Epidemiology and Drug Resistance of Pathogens Isolated from Cerebrospinal Fluids at a Children’s Medical Center in Eastern China During 2006–2020

Dan Li, Xin Zhang, Yunzhong Wang, Jian Xue, Xueqiang Ji, Xuejun Shao, Yang Li

1Department of Clinical Laboratory, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, 215004, People’s Republic of China; 2Department of Clinical Laboratory, Children’s Hospital of Soochow University, Suzhou, Jiangsu, 215025, People’s Republic of China; 3Institute of Pediatric Research, Children’s Hospital of Soochow University, Suzhou, Jiangsu, 215025, People’s Republic of China; 4Clinical Medical College of Pediatrics, Soochow University, Suzhou, Jiangsu, 215025, People’s Republic of China

*These authors contributed equally to this work

Objective: To investigate the epidemiology and drug resistance of pathogens isolated from cerebrospinal fluid samples at a children’s medical center in eastern China and provide the basis for anti-infection treatments.

Methods: In all, 307 non-duplicated strains of pathogens were isolated from cerebrospinal fluid samples in the Children’s Hospital of Soochow University from January 2006 to December 2020. Mass spectrometry was used for pathogen identification. The VITEK 2 Compact system and Kirby-Bauer method were applied to determine antimicrobial susceptibility.

Results: Among the 307 isolates, gram-positive bacteria, gram-negative bacteria and fungi accounted for 60.26%, 34.53%, and 5.21%, respectively. The most prevalent pathogens were Streptococcus pneumoniae (26.06%), Escherichia coli (20.20%) and Streptococcus agalactiae (17.26%). The number of isolates was highest in winter. The most prevalent gram-positive bacterium in children <6 months old was Streptococcus agalactiae, while Streptococcus pneumoniae was the most in children >6 months old. The drug resistance of gram-positive bacteria, fungi and Haemophilus influenzae were not high. In addition, 35 strains of gram-negative bacteria produced extended-spectrum β-lactamases (ESBLs) and 6 strains were identified as multidrug-resistant (MDR) bacteria. These strains showed much higher resistance to the antibiotics than other strains.

Conclusion: Cases of meningitis among children have increased in the past 15 years and MDR bacteria were also identified. The emergence of MDR bacteria is a cause for great concern and requires further investigation.

Keywords: cerebrospinal fluids, infection, children, drug resistance, epidemiology

Introduction

Meningitis is one of the most severe infectious diseases that has acute onset and is difficult to treat.1,2 The World Health Organization (WHO) reported that meningitis is responsible for about 250,000 deaths annually worldwide, and a great majority of these involve children.3 In China, the estimated annual incidence (per 100,000) of meningitis in children ranged from 6.95 to 22.30.4 Many types of pathogens are known to cause meningitis, among which bacteria are the most prevalent.5 Escherichia coli and Staphylococcus aureus were reportedly the primary pathogens in adult meningitis,5,6 while Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae were the most prevalent causative agents among children.7,8 However, the prevalence of meningitis pathogens may change with respect to

Correspondence: Yang Li; Xuejun Shao
Email: xjshao@suda.edu.cn

Received: 16 October 2021
Accepted: 3 December 2021
Published: 16 December 2021
different areas and seasons. In recent times, the reports of fungal meningitis are also increasing.8

The drug resistance of pathogens is a global public issue owing to the overuse and abuse of antimicrobial agents.9,10 The emergence of multidrug resistant (MDR) bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA), carbapenem-resistant Enterobacteriaceae (CRE), carbapenem-resistant Pseudomonas aeruginosa (CRPA) and carbapenem-resistant Acinetobacter baumannii (CRAB), have raised significant healthcare concerns and brought great challenges to clinical treatment.11–13 To date, few studies have reported the characteristics of pathogens isolated from cerebrospinal fluid (CSF) samples among children in eastern China. Therefore, we investigated the epidemiology and drug resistance of pathogens isolated from CSF at a children’s medical center in eastern China.

Materials and Methods

Study Site

The Children’s Hospital of Soochow University (CHSU) is a children’s medical center in Jiangsu province, eastern China. In 2020, it had about 1,920,000 outpatients and 70,000 inpatients, with over 20,000 cases of surgery. This study was approved by the Ethics Committee of CHSU (No. 2020CS099). Written informed consent and confidentiality agreements were obtained from the parents of pediatric patients. This study was conducted in accordance with the guidelines of the Declaration of Helsinki.

Isolation of Strains

Non-duplicated pathogens were isolated and collected from CSF culture at CHSU from January 1, 2006 to December 31, 2020. Briefly, CSF samples from pediatric patients with suspected meningitis were inoculated into BACTEC vials and cultivated in the BACTEC FX system (BD). When the vials were reported as positive, subcultures were inoculated on Columbia blood agar plate and chocolate agar plate, and incubated overnight to observe the results.14

Identification of Strains

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, BRUKER) was used to identify the strains on the plates according to previous literatures.15 Identification scores were interpreted according to the manufacturer’s guidelines. A score over 2.000 indicated highly probable species-level identification and a score of 1.700–1.999 suggested highly probable genus level identification. A score of <1.700 was interpreted as “not reliable identification”. In addition, Escherichia coli (ATCC25922), Pseudomonas aeruginosa (ATCC27853), Enterococcus faecalis (ATCC29212) and Staphylococcus aureus (ATCC29213) were used as the standard strains for MALDI-TOF MS calibration.15,16

Inclusion and Exclusion Criteria of Pathogens

CSF culture was used as the gold standard method to detect meningeal pathogens. In this study, the inclusion criteria of isolates from meningitis patients included increased leukocytes counts, protein concentration >100 mg/dL and hypoglycorrhachia in the CSF, which persisted during the onset as described previously.17 The pathogens isolated from patients who had no these abnormal CSF findings were excluded.17

Antimicrobial Susceptibility Assay and Identification of MDR Pathogens

The antimicrobial susceptibility was determined using Vitek 2 Compact system (bioMérieux) and Kirby-Bauer (K-B) method as described previously.18 The results were analyzed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).19 Besides, Escherichia coli (ATCC25922), Pseudomonas aeruginosa (ATCC27853), Enterococcus faecalis (ATCC29212) and Staphylococcus aureus (ATCC29213) were used as the standard strains in the susceptibility assay as control. In addition, the MDR pathogens were defined as resistant to ≥ 1 agent in ≥ 3 antimicrobial classes according to previous studies.17,20

Data Analysis

WHONET software (version 5.6), developed by the World Health Organization Collaborating Centre for Surveillance of Antimicrobial Resistance, was used to extract the data from CSF testing results and to analyze the characteristics of pathogens and antibiotic resistance data.

Results

Clinical Characteristics and Epidemiology of Cerebrospinal Fluid Infection

In all, 307 pathogenic strains were isolated from the CSF in pediatric patients from January 1, 2006 to December 31, 2020. The annual trend of CSF infection in this study is shown in Figure 1. There was an increase in the overall cases during the period of 2006–2010, 2011–2015 and
In addition, the number of pathogens isolated from the CSF in winter (December-February) was the most during this 15-year study. However, the number of pathogens in summer (June-August) was the least (Figure 2). The clinical characteristics of the population are summarized in Table 1. Among the 307 children, 168 (54.72%) were male and there were always more male patients than female patients. The median age of these patients was 5 months (range: 1 day to 15 years) and most of them were <3 years old (78.50%, 241/307). Of note, 194 children (63.19%) were aged <1 year. Most pathogens were isolated from the departments of ICU (24.10%, 74/307), followed by neonatology (22.15%, 68/307) and infectious disease (16.61%, 51/307).

Characteristics of Pathogens Isolated from CSF

Among the pathogens isolated from the CSF, 291 strains (94.79%) were bacteria and 16 strains (5.21%) were fungi. More gram-positive than gram-negative bacteria were isolated during different time periods and seasons, and the number of fungi was very small (Table 2 and Figure 3). In addition, the number and percentage of gram-positive bacteria were both increased during different time periods, while the percentage of gram-negative bacteria was decreased (Table 2). The percentage of gram-positive bacteria was the most in autumn and the percentage of gram-negative bacteria was the most in summer (Table 2 and Figure 4).

In this 15-year study, the most prevalent pathogens were *Streptococcus pneumoniae* (26.06%, 80/307), *Escherichia coli* (20.20%, 62/307) and *Streptococcus agalactiae* (17.26%, 53/307) (Table 3). In addition, the most prevalent gram-positive bacterium was *Streptococcus pneumoniae* in the period from 2006 to 2015 and different seasons (Tables 3 and 4), while *Streptococcus agalactiae* was the most prevalent, followed by *Streptococcus pneumoniae* in the period from 2016 to 2020 (Table 3).
Moreover, the most prevalent gram-positive bacterium of children under 6 months was *Streptococcus agalactiae* (Table 5). However, *Escherichia coli* was always the dominant pathogen in the period from 2006 to 2020, across different seasons and patient ages among the gram-negative bacteria. Besides, all 16 strains of fungi were *Candida albicans* (Tables 3–5).

**Antimicrobial Susceptibility of Pathogens in the CSF**

The antimicrobial susceptibility of gram-positive bacteria is summarized in Table 6. *Streptococcus pneumoniae* exhibited high resistance to sulfamethoxazole trimethoprim, erythromycin, clindamycin, quinupristin/dalfopristin and tetracycline, while it showed high sensitivity to amoxicillin, cefotaxime, levofloxacin, rifampicin, linezolid, chloramphenicol and vancomycin. Similar to *Streptococcus pneumoniae*, *Streptococcus agalactiae* showed high resistance to erythromycin, clindamycin and tetracycline, and high sensitivity to other antibiotics. In addition, *Enterococcus faecium* showed high resistance to ampicillin, levofloxacin, rifampicin and minocycline. Moreover, while both *Staphylococcus epidermidis* and *Staphylococcus aureus* were highly resistant to

---

### Table 1 Clinical Characteristics and Epidemiology of CSF Infection at Children’s Hospital of Soochow University from 2006 to 2020

|                   | 2006–2010 (n=36) | 2011–2015 (n=128) | 2016–2020 (n=143) | Total (n=307) |
|-------------------|------------------|------------------|------------------|---------------|
| **Gender, n (%)** |                  |                  |                  |               |
| Male              | 19 (52.78)       | 67 (52.34)       | 82 (57.34)       | 168 (54.72)   |
| Female            | 17 (47.22)       | 61 (47.66)       | 61 (42.66)       | 139 (45.28)   |
| **Age, y**        |                  |                  |                  |               |
| Median            | 1 y              | 6 m              | 4 m              | 5 m           |
| Range             | 1 d–12 y         | 1 d–11 y         | 1 d–15 y         | 1 d–15 y      |
| **Age, n (%)**    |                  |                  |                  |               |
| <3 y              | 20 (55.56)       | 110 (85.94)      | 111 (77.62)      | 241 (78.50)   |
| <1 m              | 2 (5.56)         | 26 (20.31)       | 43 (30.07)       | 71 (23.13)    |
| 1–6 m             | 12 (33.33)       | 41 (32.03)       | 42 (29.37)       | 95 (30.94)    |
| 7–12 m            | 3 (8.33)         | 12 (9.38)        | 13 (9.09)        | 28 (9.12)     |
| 13–35 m           | 3 (8.33)         | 31 (24.22)       | 13 (9.09)        | 47 (15.31)    |
| 3–6 y             | 11 (30.56)       | 13 (10.16)       | 19 (13.29)       | 43 (14.01)    |
| 7–12 y            | 5 (13.89)        | 5 (3.91)         | 12 (8.39)        | 22 (7.17)     |
| 13–18 y           | 0 (0)            | 0 (0)            | 1 (0.70)         | 1 (0.33)      |
| **Wards, n (%)**  |                  |                  |                  |               |
| ICU               | 12 (33.33)       | 36 (28.13)       | 26 (18.18)       | 74 (24.10)    |
| Neonatology       | 1 (2.78)         | 17 (13.28)       | 50 (34.97)       | 68 (22.15)    |
| Infectious disease| 14 (38.89)       | 23 (17.97)       | 14 (9.79)        | 51 (16.61)    |
| Neurology         | 0 (0)            | 5 (3.91)         | 21 (14.69)       | 26 (8.47)     |
| Hematology        | 3 (8.33)         | 18 (14.06)       | 3 (2.10)         | 24 (7.82)     |
| Nephrology        | 4 (11.11)        | 19 (14.84)       | 0 (0)            | 23 (7.49)     |
| Neurosurgery      | 0 (0)            | 3 (2.34)         | 20 (13.99)       | 23 (7.49)     |
| Others            | 2 (5.56)         | 7 (5.47)         | 9 (6.29)         | 18 (5.86)     |

**Notes:** *: d, days; m, months; y, years. Others, including the departments of respiratory medicine, gastroenterology, endocrinology, cardiology and emergency medicine.

**Abbreviation:** ICU, intensive care unit.

### Table 2 An Overview of the Pathogens Isolated from CSF at Different Time Periods and Seasons in This Study

|                   | 2006–2010 (n=36) | 2011–2015 (n=128) | 2016–2020 (n=143) | Total (n=307) |
|-------------------|------------------|------------------|------------------|---------------|
| **Gram-positive bacteria** |                  |                  |                  |               |
| Gram-positive bacteria | 19 (52.78)       | 70 (54.69)       | 96 (67.13)       | 165 (68.26)   |
| Gram-negative bacteria | 17 (47.22)       | 50 (39.06)       | 39 (27.27)       | 106 (41.74)   |
| Fungi              | 0 (0)            | 8 (6.25)         | 8 (5.59)         | 16 (5.21)     |
| **Gram-negative bacteria** |                  |                  |                  |               |
| Gram-negative bacteria | 17 (47.22)       | 50 (39.06)       | 39 (27.27)       | 106 (41.74)   |
| Fungi              | 0 (0)            | 8 (6.25)         | 8 (5.59)         | 16 (5.21)     |
| **Fungi**          | 0 (0)            | 8 (6.25)         | 8 (5.59)         | 16 (5.21)     |

Moreover, the most prevalent gram-positive bacterium of children under 6 months was *Streptococcus agalactiae* (Table 5). However, *Escherichia coli* was always the dominant pathogen in the period from 2006 to 2020, across different seasons and patient ages among the gram-negative bacteria. Besides, all 16 strains of fungi were *Candida albicans* (Tables 3–5).
erythromycin and clindamycin, *Staphylococcus epidermidis* also showed high resistance to sulfamethoxazole trimethoprim.

The antimicrobial susceptibility of gram-negative bacteria is summarized in Table 7. *Escherichia coli* exhibited all sensitivity to amoxicillin/clavulanic acid, amikacin, cefotetan, cefoxitin and nitrofurantoin, and showed low resistance to aztreonam, piperacillin/tazobactam, tobramycin, ceftazidime, cefepime, ceftizoxime, cefotaxime, cefoperazone/sulbactam, meropenem, imipenem and ertapenem. *Klebsiella pneumoniae* showed all sensitivity to sulfamethoxazole trimethoprim, ciprofloxacin, tobramycin, levofloxacin and nitrofurantoin, and low resistance to amoxicillin/clavulanic acid, amikacin, ampicillin/sulbactam, aztreonam, gentamicin, piperacillin, piperacillin/tazobactam, cefotetan, cefotaxime, meropenem, imipenem and ertapenem. *Haemophilus influenza* exhibited high resistance to ampicillin and sulfamethoxazole trimethoprim, but showed high sensitivity to other antibiotics. *Pseudomonas aeruginosa* showed high resistance to ampicillin, ampicillin/sulbactam, sulfamethoxazole trimethoprim, ceftriaxone, cefazolin and cefoxitin, and *Acinetobacter baumannii* showed high resistance to amikacin, ampicillin, ampicillin/sulbactam, sulfamethoxazole trimethoprim, ciprofloxacin, gentamicin, piperacillin, piperacillin/tazobactam, tobramycin, cefotetan, ceftriaxone, cefepime, cefazolin, meropenem, imipenem, ertapenem and nitrofurantoin. Moreover, 30 strains of *Escherichia coli* and 5 strains of *Klebsiella pneumoniae* produced extended-spectrum β-lactamases (ESBLs).

The antimicrobial susceptibility of fungi is summarized in Table 8. 16 strains of *Candida albicans* showed sensitivity to 5-fluorocytosine and amphotericin B, and low resistance to voriconazole, fluconazole and itraconazole. Only 1 strain of *Candida albicans* showed resistance to voriconazole, fluconazole and itraconazole.

**Figure 3** The annual number of gram-positive bacteria, gram-negative bacteria and fungi isolated from CSF in this study.

**Figure 4** The percentage of gram-positive bacteria, gram-negative bacteria and fungi isolated from CSF at different seasons in this study.

**Characteristics of Multidrug Resistant Bacteria**

The emergence of MDR bacterial pathogens is an important global public health issue. In this study, 6 strains of pathogens isolated from CSF were identified as MDR bacteria, including methicillin-resistant *Staphylococcus*
Table 3 The Pathogens Isolated from CSF at Different Time Periods in This Study

|                      | 2006–2010 (n=36) | 2011–2015 (n=128) | 2016–2020 (n=143) | Total (n=307) |
|----------------------|------------------|-------------------|------------------|--------------|
| **Gram-positive bacteria** |                  |                   |                  |              |
| Streptococcus pneumoniae | 11               | 37                | 35               | 80           |
| Streptococcus agalactiae | 3                | 15                | 17               | 32           |
| Staphylococcus aureus | 2                | 5                 | 7                | 12           |
| Staphylococcus epidermidis | 2               | 5                 | 2                | 9            |
| Listeria monocytogenes | 1                | 4                 | 1                | 6            |
| **Gram-negative bacteria** |                  |                   |                  |              |
| Escherichia coli | 5                | 10                | 2                | 17           |
| Pseudomonas aeruginosa | 5                | 6                 | 3                | 14           |
| Klebsiella pneumoniae | 2                | 1                 | 3                | 6            |
| Haemophilus influenzae | 2                | 4                 | 2                | 8            |
| Others (5) |                   |                   |                  |              |
| **Fungi** |                   |                   |                  |              |
| Candida albicans | (8)              |                   | Candida albicans | (8)          |

Table 4 The Pathogens Isolated from CSF at Different Seasons in This Study

|          | Spring (n=77) | Summer (n=66) | Autumn (n=76) | Winter (n=88) | Total (n=307) |
|----------|---------------|---------------|---------------|---------------|--------------|
| **Gram-positive bacteria** |                  |               |               |               |              |
| Streptococcus pneumoniae | 23              | 14            | 24            | 19            | 80           |
| Streptococcus agalactiae | 11              | 8             | 17            | 17            | 53           |
| Enterococcus faecium | 6               | 5             | 3             | 6             | 20           |
| Staphylococcus epidermidis | 2               | 3             | 2             | 2             | 12           |
| Listeria monocytogenes | 2               | 3             | Others (5)    | Others (1)    | 6            |
| **Gram-negative bacteria** |                  |               |               |               |              |
| Escherichia coli | 18              | 15            | 8             | 21            | 62           |
| Pseudomonas aeruginosa | 3               | 4             | 3             | 2             | 10           |
| Klebsiella pneumoniae | 3               | 5             | Others (4)    | Others (2)    | 7            |
| Haemophilus influenzae | 3               | 3             | 2             | 9             | 16           |
| Others (3) |                   |               | Others (6)    |               |              |
| **Fungi** |                   | Candida albicans (1) | Candida albicans (3) | Candida albicans (7) | Candida albicans (16) |

https://doi.org/10.2147/IDR.S344720

*Infection and Drug Resistance 2021:14*
Table 5 The Pathogens Isolated from CSF at Different Ages Among Children in This Study

|                   | <1 m    | 1–6 m   | 7–12 m  | 13–35 m | Total (n=307) |
|-------------------|---------|---------|---------|----------|---------------|
| **Gram- positive bacteria** |         |         |         |          |               |
| Streptococcus agalactiae | 27      |         |         |          |               |
| Streptococcus pneumoniae | 11      | 18      | 45      | 11       |               |
| Staphylococcus aureus | 14      | 20      | 12      | 44       |               |
| Staphylococcus epidermidis | 10     | 3       | 3       | 19       |               |
| Listeria monocytogenes | 2       | 1       |         | 2        |               |
| **Gram- negative bacteria** |         |         |         |          |               |
| Escherichia coli | 24      | 22      | 3       | 56       |               |
| Pseudomonas aeruginosa | 5       |         | 3       | 8        |               |
| Klebsiella pneumoniae | 4       |         | 4       | 8        |               |
| Haemophilus influenzae | 4       |         |         | 4        |               |
| Others | 1       | 1       | 1       | 3        |               |
| **Fungi** |         |         |         |          |               |
| Candida albicans | 2       | 6       |         | 13       |               |
| Abbreviations: m, months; y, years.
aureus (MRSA), carbapenem-resistant *Escherichia coli* (CREC), carbapenem-resistant *Klebsiella pneumoniae* (CRKP), carbapenem-resistant *Acinetobacter baumannii* (CRAB) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) (Table 9). These strains were isolated from the department of hematology, neurosurgery and ICU, and associated with the diseases such as acute lymphocytic leukemia, spinal cord embolism, sepsis, craniocerebral trauma and hydrocephalus. Furthermore, these MDR bacteria were resistant to most of the antimicrobial drugs (Table 9).

**Discussion**

Meningitis is one of the most common clinical infectious diseases among children, which is often characterized by high morbidity and mortality. Cerebrospinal fluid culture is the conventional method to detect pathogens from patients with meningitis. In this study, children aged <3 years old were more susceptible to meningitis, accounting for 78.50%. Meningitis was more common in winter than summer, which meant that children are more susceptible in the colder months. Besides, meningitis is often exhibited as acute onset and pediatric patients are mainly from the ICU, the departments of neonatology and infectious disease.

Previous reports showed that *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenza* were the most prevalent in meningitis among children globally. In this study, the most prevalent bacteria were *Streptococcus pneumoniae* (26.06%), *Escherichia coli* (20.20%) and *Streptococcus agalactiae* (17.26%). Our results are consistent with previous studies among children in China. The most prevalent gram-negative bacterium was always *Escherichia coli* in this 15-year study, while the most prevalent gram-positive bacterium changed during this period. In 2006–2015, the most prevalent gram-positive bacterium was

| Antibiotics | *Streptococcus pneumoniae* (n=80) | *Streptococcus agalactiae* (n=53) | *Enterococcus faecium* (n=20) | *Staphylococcus epidermidis* (n=12) | *Staphylococcus aureus* (n=7) |
|-------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Amoxicillin | 2 (2.50) | – | – | – | – |
| Ampicillin  | – | 0 (0) | 20 (100.00) | – | – |
| Ampicillin/sulbactam | – | – | 2 (16.67) | – | – |
| Sulfamethoxazole | 51 (63.75) | 1 (1.89) | – | 5 (41.67) | 1 (14.29) |
| trimethoprim | – | – | – | – | – |
| Ciprofloxacin | – | 1 (1.89) | 4 (20.00) | 2 (16.67) | 1 (14.29) |
| Gentamicin | – | 0 (0) | 5 (25.00) | 3 (25.00) | 1 (14.29) |
| Ceftriaxone | – | 0 (0) | – | – | – |
| Cefepime | – | 0 (0) | – | – | – |
| Cefazolin | – | – | – | 1 (8.33) | – |
| Cefotaxime | – | – | – | 0 (0) | 1 (14.29) |
| Cefuroxime | 26 (32.50) | 0 (0) | – | – | – |
| Levofloxacin | 0 (0) | 21 (39.62) | 15 (75.00) | 1 (8.33) | 1 (14.29) |
| Erythromycin | 78 (97.50) | 43 (81.13) | 2 (10.00) | 9 (75.00) | 5 (71.43) |
| Clindamycin | 77 (96.25) | 40 (75.47) | 2 (10.00) | 5 (41.67) | 5 (71.43) |
| Quinupristin/dalfopristin | 53 (66.25) | 9 (16.98) | 0 (0) | 0 (0) | 0 (0) |
| Rifampicin | 2 (2.50) | 0 (0) | 16 (80.00) | 1 (8.33) | 1 (14.29) |
| Linezolid | 0 (0) | 0 (0) | 0 | 0 | 0 (0) |
| Chloramphenicol | 7 (8.75) | 7 (13.21) | – | – | 2 (28.57) |
| Tetracycline | 65 (81.25) | 41 (77.36) | 4 (20.00) | 1 (8.33) | 2 (28.57) |
| Vancomycin | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Moxifloxacin | – | 1 (1.89) | 2 (10.00) | 0 (0) | 0 (0) |
| Tigecycline | – | 0 (0) | – | 0 (0) | 0 (0) |
| Nitrofurantoin | – | – | 0 (0) | 0 (0) | – |
| Minocycline | – | – | 12 (60.00) | – | – |
| Teicoplanin | – | – | 0 (0) | 0 (0) | 0 (0) |
Streptococcus pneumoniae, while Streptococcus agalactiae was the most prevalent in 2016–2020. In addition, children aged <6 months were more susceptible to Streptococcus agalactiae and it was different from those aged >6 months, which means that empiric therapy may be different in children of different ages.

In this 15-year study, Streptococcus pneumoniae was the main causative pathogen for meningitis in children among all the isolates. Previous studies also reported that

### Table 7 The Antimicrobial Resistance of Gram-Negative Bacteria in This Study

| Antibiotics                        | **Escherichia coli** (n=62) | **Klebsiella pneumoniae** (n=10) | **Haemophilus influenzae** (n=9) | **Pseudomonas aeruginosa** (n=8) | **Acinetobacter baumannii** (n=3) |
|------------------------------------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|----------------------------------|
| Amoxicillin/clavulanic acid        | 0 (0)                       | 1 (10.00)                       | 0 (0)                           | –                               | –                                |
| Amikacin                           | 0 (0)                       | 1 (10.00)                       | –                               | 0 (0)                           | 2 (66.67)                        |
| Ampicillin                         | 47 (75.81)                  | 10 (100.00)                     | 3 (33.33)                       | 8 (100.00)                      | 3 (100.00)                       |
| Amoxicillin/sulbactam              | 20 (32.26)                  | 2 (20.00)                       | 0 (0)                           | 3 (37.50)                       | 2 (66.67)                        |
| Aztreonam                          | 15 (24.19)                  | 2 (20.00)                       | –                               | 1 (12.50)                       | 0 (0)                            |
| Sulfamethoxazole                   | 30 (48.39)                  | 0 (0)                           | 5 (55.56)                       | 3 (37.50)                       | 2 (66.67)                        |
| Ampicillin/macrolides              |                            |                                 |                                 |                                 |                                  |
| Ciprofloxacin                      | 23 (37.10)                  | 0 (0)                           | 0 (0)                           | 1 (12.50)                       | 2 (66.67)                        |
| Gentamicin                         | 19 (30.65)                  | 2 (20.00)                       | –                               | 1 (12.50)                       | 2 (66.67)                        |
| Piperacillin                        | 23 (37.10)                  | 2 (20.00)                       | –                               | 0 (0)                           | 2 (66.67)                        |
| Piperacillin/macrolides            |                            |                                 |                                 |                                 |                                  |
| Tobramycin                         | 3 (4.84)                    | 0 (0)                           | –                               | 1 (12.50)                       | 2 (66.67)                        |
| Cefotetan                          | 0 (0)                       | 1 (10.00)                       | –                               | 2 (25.00)                       | 3 (100.00)                       |
| Ceftazidime                        | 14 (22.58)                  | 5 (50.00)                       | 0 (0)                           | 2 (25.00)                       | 0 (0)                            |
| Cefotaxime                         | 26 (41.94)                  | 3 (30.00)                       | 0 (0)                           | 3 (37.50)                       | 2 (66.67)                        |
| Cefepime                           | 12 (19.35)                  | 4 (40.00)                       | –                               | 2 (25.00)                       | 2 (66.67)                        |
| Cefazolin                          | 34 (54.84)                  | 6 (60.00)                       | –                               | 6 (75.00)                       | 1 (33.33)                        |
| Cefoxitin                          | 0 (0)                       | 3 (30.00)                       | –                               | 5 (62.50)                       |                                  |
| Cefuroxime                         | 5 (8.06)                    | 1 (10.00)                       | –                               | –                               |                                  |
| Cefuroximic acid                   |                            |                                 |                                 |                                 |                                  |
| Ceftriaxone                        | 24 (38.71)                  | 3 (30.00)                       | 1 (11.11)                       | 1 (12.50)                       |                                  |
| Cefotaxime acid                    | 14 (22.58)                  | 4 (40.00)                       | –                               | 2 (25.00)                       |                                  |
| Cefoperazone/sulbactam             | 1 (1.61)                    | 3 (30.00)                       | –                               | 1 (12.50)                       | 0 (0)                            |
| Meropenem                          | 1 (1.61)                    | 2 (20.00)                       | 0 (0)                           | 1 (12.50)                       | 1 (33.33)                        |
| Imipenem                           | 1 (1.61)                    | 2 (20.00)                       | 0 (0)                           | 1 (12.50)                       | 1 (33.33)                        |
| Ertapenem                          | 1 (1.61)                    | 2 (20.00)                       | 0 (0)                           | 1 (12.50)                       | 1 (33.33)                        |
| Levofloxacin                       | 27 (43.55)                  | 0 (0)                           | 0 (0)                           | 1 (12.50)                       | 0 (0)                            |
| Aztreonam                          | –                           | –                               | 1 (11.11)                       | –                               | –                                |
| Chloramphenicol                    | –                           | –                               | 2 (22.22)                       | –                               | –                                |
| Tetracycline                       | –                           | –                               | 2 (22.22)                       | –                               | –                                |
| Cefaclor                           | –                           | –                               | 1 (11.11)                       | –                               | –                                |
| Nitrofurantoin                     | 0 (0)                       | 0 (0)                           | –                               | 2 (25.00)                       | 1 (33.33)                        |
| Ticarcillin                        | –                           | –                               | –                               | 0 (0)                           | –                                |
| Minocycline                        | –                           | –                               | –                               | –                               | 0 (0)                            |
| ESBL (+)                           | 30 (48.39)                  | 5 (50.00)                       | 0 (0)                           | –                               | –                                |

### Table 8 The Antimicrobial Resistance of Fungi in This Study

| Antibiotics | **S** (n, %) | **I** (n, %) | **R** (n, %) |
|-------------|--------------|--------------|--------------|
| 5-Fluorocytosine | 16 (100.00) | 0 (0) | 0 (0) |
| Voriconazole | 15 (93.75) | 0 (0) | 1 (6.25) |
| Fluconazole | 15 (93.75) | 0 (0) | 1 (6.25) |
| Amphotericin B | 16 (100.00) | 0 (0) | 0 (0) |
| Itraconazole | 15 (93.75) | 0 (0) | 1 (6.25) |
children were more susceptible to *Streptococcus pneumoniae* infection, and this is probably due to the high colonization rate and immature immune system of children.\textsuperscript{14} In 2017, a 13-valent pneumococcal conjugate vaccine against *Streptococcus pneumoniae* was introduced in China\textsuperscript{27} and the isolated number of *Streptococcus pneumoniae* was slightly decreased in 2016–2020 in this study. However, it was listed as a category II vaccine and was not mandatorily prescribed in China.\textsuperscript{27} More efforts are still needed to increase the coverage of this vaccine and reduce pneumococcal infection.

The drug resistance of pathogens is a global public health problem.\textsuperscript{9,10} The pathogens isolated from the CSF are often more virulent than those isolated from other infection sites and will cause more serious symptoms.\textsuperscript{26,29} If the pathogens are also MDR, it will be greatly challenging to treat and the outcomes will be worse.\textsuperscript{21,30,31} In this study, the drug resistance of the gram-positive bacteria, fungi and *Haemophilus influenza* were not high, while the other gram-negative bacteria exhibited higher resistance to antibiotics. In addition, 30 strains of *Escherichia coli* and 5 strains of *Klebsiella pneumoniae* produced ESBLs. Furthermore, 6 strains of pathogens isolated from the CSF were identified as MDR bacteria (MRSA, CREC, CRKP, CRPA and CRAB). These strains showed resistance to most of the antibiotics and clinicians had limited treatment options for patients. More attention should be paid and further research is needed to address these challenges.

### Conclusions

This study revealed the epidemiology and drug resistance of pathogens isolated from CSF samples of children in eastern China. There was an increase in the overall cases of meningitis among children in the recent 15 years, and *Streptococcus pneumoniae*, *Escherichia coli* and *Streptococcus agalactiae* were the most prevalent isolates. Several MDR bacteria were also identified, which merits further investigation to address these issues.

### Ethical Approval

This study was approved by the Ethics Committee of Children’s Hospital of Soochow University (No. 2020CS0999). Written informed consent and confidentiality agreements were obtained from the parents of all pediatric patients. This study was conducted in accordance with the guidelines of the Declaration of Helsinki.

### Acknowledgments

This work was supported by the Special Foundation for National Science and Technology Basic Research Program

| Number | Multidrug Resistant Bacteria | Wards       | Diagnosis                      | Drug Resistant Antibiotics                                                                 |
|--------|------------------------------|-------------|--------------------------------|---------------------------------------------------------------------------------------------|
| 1      | Methicillin-resistant        | Hematology  | Acute lymphocytic leukemia     | Sulfamethoxazole trimethoprim, Erythromycin, Ciprofloxacin, Rifampicin, Gentamicin, Cefoxitin, Levofloxacin |
|        | Staphylococcus aureus, MRSA  |             |                                |                                                                                             |
| 2      | Carbenapen-resistant         | Neurosurgery| Spinal cord embolism           | Ampicillin, Cefuroxime, Ceftriaxone, Cefotaxime, Cefazolin, Ampicillin/sulbactam, Meropenem, Imipenem, Ertapenem, Piperacillin/tazobactam, Cefepime, Cefoperazone/sulbactam |
|        | *Escherichia coli*, CREC     |             |                                |                                                                                             |
| 3      | Carbenapen-resistant         | Neurosurgery| Spinal cord embolism           | Ampicillin, Cefuroxime, Ceftriaxone, Cefotaxime, Cefazolin, Ampicillin/sulbactam, Meropenem, Imipenem, Ertapenem, Piperacillin/tazobactam, Cefepime, Cefoperazone/sulbactam |
|        | *Klebsiella pneumoniae*, CRKP|             |                                |                                                                                             |
| 4      | Carbenapen-resistant         | ICU         | Sepsis                         | Ampicillin, Tobramycin, Ciprofloxacin, Sulfamethoxazole trimethoprim, Ampicillin/sulbactam, Piperacillin/tazobactam, Cefotetan, Ceftriaxone, Cefepime, Meropenem, Imipenem, Ertapenem, Gentamicin, Amikacin, Piperacillin |
|        | *Klebsiella pneumoniae*, CRKP|             |                                |                                                                                             |
| 5      | Carbenapen-resistant         | ICU         | Craniocerebral trauma          | Ampicillin, Tobramycin, Ciprofloxacin, Sulfamethoxazole trimethoprim, Ampicillin/sulbactam, Piperacillin/tazobactam, Cefotetan, Ceftriaxone, Cefepime, Meropenem, Imipenem, Ertapenem, Gentamicin, Amikacin, Piperacillin |
|        | *Acinetobacter baumannii*, CRAB|         |                                |                                                                                             |
| 6      | Carbenapen-resistant         | ICU         | Hydrocephalus                  | Ampicillin, Cefazolin, Cefepime, Cefotaxime, Cefazidime, Cefuroxime, Meropenem, Imipenem, Ertapenem, Piperacillin/tazobactam, Cefepime, Cefotaxim, Cefoxitin, Cefazidime |
|        | *Pseudomonas aeruginosa*, CRPA|             |                                |                                                                                             |
of China (2019FY101200), the National Natural Science Foundation of China (82020106), the High-level Innovative and Entrepreneurial Talents Introduction Program of Jiangsu Province (2020-30186, 2020-30191), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (20KJB310009, 20KJB310012), the Medical Research Project of Jiangsu Commission of Health (M20200227), the Science and Technology Program of Suzhou (SKJY2021092, SYS2020163, SYS2019120, SL2021904, SS201867).

Disclosure
The authors report no conflicts of interest in this work.

References
1. Gonzalez-Granado LI. Acute bacterial meningitis. Lancet Infect Dis. 2010;10(9):596. doi:10.1016/S1473-3099(10)70184-5
2. Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev. 2010;23(3):467–492. doi:10.1128/CMR.00070-09
3. WHO and partners call for urgent action on meningitis; 2021. Available from: https://www.who.int/news/item/28-09-2021-who-and-partners-call-for-urgent-action-on-meningitis. Accessed September 28, 2021.
4. Li Y, Yin Z, Shao Z, et al. Population-based surveillance for bacterial meningitis in China, September 2006-December 2009. Emerg Infect Dis. 2014;20(1):61–69. doi:10.3201/eid2001.120375
5. Zhou T, Kuang M, Huang S, et al. Epidemiological characteristics and drug resistance analysis of cerebrospinal fluid microbial infections in Wenzhou Area. Infect Drug Resist. 2021;14:2091–2103. doi:10.2147/IDR.S312175
6. Chang JB, Wu H, Wang H, et al. Prevalence and antibiotic resistance of bacteria isolated from the cerebrospinal fluid of neurosurgical patients at Peking Union Medical College Hospital. Antimicrob Resist Infect Control. 2018;7:41. doi:10.1186/s13756-018-0323-3
7. Jiang H, Su M, Kui L, et al. Prevalence and antibiotic resistance profiles of cerebrospinal fluid pathogens in children with acute bacterial meningitis in Yunnan province, China, 2012–2015. PLoS One. 2017;12(6):e0180161. doi:10.1371/journal.pone.0180161
8. Sanguinetti M, Postaroro B, Beigelman-Aubry C, et al. Diagnosis and treatment of invasive fungal infections: looking ahead. J Antimicrob Chemother. 2019;74(Suppl2):ii27–ii57. doi:10.1093/jac/dkz041
9. Christaki E, Marcou M, Tofarides A. Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. J Mol Evol. 2020;88(1):26–40. doi:10.1007/s00239-019-09914-3
10. Hu F, Zhu D, Wang F, et al. Current status and trends of antibiotic resistance in China. Clin Infect Dis. 2018;67(suppl2):S128–S134. doi:10.1093/cid/ciy557
11. Algammal AM, Hetta HF, Elkelifs A, et al. Methicillin-resistant Staphylococcus aureus (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist. 2020;13:3255–3265. doi:10.2147/IDR.S272733
12. Miao M, Wen H, Xu P, et al. Genetic diversity of carbapenem-resistant Enterobacteriaceae (CRE) clinical isolates from a tertiary hospital in eastern China. Front Microbiol. 2019;9:3341. doi:10.3389/fmicb.2018.03341
13. Makhartia RR, El-Kholy I, Hetta HF, et al. Antibiogram and genetic characterization of carbapenem-resistant gram-negative pathogens incurred in healthcare-associated infections. Infect Drug Resist. 2020;13:3991–4002. doi:10.2147/IDR.S276975
14. Houri H, Tabatabaei SR, Saeey Y, et al. 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. doi:10.1016/j.ijid.2017.01.020
15. Yi M, Wang L, Xu W, et al. Species distribution and antibiotic susceptibility of Nocardia isolates from Yantai, China. Infect Drug Resist. 2019;12:3653–3661. doi:10.2147/IDR.S32098
16. AbdulWahab A, Taj-Aldeen SJ, Ibrahim EB, et al. Discrepancy in MALDI-TOF MS identification of uncommon Gram-negative bacteria from lower respiratory secretions in patients with cystic fibrosis. Infect Drug Resist. 2015;8:83–88. doi:10.2147/IDR.S80341
17. Houri H, Pormohammad A, Riahi SM, et al. Acute bacterial meningitis in Iran: systematic review and meta-analysis. PLoS One. 2017;12(12):e0169617. doi:10.1371/journal.pone.0169617
18. Kang H, Zheng W, Kong Z, et al. Disease burden and molecular epidemiology of carbapenem-resistant Klebsiella pneumoniae infection in a tertiary hospital in China. Ann Transl Med. 2020;8(9):605. doi:10.21037/atm.2020.03.122
19. Gu B, Bi R, Cao X, et al. Clonal dissemination of KPC-2-producing Klebsiella pneumoniae ST11 and ST48 clone among multiple departments in a tertiary teaching hospital in Jiangsu Province, China. Ann Transl Med. 2019;7(23):716. doi:10.21037/atm.2019.12.01
20. Mgiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–281. doi:10.1111/j.1469-0691.2011.03570.x
21. Li Y, Li D, Xue J, et al. The epidemiology, virulence and antimicrobial resistance of invasive Klebsiella pneumoniae at a children’s medical center in eastern China. Infect Drug Resist. 2021;14:3737–3752. doi:10.2147/IDR.S32353
22. Algammal AM, Hashem HR, Alftti KJ, et al. atpD gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-Proteus mirabilis. Sci Rep. 2021;11(1):9476. doi:10.1038/s41598-021-88861-w
23. Shen H, Zhu C, Liu X, et al. The etiology of acute meningitis and encephalitis syndromes in a sentinel pediatric hospital, Shenzhen, China. BMC Infect Dis. 2019;19(1):560. doi:10.1186/s12879-019-4162-5
24. Gaschignard J, Levy C, Romain O, et al. Neonatal bacterial meningitis: 444 Cases in 7 years. Pediatr Infect Dis J. 2011;30(3):212–217. doi:10.1097/inf.0b013e3181f8ab1e7
25. Sadaranani M, Pollard AJ. Bacterial meningitis in childhood. Adv Exp Med Biol. 2011;719:185–199. doi:10.1007/978-1-4614-0204-6_16
26. Madhi SA. Pneumococcal conjugate vaccine and changing epidemiology of childhood bacterial meningitis. J Pediatr. 2015;91(2):108–110. doi:10.1016/j.jpeds.2014.11.001
27. Chen K, Zhang X, Yao T, et al. Hospitalization for invasive pneumococcal diseases in young children before use of 13-valent pneumococcal conjugate vaccine, Suzhou, China. Emerg Infect Dis. 2021;27(1):69–75. doi:10.3201/eid2701.181415
28. McCormick DW, Wilson ML, Mankhambo L, et al. Risk factors for death and severe sequelae in Malawian children with bacterial meningitis, 1997–2010. Pediatr Infect Dis J. 2013;32(2):e54–61. doi:10.1097/INF.0b013e31826fa5f5a
29. Namani S, Milenkovic Z, Kuchar E, et al. Mortality from bacterial meningitis in children in Kosovo. J Child Neurol. 2012;27(1):46–50. doi:10.1177/0883073811413280
30. Edmond K, Clark A, Korczak VS, et al. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. Lancet Infect Dis. 2010;10(5):317–328. doi:10.1016/S1473-3099(10)70048-7
31. Okike IO, Johnson AP, Henderson KL, et al. Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United Kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. Clin Infect Dis. 2014;59(10):e150–e157. doi:10.1093/cid/ciu514
