ORIGINAL RESEARCH

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

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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 were >6 months old. The drug resistance of gram-positive bacteria, fungi and *Haemophilus influenza* 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

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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 influenza* were the most prevalent causative agents among children.[7] However, the prevalence of meningitis pathogens may change with respect to

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
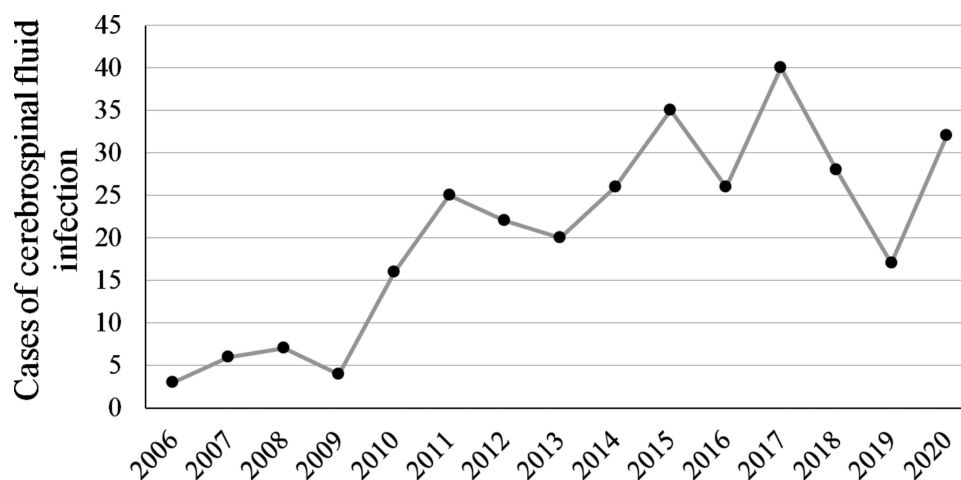
**Figure 1** The annual trends of CSF infection from 2006 to 2020 in this study.

2016–2020. 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).
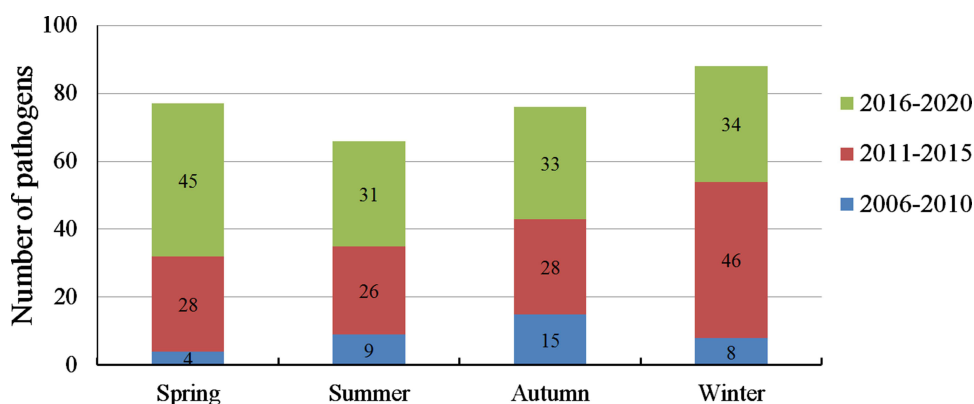
Figure 2 The number of pathogens isolated from CSF at different seasons in this study.

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*				
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

	Years			Seasons				Total (n=307)
	2006–2010 (n=36)	2011–2015 (n=128)	2016–2020 (n=143)	Spring (n=77)	Summer (n=66)	Autumn (n=76)	Winter (n=88)	
Gram-positive bacteria	19 (52.78)	70 (54.69)	96 (67.13)	46 (59.74)	38 (57.58)	54 (71.05)	47 (53.41)	185 (60.26)
Gram-negative bacteria	17 (47.22)	50 (39.06)	39 (27.27)	26 (33.77)	27 (40.91)	19 (25.00)	34 (38.64)	106 (34.53)
Fungi	0 (0)	8 (6.25)	8 (5.59)	5 (6.49)	1 (1.52)	3 (3.95)	7 (7.95)	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).
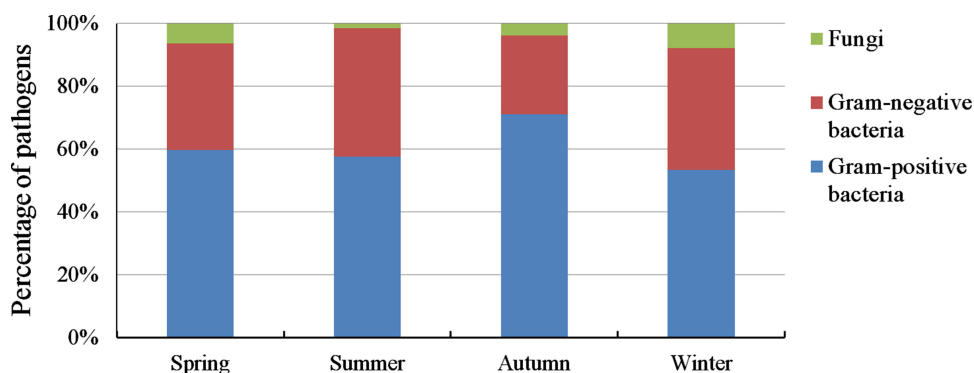
Antimicrobial Susceptibility of Pathogens in the CSF

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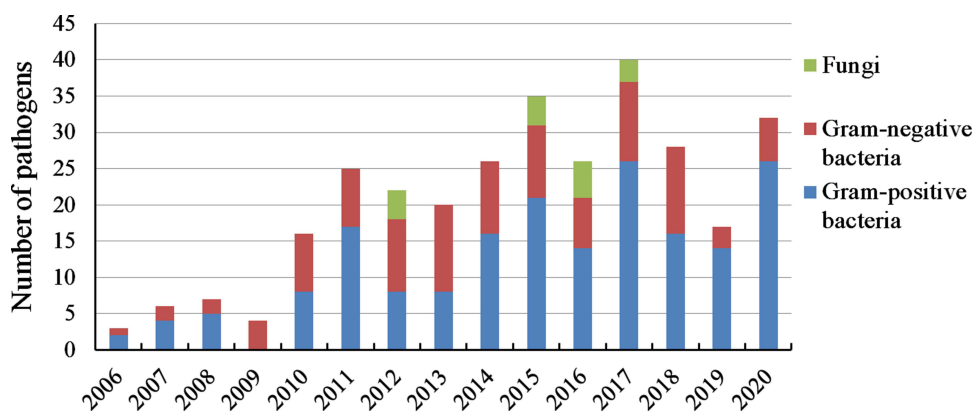
Figure 3 The annual number of gram-positive bacteria, gram-negative bacteria and fungi isolated from CSF in this study.

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

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, ceftizoxime, 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.

Characteristics of Multidrug Resistant Bacteria

The emergence of MDR bacterial pathogens is an important global public health issue.[21,22] 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) Streptococcus agalactiae (3) Staphylococcus aureus (2) Staphylococcus epidermidis (2) Listeria monocytogenes (1)	Streptococcus pneumoniae (37) Streptococcus agalactiae (15) Staphylococcus epidermidis (5) Listeria monocytogenes (4) Enterococcus faecium (4) Others (5)	Streptococcus agalactiae (35) Streptococcus pneumoniae (32) Enterococcus faecium (16) Staphylococcus epidermidis (5) Staphylococcus aureus (3) Others (5)	Streptococcus pneumoniae (80) Streptococcus agalactiae (53) Enterococcus faecium (20) Staphylococcus epidermidis (12) Staphylococcus aureus (7) Others (13)
Gram-negative bacteria	Escherichia coli (5) Pseudomonas aeruginosa (5) Klebsiella pneumoniae (2) Haemophilus influenzae (2) Others (3)	Escherichia coli (30) Klebsiella pneumoniae (6) Acinetobacter baumannii (3) Haemophilus influenzae (3) Pseudomonas aeruginosa (3) Others (5)	Escherichia coli (27) Haemophilus influenzae (4) Klebsiella pneumoniae (2) Stenotrophomonas maltophilia (2) Others (4)	Escherichia coli (62) Klebsiella pneumoniae (10) Haemophilus influenzae (9) Pseudomonas aeruginosa (8) Acinetobacter baumannii (3) Others (14)
Fungi	-	Candida albicans (8)	Candida albicans (8)	Candida albicans (16)

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) Streptococcus agalactiae (11) Enterococcus faecium (6) Staphylococcus aureus (2) Staphylococcus epidermidis (2) Others (2)	Streptococcus pneumoniae (14) Streptococcus agalactiae (8) Enterococcus faecium (5) Listeria monocytogenes (3) Staphylococcus epidermidis (3) Others (5)	Streptococcus pneumoniae (24) Streptococcus agalactiae (17) Staphylococcus epidermidis (7) Enterococcus faecium (3) Staphylococcus aureus (2) Others (1)	Streptococcus pneumoniae (19) Streptococcus agalactiae (17) Enterococcus faecium (6) Enterococcus faecalis (3) Others (2)	Streptococcus pneumoniae (80) Streptococcus agalactiae (53) Enterococcus faecium (20) Staphylococcus epidermidis (12) Staphylococcus aureus (7) Others (13)
Gram-Negative Bacteria	Escherichia coli (18) Haemophilus influenzae (3) Stenotrophomonas maltophilia (2) Others (3)	Escherichia coli (15) Pseudomonas aeruginosa (4) Enterobacter cloacae (2) Others (6)	Escherichia coli (8) Klebsiella pneumoniae (4) Pseudomonas aeruginosa (3) Haemophilus influenzae (2) Others (2)	Escherichia coli (21) Klebsiella pneumoniae (5) Haemophilus influenzae (3) Acinetobacter baumannii (2) Others (3)	Escherichia coli (62) Klebsiella pneumoniae (10) Haemophilus influenzae (9) Pseudomonas aeruginosa (8) Acinetobacter baumannii (3) Others (14)
Fungi	Candida albicans (5)	Candida albicans (1)	Candida albicans (3)	Candida albicans (7)	Candida albicans (16)

Table 5 The Pathogens Isolated from CSF at Different Ages Among Children in This Study

	<3 y					3–6 y	7–12 y	13–18 y	Total (n=307)
	<1 m	1–6 m	7–12 m	13–35 m	Total				
Gram-positive bacteria	Streptococcus agalactiae (27) Enterococcus faecium (9) Listeria monocytogenes (2) -	Streptococcus agalactiae (21) Streptococcus pneumoniae (16) Enterococcus faecium (6) Staphylococcus epidermidis (5) Enterococcus faecalis (3) Others (1)	Streptococcus pneumoniae (11) Listeria monocytogenes (2) Staphylococcus epidermidis (2) -	Streptococcus pneumoniae (18) Streptococcus agalactiae (4) Enterococcus faecium (4) Staphylococcus epidermidis (3) Streptococcus oralis (3) Others (3)	Streptococcus agalactiae (52) Streptococcus pneumoniae (45) Enterococcus faecium (19) Staphylococcus epidermidis (10) Listeria monocytogenes (6) Others (8)	Streptococcus pneumoniae (24) Staphylococcus aureus (5) Enterococcus faecium (1) -	Streptococcus pneumoniae (10) Staphylococcus epidermidis (2) Streptococcus agalactiae (1) Enterococcus faecalis (1) -	Streptococcus pneumoniae (1) -	Streptococcus pneumoniae (80) Streptococcus agalactiae (53) Enterococcus faecium (20) Staphylococcus epidermidis (12) Staphylococcus aureus (7) Others (13)
Gram-negative bacteria	Escherichia coli (24) Klebsiella pneumoniae (2) Others (5)	Escherichia coli (22) Pseudomonas aeruginosa (5) Klebsiella pneumoniae (4) Haemophilus influenzae (4) Others (2)	Escherichia coli (3) Klebsiella pneumoniae (2) Enterobacter aerogenes (1) Haemophilus influenzae (1) Stenotrophomonas maltophilia (1) -	Escherichia coli (7) Pseudomonas aeruginosa (3) Yersinia enterocolitica (1) Acinetobacter johnsonii (1) -	Escherichia coli (56) Klebsiella pneumoniae (8) Pseudomonas aeruginosa (8) Haemophilus influenzae (5) Enterobacter aerogenes (2) Others (9)	Escherichia coli (4) Haemophilus influenzae (4) Acinetobacter baumannii (2) Others (3)	Escherichia coli (2) Klebsiella pneumoniae (1) Acinetobacter pittii (1) Stenotrophomonas maltophilia (1) -	-	Escherichia coli (62) Klebsiella pneumoniae (10) Haemophilus influenzae (9) Pseudomonas aeruginosa (8) Acinetobacter baumannii (3) Others (14)
Fungi	Candida albicans (2)	Candida albicans (6)	Candida albicans (5)	-	Candida albicans (13)	-	Candida albicans (3)	-	Candida albicans (16)

Abbreviations: m, months; y, years.

Table 6 The Antimicrobial Resistance of Gram-Positive Bacteria in This Study

Antibiotics	*Streptococcus pneumoniae* (n=80)	*Streptococcus agalactiae* (n=53)	*Enterococcus faecium* (n=20)	*Staphylococcus epidermidis* (n=12)	*Staphylococcus aureus* (n=7)
	R (n, %)	R (n, %)	R (n, %)	R (n, %)	R (n, %)
Amoxicillin	2 (2.50)	–	–	–	–
Ampicillin	–	0 (0)	20 (100.00)	–	–
Ampicillin/sulbactam	–	–	–	2 (16.67)	–
Sulfamethoxazole trimethoprim	51 (63.75)	1 (1.89)	–	5 (41.67)	1 (14.29)
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)	–
Cefoxitin	–	–	–	0 (0)	1 (14.29)
Cefotaxime	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)

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.[4,23] Cerebrospinal fluid culture is the conventional method to detect pathogens from patients with meningitis.[24] 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.[7,25,26] 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

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)
	R (n, %)	R (n, %)	R (n, %)	R (n, %)	R (n, %)
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)
Ampicillin/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 trimethoprim	30 (48.39)	0 (0)	5 (55.56)	3 (37.50)	2 (66.67)
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/tazobactam	1 (1.61)	2 (20.00)	–	1 (12.50)	2 (66.67)
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)
Ceftriaxone	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)	–
Ceftizoxime	5 (8.06)	1 (10.00)	–	–	–
Cefuroxime	24 (38.71)	3 (30.00)	1 (11.11)	1 (12.50)	–
Cefotaxime	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)
Azithromycin	–	–	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	I	R
	(n, %)	(n, %)	(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)

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 9 The Characteristics of MDR Bacteria in This Study

Number	Multidrug Resistant Bacteria	Wards	Diagnosis	Drug Resistant Antibiotics
1	Methicillin-resistant *Staphylococcus aureus*, MRSA	Hematology	Acute lymphocytic leukemia	Sulfamethoxazole trimethoprim, Erythromycin, Ciprofloxacin, Rifampicin, Gentamicin, Cefoxitin, Levofloxacin
2	Carbapenem-resistant *Escherichia coli*, CREC	Neurosurgery	Spinal cord embolism	Ampicillin, Cefuroxime, Ceftriaxone, Cefotaxime, Cefazolin, Ampicillin/sulbactam, Meropenem, Imipenem, Ertapenem, Piperacillin/tazobactam, Cefepime, Cefoperazone/sulbactam
3	Carbapenem-resistant *Klebsiella pneumoniae*, CRKP	Neurosurgery	Spinal cord embolism	Ampicillin, Cefuroxime, Ceftriaxone, Cefotaxime, Cefazolin, Ampicillin/sulbactam, Meropenem, Imipenem, Ertapenem, Piperacillin/tazobactam, Cefepime, Cefoperazone/sulbactam
4	Carbapenem-resistant *Klebsiella pneumoniae*, CRKP	ICU	Sepsis	Ampicillin, Cefazolin, Cefepime, Cefotaxime, Ceftazidime, Cefuroxime, Meropenem, Imipenem, Ertapenem, Piperacillin/tazobactam
5	Carbapenem-resistant *Acinetobacter baumannii*, CRAB	ICU	Craniocerebral trauma	Ampicillin, Tobramycin, Ciprofloxacin, Sulfamethoxazole trimethoprim, Ampicillin/sulbactam, Piperacillin/tazobactam, Cefotetan, Ceftriaxone, Cefepime, Meropenem, Imipenem, Ertapenem, Gentamicin, Amikacin, Piperacillin
6	Carbapenem-resistant *Pseudomonas aeruginosa*, CRPA	ICU	Hydrocephalus	Meropenem, Imipenem, Ertapenem, Cefoperazone/sulbactam, Ampicillin, Cefazolin, Cefepime, Cefotaxime, Cefoxitin, Ceftazidime, Cefuroxime

children were more susceptible to *Streptococcus pneumoniae* infection, and this is probably due to the high colonization rate and immature immune system of children.[14] In 2017, a 13-valent pneumococcal conjugate vaccine against *Streptococcus pneumoniae* was introduced in China[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.[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.[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.[28,29] If the pathogens are also MDR, it will be greatly challenging to treat and the outcomes will be worse.[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. 2020CS099). 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.

Disclosure

The authors report no conflicts of interest in this work.

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