International Journal of Infectious Diseases 50 (2016) 38–43

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Clinical and pathogenic analysis of 507 children with bacterial meningitis in Beijing, 2010–2014

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 15 July 2016
Accepted 15 July 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Bacterial meningitis
Children
Pathogen
Antibiotic susceptibility

SUMMARY

Objectives: To explore the clinical characteristics and analyze the pathogens of bacterial meningitis in children.
Methods: Bacterial meningitis cases occurring from January 2010 through December 2014 at Beijing Children's Hospital were reviewed retrospectively. The records of all patients, including data on clinical features and laboratory information, were obtained and analyzed.
Results: In total, the cases of 507 pediatric patients seen over a 5-year period were analyzed; 220 of these cases were etiologically confirmed. These patients were classified into four age groups: 29 days to 1 year (n = 373, 73.6%), 1–3 years (n = 61, 12.0%), 3–6 years (n = 41, 8.1%), and >6 years (n = 32, 6.3%). The main pathogens identified in this study were *Streptococcus pneumoniae* (n = 73, 33.2%), *Escherichia coli* (n = 24, 10.9%), Enterococcus (n = 22, 10.0%), and group B Streptococcus (n = 18, 8.2%). All Gram-positive bacteria were sensitive to vancomycin and linezolid. All Gram-negative bacteria were sensitive to meropenem. The total non-susceptibility rate of *S. pneumoniae* to penicillin was 47.6% (20/42). The resistance rates to ceftriaxone, cefepime, and ceftazidime were 75% (9/12), 55.6% (5/9), and 40% (4/10), respectively.
Conclusions: The main pathogen of bacterial meningitis in this study was *S. pneumoniae*. The antibiotic resistance rates among children with bacterial meningitis are of serious concern.

1. Introduction

Bacterial meningitis continues to be a major cause of illness and death among neonates and children throughout the world.[1] In population-based studies, the incidence of acute bacterial meningitis in China ranges from 6.95 to 22.3 cases/100 000 children < 5 years of age.[2–4] Neurological sequelae are relatively common in survivors.[5–10] Available studies have shown the main causal pathogens of bacterial meningitis to be *Neisseria meningitidis*, *Haemophilus influenzae* type b (Hib), and *Streptococcus pneumoniae*.[11–15]

The incidence of bacterial meningitis has decreased since the introduction of conjugated vaccines targeting Hib, *S. pneumoniae*, and *N. meningitidis*. The incidence of bacterial meningitis changed by −31% in the USA during the years 1998–2007, from 2.00 cases per 100 000 population in 1998–1999 to 1.38 cases per 100 000 population in 2006–2007.[14] The epidemiology of meningitis in Canada has been influenced dramatically by universal immunization programs for Hib, *N. meningitidis*, and *S. pneumoniae*.[15–17] However, the incidence of this disease and associated deaths in resource-limited countries continue to grow.[18] Accurate monitoring of the pathogen-specific estimates of the number of bacterial meningitis cases is challenging in many countries because of limited laboratory-based surveillance and the misuse

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http://dx.doi.org/10.1016/j.ijid.2016.07.010

of antibiotics. In this study, hospital-based data on bacterial meningitis were used to describe the distribution of pathogens in order to provide a baseline for the evaluation of this severe disease in China.

2. Patients and methods

2.1. Study population

Cases of bacterial meningitis occurring in patients younger than 16 years of age at Beijing Children's Hospital from January 2010 through December 2014 were reviewed retrospectively. This tertiary care hospital is a National Children's Medical Center with 970 beds and treats more than three million outpatients and 70 000 hospitalized patients every year. The records of all patients with probable or confirmed bacterial meningitis during this 5-year period were obtained. Demographic data, underlying diseases, clinical features in the patient history, laboratory findings, treatments, and outcomes were reviewed.

2.2. Diagnosis of bacterial meningitis

Any child with a sudden onset of fever (>38.5 °C rectal or >38.0 °C axillary) and with neck stiffness, altered consciousness, or other meningeal symptoms was considered a suspected bacterial meningitis patient. A case that was laboratory-confirmed, with the identification of a bacterial pathogen (Hib, *S. pneumoniae*, meningococcus, or others) in the cerebrospinal fluid (CSF) or blood of a child with clinical symptoms consistent with bacterial meningitis, was considered a 'proven' case. A suspected case with a CSF examination showing at least one of the following was considered a 'probable' case: turbid CSF appearance, leukocytosis $>100 \times 10^6$ cells/l, and leukocytosis of $10–100 \times 10^6$ cells/l with either an elevated protein level (>100 mg/dl) or decreased glucose level (<40 mg/dl). These criteria are consistent with the World Health Organization (WHO) case definition.[19]

2.3. Sample collection

CSF samples were obtained aseptically from each participant through lumbar puncture. Up to 1 ml of CSF was collected into a sterile tube. Samples were sent immediately to the hospital laboratory for a cell count, Gram staining, and bacterial culture, as well as to measure glucose and protein levels. Twenty-four hours after incubation at 35 ± 2 °C in 5% CO_2, bacterial isolates were identified by colony morphology analysis and growth requirements. After identification, isolates of *S. pneumoniae* were subsequently stored at −80 °C until further investigation. An antibiotic susceptibility test for each isolate was performed using the disk diffusion method or broth microdilution method for the minimum inhibitory concentration (MIC) values. The results were interpreted according to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing.[20] *Staphylococcus aureus* isolates that were resistant to either oxacillin or cefoxitin were considered methicillin-resistant *S. aureus* (MRSA). Clinical and demographic data and the therapeutic history were collected using a standard case investigation form.

2.4. Bacterial isolates

All *S. pneumoniae* isolates were identified based on typical colony morphology, Gram staining, an optochin sensitivity test (Oxoid Company, Basingstoke, UK), and an Omni serum assay (Statens Serum Institute, Copenhagen, Denmark). All isolates were stored at −80 °C until further study.

2.5. Serotyping of S. pneumoniae

The serogroups of *S. pneumoniae* were tested using the Quellung reaction with Pneumotest kits, and the serotypes were tested with factor antisera (Statens Serum Institute). The interpretation of the serotyping depended on the capsular swelling seen under phase-contrast microscopy with an oil immersion lens (magnification, 100×), as described in the literature.[10]

2.6. Ethics statement

This study was reviewed and approved by the Ethics Committee of Beijing Children's Hospital Affiliated to Capital Medical University.

2.7. Statistical analysis

Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables within two groups were compared using the independent *t*-test for parametric data and the Mann–Whitney *U*-test for non-parametric data. *p*-Values of <0.05 were considered statistically significant. All statistical analyses were conducted using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Study population

A total of 507 children were included in the study; their median age was 5 months (range 29 days to 15 years). There were 326 (64.3%) boys and 181 (35.7%) girls, giving a male to female ratio of 1.8:1. These cases were classified into four age groups: 29 days to 1 year (n = 373, 73.6%), 1–3 years (n = 61, 12.0%), 3–6 years (n = 41, 8.1%), and >6 years (n = 32, 6.3%). The number of cases diagnosed each year increased over time, especially in 2014 with 136 patients, and *S. pneumoniae* was the predominant pathogen (Figures 1 and 2). A pathogen was identified in 220 (43.4%) cases, with positive results in CSF and/or blood.

3.2. Comparison of the pathogen-positive and pathogen-negative groups

The pathogen-positive group comprised 220 (43.4%) patients who had at least one pathogenic bacterial strain identified in either CSF or blood culture. The pathogen-negative group comprised 287 cases. Patients in the latter group were generally younger than those in the pathogen-positive group (median age 208 vs. 133 days, p = 0.003). The two groups of patients did not differ significantly in sex distribution or blood white blood cell (WBC) count. With

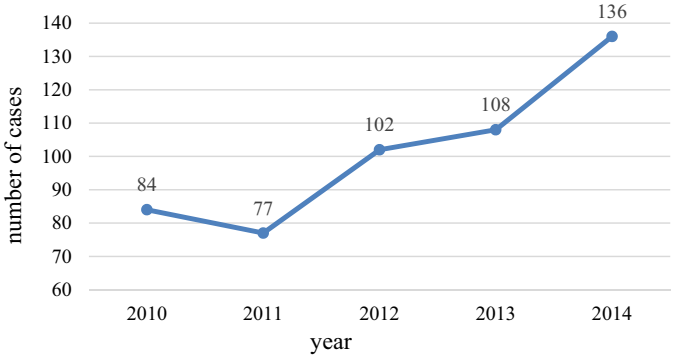

Figure 1. Number of bacterial meningitis patients diagnosed per year.

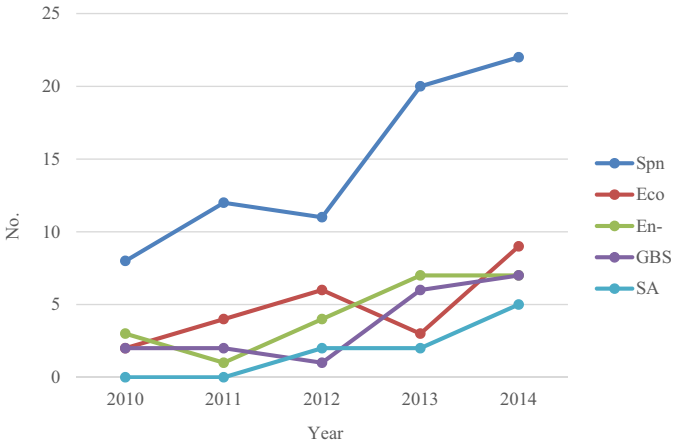

Figure 2. Number of bacterial meningitis cases caused by the main pathogens per year. (Note: Spn, *Streptococcus pneumoniae*; Eco, *Escherichia coli*; En, Enterococcus; GBS, group B Streptococcus; SA, *Staphylococcus aureus*.).

regard to other blood inflammatory indices, patients in the pathogen-positive group were frequently found to have higher C-reactive protein (70 vs. 40 mg/l, $p = 0.005$) and procalcitonin levels (2 vs. 0.75 ng/ml, $p = 0.008$) than those in the pathogen-negative group. In terms of CSF indices, patients in the pathogen-positive group had an apparently lower glucose level (1.7 vs. 2.3 mmol/l, $p = 0.019$) but higher protein level (1630 vs. 1353 mg/l, $p = 0.0$) than those in the pathogen-negative group. There was no significant difference in the CSF WBC count between members of these two groups, and they had similar complications, sequelae, and mortality rates. Antibiotic usage before the first lumbar puncture was also similar in the pathogen-positive and pathogen-negative groups (61.8% vs. 67.3%, $p = 0.22$) (Table 1).

3.3. Bacterial pathogen composition

The positive rate from the CSF samples was 31% (157/507), and 69 patients also had positive results from blood culture. In addition, 63 patients had negative results for CSF culture but positive blood culture results. Positive pathogens were calculated on the basis of a positive result obtained in the CSF and/or blood. *S. pneumoniae* ($n = 73$, 33.2%), *Escherichia coli* ($n = 24$, 10.9%), Enterococcus ($n = 22$, 10.0%), and group B Streptococcus (GBS) ($n = 18$, 8.2%) were the most frequently detected pathogens in this study (Figure 2). In addition, there were nine instances of *S. aureus* (4.1%), nine of *Pseudomonas aeruginosa* (4.1%), and eight of Salmonella (3.6%). There were only five cases of *H. influenzae* and one of *N. meningitidis* during the 5-year period. The number of cases diagnosed each year increased over time, and *S. pneumoniae* was the predominant pathogen with eight to 22 cases per year. In terms of the pathogen composition by age group, it was notable that *S. pneumoniae* was predominant in all age groups (from 21.3% to 64.0%), except the <1 year age group (Figure 3).

In the group of patients aged 29 days to 1 year, *E. coli* and GBS were the second and third most prevalent pathogens, respectively. Of the patients with *E. coli*, 91.7% (22/24) were less than 1 year old. Patients with a GBS infection were all infants between 1 and 4 months old.

3.4. Antimicrobial susceptibility testing

Penicillin susceptibility results were available for 42 *S. pneumoniae* isolates. The total non-susceptibility (intermediate and resistant) rate to penicillin was 47.6% (20/42), as defined by the parenteral meningitis breakpoint (susceptible ≤0.06 mg/l, resistant ≥0.12 mg/l). The incidence of *S. pneumoniae* showed an increasing trend each year (from eight to 22 cases), with steady non-susceptibility rates (33.3–57.1%) (Figure 4). The susceptibility of the 42 *S. pneumoniae* isolates to antibiotics is shown in Figure 5. The non-susceptibility rates to ceftriaxone, cefotaxime, and cefepime were 25%, 46.9%, and 51.9%, respectively. There were no *S. pneumoniae* isolates that were resistant to vancomycin or linezolid (Table 2).

There were 24 isolates of *E. coli*. All of the isolates were resistant to ampicillin and cefuroxime. The resistance rates to ceftriaxone, cefepime, and ceftazidime were 75% (9/12), 55.6% (5/9), and 40% (4/10), respectively. There were no *E. coli* isolates that were resistant to carbapenems (meropenem, ertapenem, and imipenem), amikacin, cefoperazone, or sulbactam. There were 18 isolates of GBS. There were no GBS isolates that were resistant to vancomycin, linezolid, penicillin G, ceftriaxone, or cefepime. There were nine isolates of *S. aureus*, including three isolates of MRSA and four isolates of methicillin-sensitive *S. aureus* (MSSA). The resistance rates to penicillin G, oxacillin, and tetracycline were 85.7% (6/7), 33.3% (2/6), and 33.3% (2/6), respectively. There were no *S. aureus* isolates that were resistant to vancomycin, teicoplanin, linezolid, or levofloxacin (Table 2).

3.5. S. pneumoniae *serotype distribution and vaccine coverage*

A total of five serotypes were identified among the 12 *S. pneumoniae* isolates. There were four isolates of serotype 19F, three isolates of 23F, two isolate each of 19A and 14, and one isolate of 6A . The total coverage rates of pneumococcal conjugate vaccines PCV7, PCV10, and PCV13 were 75.0% (9/12), 75.0% (9/12), and 100% (12/12), respectively.

Table 1
Comparison of pathogen-positive and positive-negative groups[a]

	Total N=507	Pathogen-positive n=220	Pathogen-negative n=287	Chi-square	p-Value
Age(days)	151(74–398)	208(86–527)	133 (70–308)		0.003
Male(%)	64.3%	60.4%	67.4%	2.67	0.113
Blood WBC count ($\times 10^9$/l)	11.8(7.8–16.9)	11.2(7.1–17.0)	12.4 (8.6–16.8)		0.1
Blood CRP (mg/l)	57(8–135)	70(15–160)	40 (8–115)		0.005
PCT (ng/ml)	1(0.3–10)	2(0.38–11)	0.75 (0.2–5.78)		0.008
CSF WBC count ($\times 10^6$/l)	390(110–1600)	395(95–1633)	390 (120–1600)		0.521
CSF glucose (mmol/L)	2.2(0.88–2.96)	1.7(0.4–2.7)	2.4(1.4–3.1)		0.019
CSF protein (mg/L)	1469.5(849.3–2361.3)	1630(970–2600)	1353 (807–2155)		0
Complications (%)	54.0%	57.3%	51.4%	1.746	0.206
Sequela (%)	29.0%	29.8%	28.4%	0.117	0.767
Mortality (%)	0.8%	1.4%	0.4%		0.322
Antibiotic use before lumbar puncture (%)	64.9%	61.8%	67.3%	1.634	0.22

WBC, white blood cell;CRP, C-reactive protein; PCT, procalcitonin; CSF, cerebrospinal fluid.
[a] Results are reported as the median (interquartile range), or as the percentage.

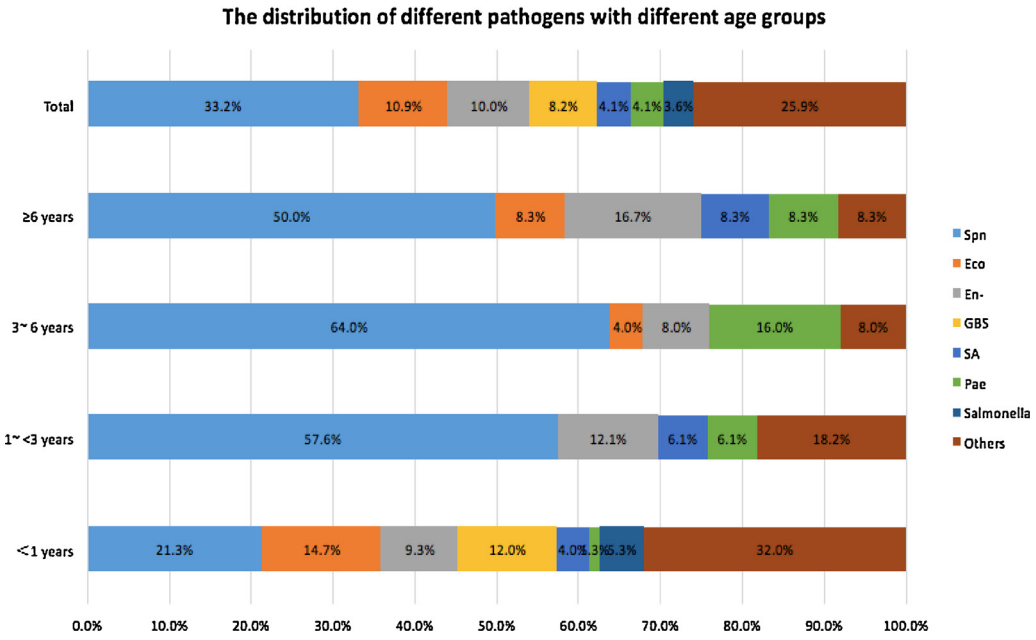

Figure 3. The distribution of different pathogens by age group. (Note: Spn, *Streptococcus pneumoniae*; Eco, *Escherichia coli*; En, Enterococcus; GBS, group B Streptococcus; SA, *Staphylococcus aureus*; Pae, *Pseudomonas aeruginosa*.).

4. Discussion

Since the introduction of conjugated vaccines targeting Hib, *S. pneumoniae*, and *N. meningitidis*, there has been a decreasing incidence of bacterial meningitis in infants and children in the USA and Canada. However, the situation in China is markedly different. A recent study reported that for children <5 years of age, the estimated incidence was 6.95–22.3 cases/100 000 children,[4] which was similar to that found previously in other studies performed in China (from 12.4 to 19.2 cases/100 000 children[2,3]).

In the present study covering the period January 2010 to December 2014, there were 507 cases of suspected bacterial meningitis in the hospital, and the number of cases enrolled per year increased over this 5-year period. Furthermore, the pathogen was identified and confirmed in 220 patients by blood and/or CSF culture, in accordance with the WHO criteria.

Of note, the proportion of cases caused by *S. pneumoniae* (33.2%) was relatively high (ranging from 21.3% to 64.0% across age groups). Similar to other countries, low incidences of *H. influenzae* (five cases) and Neisseria infection (one case) were found during the 5-year period. The factors related to the low positive rates for these two pathogens of bacterial meningitis are complex. One key contributing factor is that a meningococcal vaccine has been part of the National Immunization Program (NIP) since the 1980s; the coverage of group A meningococcal polysaccharide reported in 2012 was 91.22%.[21] The Hib vaccine has been in use since the 1990s with a coverage of 50.9%,[22] even though it has not been included in the NIP. The other key factor is the high rate of antimicrobial drug use in clinical practice in China. Of the bacterial meningitis patients in the present study, 63.9% had used antibiotics before clinical specimens were collected. This finding is similar to that of a previous study in China.[4] Antibiotic abuse is an increasing threat in China. According to a survey performed at Beijing Children's Hospital, more than 98% of patients in the outpatient department who were diagnosed with the common cold were given antibiotics by physicians. More than one-third of the patients had been taking antibiotics before coming to the hospital.[23]

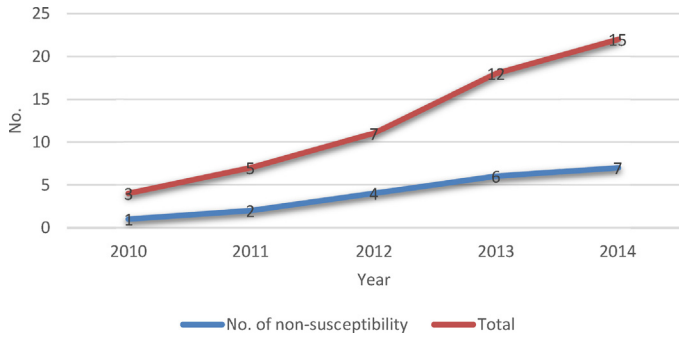

Figure 4. Number of *Streptococcus pneumoniae* isolates with non-susceptibility to penicillin.

The World Health Organization has sounded the alarm on the abuse of antibiotics in China. The use of antibiotics in this country is very high. Over the past decade, more than half of outpatients in China have been prescribed antibiotics—far above the level recommended by the WHO (less than 30%). Antibiotic treatment reduces the positive rate from CSF samples in bacterial meningitis cases. Dalton and Allison showed that antibiotic treatment reduced the number of positive cultures recovered from the CSF by approximately 30%.[24,25]

S. pneumoniae infection is a serious but vaccine-preventable cause of meningitis. The *S. pneumoniae* vaccine was introduced in China at the end of 2008 and was stopped at the end of 2014; it has not been introduced into the NIP.[26] The immunization rates with this vaccine were low, primarily because of its high price (860 RMB/dose, approximately US$ 138.5/dose). One study performed in 2014 in Shanghai showed a PCV vaccine coverage of 11.4% from 28 141 abstracted pediatric records.[22]

In the present study, a total of five serotypes were identified among 12 *S. pneumoniae* isolates. There were four isolates of serotype 19F, three isolates of 23F, two isolates each of 19A and 14, and one isolate of 6A. The total coverage rates of PCV7, PCV10, and PCV13 were 75.0%, 75.0%, and 100%, respectively. Many studies have shown that certain *S. pneumoniae* serotypes, especially vaccine serotypes, are related to more severe invasive pneumococcal infections. Serotypes 19F and 19A were found to be the most common serotypes associated with severe *S. pneumoniae*

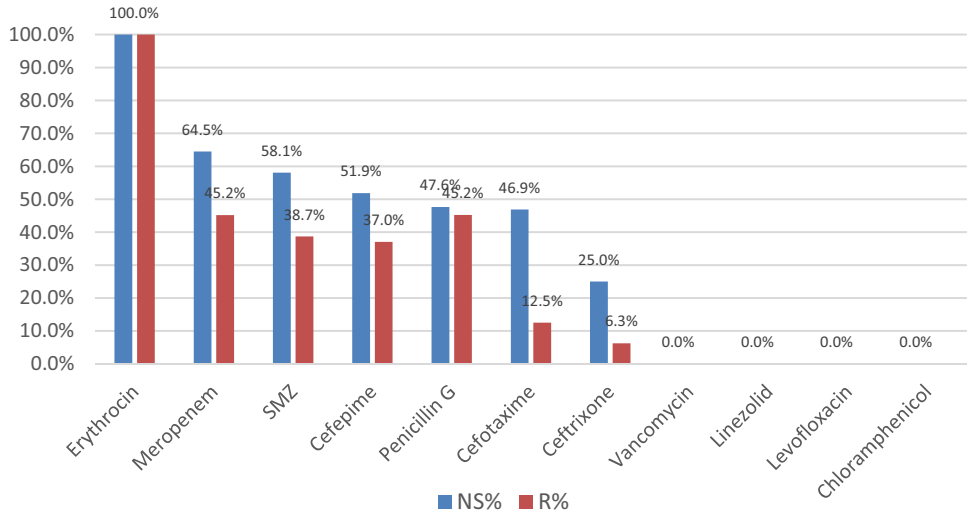

Figure 5. Susceptibility of the *Streptococcus pneumoniae* isolates to antibiotics. (Note: NS, non-susceptible; R, real resistance; SMZ, sulfamethoxazole.) .

disease, partly because antibiotic resistance was so high.[27,28] A recent study from the USA showed that serotype 19A was the major multidrug-resistant *S. pneumoniae* (MDRSP) (38.5%).[29] Another study in China including 171 *S. pneumoniae* isolates from invasive *S. pneumoniae* diseases in children, showed that the most common serotypes were 19F (19.9%), 14 (19.3%), and 19A (18.1%). Thus, the incidence of invasive *S. pneumoniae* infections, especially pneumococcal meningitis, may decrease significantly following the complete introduction of PCV (especially PCV13) in China.

A high level of antibiotic resistance in *S. pneumoniae* isolates was found in the present study. The penicillin non-susceptibility rate was 47.6%, which is significantly higher than rates reported in developed countries (24.4% in Europe,[30] 38.9–42.7% in the U.S.,[31] and 3.9% in Canada[15]), but lower than the rate reported in a study from Shanghai, China[34] (70%). The non-susceptibility rates to penicillin are increasing, and this finding coincides with global trends.[32,33] No *S. pneumoniae* isolates were found to be resistant to vancomycin or linezolid, which is consistent with the findings of another study.[34]

This study has several limitations. It was a hospital-based retrospective review. Because some patients only had positive culture results at other hospitals before admission, the resistance data for the different antibiotics are incomplete. For the same reason, only 12 *S. pneumoniae* isolates were available for serotyping. Thus, this study might not represent overall epidemic trends in China. Future longitudinal, prospective and multicenter surveillance for pathogens of bacterial meningitis should be conducted. Moreover, the serotype distribution, antimicrobial susceptibility, and molecular epidemiology of *S. pneumoniae* are required.

In conclusion, the main pathogen of bacterial meningitis in this study was *S. pneumoniae*. The antibiotic resistance rates among children with bacterial meningitis are of serious concern.

Table 2
Real resistance of the main pathogenic bacterial meningitis strains (*n*/*N*)

Antibiotics	Spn	Eco	GBS	SA
Penicillin G	19/42	–	0/14	6/7
Ampicillin	0/4	8/9	–	–
Amoxicillin–clavulanic acid	0/1	1/10	–	–
Piperacillin	–	1/10	–	–
Oxacillin	0/2	–	–	2/6
Cefoxitin	–	0/9	–	–
Cefuroxime	–	9/11	–	–
Ceftazidime	–	4/10	–	–
Cefoperazone–sulbactam	–	0/12	–	–
Cefotaxime	4/32	–	–	–
Ceftriaxone	1/16	9/12	0/10	1/1
Cefepime	10/27	5/9	0/9	–
Vancomycin	0/56	–	0/14	0/7
Linezolid	0/29	–	–	0/7
Teicoplanin	–	–	–	0/4
Ciprofloxacin	–	5/9	–	0/7
Levofloxacin	0/14	–	–	0/6
Amikacin	–	0/9	–	–
Meropenem	14/31	0/15	–	–
Ertapenem	0/3	0/3	–	–
Imipenem	–	0/11	–	–
Gentamicin	–	4/9	–	–
Chloramphenicol	0/28	–	–	1/1
Rifampicin	0/3	–	–	0/7
Tetracycline	4/6	–	–	2/6
Sulfamethoxazole	12/31	8/9	–	1/6

Spn, Streptococcus pneumoniae; Eco, Escherichia coli; GBS, group B Streptococcus; SA, Staphylococcus aureus.

5. Contributors

All of the authors had access to the full dataset (including the statistical reports and tables) and take responsibility for the integrity of the data and the accuracy of the data analysis. GLY, ZZX, YKH, YYH, and LG conceived and designed the study. GLY, ZZX, WX, ZPP, SW, LLL, LG, and YYH collected the data and designed the analysis. GLY, ZZX, LG, and YYH interpreted the data. GLY and ZZX wrote the first draft of the paper. GLY, YYH, and LG reviewed and approved the final report.

Acknowledgements

The National Natural Science Foundation of China (grant number 81371853) supported this study financially. We thank the Department of Medical Records and Statistics and the Clinical Laboratory of Beijing Children's Hospital for their contribution to this study.

Conflict of interest: No conflicts of interest are declared with regard to this article.

References

1. Kim KS. Acute bacterial meningitis in infants and children. *Lancet Infect Dis* 2010;**10**:32–42.

2. Yang Y, Leng Z, Shen X, Lu D, Jiang Z, Rao J, et al. Acute bacterial meningitis in children in Hefei, China 1990-1992. *Chin Med J (Engl)* 1996;**109**:385–8.
3. Dong BQ, Tang ZZ, Lin M, Li CY, Tan DM, Liang DB, et al. Epidemiologic surveillance for bacterial meningitis in 140000 children under 5 years of age in Nanning District, Guangxi Province [in Chinese]. *Zhonghua Liu Xing Bing XueZaZhi* 2004;**25**:391–5.
4. Li Y, Yin Z, Shao Z, Li M, Liang X, Sandhu HS, et al. Acute Meningitis and Encephalitis Syndrome Study Group. Population-based surveillance for bacterial meningitis in China, September 2006–December 2009. *Emerg Infect Dis* 2014;**20**:61–9.
5. Klinger G, Chin CN, Beyene J, Perlman M. Predicting the outcome of neonatal bacterial meningitis. *Pediatrics* 2000;**106**:477–82.
6. Stevens JP, Eames M, Kent A, Halkets S, Holt D, Harvey D. Long-term outcome of neonatal meningitis. *Arch Dis Child Fetal Neonatal Ed* 2003;**88**:F179–84.
7. Chang CJ, Chang WN, Huang LT, Huang SC, Chang YC, Hung PL, et al. Bacterial meningitis in infants: the epidemiology, clinical features, and prognostic factors. *Brain Dev* 2004;**26**:168–75.
8. De Louvois J, Halket S, Harvey D. Neonatal meningitis in England and Wales: sequelae at 5 years of age. *Eur J Pediatr* 2004;**7**:730–4.
9. Roine I, Pelyola H, Fernandez J, Zavala I, González Mata A, González Ayala S, et al. Influence of admission findings on death and neurological outcome from childhood bacterial meningitis. *Clin Infect Dis* 2008;**46**:1248–52.
10. Arditi M, Mason Jr EO, Bradley JS, Tan TQ, Barson WJ, Schutze GE, et al. Three-year multicenter surveillance of pneumococcal meningitis in children: clinical characteristics, and outcome related to penicillin susceptibility and dexamethasone use. *Pediatrics* 1998;**102**:1087–97.
11. Shen X, Yang Y, Zhang G, Bai H, Xu S, Li Y, et al. Etiologic analysis of 128 bacterial meningitis cases [in Chinese]. *Chinese Journal of Infectious Diseases* 1991;**9**:230–2.
12. Shen X, Li Y, Tong Y, Gao W, Yang Y, Wang Y. Etiologic analyses for 60 cases of purulent meningitis [in Chinese]. *National Medical Journal of China* 1998;**78**:466.
13. Yi W, Chuanqing W, Xiaohong W. Etiology and antimicrobial susceptibility of children with bacterial meningitis [in Chinese]. *Journal of Applied Clinical Pediatrics* 2006;**21**:355–6. 366.
14. Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL, et al. ; Emerging Infections Programs Network. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med* 2011;**364**:2016–25.
15. Le Saux N. Canadian Paediatric Society, Infectious Diseases and Immunization Committee. Guidelines for the management of suspected and confirmed bacterial meningitis in Canadian children older than one month of age. *Paediatr Child Health* 2014;**19**:141–52.
16. Bettinger JA, Scheifele DW, Kellner JD, Halperin SA, Vaudry W, Law B, et al. The effect of routine vaccination on invasive pneumococcal infections in Canadian children, Immunization Monitoring Program, Active 2000-2007. *Vaccine* 2010;**28**:2130–6.
17. Bettinger JA, Scheifele DW, Halperin SA, Kellner JD, Vanderkooi OG, Schryvers A, et al. Evaluation of meningococcal serogroup C conjugate vaccine programs in Canadian children: interim analysis. *Vaccine* 2012;**30**:4023–7.
18. Molyneux E, Riordan FA, Walsh A. Acute bacterial meningitis in children presenting to the Royal Liverpool Children's Hospital, Liverpool, UK and the Queen Elizabeth Central Hospital in Blantyre, Malawi: a world of difference. *Ann Trop Paediatr* 2006;**26**:29–37.
19. World Health Organization. WHO-recommended standards for surveillance of selected vaccine-preventable diseases; 2003, May. WHO/V&B/03. 01. Geneva: WHO; 2013 , Available at: http://www.measles rubellainitiative.org/wp-content/uploads/2013/06/WHO- surveillance-standard.pdf (accessed November 5, 2013)
20. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 34th informational supplement. M100-S24. Wayne, PA: CLSI; 2014.
21. Cao L, Wang HQ, Zheng JS, Yuan P, Cao LS, Zhang GM. National immunization coverage survey in China after integrated more vaccines into EPI since 2008 [in Chinese]. *Chinese Journal of Vaccine and Immunization* 2012;**18**:419–24.
22. Wagner AL, Sun X, Montgomery JP, Huang Z, Boulton ML. The impact of residency and urbanicity on *Haemophilus influenzae* type b and pneumococcal immunization in Shanghai children: a retrospective cohort study. *PLoS One* 2014;**9**:e97800.
23. Yang YH, Fu SG, Peng H, Shen AD, Yue SJ, Go YF, et al. Abuse of antibiotics in China and its potential interference in determining the etiology of pediatric bacterial diseases. *Pediatr Infect Dis J* 1993;**12**:986–8.
24. Dalton HP, Allison MJ. Modification of laboratory results by partial treatment of bacterial meningitis. *Am J Clin Pathol* 1968;**49**:410–3.
25. Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* 2001;**108**:1169–74.
26. Implementation plan for national expanded immunization programs. Gazette of the Ministry of Health of People's Republic of China 2008; 3:17-21
27. Isaacman DJ, McIntosh ED, Reinert RR. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. *Int J Infect Dis* 2010;**14**:e197–209.
28. Lamb KE, Flasche S, Diggle M, Inverarity D, Greenhalgh D, Jefferies JM, et al. Trends in serotypes and sequence types among cases of invasive pneumococcal disease in Scotland, 1999-2010. *Vaccine* 2014;**32**:4356–63.
29. Richter SS, Diekema DJ, Heilmann KP, Dohrn CL, Riahi F, Doern GV. Changes in pneumococcal serotypes and antimicrobial resistance after introduction of the 13-valent conjugate vaccine in the United States. *Antimicrob Agents Chemother* 2014;**58**:6484–9.
30. Navarro Torné A, Dias JG, Quinten C, Hruba F, Busana MC, Lopalco PL, et al. European enhanced surveillance of invasive pneumococcal disease in 2010: data from 26 European countries in the post-heptavalent conjugate vaccine era. *Vaccine* 2014;**32**:3644–50.
31. Mendes RE, Costello AJ, Jacobs MR, Biek D, Critchley IA, Jones RN. Serotype distribution and antimicrobial susceptibility of USA *Streptococcus pneumoniae* isolates collected prior to and post introduction of 13-valent pneumococcal conjugate vaccine. *Diagn Microbiol Infect Dis* 2014;**80**:19–25.
32. Hackel M, Lascols C, Bouchillon S, Hilton B, Morgenstern D, Purdy J. Serotype prevalence and antibiotic resistance in *Streptococcus pneumoniae* clinical isolates among global populations. *Vaccine* 2013;**31**:4881–7.
33. Chiba N, Morozumi M, Shouji M, Wajima T, Iwata S, Ubukata K. Invasive Pneumococcal Diseases Surveillance Study Group. Changes in capsule and drug resistance of pneumococci after introduction of PCV7, Japan, 2010-2013. *Emerg Infect Dis* 2014;**20**:1132–9.
34. Zhang L, Wang C, Wang Y. Clinical and pathogenic analysis of bacterial meningitis with pathogen-identified in 146 children (in Chinese). *Chinese Journal of Evidence Based Pediatrics* 2013;**8**:161–6.