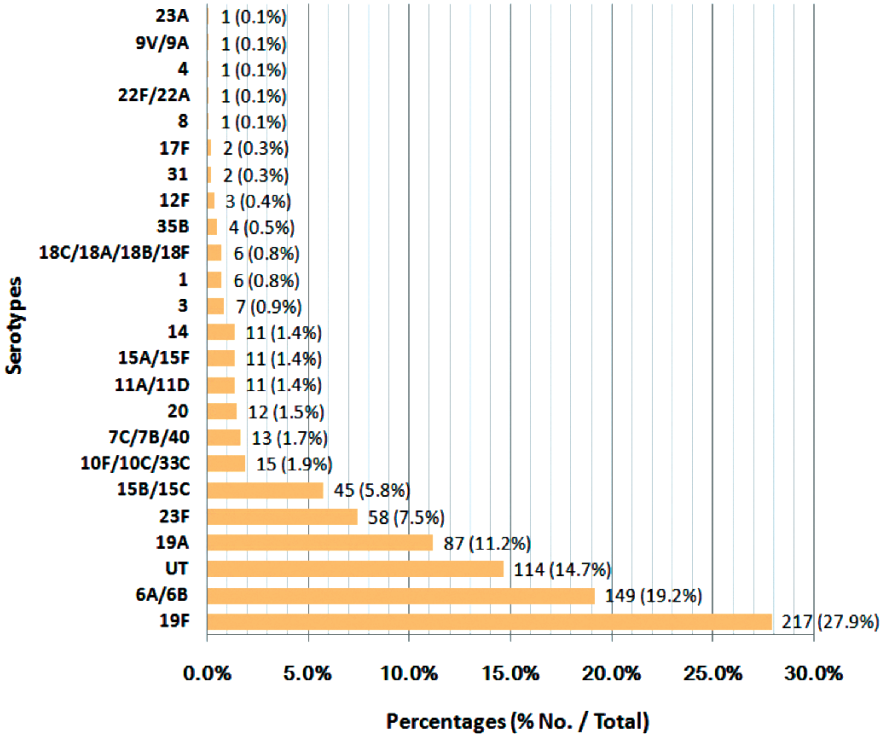


**Figure 1 -** Serotype distribution of 778 pneumococcal isolates. Serotypes 6A/6B, 11A/11D, 15A/15F, 15B/15C, 18C/18A/18B/18F, 9V/9A, 7C (7B, 40), 22F (22A), 12F (12A), and 10F/10C/33C are not discriminated from other serotypes by multiplex polymerase chain reaction.

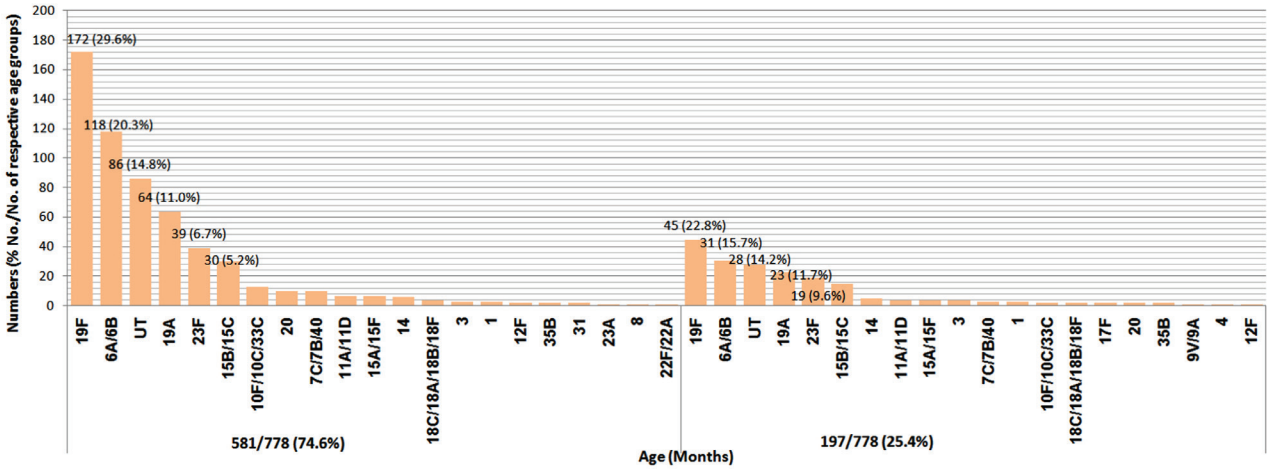


**Figure 2 -** Prevalence of pneumococcal serotypes in different age groups. Serotypes 6A/6B, 11A/11D, 15A/15F, 15B/15C, 18C/18A/18B/18F, 9V/9A, 7C (7B, 40), 22F (22A), 12F (12A), and 10F/10C/33C are not discriminated from other serotypes by multiplex polymerase chain reaction. UT - un-typeable.

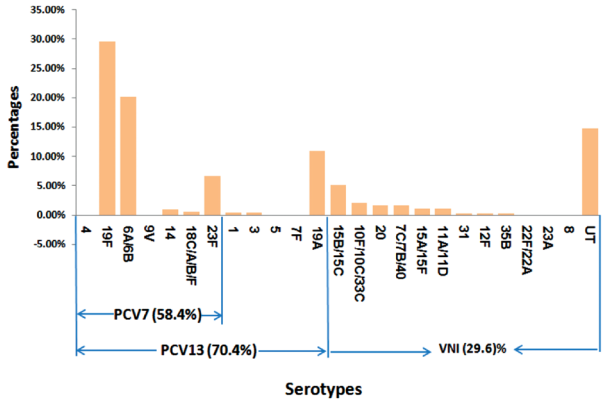


**Figure 3 -** Vaccine coverage in children less than 2 years old. Serotypes 6A/6B, 11A/11D, 15A/15F, 15B/15C, 18C/18A/18B/18F, 9V/9A, 7C (7B, 40), 22F (22A), 12F (12A), and 10F/10C/33C are not discriminated from other serotypes by multiplex polymerase chain reaction. UT - un-typeable. VNI - vaccine not included.

**Table 1 -** Rates of susceptibility, intermediate, and resistance of 778 *Streptococcus pneumoniae (S. pneumoniae)* isolates.

| Antibiotics | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Ertapenem | 99.4 | 0.6 | 0 |
| Tetracycline | 4.4 | 5.0 | 90.6 |
| Trimethoprim/ sulfamethoxazole | 7.5 | 11.7 | 80.9 |
| Ofloxacin | 98.5 | 1.2 | 0.4 |
| Vancomycin | 100 | 0 | 0 |
| Levofloxacin | 99.2 | 0.4 | 0.4 |
| Chloramphenicol | 92.7 | 0 | 7.3 |
| Meropenem | 94.0 | 3.3 | 2.7 |
| Moxifloxacin | 100 | 0 | 0 |
| Linezolid | 100 | 0 | 0 |
| Erythromycin | 3.3 | 0.1 | 96.5 |
| Penicillin | 96.0 | 2.8 | 1.2 |
| Ceftriaxone | 95.8 | 3.5 | 0.8 |
| Cefotaxime | 93.8 | 5.7 | 0.5 |

Values are expressed as percentage

alone accounted for 27.9% (217) of all isolates, followed by serotypes 6A/6B (19.2%, 149), 19A (11.2%, 87), 23F (7.5%, 58), and 15B/15C (5.8%, 45).

**Figure 2** shows the distribution of pneumococcal serotypes in different age groups. Of all isolates, 74.6% (581/778) were from children <2 years of age and 8.5% (66/778) were from adults >65 years of age. The 5 most frequent serotypes did not differ when grouped by age. Nevertheless, serotypes 31, 8, and 23A were not detected in the population aged 2 years or older, and serotypes 9V/9A were not detected in children less than 2 years old. Significant differences in the prevalence of serotypes between the 2 age groups were not found (Fisher's exact test).

***Polysaccharide conjugate vaccine-7 and PCV13 vaccine coverage.*** Only pneumococcal isolates (581 strains) recovered from children less than 2 years old were used to estimate the vaccine impact. **Figure 3** shows that the coverage rates for PCV7 and PCV13 would be 58.4% and 70.4%, respectively.

***Antimicrobial susceptibility.*** **Table 1** documents the frequencies of antimicrobial susceptibility of

**Table 2 -** Antimicrobial resistance patterns of pneumococcal isolates grouped by serotypes.

| Serotypes | N | PCN | CRO | CTX | MEM | ETP | ERYC | TET | CAM | OFL | LVF | TMP-SMX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | 1 | 0 (0) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 22F/22A* | 1 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) |
| 23A | 1 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 1 (100) |
| 9V/9A8 | 1 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 1 (100) |
| 31 | 2 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (100) | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 2 (100) |
| 17F | 2 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (100) | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 2 (100) |
| 12F /12A | 3 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (66.7) | 2 (66.7) | 0 (0) | 0 (0) | 0 (0) | 2 (66.7) |
| 35B | 4 | 0 (0) | 1 (25.0) | 1 (25.0) | 0 (0) | 0 (0) | 4 (100) | 4 (100) | 1 (25.0) | 0 (0) | 0 (0) | 4 (100) |
| 11A/11D* | 11 | 1 (9.1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 11 (100) | 11 (100) | 0 (0) | 1 (9.1) | 1 (9.1) | 11 (100) |
| 15A/15F* | 11 | 1 (9.1) | 0 (0) | 1 (9.1) | 0 (0) | 0 (0) | 11 (100) | 9 (81.8) | 0 (0) | 0 (0) | 0 (0) | 4 (36.4) |
| 20 | 12 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 10 (83.3) | 11 (91.7) | 1 (8.3) | 0 (0) | 0 (0) | 11 (91.7) |
| 7C /7B /40* | 13 | 0 (0) | 0 (0) | 0 (0) | 1 (7.7) | 0 (0) | 12 (92.3) | 11 (84.6) | 0 (0) | 1 (7.7) | 1 (7.7) | 8 (61.5) |
| 10F/10C/33C* | 15 | 0 (0) | 0 (0) | 1 (6.7) | 0 (0) | 0 (0) | 15 (100) | 15 (100) | 1 (6.7) | 1 (6.7) | 0 (0) | 12 (80.0) |
| 15B/15C* | 45 | 2 (4.4) | 5 (11.1) | 3 (6.7) | 4 (8.9) | 2 (4.44) | 42 (93.3) | 42 (93.3) | 1 (2.2) | 1 (2.2) | 2 (4.4) | 44 (97.8) |
| UT | 114 | 5 (4.4) | 3 (2.6) | 3 (2.6) | 5 (4.4) | 0 (0) | 109 (95.6) | 106 (93.0) | 8 (7.0) | 2 (1.8) | 1 (0.9) | 97 (85.1) |
| 4 | 1 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 1 (100) |
| 1 | 6 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 6 (100) | 5 (83.3) | 1 (16.7) | 0 (0) | 0 (0) | 2 (33.3) |
| 18C/ 18A/ 18B/ 18F* | 6 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 6 (100) | 6 (100) | 1 (16.7) | 0 (0) | 0 (0) | 6 (100) |
| 3 | 7 | 0 (0) | 0 (0) | 0 (0) | 1 (14.3) | 0 (0) | 6 (85.7) | 6 (85.7) | 0 (0) | 0 (0) | 0 (0) | 4 (57.1) |
| 14 | 11 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 11 (100) | 11 (100) | 1 (9.1) | 1 (9.1) | 0 (0) | 9 (81.8) |
| 23F | 58 | 2 (3.5) | 2 (3.5) | 2 (3.5) | 6 (10.3) | 0 (0) | 57 (98.3) | 58 (100) | 3 (5.2) | 1 (1.7) | 0 (0) | 58 (100) |
| 19A | 87 | 3 (3.5) | 1 (1.2) | 5 (5.8) | 6 (6.9) | 0 (0) | 85 (97.7) | 87 (100) | 1 (1.2) | 2 (2.3) | 0 (0) | 85 (97.7) |
| 6A/6B* | 149 | 2 (1.3) | 3 (2.0) | 3 (2.0) | 7 (4.7) | 0 (0) | 142 (95.3) | 139 (93.3) | 32 (21.5) | 1 (0.7) | 1 (0.7) | 144 (96.6) |
| 19F | 217 | 15 (6.9) | 18 (8.3) | 29 (13.4) | 16 (7.4) | 3 (1.4) | 214 (98.6) | 212 (97.7) | 5 (2.3) | 1 (0.5) | 0 (0) | 212 (97.7) |

*are not discriminated from other serotypes by multiplex polymerase chain reaction. PCN - Penicillin, CRO - Ceftriaxone, CTX - Cefotaxime, MEM - Meropenem, ETP - Ertapenem, ERYC - Erythromycin, TET - Tetracycline, CAM - Chloramphenicol, OFL - Ofloxacin, LVF - Levofloxacin, TMP-SMX - Trimethoprim/Sulfamethoxazole

*S. pneumoniae* isolates. The prevalence of penicillin-resistant *S. pneumoniae* (PRSP) and penicillin-intermediate *S. pneumoniae* (PISP) were 1.2% and 2.8%, respectively. The non-susceptibility rates for ceftriaxone and cefotaxime were 4.2% and 6.2%, respectively. All isolates were highly resistant to erythromycin, tetracycline, and trimethoprim/sulfamethoxazole, with resistance rates of 96.5%, 90.6%, and 80.9%, respectively, and 94%-99.4% of isolates were susceptible to levofloxacin, ofloxacin, ertapenem, and meropenem. All isolates were completely susceptible to vancomycin, moxifloxacin, and linezolid.

**Table 2** documents the antimicrobial resistance patterns of pneumococcal isolates according to serotype. Among all serotypes, serotype 19F predominated in resistance to beta-lactams. In addition, serotype 19F showed significantly higher resistance frequency to penicillin than serotypes 6A/6B ($p$=0.012 by Fisher's exact test). Except for tetracycline and trimethoprim/sulfamethoxazole, there was no significant difference between the PCV13 and non-PCV13 groups regarding their antimicrobial susceptibility ($p$>0.05 by the $\chi^2$ test). Only serotypes 15B/15C and 19F were resistant to ertapenem, while serotypes 15B/15C, 19F, 19A, 23F, and 6A/6B were resistant to meropenem. For isolates resistant to penicillin, ceftriaxone, cefotaxime, and meropenem,[22-39] (70%-81.3%) of the strains belonged to PCV13 serotypes.

**Discussion.** The present study presented baseline information for further surveillance of pneumococcal serotypes before or after the widespread use of pneumococcal vaccines in Zunyi city, a less-developed city in China. The epidemiological data is somewhat

different from those obtained in other areas. Global data have shown that serotypes 23F, 19F, 14, 6A, 6B, 1, and 5 are the most common serotypes.[16] Serotypes 19A, 19F, 3, 23F, 5, 6, and 14 were the main serotypes prevalent in other regions of China.[5,17] The present study increases our knowledge on pneumococcal serotype prevalence in China, and indicates that in addition to serotypes 19F, 6A/6B, 19A and 23F, serotype 15B/15C was common among the analyzed population.

Most isolates were recovered from male patients. It is interesting that this pattern of pneumococcal infection is quite like that of enterovirus infection,[10] which is also more common in males than females. Moreover, a report by Peltola et al[18] also suggested the association between invasive pneumococcal infection and enterovirus infection in children. Thus, it seems likely that pneumococcal infection might be a complication of enterovirus infection in this region. Nevertheless, the direct association between an enterovirus infection and a pneumococcal infection requires further investigation.

Vaccination is an effective way to reduce the burden of pneumococcal diseases. Currently available pneumococcal vaccines are based on the serotype-specific CPS. Although the PCV7 has been present in China for years, it was seldom used in children in the Zunyi region.[9,19] However, with the improvement in the socioeconomic status of this region, some families can bear the expense on pneumococcal vaccination. Thus, it is important to have the local pneumococcal serotype information. Our data showed that 70.4% of the pneumococcal serotypes isolated from children less than 2 years of age belonged to PCV13 serotypes, suggesting that introducing this vaccine would be effective in preventing pneumococcal infections in this region. However, we also observed the emergence of serotype 15B/C and other non-vaccine serotypes (29.6%), which are not covered by the PCV13, suggesting that further strategies including expansion of PCV serotypes and development of protein-based vaccines are necessary.

The prevalence of resistance to penicillin varies considerably between regions in China. There are a few studies of the prevalence of antibiotic resistance among *S. pneumoniae* in China; several hospital-based studies in Chongqing[20] (38.5%) and Shanghai reported distinct penicillin resistance rates for *S. pneumoniae* (10.7%). However, our findings show a much lower frequency of pneumococcal isolates resistant to penicillin. This inconsistency may partially be explained by the application of the new non-meningeal breakpoints of penicillin. In this study, ≤2 mg/ml penicillin was used as the susceptibility breakpoint for non-meningeal pneumococcal infections, which had led to a significant increase in susceptibility rates.[21] We observed a higher penicillin resistance frequency for serotype 19F than serotype 6A/B, which may partially be explained by their difference in prevalence and, hence, the exposure to penicillin drugs. In agreement with the situation in other cities of China,[8,9] nearly all strains were resistant to erythromycin. This might be related to the very common use of macrolides and their derived drugs, such as azithromycin, to treat other bacterial infections in children.

***Study limitation.*** First, since the data we used were only obtained through a laboratory information database, it is uncertain whether all pneumococcal isolates were truly responsible for the infections, especially in patients with upper respiratory tract infections which are sometimes co-infected by viruses and other bacteria. Secondly, we were not able to discriminate between serotypes 6A/B and serotypes 18C/18A/18B/18F, which led to bias regarding the estimation of the impact of the PCV7 vaccine. Additionally, several other serotypes could not be differentiated, such as 11A/11D, 15A/15F, 15B/15C, and 18C/18A/18B/18F among others. Finally, 114 isolates were not typed by the MP-PCR, and serotype identification using antisera is needed.

In the face of the increasingly serious problem of drug resistance, it is important to continue monitoring *S. pneumoniae* capsular serotypes and drug resistance patterns geographically and over time. It is also important to continue to study resistance mechanisms in order to slow down the selection of drug resistant *S. pneumoniae*. In addition, clinicians should strictly follow the guidelines for the use of antimicrobial agents and recommend vaccinations in children to control pneumococcal infections.

In conclusion, the present study shows that serotypes 19F, 6A/6B, 19A, 23F, and 15B/15C were the most frequent pneumococcal strains in Zunyi, China. PCV13 serotypes accounted for 70-81.3% of the pneumococcal isolates circulating in the region investigated. Pneumococcal serotypes varied in resistance to several antibiotics, but penicillin remains a good choice for treatment of pneumococcal diseases.

**Acknowledgment.** *This project was supported by the Scientific Project of Health and Family Planning Commission of Guizhou Province, "15851 talents elite project" of Zunyi city, Innovation Talent Team of Science and Technology jointly provided by Zunyi city and the First People's Hospital, and the Talent Team Project provided by Guizhou Province and Zunyi city, China.*

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