Pathogen Analysis of Central Nervous System Infections in a Chinese Teaching Hospital from 2012–2018: A Laboratory-based Retrospective Study*

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Summary: Central nervous system (CNS) infections are associated with high mortality rates. The clinical presentation of many CNS infections by different pathogens is difficult to distinguish, but the definite diagnosis of the etiology is critical for effective therapy and prognosis. The aim of this study was to explore the etiology of CNS infections with definite diagnoses based on data from a clinical microbiology laboratory in Tongji Hospital, a teaching hospital in China, obtained over a six-year period. We conducted a retrospective study on all cerebrospinal fluid (CSF) specimens submitted to our clinical microbiology laboratory from September, 2012 to December, 2018. The etiology of CNS infections caused by *Cryptococcus neoformans*, *Mycobacterium tuberculosis* and common bacteria was analyzed. Antimicrobial susceptibility testing was conducted on all isolates. The results showed that 1972 cases of CNS infections were identified from 18 300 CSF specimens. Common bacterial meningitis (BM), cryptococcal meningitis (CM) and tuberculous meningitis (TM) accounted for 86.3% (677/785), 9.4% (74/785) and 4.3% (34/785) respectively of cases over the six-year period. BM was the most common among the different age groups, followed by CM. Of the TM cases, 44.1% (15/34) were distributed within the age group of 15–34 years, whereas for CM cases, 52.7% (39/74) occurred within the 35–54-year age group, and the age distribution of BM cases was fairly even. Among the bacterial pathogens isolated, *Staphylococcus epidermidis* was the most common, accounting for 12.5% (98/785), followed by *Acinetobacter baumannii* (ABA) and *Staphylococcus aureus* (SAU), accounting for 11.8% (93/785) and 7.6% (60/785) respectively. The resistance rates to antibiotics were >75%, with the exception of the resistance rate of ABA to tegafycline, which was <3%. More than 60% of SAU strains displayed resistance to penicillin, oxacillin, ampicillin/sulbactam, cefazolin, cefuroxime, gentamycin, tobramycin, erythromycin and levofloxacin, whereas more than 90% of SAU strains showed susceptibility to trimethoprim/sulfamethoxazole, tegafycline, vancomycin, teicoplanin and linezolid. For *C. neoformans*, the susceptibility rates to amphotericin B, 5-fluorocytosine, fluconazol and voriconazole were >95%. Analysis of samples from patients with CNS infection in a clinical microbiology laboratory at a teaching hospital in China over a six-year period indicated that the most common etiological agents were the bacteria ABA and SAU. The antibiotic resistance levels of ABA were found to be high and of concern, whereas isolates of *C. neoformans* were found to be sensitive to antifungal antibiotics.
Key words: central nervous system infection; bacterial meningitis; cryptococcal meningitis; tuberculous meningitis; antimicrobial resistance

Central nervous system (CNS) infections are relatively common and can result in serious disease and high mortality rates. Pathogens responsible for such infections include bacteria, viruses, fungi and parasites. However, the initial symptoms associated with infections by these different pathogens are difficult to distinguish[1]. Therefore, precise diagnoses of CNS infections with a particular pathogen are often lacking and information regarding the etiological agents of CNS infections is limited particularly in low- and middle-

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income countries[2]. Biopsy and culturing of brain tissue can provide definite pathogen evidence, but obtaining brain tissue is highly invasive. Instead, cerebrospinal fluid (CSF) specimens are commonly examined to determine the etiology of CNS infections[3–5]. Definite diagnosis of the etiology of meningitis is crucial for effective therapy and a positive prognosis.

CNS infections by viruses are the most common but are generally mild and self-limiting. CNS infections caused by bacteria, by contrast, are acute and often fatal[1]. Bacterial meningitis (BM) is a serious infectious disease with high rates of morbidity and a reported mortality rate of 15%–30%[6, 7]. It was reported that about 1.2 million cases of BM occur every year worldwide. However, the rates of incidence vary by country[8]. In the USA, the incidence of BM was 2.6 to 6 cases per 100 000 adults annually[9]. In the UK and western Europe the incidence of BM was 1–2 cases per 100 000 people per year, but in the Sahel region of Africa this rate could reach 1000 cases per 100 000 people per year[10–12]. In the pre-vaccine era, the most common pathogens to cause BM were *Haemophilus influenzae* type b, *Streptococcus pneumonia* and *Neisseria meningitides*[13]. Following the introduction of effective conjugate vaccines, the incidence rates of BM caused by *Haemophilus influenzae* type b, *S*. pneumoniae and *N*. *meningitides* decreased significantly[14]. Many reports then indicated that *Staphylococcus aureus* and gram-negative bacteria including *Acinetobacter*, *Klebsiella pneumoniae*, *Escherichia coli* and non-typhoidal *Salmonella*, were the leading causes of BM[15–17]. Meanwhile, reports from Africa and India showed that antibiotic resistance in gram-negative bacteria was increasing alarmingly[17, 18].

To date, limited data have been reported on the etiology of BM in central China. Wuhan is the capital city of Hubei province, located in the middle of central China. Tongji Hospital (including three branch hospitals), which has more than 6000 beds, is the largest teaching hospital in central China, located in Wuhan. The aim of this study was to describe the etiology of CNS infection and determine the levels of antibiotic resistance in the associated pathogens in this region of China.

1 MATERIALS AND METHODS

1.1 Ethical Aspects

The study protocol was approved by the Tongji Hospital Ethics Committee for Research in Health.

1.2 Study Population and Study Design

The study population included all patients for whom a lumbar puncture had been performed and a CSF specimen had been sent to the clinical microbiology laboratory in Tongji Hospital from September, 2012 to December, 2018. Each CSF specimen was examined for bacteria, fungus and *Mycobacterium tuberculosis* (TB) by culturing on different media using a smear method.

1.3 Isolation of Strains and Identification Methods

Columbia blood agar plates, chocolate plates and brain-heart infusion broth were used for bacterial culture. After 48 h of culture, the brain-heart infusion broth was transferred to Columbia blood agar plates. After 72 h of culture, if no bacterial growth was detected on the Columbia blood agar plate, chocolate plate or brain-heart infusion broth, the result was considered negative. However, if bacteria were isolated from any of these media, the result was considered positive. Strains that could not easily be identified were further investigated by manual biochemical reaction methods and/or instruments (i.e. VITEK-2 COMPACT, Biomerieux, France, or IVD-MALDIBIOTYPER velocitron, Bruker, Germany). Fungus was cultured on Sabouraud medium for 7 days, and *Crytococcus neoformans* was identified using the VITEK-2 COMPACT system and the IVD-MALDIBIOTYPER velocitron. A positive result with India ink stain was also considered confirmation of the presence of *C. neoformans*. TB was cultured by L-J media and liquid media (MGIT 960, BD Biosciences, USA). MPT-64-based rapid immunochromatographic tests (M. tuberculosis Diagnostic Kit, Colloidal Gold, Xinchuang Corporation, Hangzhou, China) were used for *Mycobacterium* identification and only TB was detected.

1.4 Antibiotic Resistance Testing

All of the bacterial isolates were tested by the Kirby-Bauer method for antimicrobial susceptibility according to the Clinical and Laboratory Standards Institute (CLSI) Guidelines, 2018[19]. All of the antibiotics tested were from Oxoid Corporation, UK. ATCC 25922, 25923, 35218, 700603 and 27853 were used as quality control strains. Antibiotic susceptibility was interpreted according to the CLSI guidelines, 2018[19]. The broth microdilution method was used for *C. neoformans* to test the susceptibility using ATB FUNGUS 3 (Biomerieux, France). ATCC 22019 was used as a quality control strain for ATB FUNGUS 3. The antibiotic resistance profile of *C. neoformans* was interpreted according to the manufacturer's instructions for ATB FUNGUS 3.

1.5 Statistical Analysis

The antibiotic susceptibility data were statistically analyzed using the software WHONET 5.6 (WHO).

2 RESULTS

2.1 Spectrum of Etiologies of CNS Infection

A total of 18 300 samples of CSF specimens were sent to the clinical laboratory from September, 2012 to December, 2018. Among these samples, 16 328

specimens were negative by culture for TB, *C. neoformans* and common bacteria. The other 1972 specimens had a preliminary etiological diagnosis of TB, *C. neoformans* or common bacteria. Of these specimens, 1187 were from repeat patients, leaving 785 non-duplicated samples with a preliminary etiological diagnosis. The number of samples diagnosed with cryptococcal meningitis (CM), tuberculous meningitis (TM) and BM was 74, 34 and 677, respectively.

2.2 Incidence of CNS Infection among Different Age groups

Of those specimens with a mixed infection, the proportions of the different pathogens, *C. neoformans*, TB and common bacteria, were 9.4% (74/785), 4.3% (34/785) and 86.3% (677/785), respectively. The study population was divided into four different age groups: <15, 15–34, 35–54 and ≥55 years. The proportions of the specimens from patients belonging to the above age groups were 15.8% (124/785), 26.6% (209/785), 37.0% (290/785) and 20.6% (162/785), respectively. Bacterial infections were the most common, with the above four age groups having the following proportions 17.7% (120/677), 26.3% (178/677), 35.9% (243/677) and 20.1% (136/677), respectively. For each type of pathogenic meningitis, the distribution of the age groups of the patients is shown in fig. 1. For CM, 35–54 years group was the most common age group infected, accounting for 52.7%. For TM, 15–34 years group, was the most common age group infected, accounting for 44.1%.

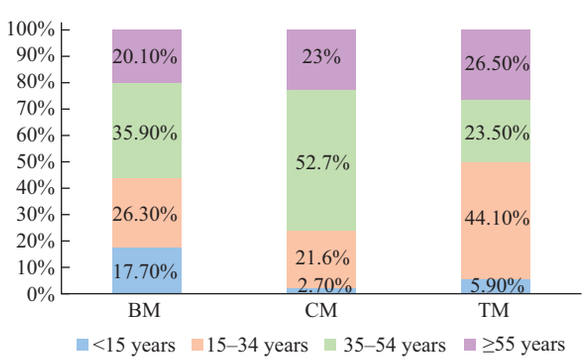

Fig. 1 The distribution of age groups in meningitis of different pathogens

Among bacterial infections, the most common pathogen was *Staphylococci epidermidis* accounting for 12.5% (98/785), followed by *Acinetobacter Bauman* (ABA) and *S. aureus* (SAU) accounting for 11.8% (93/785) and 7.6% (60/785), respectively (table 1).

2.3 Antimicrobial Resistance and Susceptibility

Of the 93 strains of ABA detected, the resistance rates to the common antibiotics were between 74.2% and 96.8%, with the exception of tegafycline that showed a resistance rate of 2.9%. The susceptibility rates to the common antibiotics were between 2.2% and 25.8%, with the exception of tegafycline that showed a susceptibility rate of 72.5% (table 2). For SAU, the highest resistance rate of >60% was detected for penicillin, oxacillin, ampicillin/sulbactam, cefazolin, cefuroxime, gentamycin, tobramycin, erythromycin and levofloxacin, and a rate of between 30% and 60% was detected for fosfomycin, clindamycin and rifampicin. The susceptibility rates to vancomycin, teicoplanin, linezolid and trimethoprim/sulfamethoxazole were >90% (table 3). For *C. neoformans*, the susceptibility rates to amphotericin B, 5-fluorocytosine, fluconazol and voriconazole were >95%, but the susceptibility rate to itraconazole was 73% (table 4).

Table 1 Composition of the main pathogenic bacteria causing meningitis

Pathogenic bacteria	*n*	%
Gram-positive cocci		
Staphylococcus epidermidis	98	12.5
Staphylococcus aureus	60	7.6
Staphylococcus haemolyticus	40	5.1
Coagulase-negative *Staphylococcus*	38	4.8
Enterococcus faecium	36	4.6
Staphylococcus hominis	27	3.4
Staphylococcus capitis	25	3.2
Enterococcus faecalis	24	3.1
Gram-negative bacilli		
Acinetobacter baumannii	93	11.8
Klebsiella pneumoniae	31	3.9
Pseudomonas aeruginosa	23	2.9
Escherichia coli	13	1.7
Fungus		
Cryptococcus neoformans	74	9.4
Candida parapsilosis	3	0
Candida albicans	2	0
Candida famata	1	0
Candida guilliermondii	1	0
Candida parapsilosis	1	0
Mycobacterium		
Mycobacterium tuberculosis	34	4.3

3 DISCUSSION

In our surveillance over six years, BM was the leading cause of laboratory-confirmed CNS infection, followed by CM and then TM. However, our report is not consistent with that reported for Gauteng province in South Africa from 2009 through to 2012, which showed that CM was the leading cause of laboratory-confirmed CNS infection, followed by TM[20]. The low detection rates of CM and TM may be related to the different detection methods employed. In our study, India ink staining and CSF culture were used for screening CM, and cryptococcal antigen was not

Table 2 Antimicrobial susceptibility rates and resistance rates (%) of *Acinetobacter baumannii*

Antimicrobial agent	*n*	Resistance rate (%)	Intermediary rate (%)	Susceptibility rate (%)
Piperacillin	93	88.2	7.5	4.3
Cefoperazone/sulbactam	92	76.1	7.6	16.3
Ampicillin/sulbactam	93	83.9	1.1	15.1
Piperacillin/tazobactam	93	83.9	4.3	11.8
Ceftazidime	93	87.1	1.1	11.8
Cefepime	93	86	0	14
Aztreonam	93	96.8	1.1	2.2
Imipenem	93	86	0	14
Meropenem	93	84.9	0	15.1
Amikacin	93	74.2	0	25.8
Gentamicin	93	88.2	0	11.8
Tobramycin	92	83.7	2.2	14.1
Ciprofloxacin	93	83.9	0	16.1
Levofloxacin	93	79.6	4.3	16.1
Trimethoprim-sulfamethoxazole	93	81.7	0	18.3
Tegafycline	69	2.9	24.6	72.5

Table 3 Antimicrobial susceptibility rates and resistance rates (%) of *Staphylococcus aureus*

Antimicrobial agent	*n*	Resistance rate (%)	Intermediary rate (%)	Susceptibility rate (%)
Penicillin G	60	96.7	0	3.3
Oxacillin	60	78.3	0	21.7
Ampicillin/sulbactam	60	61.7	11.7	26.7
Cefazolin	60	78.3	0	21.7
Cefuroxime	60	78.3	0	21.7
Gentamicin	60	66.7	0	33.3
Tobramycin	60	70	0	30
Rifampicin	60	31.7	1.7	66.7
Levofloxacin	60	68.3	0	31.7
Trimethoprim-sulfamethoxazole	60	0	1.7	98.3
Fosfomycin	57	35.1	3.5	61.4
Clindamycin	60	56.7	3.3	40
Erythromycin	60	70	0	30
Linezolid	60	0	0	100
Vancomycin	60	0	0	100
Teicoplanin	59	0	0	100
Tegafycline	33	3	3	93.9

Table 4 Antimicrobial susceptibility rates and resistance rates (%) of *Cryptococcus neoformans*

Antimicrobial agent	*n*	Resistance rate (%)	Intermediary rate (%)	Susceptibility rate (%)
Amphotericin B	74	1.4	0	98.6
5-fluorocytosine	74	0	2.7	97.3
Fluconazol	74	0	0	100
Itraconazole	74	0	27	73
Voriconazole	74	0	0	100

adopted. A study from Beijing involving specimens from 2002–2013 indicated that the sensitivity of detection by analysis of the CSF cryptococcal antigen, India ink staining and CSF culture was 81.5%, 85.3% and 82.4%, respectively. By combining these tests, the sensitivity increased to 91.2%[21]. As for TM, the Xpert MTB/RIF assay was not included in this analysis, which was recommended over conventional tests for the diagnosis of TB meningitis by the WHO[22].

In our study, *Staphylococcus epidermidis* accounted for the largest proportion of the bacteria isolated, but this organism constitutes part of the normal flora on the skin and mucous membranes and may therefore be considered a bacterial contaminant. SAU and ABA were the main pathogenic bacteria detected. A report from France from 2001 to 2013 indicated that *Streptococcus agalactiae* was the leading cause of BM, accounting for 55.8% of cases, followed by *E. coli*, accounting for 27.9%[23]. Another study from Tehran, Iran, from 2007 to 2010 revealed

that the most commonly isolated microorganism from CSF was *S. pneumoniae* (33.33%), followed by *N. meningitidis* (27.78%) and *H. influenzae* (16.67%)[24]. These discrepancies may be due to different vaccine use, and distinct social, economic and environmental factors in these regions.

CM has been reported to be the most common fungal infection of the CNS and the average annual incidence of CM was about 0.43/100 000 in China[25]. Our data indicated that CM was most common in the 35–54-year-old age group. Data from the USA from 1986–2012 showed that 41.4% of CM cases occurred in the 30–40-year-old age group[26]. This age distribution of CM patients might correlate with the prevalence of HIV infection. HIV-associated CM has been reported to have a high global burden[27]. Among CNS opportunistic infections, HIV-associated CM was the leading type and 15%–20% of deaths were caused by this infection, with even higher mortality rates (up to 70%) being reported in certain populations[28]. Accurate diagnosis of CNS infections is crucial for effective therapy and a positive prognosis. The method of detecting cryptococcal antigen in the CSF has high sensitivity (92%–100%) and specificity (83%–98%), with slightly lower levels of sensitivity for India ink staining of CSF (75%–86%)[27].

TM accounted for about 1% of all cases of TB and was the most severe form of TB, resulting in death or severe disability in around 50% of those with the disease[29]. According to the uniform case definition of TM by the Journal of Lancet Infectious Diseases, definitive diagnosis of TM should be confirmed by microbiological testing (AFB smear positive or culture positive) and the detection of acid-fast bacilli along with histological changes consistent with TB in the brain or spinal cord and suggestive symptoms or signs, accompanied by CSF changes, or visible CNS infection (on autopsy)[30]. Cases of probable or possible TM should be determined by using clinical criteria, CSF criteria and cerebral imaging criteria combined[30]. The TM cases in our study were all definite TM, confirmed by microbiological testing (AFB smear positive or culture positive), and did not include probable or possible TM.

Our resistance and susceptibility test results showed that ABA was highly resistant (>70%) to common antibiotics, with the exception of tegafycline. The global antimicrobial surveillance study and the Tigecycline Evaluation and Surveillance Trial (T.E.S.T) showed that meropenem resistant *Acinetobacter spp.* increased from 17.7% to 33.0% and multidrug-resistant *Acinetobacter spp.* increased from 25.6% to 49.7% in 2005–2007 to 2008–2012[31]. Antibiotic resistance levels were significant for ABA, but not for *C. neoformans*. Our data showed that sensitivity to the five anti-fungal drugs was >95% in all cases. In our study, SAU was most sensitive to vancomycin (100%), teicoplanin (100%), linezolid (100%), trimethoprim/sulfamethoxazole (98.3%) and tegafycline (93.9%).

This study had several limitations. We analyzed the etiology of CM, TM and BM, but viral causes (such as Japanese encephalitis virus, herpes simplex virus, rabies, varicella zoster virus, chikungunya virus, cytomegalovirus and dengue virus) and parasitic causes (such as malaria, neurocysticercosis, neuroschistosomiasis and soil-transmitted helminths) were not included. For CM, cryptococcal antigen detection was not performed, and therefore, the morbidity of CM might be underestimated.

In conclusion, bacterial pathogens were found to be the main etiological agents of CNS infection in this region of China. Interestingly, the main bacterial pathogens were no longer found to be *S. pneumoniae*, *H. influenzae* type b and *N. meningitides*, but instead were ABA and SAU. HIV-associated CM was detected at a high rate and therefore deserves close attention. ABA showed a high level of antibiotic resistance *in vitro*, unlike *C. neoformans*. Antibiotics should be carefully selected in clinic practice according to the results of *in vitro* susceptibility testing.

Conflict of Interest Statement

The authors declare that they have no competing interest.

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