

Experimental research

The microbiology and clinical characteristics of bacterial and fungal meningitis in a Chinese hospital

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Abstract

Introduction: The aim was to investigate the distribution, antibiotic resistance, initial clinical and laboratory characteristics of pathogens isolated from the cerebrospinal fluid samples (CSF).

Material and methods: The CSF were subjected for identification. The minimum inhibitory concentrations (MICs) of antimicrobial agents were tested. The initial clinical and laboratory characteristics were collected.

Results: The rate of positive yield of pathogen meningitis in our study was 8.16% . Among the 1994 non-repetitive pathogens, Gram-positive pathogen was the most common (71.71%), followed by Gram-negative pathogen (20.36%), fungal (6.62%) and *Mycobacterium tuberculosis* (1.30%). Coagulase-negative staphylococci (CNS) was the most common Gram-positive pathogen and *Acinetobacter baumannii* (Ab) was the most common Gram-negative pathogen. As to fungal pathogen, *Cryptococcus neoformans* (CN) was the most common. The main CNS showed higher antimicrobial resistant rate for Penicillin G, oxacillin, clindamycin and erythromycin. *Acinetobacter baumannii* resistant to the most tested antibiotics and only showed considerable rate of resistance to cefoperazone/sulbactam, tigecycline and amikacin. All CN were susceptible to 5-fluorocytosine and only 3.6% CN were resistance to fluconazole. Long days of hospital stay, fever and meningeal irritation signs were common symptoms in the bacterial and fungal meningitis. Head injury, disturbance of consciousness, the pupil size of both eyes was not equal, pupillary reaction to light was slow or absent were more common in the Ab meningitis. The CSF protein concentration and white blood cells count as well as glucose concentration can be used in guiding initial treatment and further microbiological investigation.

Conclusions: The main pathogens showed higher antimicrobial resistant rate and the selection of empiric antibiotics should take into consideration local epidemiology, antibiotic resistance patterns and the suspected causative microorganism.

Key words: cerebrospinal fluid, central nervous system infections, bacterial meningitis, fungal meningitis, drug resistance, clinical characteristics.

Introduction

Bacterial and fungal meningitis, an infection of the meninges and cerebrospinal fluid surrounding the brain and spinal cord, is a serious threat to global health. Bacterial meningitis kills about a fifth of people

with the disease and up to half of the survivors suffer debilitating sequelae [1, 2]. It is a major cause of death and disability worldwide [3].

The main pathogens of bacterial and fungal meningitis are known to be different in different eras and geographic areas [4, 5]. Epidemiologic trends between western and eastern countries appear to differ in the percentage contributions of the major species inducing bacterial and fungal meningitis. In surveys from western countries, *Escherichia coli* was the leading pathogen among aerobic Gram-negative bacilli, which account for 4.3–12.3% of the pathogens causing bacterial meningitis [6]. In Asia, *Klebsiella pneumoniae* was the most common among the implicated Gram-negative pathogens and the overall incidence of meningitis caused by Gram-negative bacilli is 7.6–28.8% [6, 7], which is about twice the incidence in western countries.

To reduce the mortality and improve the cure rates, it is vital to screen and identify pathogens in clinical specimens from patients as early as possible [8, 9]. Early and accurate diagnosis will support physicians with the selection of the appropriate antimicrobial agents [10]. Most laboratory methods are mainly based on the identification of pathogens by staining or cultures, the detection of specific antigens and antibodies, or the examination of pathogen nucleic acids with polymerase chain reaction (PCR) in CSF [11, 12]. Although diagnosis based on culture is the gold standard for the diagnosis of bacterial and fungal meningitis, the clinical value of culture technique is limited due to its low sensitivity and long time required [13]. Early clinical suspicion and implementation of appropriate antimicrobial therapy are critical to minimize adverse outcomes. The decision to commence antibiotics is often based on clinical symptoms as well as on the preliminary laboratory results, which are readily available within an hour. The initial clinical and laboratory characteristics of patients can guide initial treatment and further microbiological investigation.

In spite of the harm caused by bacterial and fungal meningitis, the contribution of pathogens to meningitis remains poorly described in many regions of the various developing countries [14]. So we conducted the present study to investigate the frequency and antibiotic resistance of pathogens isolated from the cerebrospinal fluid samples (CSF) of patients admitted to Xiangya Hospital of Central South University with suspected meningitis and analyze the clinical and laboratory characteristics of bacterial and fungal meningitis to determine the utility of clinical and laboratory parameters in assessing the suspected causative microorganism.

Material and methods

Patients

This study was conducted at Xiangya Hospital of Central South University, a 3500-bed tertiary care facility with an annual admission of more than 100,000 inpatients, located in Hunan, China. Only the first strain isolated from CSF was collected when duplicate strains were from the same patient from 2012 to 2016. All isolates were identified to the species level by the Vitek-2 Compact system (bioMérieux, Durham, NC). The MICs of antimicrobial agents were determined by Vitek AST-GN13, AST-GN16 or AST-GP cards (bioMérieux, Durham, NC) for bacterial and by ATB FUNGUS 3 (bioMérieux, Durham, NC) for fungi. The results were interpreted according to the breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI, 2016) [15]. *Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as a quality control strain.

Data collection

Clinical information was obtained by reviewing patients' medical charts. The clinical data collected in the study included sex, age, days of hospital stay, head injury before hospitalization, invasive operation in brain, underlying diseases (diabetes, hypertension, chronic pulmonary disease, neurologic disease and chronic liver disease), bacteremia, the main clinical manifestations (fever, headache, nausea/vomiting, meningeal irritation signs, disturbance of consciousness, the pupil size of both eyes not being equal, pupillary reaction to light being slow or absent, twitch), the prescribed antimicrobial/immunosuppressant/hormone received within 7 days prior to positive culture of pathogen, laboratory results including CSF white blood cells (WBC) count and classification, CSF glucose concentration, CSF protein concentration, peripheral WBC and platelet count. Laboratory data were taken on the day of the first strain isolated from the CSF. This study was approved by the Xiangya Hospital Ethics Committee.

Statistical analysis

All the results were analyzed by the statistical package for social science software (version 20.0; SPSS, Chicago, IL, USA). Numerical variables with normal distribution were presented as the mean \pm standard deviation ($\bar{x} \pm SD$), and those without normal distribution were described as the median with range. Categorical variables were presented as the frequency (percentage). One-way ANOVA and least significant difference (LSD) t-test were used to compare continuous variables as appropriate. χ^2 test and χ^2 segmentation were used

to compare categorical variables as appropriate. A *p*-value of less than 0.05 was considered statistically significant (except for χ^2 segmentation), and all probabilities were two tailed.

Results

Distribution of pathogens in cerebrospinal fluid samples

A total of 1994 non-repetitive pathogens were isolated from 24 423 CSF samples obtained at the Xiangya Hospital of Central South University during 2012–2016 over a period of 5 years. The rate of positive yield of pathogen meningitis in our study was 8.16% (1994/24423). Most strains were identified from neurosurgery, neurology and intensive care unit (ICU). Men accounted for 64.0% of all patients, and the median age was 42 years (range: 0–81 years). Among the 1994 non-repetitive pathogens found in the CSF, Gram-positive pathogens were the most common (1430, 71.71%), followed by Gram-negative pathogens (406, 20.36%), fungal (132, 6.62%) and *Mycobacterium tuberculosis* (26, 1.30%). As to the Gram-positive pathogen, central nervous system (CNS) was the most common, followed by *Streptococcus pneumoniae*, *Enterococcus faecium*, *Corynebacterium* and *Staphylococcus aureus* (SA). As to the Gram-negative pathogens, Ab was the most common, followed by *Klebsiella pneumoniae* (Kp), *Escherichia coli* (Ec), other *Acinetobacter* and *Pseudomonas aeruginosa* (PA). As to the fungal pathogens, CN was the most common, followed by *Candida albicans* (Table I).

Antibiotic resistance rate of the main pathogens

Antibiotic resistance rate of the major *Staphylococcus*

Among the various antibiotics tested, linezolid, vancomycin, Quinupristin/Dalfopristin syncercid, tigecycline and nitrofurantoin were found most effective against the major *Staphylococcus*. The major *Staphylococcus* showed a higher antimicrobial resistant rate for penicillin G, oxacillin, clindamycin and erythromycin (Table II).

Antibiotic resistance rate of the *Streptococcus pneumoniae*

Among the various antibiotics tested, linezolid, vancomycin, ofloxacin, meropenem and ertapenem were found most effective against *Streptococcus pneumoniae*. *Streptococcus pneumoniae* showed a higher antimicrobial resistant rate for penicillin, erythromycin, tetracycline, clindamycin, trimethoprim/sulfamethoxazole and cefotaxime (Table III).

Antibiotic resistance rate of *Enterococcus faecium*

Among the various antibiotics tested, linezolid, vancomycin, Quinupristin/Dalfopristin syncercid and tigecycline were found most effective against *Enterococcus faecium*. *Enterococcus faecium* showed a higher antimicrobial resistant rate for penicillin G, ampicillin, erythromycin, tetracycline, ciprofloxacin and levofloxacin (Table IV).

Antibiotic resistance rate of the major Gram-negative bacteria

Ab was resistant to most tested antibiotics and only showed a considerable rate of resis-

Table I. Pathogen distribution of cerebrospinal fluid specimens

Organisms	Number	Percentage
Gram-positive bacteria:	1430	71.72
Coagulase-negative staphylococci	1016	50.95
<i>Streptococcus pneumoniae</i>	115	5.77
<i>Enterococcus faecium</i>	48	2.41
<i>Corynebacterium</i>	48	2.41
<i>Staphylococcus aureus</i>	40	2.01
<i>Enterococcus faecalis</i>	38	1.91
<i>Micrococcus lutea</i>	36	1.81
Other Gram-positive bacteria	89	4.46
Gram-negative bacteria:	406	20.36
<i>Acinetobacter baumannii</i>	148	7.42
Other <i>Acinetobacter</i>	31	1.55
<i>Klebsiella pneumoniae</i>	47	2.36
<i>Escherichia coli</i>	34	1.71
<i>Pseudomonas aeruginosa</i>	26	1.30
Other <i>Pseudomonas</i>	17	0.85
<i>Bacillus</i> genus	17	0.85
Other Gram-negative bacteria	86	4.31
Fungi and others:	158	7.92
<i>Cryptococcus neoformans</i>	92	4.61
<i>Candida albicans</i>	22	1.10
Other fungi	18	0.90
<i>Mycobacterium tuberculosis</i>	26	1.30
Total	1994	100.00

Table II. Antibiotic resistance rate of the major *Staphylococcus* species

Antibiotic	<i>Staphylococcus epidermidis</i>		<i>Staphylococcus haemolyticus</i>		<i>Staphylococcus capitis</i>		<i>Staphylococcus hominis</i>		<i>Staphylococcus aureus</i>	
	R (%)	I (%)	R (%)	I (%)	R (%)	I (%)	R (%)	I (%)	R (%)	I (%)
Penicillin G	94.3	0.0	91.3	0.7	78.6	0.0	92.3	0.0	95.5	0.00
Oxacillin	80.9	0.0	87.8	0.0	54.8	0.0	80.5	0.0	47.1	0.00
Gentamicin	17.2	11.3	55.6	7.7	16.0	19.3	0.8	6.5	23.5	1.5
Rifampicin	13.8	1.1	26.1	0.0	1.1	0.5	15.3	1.6	23.5	0.00
Ciprofloxacin	33.7	8.3	76.1	4.9	41.1	98.5	48.0	2.4	29.9	0.00
Levofloxacin	34.0	7.2	69.5	7.0	36.6	6.4	43.8	5.8	30.4	0.00
Moxifloxacin	10.9	22.9	41.2	28.6	21.7	16.1	40.0	3.3	25.0	4.4
Trimethoprim/ sulfamethoxazole	58.8	0.0	27.3	0.0	9.0	0.0	56.5	0.0	1.4	0.00
Clindamycin	44.2	0.6	61.8	0.0	34.4	9.1	78.0	0.9	50.7	1.4
Erythromycin	70.5	3.3	89.2	1.0	42.0	3.2	90.3	0.0	55.1	0.00
Linezolid	0.0	0.0	0.7	0.0	1.8	0.0	0.0	0.0	0.00	0.00
Vancomycin	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
Quinupristin/ Dalfopristin syncercid	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.00	0.00
Tetracycline	29.6	1.0	26.7	0.5	6.4	2.2	37.3	0.4	39.7	0.00
Tigecycline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
Nitrofurantoin	0.0	0.0	0.0	3.3	0.0	0.0	1.9	0.0	3.2	3.2

Table III. Antibiotic resistance rate of *Streptococcus pneumoniae*

Antibiotic	R (%)	I (%)	S (%)
Vancomycin	0.00	0.00	100
Linezolid	0.00	0.00	100
Ceftriaxone	13.6	9.1	77.3
Levofloxacin	12.9	0.00	87.1
Ofloxacin	0.00	0.00	100
Meropenem	0.00	0.00	100
Ertapenem	0.00	11.8	88.2
Erythromycin	86.5	2.7	10.8
Chloramphenicol	12.0	0.00	88.0
Tetracycline	80.0	0.00	20.0
Clindamycin	95.2	0.00	4.8
Cefotaxime	42.9	28.6	28.5
Penicillin	71.4	0.00	28.6
Telithromycin	5.9	0.00	94.1
Trimethoprim/ sulfamethoxazole	66.7	7.4	25.9

Table IV. Antibiotic resistance rate of *Enterococcus faecium*

Antibiotic	R (%)	I (%)	S (%)
Ciprofloxacin	83.9	12.9	3.2
Erythromycin	88.7	9.7	1.6
Ampicillin	89.1	0.0	10.9
Tetracycline	67.2	0.0	32.8
Penicillin G	85.5	0.0	14.5
High concentration gentamicin	22.7	0.0	77.3
Levofloxacin	80.8	0.0	19.2
Clindamycin	100 ^a	0.0	0.0
Linezolid	0.0	0.0	100
Vancomycin	3.1	6.3	90.6
Nitrofurantoin	33.3	60.0	6.7
Quinupristin/ dalfopristin	0.0	0.0	100
Tigecycline	0.0	0.0	100

Table V. Antibiotic resistance rate of the major Gram-negative bacteria

Antibiotic	<i>Acinetobacter baumannii</i>		<i>Klebsiella pneumoniae</i>		<i>Escherichia coli</i>	
	R (%)	I (%)	R (%)	I (%)	R (%)	I (%)
Ampicillin	100.0 ^a	0.0	100 ^a	0.00	96.6	0.00
Cefoperazone/sulbactam	13.2	31.3	42.2	11.9	1.9	3.7
Piperacillin/tazobactam	88.1	2.5	34.5	6.4	0.00	0.00
Aztreonam	100 ^a	0.0	71.3	0.00	55.2	0.00
Ampicillin/sulbactam	85.2	4.3	73.5	3.1	78.4	13.7
Ceftriaxone	90.0	8.7	75.7	0.00	79.3	0.00
Ceftazidime	87.1	1.7	61.8	3.9	34.5	1.8
Cefepime	90.6	0.4	51.1	10.6	38.1	0.00
Cefoxitin	100.0	0.0	58.3	0.00	0.00	0.00
Imipenem	86.5	0.9	34.8	0.00	1.7	0.00
Tigecycline	5.3	42.8	7.7	3.8	0.00	0.00
Gentamicin	81.3	3.1	51.8	0.00	65.5	0.00
Amikacin	47.1	1.3	31.6	0.00	6.9	0.00
Tobramycin	79.4	1.2	51.8	0.00	65.5	0.00
Levofloxacin	61.5	23.3	39.5	5.3	53.4	1.7
Ciprofloxacin	86.8	0.0	40.0	7.0	55.2	8.6
Trimethoprim/ Sulfamethoxazole	68.8	0.0	36.5	0.00	82.8	0.00
Nitrofurantoin	100.0	0.0	44.0	40.0	5.2	5.2
Cefazolin	100.0	0.0	79.7	0.00	82.9	4.9
Cefotetan	100.0	0.0	34.7	0.00	0.00	7.8

^aNatural resistance.

tance to cefoperazone/sulbactam, tigecycline and amikacin. *Klebsiella pneumoniae* showed a considerable rate of resistance to most tested antibiotics and was highly susceptible to tigecycline. *Escherichia coli* showed a higher antimicrobial resistant rate for ampicillin, ampicillin/sulbactam, ceftriaxone, gentamicin, tobramycin, trimethoprim/sulfamethoxazole, cefazolin and showed highly susceptible to cefoperazone/sulbactam, piperacillin/tazobactam, cefoxitin, imipenem, tigecycline, amikacin, nitrofurantoin and cefotetan (Table V).

Antibiotic resistance rate of *Cryptococcus neoformans* (CN)

All *Cryptococcus neoformans* were susceptible to 5-fluorocytosine and only 3.6% CN were resistance to fluconazole. There are no approved interpretive breakpoints available for amphotericin B, itraconazole and voriconazole (Table VI).

Table VI. Antibiotic resistance rate of *Cryptococcus neoformans*

Antibiotic	R (%)	I (%)	S (%)
5-Fluorocytosine	0.0	0.0	100.0
Amphotericin B	N	N	N
Fluconazole	3.6	6.5	89.9
Itraconazole	N	N	N
Voriconazole	N	N	N

Initial clinical and laboratory characteristics of study patients

The comparative results in the study patients infected with Coagulase-negative staphylococci (CNS), *Acinetobacter baumannii* and CN are listed in Table VII. The patients with *Mycobacterium tuberculosis* was too few for analysis. There was a statistically significant difference ($p < 0.05$) in head injury, invasive operation, fever, headache,

Table VII. Initial clinical and laboratory characteristics of study patients infected with CNS, Ab and CN

Parameter	CNS	AB	CN	χ^2 or <i>F</i>	<i>P</i> -value
Sex (male)	34 (68.0%)	30 (60.0%)	29 (58.0%)	$\chi^2 = 1.188$	0.552
Age [years]	41.44 ±20.90	44.06 ±17.39	42.26 ±19.38	<i>F</i> = 0.241	0.786
Days of hospital stay [days]	31.84 ±25.42	28.36 ±18.03	37.32 ±33.09	<i>F</i> = 1.481	0.231
Head injury	9 (18.0%)	20 (40.0%)	1 (2.0%)	$\chi^2 = 22.750$	< 0.001
Invasive operation	35 (70.0%)	43 (86.0%)	2 (4.0%)	$\chi^2 = 75.911$	< 0.001
Underlying diseases	9 (18.0%)	6 (12.0%)	9 (18.0%)	$\chi^2 = 0.893$	0.640
Bacteremia	2 (4.0%)	5 (10.0%)	8 (16.0%)	$\chi^2 = 4.000$	0.135
Fever	23 (46.0%)	32 (64.0%)	35 (70.0%)	$\chi^2 = 6.500$	0.039
Headache	24 (48.0%)	9 (18.0%)	41 (82.0%)	$\chi^2 = 41.021$	< 0.001
Nausea/vomiting	13 (26.0%)	10 (20.0%)	27 (54.0%)	$\chi^2 = 14.820$	0.001
Meningeal irritation signs	22 (44.0%)	27 (54.0%)	24 (48.0%)	$\chi^2 = 1.014$	0.602
Disturbance of consciousness	21 (42.0%)	39 (78.0%)	2 (4.0%)	$\chi^2 = 56.470$	< 0.001
The pupil size of both eyes was not equal	7 (14.0%)	18 (36.0%)	1 (2.0%)	$\chi^2 = 20.751$	< 0.001
Pupillary reaction to light was slow or absent	18 (36.0%)	38 (76.0%)	3 (6.0%)	$\chi^2 = 51.686$	< 0.001
Twitch	5 (10.0%)	5 (10.0%)	2 (4.0%)	$\chi^2 = 1.630$	0.443
The prescribed antimicrobial received	41 (82.0%)	35 (70.0%)	8 (16.0%)	$\chi^2 = 50.162$	< 0.001
The prescribed immunosuppressant/hormone received	23 (46.0%)	20 (40.0%)	7 (14.0%)	$\chi^2 = 13.020$	0.001
CSF WBC counts [$\times 10^6/l$]	550.73 ±1167.02	2480.89 ±3902.39	89.59 ±85.61	<i>F</i> = 14.129	< 0.001
CSF polymorphonuclear cell ratio (%)	62.66 ±34.92	79.24 ±27.10	41.76 ±30.05	<i>F</i> = 14.901	< 0.001
CSF glucose [mmol/l]	3.78 ±1.73	1.81 ±3.30	2.21 ±1.52	<i>F</i> = 15.272	< 0.001
CSF protein concentration [g/l]	1.27 ±0.87	2.03 ±1.09	0.99 ±0.72	<i>F</i> = 17.258	< 0.001
Peripheral blood WBC count [$\times 10^9/l$]	11.44 ±5.27	13.07 ±6.22	9.64 ±4.86	<i>F</i> = 5.653	0.004
Peripheral blood PLT count [$\times 10^9/l$]	255.14 ±159.72	266.94 ±139.74	244.64 ±141.47	0.287	0.751

nausea/vomiting, disturbance of consciousness, the pupil size of both eyes not being equal, pupillary reaction to light being slow or absent, CSF WBC counts, CSF polymorphonuclear cell ratio, CSF glucose concentration, CSF protein concentration, peripheral blood WBC counts, the prescribed antimicrobial received and the prescribed immunosuppressant/hormone received in the three groups.

Long duration of hospital stay (31.84 ±25.42 vs. 28.36 ±18.03 vs. 37.32 ±33.09 days, $p = 0.231$), fever (46.0% vs. 64.0% vs. 70.0%, $p = 0.039$) and meningeal irritation signs (44.0% vs. 54.0% vs. 48.0%, $p = 0.602$) were common symptoms in the three groups. There was no statistically significant difference ($p > 0.05$) in sex, age, underlying diseases, bacteremia, twitch and peripheral blood PLT counts among the three groups.

When compared to the CNS infection group, head injury, disturbance of consciousness, the pupil size of both eyes not being equal, pupillary reaction to light being slow or absent, high CSF WBC counts, high ratio of CSF polymorphonuclear cell and high CSF protein concentration were more common in the Ab infection group, while headache was more common in the CNS infection group compared to the Ab infection group (Table VIII).

When compared to the CN infection group, head injury, invasive operation, disturbance of consciousness, the pupil size of both eyes not being equal, pupillary reaction to light being slow or absent, the prescribed antimicrobial received, the prescribed immunosuppressant/hormone received and higher ratio of CSF polymorphonuclear cell were more common in the CNS and Ab infection group. Fever, headache and nausea/vomiting

were more common in the CN infection group compared to the CNS and Ab infection group. The CSF glucose in the CN infection group was lower than in the CNS infection group but higher than in the Ab infection group (Table IX).

Discussion

Central nervous system infections continue to afflict populations worldwide, especially due to their associations with mortality and long-term disability [16]. The prevalence rates for pathogens causing meningitis vary with time, geographical distribution, age, underlying medical or surgical conditions, and mode of infection [17, 18]. The rate of positive yield of pathogen meningitis in our study was 8.16%, which was higher than the results of previous studies [19–21]. The male-to-female ratio was 1.78 : 1 in our patients, indicating that males are prone to have meningitis. A similar sex discrepancy in meningitis can be found in other countries [22, 23]. The reasons for this may be the differences in the immune, endocrine and reproductive systems of males and females [24]. Almost

all microbes that are pathogenic to human beings have a potential to cause meningitis, but a relatively small number of pathogens account for most cases, although the reasons for this association remain partly understood [25]. An obvious increase in Gram-positive pathogens isolated from the CSF specimens can be found in our study. Gram-positive pathogens were responsible for 71.71% of the total isolates. The ratio of Gram-positive pathogens to Gram-negative pathogens was about 3.52 : 1, which appeared much higher than that observed in studies from other countries [19, 20, 26]. Reasons for this difference may include population differences in colonization, genetic differences in immune response and possibly geographic differences in laboratory techniques for pathogen isolation and reporting [27]. Staphylococcal, streptococcal and enterococcal bacteria were the common ones among Gram-positive pathogens, which were the common Gram-positive pathogens in other reports [7, 28, 29]. This finding highlights that treatment and management programs have been designed to address these bacteria. The reason for the low

Table VIII. χ^2 segmentation method

Variable	CNS vs. AB		CNS vs. CN		AB vs. CN	
	χ^2 value	P-value	χ^2 value	P-value	χ^2 value	P-value
Head injury	5.877	0.015	7.111	0.008	21.760	< 0.001
Invasive operation	3.730	0.053	46.718	< 0.001	67.919	< 0.001
Fever	3.273	0.070	5.911	0.015	0.407	0.523
Headache	10.176	0.001	12.703	< 0.001	40.960	< 0.001
Nausea/vomiting	0.508	0.476	8.167	0.004	12.398	< 0.001
Disturbance of consciousness	13.500	< 0.001	20.384	< 0.001	56.594	< 0.001
The pupil size of both eyes was not equal	6.453	0.011	4.891	0.027	18.778	< 0.001
Pupillary reaction to light was slow or absent	16.234	< 0.001	13.562	< 0.001	50.641	< 0.001
The prescribed antimicrobial received	1.974	0.160	43.577	< 0.001	29.743	< 0.001
The prescribed immunosuppressant/hormone received	0.367	0.545	12.190	< 0.001	8.574	0.003

The redefined P level was 0.0167 (0.05÷3 = 0.0167) after Bonferroni correction.

Table IX. LSD t-test

Parameter	CNS vs. AB		CNS vs. CN		AB vs. CN	
	T-value	P-value	T-value	P-value	T-value	P-value
CSF WBC count [$\times 10^6/l$]	4.038	< 0.001	0.975	0.331	5.029	< 0.001
CSF polymorphonuclear cell ratio (%)	2.305	0.023	2.853	0.005	5.454	< 0.001
CSF glucose [mmol/l]	5.185	< 0.001	4.216	< 0.001	1.035	0.302
CSF protein concentration [g/l]	4.192	< 0.001	1.519	0.131	5.688	< 0.001
Peripheral blood WBC counts [$\times 10^9/l$]	1.596	0.113	1.764	0.080	3.360	0.001

identification of *Streptococcus pneumoniae*, which has been reported as the most common cause of bacterial meningitis [30, 31], could be that less attention is given to the laboratory diagnosis of this pathogen. Gram-negative bacilli usually cause meningitis after head trauma or neurosurgery. Ab, KP and Ec were common etiological agents of meningitis, which is in accordance with most of the published studies [32–34]. Therefore the epidemiologic changes in meningitis should alter clinicians' behavior regarding antibiotic prescription [35, 36].

There should be no delay in starting appropriate empiric antibiotics once the bacterial or fungal meningitis is suspected, and this requires knowledge of the likely pathogens and their antibiotic susceptibilities. Antibiotics such as linezolid, vancomycin, Quinupristin/Dalfopristin syncercid, tigecycline and nitrofurantoin were found to be most effective against staphylococcal meningitis. Penicillin G, oxacillin, clindamycin and erythromycin were the least effective antibiotics against the staphylococcal isolates. Therefore, the choice of vancomycin or linezolid as the antimicrobial agent for staphylococcal infection-related meningitis was required. Vancomycin is recommended for methicillin-resistant staphylococcal meningitis. Linezolid may be chosen in cases of vancomycin resistance (MIC > 2 µg/ml) or in cases of contraindications to vancomycin [37]. Rifampicin could also be considered as supplementary therapy together with vancomycin or linezolid. Rifampicin must not be used as monotherapy to avoid the development of resistance [38].

After identification of the pathogen through culture and antibiotic susceptibility testing, the antibiotic treatment can be optimized. Linezolid, vancomycin, ofloxacin, meropenem and ertapenem were found to be the most effective drugs against *Streptococcus pneumoniae*. Reduced susceptibility to penicillin and third-generation cephalosporins of *Streptococcus pneumoniae* is a growing problem in China although resistance rates vary considerably between countries [2]. Some experts advise that when *Streptococcus pneumoniae* has been identified and susceptibility testing is pending or not available, empiric treatment should include vancomycin or rifampicin, although there is uncertainty regarding the benefit of adding vancomycin or rifampicin to a third-generation cephalosporin in *Streptococcus pneumoniae* meningitis patients [39–41]. Erythromycin, tetracycline, clindamycin, penicillin and trimethoprim/sulfamethoxazole were the least effective antibiotics against *Streptococcus pneumoniae*. By contrast, a higher resistance rate (92.1%) to macrolide antibiotics was also reported by Tsai *et al.* from Taiwan [42]. The increased rate of macrolide antibiotic resistance can possibly be correlated with the wide use of this antibiotic in

the communities because of its dose convenience, cost effectiveness, easy availability and the practice of prescribing it to treat suspected bacterial pneumonia cases.

Third-generation cephalosporin-resistant strains have become increasingly common in our study, which was also found in recent studies [43–45]. The resistance of Ab and KP to ceftriaxone/cef-tazidime was 90.0%/87.1% and 75.7%/61.8%, which is in accordance with the other studies [35, 46]. The third-generation cephalosporins have been widely used for the treatment of patients with meningitis for their penetrance well into the CSF after intravenous administration and have resulted in a dramatic decrease in meningitis-related mortality [47]. The increasing number of third-generation cephalosporin-resistant Gram-negative pathogens not only challenges the existing therapeutic options for serious central nervous system infection but also influences the choice of empiric antibiotics. Empiric use of antibiotics requires clinical decision-making taking into account both the risk of the antibiotics not being covered appropriately for a certain pathogen and the risks associated with the overuse of antibiotics. Based on this study, empiric use of third-generation cephalosporins should be reassessed in patients with meningitis, and it would be sensible to initiate further studies of other potentially effective agents such as carbapenem as the early empiric therapy, although there need to be further investigations to ensure that the empiric use of carbapenem can improve the survival of patients with meningitis.

In bacterial and fungal meningitis, clinical deterioration can occur rapidly and is often difficult to predict [48]. The decision to commence antibiotics is often based on clinical symptoms as well as on the preliminary CSF results, which are readily available within an hour. In our current study, we investigated the differences in the initial clinical and laboratory findings between patients associated with CNS, Ab and CN. Multiple studies have shown that headache, fever and neck stiffness were common signs and symptoms in patients with bacterial meningitis [1, 18, 49–51]. Our study also showed that fever and meningeal irritation signs were common symptoms in the three groups. Head injury, invasive operation, fever, headache, nausea/vomiting, disturbance of consciousness, the pupil size of both eyes not being equal, pupillary reaction to light being slow or absent, the prescribed antimicrobial received and the prescribed immunosuppressant/hormone received were predictive of a diagnosis of meningitis. Several studies have confirmed these findings [52–54]. In our study, of these factors, only head injury, headache, disturbance of conscious-

ness and pupillary reaction to light being slow or absent were significantly different among the three groups. Fever, headache and nausea/vomiting were more common in the CN infection group compared to the other two groups. Head injury, disturbance of consciousness, the pupil size of both eyes not being equal, and pupillary reaction to light being slow or absent were more common in the Ab infection group compared to the other two groups.

Another study noted that the extent of CSF abnormalities depends on the causative microorganism and can assess the cause of suspected meningitis [40]. Our study tried to assess the utility of CSF WBC, protein and glucose concentration in assessing the cause of suspected meningitis. There was a significant difference between the CSF parameters in Ab meningitis compared to CNS and CN meningitis. Abnormalities of CSF composition in Ab meningitis include pleocytosis of mainly polymorphic leukocytes, low glucose concentration and elevated protein levels. Abnormalities of CSF composition in CN meningitis are high WBC of normal polymorphic leukocytes, low glucose concentration and high protein levels. Abnormalities of CSF composition in CNS meningitis are high WBC of mainly polymorphic leukocytes, a normal glucose concentration and high protein levels. This is in accordance with some studies indicating that high CSF WBC counts, high ratio of CSF polymorphonuclear cells, high CSF protein concentration and low CSF glucose concentration indicate a potential diagnosis of bacterial meningitis when compared to CN and viral meningitis [1, 55, 56]. A previous study also showed that glucose levels lower than 1.81 mmol/l, protein levels over 2.03 g/l and leukocyte count over 2480 cells/mm³ are individual predictors of bacterial meningitis [56]. Some study have shown that both CSF protein concentration and WBC have a low specificity for distinguishing the cause of meningitis. However, this does not preclude clinical use of these CSF parameters, as definitive testing would be indicated even if a high specificity had been found. This study provides evidence that in cases of suspected meningitis, the CSF protein concentration and WBC as well as glucose concentration can be used in guiding initial treatment and further microbiological investigation.

Certain limitations and problems should be noted. First, we did not record cases of clinical meningitis without positive cultures of CSF as well as viral meningitis, which were difficulties encountered in conducting studies assessing disease in developing countries such as China. Second, our data were from a single hospital, so they are unlikely to represent the exact epidemiology of the whole area. Third, the hospital-based studies determining etiologies of meningitis were

conducted in academic tertiary hospitals where patients were likely to exhibit more severe and high previous antibiotic usage in the early stage of disease, which could lower the culture positive rate. Finally, there are few small towns and rural patients arrive who at this hospital were the main component of community-acquired bacterial meningitis; thus our data may not be representative of the spectrum of diseases in the population.

In conclusion, a variety of Gram-positive as well as Gram-negative organisms was documented. This situation calls for the longitudinal nation-wide surveillance for meningitis to document the real scenario regarding prevalent strains of pathogens. The selection of empiric antibiotics should take into consideration local epidemiology, antibiotic resistance patterns and the suspected causative microorganism according to the initial clinical and laboratory characteristics of patients. Early and focused treatment of the established disease is vital and further investigates into optimum empirical antibiotic therapy is needed.

Conflict of interest

The authors declare no conflict of interest.

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