MAJOR ARTICLE

Molecular Profile of Drug Resistance in Tuberculous Meningitis From Southwest China

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Background. Tuberculous meningitis (TBM) is the most severe form of extrapulmonary tuberculosis and causes high mortality and morbidity. Isoniazid resistance is strongly predictive of death in patients with TBM.

Methods. In the present study, using polymerase chain reaction (PCR) and Genotype MTBDRplus line-probe assay, we investigated the drug resistance in patients with TBM living in Southwest China.

Results. Our results showed that only one-third of patients with TBM had a positive result for *Mycobacterium tuberculosis* culture from cerebrospinal fluid (CSF). PCR-based detection of *M. tuberculosis* DNA in CSF is not only an alternative diagnostic approach for TBM but also can be further used for the detection of drug resistance when combined with the MTBDRplus assay, the results of which were consistent with the classic drug susceptibility test. However, it further provided the molecular profile of the mutations can be conducted much faster than the classic drug susceptibility test can (1 day vs 30–40 days, respectively). In the studied 30 CSF samples from patients with TMB, we found a rate of 64.29% for isoniazid resistance, 39.29% for rifampicin resistance, and 32.14% for multidrug-resistant tuberculosis, which is relatively higher than the reported resistance in pulmonary tuberculosis. However, the molecular profile indicated that the most frequently observed mutations in the *rpoB* and *katG* genes are also responsible for drug resistance in TBM.

Conclusions. Our data suggest that the MTBDRplus line-probe assay is capable of detecting drug resistance for the CSF samples that have a PCR-positive result. We recommend PCR-based diagnosis and drug resistance test as routine assays for patients with suspected TBM.

Tuberculous meningitis (TBM) is the most severe form of extrapulmonary tuberculosis (EPTB) and causes exceptionally high mortality and morbidity [1], even when the pathogenic organism, *Mycobacterium tuberculosis* (MTB), is sensitive to the first-line antituberculosis agents [2]. It has been shown that almost all patients infected with multidrug-resistant isolates, which are resistant to at least 2 of the most potent first-line antituberculosis drugs (rifampicin and isoniazid), are likely to die within 2 months after diagnosis [3, 4]. Rapid identification of drug-resistant MTB complex strains from cerebrospinal fluid (CSF) in high-risk patients with TBM is of crucial importance for appropriate and adequate treatment.

As the global prevalence of drug-resistant tuberculosis has increased, multidrug-resistant tuberculosis has been identified in patients with pulmonary tuberculosis (PTB) as well as EPTB [5]. Although the phenotype and genotype of drug resistance in PTB has been well documented, the status of drug resistance in EPTB has not been well studied and that in TBM even less. In developed countries, commercial molecular drug susceptibility tests (DSTs) have recently been introduced for rapid detection of multidrug-resistant PTB and have greatly contributed to the decrease of the prevalence of PTB when combined with good treatment programs [6, 7]. However, in developing countries such as China, these approaches have not been adopted in routine examination.

China is ranked second among the 22 high-burden countries that together account for 80% of the

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tuberculosis cases and ~22% of multidrug-resistant tuberculosis cases worldwide [8, 9]. Currently, there is no quick identification of and drug susceptibility program for MTB in most of the clinical microbiology laboratories in China. Our hospital, West China Hospital of Sichuan University (Chengdu, Sichuan, China), has been equipped with a platform integrating comprehensive diagnosis of tuberculosis and rapid detection of drug-resistant tuberculosis with current molecular biology techniques. In the present study, CSF samples were collected from patients with clinically suspected TBM and subjected to real-time polymerase chain reaction (PCR) detection for MTB DNA by means of a commercial kit (Qiagen, Hilden, Germany), which has previously been included in diagnostic criteria for TBM [10]. Sequentially, these CSF samples that were PCR-positive for MTB DNA were further tested for drug resistance with the Genotype MTBDRplus line-probe assay (Hain Lifescience, Nehren, Germany), which is based on DNA-Strip technology and permits the molecular genetic identification of the MTB complex and its resistance to rifampicin and/or isoniazid.

The performance of the MTBDRplus assays has been adequately validated in direct testing of smear-positive sputem samples and MTB isolates in various low-incidence settings, demonstrating excellent specificity and good concordance with phenotypic DST results [9, 11, 12]. In 2008, this commercial kit was approved by the World Health Organization for rapid screening of rifampicin and/or isoniazid resistance in settings of high-risk multidrug-resistant tuberculosis. In this kit, rifampicin resistance is identified by mutations in the *rpoB* gene (coding for the β-subunit of the RNA polymerase). The isoniazid resistance is identified by mutations in the *katG* gene (coding for catalase-peroxidase) and the promoter region of the *inhA* gene (coding for nicotinamide adenine dinucleotide, reduced enoyl-acyl carrier protein reductase). In the present study, we determined for the first time the molecular profiles of rifampicin and isoniazid resistance in patients with TBM living in Southwest China.

MATERIALS AND METHODS

From October 2009 through December 2010, there were a total of 2041 cases of suspected in TBM West China Hospital of Sichuan University, and 123 cases were diagnosed on the basis of positive results of MTB culture and/or PCR for MTB DNA of CSF samples, in combination with clinical features and radiological findings (computed tomography [CT] and magnetic resonance imaging [MRI]). This study was approved by the ethical committee of West China Hospital, Sichuan University, and signed consent forms were obtained from the 30 patients who were enrolled into this study.

Through lumbar puncture, ~10 mL of CSF was collected in a sterile bottle and subjected to 3 examinations: 2 mL was used for cellular and biochemical analysis, 6 mL for MTB culture and traditional drug resistance testing with the Bactec MGIT960 system (Becton Dickinson, Cockeysville, MD), and 2 mL for extraction of MTB DNA, which was used for PCR and MTBDRplus assay to determine the molecular profile of drug resistance. The amount of the CSF for these tests was adopted from a previous study [13] or the manufacturer's instructions. The cellular and biochemical analysis was performed with a routine CSF laboratory analysis (Modular P800; Roche Diagnostics, Basel, Switzerland). Isolation of MTB was performed using the Bactec MGIT960 liquid culture system. MTB DNA was extracted using NucliSens EasyMag (BioMérieux, Lyon, French). The standard DST was performed with the Bactec MGIT960 for isoniazid (0.1 and 0.4 μg/mL, representing low- and high-level resistance, respectively) and rifampicin (1 μg/mL). The genotype profile of drug resistance was examined using the MTBDRplus assay.

The presence of human immunodeficiency virus (HIV) antibody in serum samples was examined using the Modular Analytics E170 automated immunoassay analyzer (HIV Combi; Roche Diagnostics). Mutations in the *rpoB, katG,* and *inhA* genes associated with resistance to rifampicin and isoniazid were identified using MTBDRplus kits according to the manufacturer's instructions. Briefly, PCR (50 μL/tube; 40-cycle program) was performed using HotStar Taq DNA Polymerase (Qiagen). PCR products were analyzed in 2.0% agarose gel stained with ethidium bromide. After hybridization, membrane strips were attached to the evaluation sheet, read, and interpreted by an operator (who was blinded to the bacteriological results and vice versa), according to the manufacturer's recommendations.

The British Medical Research Council stage criteria [14] were used to grade the severity of TBM: stage I, a score of 15 for the Glasgow Coma Scale (GCS) without focal neurologic signs; stage II, signs of meningeal irritation with slight or no clouding of sensorium and minor or no neurological deficit (cranial nerve palsies;GCS, 11–14); stage III, severe clouding of sensorium, convulsions, focal neurological deficit, and involuntary movements (GCS, <10). The Glasgow Outcome Scale (GOS) [15] was assigned retrospectively to grade the outcome in follow-up: 1, death; 2, persistent vegetative state; 3, severe disability (dependent for daily support); 4, moderate disability (disabled but independent); and 5, good recovery (normal life with or without minor neurological and/or psychological deficit). Generally, a GOS score of 1–3 was considered to be a poor outcome and a score of 4 or 5 a good outcome.

Continuous variables were described with medians and ranges; categorical variables were described with numbers and percentages. Statistical analyses were performed with SPSS software (version 13.0; SPSS). The difference between groups was examined using the Fisher exact test. The differences were considered significant for $P < .05$.

Table 1. Demographic Profile, Clinical Features, and Prognoses of Patients With Tuberculous Meningitis Who Tested Positive by Cerebrospinal Fluid Culture and/or Polymerase Chain Reaction

Characteristics	No. (%) of culture-positive patients (n = 10)	No. (%) of PCR-positive patients (n = 20)	*P*	No. (%) of total patients (N = 30)
Median age, years (range)	26 (21–61)	30 (18–73)	…	29 (18–73)
Male sex	3 (30)	13 (65)	.122	16 (53)
Prior tuberculosis	4 (40)	4 (20)	.384	8 (27)
Clinical features at admission				
Headache	9 (90)	16 (80)	.640	25 (83)
Fever	6 (60)	15 (75)	.431	21 (70)
Vomiting	5 (50)	14 (70)	.425	19 (63)
Altered mentation	1 (10)	5 (25)	.633	6 (20)
Focal deficit	0 (0)	5 (25)	.140	5 (17)
Seizures	2 (20)	5 (25)	>.999	7 (23)
Meningeal signs	7 (70)	16 (80)	.657	23 (77)
Altered consciousness	0 (0)	7 (35)	.064	7 (23)
Coma	0 (0)	1 (5)	>.999	1 (3)
Cranial nerve palsy	1 (10)	6 (30)	.372	7 (23)
Hemiparesis and/or paraparesis	1 (10)	8 (40)	.204	9 (30)
BMRC severity grade				
I	8 (80)	1 (5)	<.001[a]	9 (30)
II	0 (0)	8 (40)	.030[a]	8 (27)
III	2 (20)	11 (55)	.119	13 (43)
MRI and CT abnormalities				
Hydrocephalus	1 (10)	3 (15)	>.999	4 (13)
Meningeal enhancement	3 (30)	8 (40)	.702	11 (37)
Infarction	4 (40)	4 (20)	.384	8 (27)
Tuberculoma	1 (10)	1 (5)	>.999	2 (7)
Extrameningeal tuberculosis	8 (80)	17 (85)	>.999	25 (83)
Prognosis				
Dead	0 (0)	1 (5)	>.999	1 (3)
Persistent vegetative state	0 (0)	3 (15)	.532	3 (10)
Severe disability	1 (10)	5 (25)	.633	6 (20)
Moderate disability	4 (40)	9 (45)	>.999	13 (43)
Good recovery	5 (50)	2 (10)	.026[a]	7 (23)

Abbreviations: BMRC, British Medical Research Council; CT, computed tomography; MRI, magnetic resonance imaging; PCR, polymerase chain reaction.

[a] $P < .05$.

RESULTS

All 30 patients with TBM who were enrolled in this study showed positive results for PCR amplification of MTB DNA, and 10 of them also showed positive results for culture. To dissect the value of the current drug resistance test in culture- and PCR-based diagnosis of TBM, we analyzed and presented the data for these patients in 2 separate groups: culture-positive patients and PCR-positive patients. The demographic profile, clinical features, and prognoses of patients are summarized in Table 1. The results of CSF analysis are summarized in Table 2. Although the symptoms and signs did not significantly differ between the 2 groups of patients, PCR-positive patients appeared to show a greater grade of disease severity and poorer outcome than did culture-positive patients ($P < .05$) (Table 1). Regarding the CSF analysis results, the cellular and biochemical compositions in both groups support the diagnosis of TBM. However, there was no significant difference between culture-positive and PCR-positive patients or between de novo and previously treated patients ($P > .05$ for all parameters). These data suggested that PCR detection of MTB DNA in CSF is a more sensitive alternative approach for the diagnosis of TBM.

The majority of patients with TBM presented typical clinical features (such as the triad of fever, headache, and meningeal signs) and CSF changes (such as leukocytosis, raised protein levels, and a plasma glucose level of <50% of CSF). Meningeal enhancement and infarction were the 2 most frequently observed imaging abnormalities on MRI and CT scans.

Table 2. Results of Cerebrospinal Fluid (CSF) Analysis for Patients With Tuberculous Meningitis Who Tested Positive by CSF Culture and Polymerase Chain Reaction

CSF analysis	Median (range) of culture-positive patients (n = 10)	Median (range) of PCR-positive patients (n = 20)	Median (range) of patients with de novo tuberculosis (n = 22)	Median (range) of previously treated patients (n = 8)	Median (range) of total patients (N = 30)
Opening pressure, cm H_2O	175 (95–260)	210 (80–260)	180 (80–260)	260 (140–260)	200 (80–260)
Total leukocyte count, 10^6 cells/L	260 (10–1150)	140 (8–320)	170 (8–320)	200 (90–1150)	170 (8–1150)
Lymphocyte count, %	38 (2–99)	38 (1–97)	43 (1–99)	32 (1–67)	38.0 (1–99)
Glucose level, mmol/L	1.66 (0.35–4.42)	1.635 (0.42–4.45)	1.635 (0.35–4.45)	1.815 (0.61–3.41)	1.635 (0.35–4.45)
CSF to blood ratio of glucose	0.28 (0.05–0.89)	0.28 (0.06–0.78)	0.28 (0.05–0.89)	0.26 (0.07–0.49)	0.28 (0.05–0.89)
Protein level, mg/dL	1.7 (0.34–4.15)	1.62 (0.33–5.45)	1.66 (0.33–5.45)	1.685 (1.08–2.17)	1.66 (0.33–5.45)
Chlorinate level, mmol/L	111.4 (104–125.2)	112.05 (99.4–146.6)	112.2 (99.4–146.6)	111.4 (104–119)	111.95 (99.4–146.6)
Valid GTplus results, no. (%) of patients	10 (100)	18 (90.0)	21 (95.5)	7 (87.5)	28 (93.3)

Abbreviations: GTplus, Genotype MTBDRplus line-probe assay; PCR, polymerase chain reaction.

Interestingly, acid-fast bacilli were not seen on any CSF smear and none of 30 patients was HIV-seropositive. In addition, recent history of tuberculosis contact was observed in only 8 (26.67%) of 30 patients. Concomitant extrameningeal tuberculosis was observed in 25 (83.33%) of 30 patients: 20 cases with pulmonary involvement and 5 with spine involvement.

Ten MTB isolates recovered from CSF samples were examined using the standard DST. Five (50%) of 10 strains were found to be sensitive and the remaining 5 were high-level isoniazid-resistant strains (3 multidrug-resistant strains and 2 strains monoresistant to isoniazid). Furthermore, the MTBDRplus assay was used to determine drug resistance in all 30 CSF samples. Interpretable results with no ambiguity in the hybridization pattern were obtained in samples from 28 (93.3%) of 30 patients. An unreadable result was defined as either no band at all or very weak or unreadable bands in *rpoB*, *katG*, and/or *inhA* sections; this was observed in PCR-positive 2 cases. In the culture-positive patients, the results of the MTBDRplus assay were consistent with standard DST results (Table 3). In the PCR-positive group, a drug resistance rate of 83.33% (15 of 18 patients) was observed, but it is not significantly different from the rate in the culture-positive group ($P = .091$). The drug-resistant patterns for rifampicin and isoniazid are summarized in the Table 3. Furthermore, to determine whether the drug resistance was related to previous treatment, we divided patients into de novo and previously treated groups and compared the pattern between these 2 groups. We found that although the pattern of monoresistance to rifampicin or isoniazid did not show significance, the rate of multidrug resistance was higher in the previously treated group than in the de

Table 3. Drug Resistance Patterns in Valid Results of the Genotype MTBDRplus Line-Probe Assay

Drug resistance pattern	No. of culture-positive patients (n = 10)	No. of PCR-positive patients (n = 18)	*P*	No. of patients with de novo tuberculosis (n = 21)	No. of previously treated patients (n = 7)	*P*	No. of total patients (N = 28)
Resistance to any drug, no. (%) of patients	5 (50.0)	15 (83.3)	.091	14 (66.7)	6 (85.7)	.633	20 (71.4)
Multidrug resistance	3	6	>.999	4	5	.020[a]	9
Monoresistance to rifampicin	0	2	.524	2	0	>.999	2
Monoresistance to isoniazid	2	7	.417	8	1	.371	9
Resistance to rifampicin, no. (%) of patients	3 (30.0)	8 (44.4)	.689	6 (28.6)	5 (71.4)	.076	11 (39.3)
Resistance to isoniazid,[b] no. (%) of patients	5 (50.0)	13 (72.2)	.412	12 (57.1)	6 (85.7)	.364	18 (64.3)
High	5	10	>.999	10	5	.385	15
Low	0	3	.533	2	1	>.999	3

[a] $P < .05$.

[b] The high- and low-level resistance is defined as resistance to isoniazid with a concentration of 0.1 and 0.4 μg/mL in the culture medium, corresponding to *katG* and *inhA* gene mutations, respectively.

novo group, supporting the idea that earlier exposure to antituberculosis agents may increase the chance of multidrug resistance in patients with TBM. As for the frequency of drug resistance, it appears that resistance to isoniazid is more common than that to rifampicin in all groups of patients with TBM.

Further analysis for genotypic results of the MTBDRplus assay indicated that 20 (71.43%) of 28 isolates of MTB from CSF samples harbored mutations that conferred resistance to rifampicin and/or isoniazid. Among these mutations, those at *rpoB* codons 530–533 accounted for 90.91% of the resistance to rifampicin and those at *katG315* accounted for 77.78% of resistance to isoniazid. A similar tendency was observed in the multidrug-resistant strains (Table 4). In summary, a high rate of drug resistance was observed in TBM strains. The drug resistance pattern and molecular profile for the mutations are demonstrated in Figure 1. For the details of the mutations for the hybridization pattern, please refer to Supplementary Table 1 (online only).

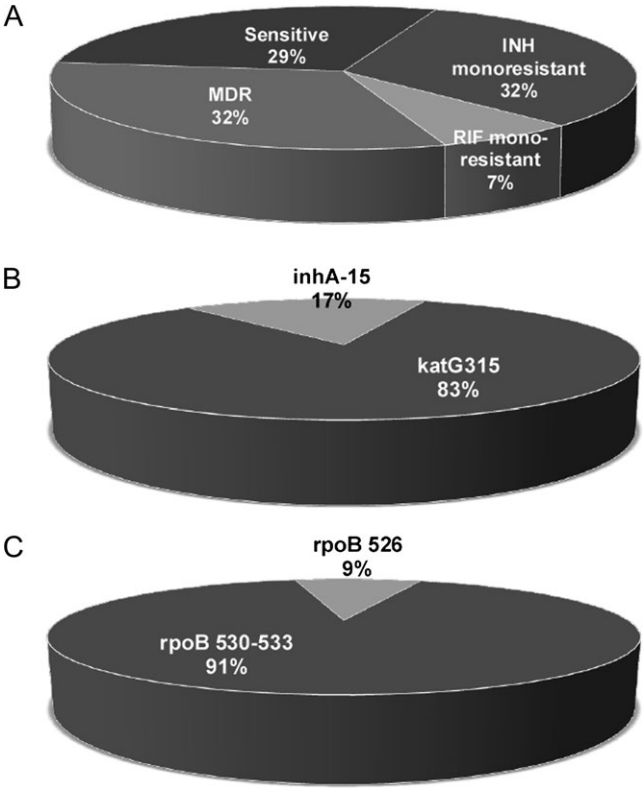

Figure 1. Phenotype and genotype of drug resistance in *Mycobacterium tuberculosis* isolates from patients with tuberculous meningitis (TBM). A total of 20 drug-resistant strains were detected in 30 cerebrospinal fluid samples from patients with TBM; the resistance is presented as the percentage of resistant isolates (*A*). Mutations in the *kat315* and *inhA* genes were detected among 18 isoniazid (INH)–resistant strains; the frequencies of the 2 mutations are shown (*B*). Mutations in *rpoB* 530-533 and *rpoB*526 were detected among rifampicin (RIF)–resistant strains; the frequencies of the 2 mutations in *rpoB* gene are shown (*C*). MDR, multidrug-resistant.

DISCUSSION

Sichuan province is located in Southwest China, bears 10% of the overall tuberculosis burden of China, and has an incidence of 64,5480 new PTB cases per year [16]. In 2008, the China Center for Disease Control and Prevention (CDC) reported that the mean prevalence of multidrug-resistant tuberculosis in China among all cases was 9.3% (5.4% among new cases and 25.6% among previously treated cases) [17]. Recently, Wu et al [12] reported that the rate of of multidrug resistance in Sichuan is 15.1%. In the present study, we found that the rate of multidrug resistance was 32.14% among 28 cases of TBM (18.2 % among new cases and 62.5 % among previously treated cases). In contrast to our findings on phenotypic resistance of 64.29% for isoniazid resistance, 39.29% for rifampicin resistance, and 32.14% for multidrug resistance among TBM strains, Wu et al [12] reported 19.12% for isoniazid resistance, 17.2% for rifampicin resistance, and 15.1% for multidrug resistance among pulmonary MTB strains. These results are similar to our acid-fast bacilli smear-positive sputum results in a recent study: 25% for isoniazid resistance, 28.8% for rifampicin resistance, and 19.2% for multidrug resistance [18]. Collectively, these results indicate that the rate of drug resistance in TBM is higher than in PTB and that different MTB strains may exist among pulmonary and meningeal tuberculosis.

Table 4. Genotypic Results of the Genotype MTBDRplus Line-Probe Assay for Detection of Rifampicin and Isoniazid Resistance

Drug, gene, codon	No. (%) of cases with any drug resistance (n = 20)	No. (%) of cases with multidrug resistance (n = 9)
Rifampicin, no. of cases	11	9
rpoB, 530–533	10 (90.91)	8 (88.89)
rpoB, 526	1 (9.09)	1 (11.11)
Isoniazid,[a] no. of cases	18	9
katG, 315	15 (83.33)	7 (77.78)
inhA, 15	4 (22.22)	2 (22.22)

[a] One case showed mutations in both *katG 315* and *inhA*.

Luo et al [19] recently reported the genotypic results of multidrug-resistant isolates from cases of PTB in the Shanghai area and showed that 65.2% of mutations in the *rpoB* gene were in codons 531–533 and 19.4% were in in codon 526. Mutations in *katG315* and *inhA* genes were observed in 72.7% and 9.9% of multidrug-resistant isolates, respectively. These results are similar to those in our previous study of acid-fast bacilli smear-positive sputum samples [18] and to the present results for multidrug-resistant strains from patients with TBM (88.89% of *rpoB* mutations in codons 531–533 and 11.11% in codon 526, and 77.78% of *katG315* and 22.22% of *inhA* mutations, respectively).

The S531L mutation in the *rpoB* gene and the S315T mutation in the *katG* gene were the 2 most frequently observed mutations among multidrug-resistant and monoresistant strains. These mutations also consistently occurred in the TBM strains studied in the present investigation. However, an unusually high percentage (77.78%) of strains monoresistant to isoniazid were observed to have a double pattern (positive hybridization with mutant and wild-type probes) in *katG315* mutants, which had been reported in previous studies of sputum samples [20, 21, 22]. According to the manufacturer's instructions, these results indicate the presence of either heterogeneous strains or mixed populations of MTB and were all interpreted as resistance to the relevant drug. The transmission of mixed strains with these mutations in PTB may be one mechanism underlying the high rate of these drug-resistant strains in overall tuberculosis cases. However, in the TBM, the possibility of superinfection with a second strain is unlikely. A recent study demonstrated that a isoniazid-resistant strain with the *katG315* mutation may be more likely to develop the multidrug-resistant capability [23]. Therefore, we speculate that the MTB strains with double patterns represent wild-type strains in the initial infection, some of which acquired these mutations in the development of TBM. Because we do not have data on the drug resistance in the primary site of infection (mostly the lungs), we do not know whether the mutation occurred before or after spreading to the meninges.

From the present study, we obtained some important information regarding TBM diagnosis and drug resistance. First, only one-third of patients with TBM showed a positive result for MTB culture, suggesting that a negative CSF culture is not a criterion to exclude TBM. Second, not only were the PCR-positive results consistent with all of the culture-positive results, but PCR also detected the presence of MTB in culture-negative CSF samples, supporting the proposal of its routine application for patients with suspected TBM [24]. Third, the results of the molecular drug resistance assay were consistent with those of the traditional standard DST method, but the molecular drug resistance assay is much faster (1 day vs 30–40 days), which is critically and especially valuable for patients with TBM as guidance for timely and adequate therapy. Finally, this is the first report on drug resistance in patients with TBM living Southwest China. We hope this study will inspire more colleagues to conduct similar research so that we can obtain the big picture of drug resistance in TBM in different regions.

In addition to the above findings, we also observed that that PCR-positive patients with TBM who lacked positive cultures from CSF had a greater disease severity and poorer prognosis (Table 1), although these patients received similar treatment: levofloxacin or amikacin plus the classic regimen (isoniazid, rifampicin, ethambutol, and pyrazinamide). One possible reason is a relatively high frequency of initial isoniazid monoresistance. Isoniazid is a cornerstone of the modern short-course chemotherapy for tuberculosis; it is unique among the first-line antituberculous agents for its dual properties of high penetration of CSF and early bactericidal activity, thereby making it a critical drug for the successful treatment of TBM [25]. CDC reported that isoniazid resistance is strongly predictive of death in patients with TBM [26]. Therefore, resistance to isoniazid could make a considerable contribution to the overall mortality and associated morbidity of TBM. Therefore, we urge all healthcare practitioners in areas with a high tuberculosis burden to be aware of drug-resistant tuberculosis in patients with suspected TBM.

There are several limitations in this study. First, our finding may not be applicable for the general population. Second, the PCR probe-based assay is not currently available for other antituberculosis drugs. Third, because this study included only 30 patients, some significant differences between groups may not have been detected during the statistical analysis. Accumulation of more phenotypic and genotypic data of drug resistance in patients with TBM from different regions is necessary to fully learn the big picture of drug resistance patterns in China, which will be helpful in developing new policies of public health administration and new therapies for patients with TBM.

In conclusion, the present study was performed on samples from patients with TBM who have tested positive for CSF culture of MTB or for MTB DNA by PCR. Our data suggest that the Genotype MTBDRplus line-probe assay is capable of detecting drug resistance in the CSF samples that have a PCR-positive result. We recommend that physicians order a nucleic acid amplification assay on CSF samples from all patients with suspected TBM. If a positive result is obtained, a molecular DST assay should be immediately requested.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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