BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Genetics and Molecular Microbiology

Comparison of culture and PCR methods in the diagnosis of bacterial meningitis

Emel Ödemiş Başpınar[a], *Saim Dayan*[b], *Muhammed Bekçibaşı*[c,*], *Recep Tekin*[b], *Celal Ayaz*[b], *Özcan Deveci*[b], *Salih Hoşoğlu*[d]

[a] *Celal Ertuğ Etimesgut State Hospital, Department of Infectious Diseases and Clinical Microbiology, Ankara, Turkey*
[b] *Dicle University School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Diyarbakır, Turkey*
[c] *Bismil State Hospital, Department of Infectious Diseases and Clinical Microbiology, Diyarbakır, Turkey*
[d] *Fatih University School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey*

ARTICLE INFO

Article history:
Received 7 January 2016
Accepted 23 June 2016
Available online 6 October 2016

Associate Editor: Ana Lucia Darini

Keywords:
Bacterial meningitis
Culture
Speed-Oligo
PCR

ABSTRACT

Our aim in this study is to compare the standard culture method with the multiplex PCR and the Speed-Oligo® Bacterial Meningitis Test (SO-BMT) – a hybridization-based molecular test method – during the CSF examination of the patients with the pre-diagnosis of acute bacterial meningitis. For the purposes of this study, patients with acute bacterial meningitis treated at the Dicle University Medical Faculty Hospital, Infectious Diseases and Clinical Microbiology Clinic between December 2009 and April 2012 were retrospectively evaluated. The diagnosis of bacterial meningitis was made based on the clinical findings, laboratory test anomalies, CSF analysis results, and the radiological images. Growth was observed in the CSF cultures of 10 out of the 57 patients included in the study (17.5%) and *Streptococcus pneumoniae* was isolated in all of them. The CSF samples of 34 patients (59.6%) were positive according to the SO-BMT and *S. pneumoniae* was detected in 33 of the samples (97.05%), while *Neisseria meningitidis* was found in 1 sample (2.95%). In a total of 10 patients, *S. pneumoniae* was both isolated in the CSF culture and detected in the SO-BMT. The culture and the SO-BMT were negative in 23 of the CSF samples. There was no sample in which the CSF culture was positive although the SO-BMT was negative. While SO-BMT seems to be a more efficient method than bacterial culturing to determine the pathogens that most commonly cause bacterial meningitis in adults, further studies conducted on larger populations are needed in order to assess its efficiency and uses.

Introduction

Bacterial meningitis is a serious infectious disease that can be fatal in children and in adults. Although its incidence has diminished due to the development of polysaccharide and conjugate vaccines in recent years, 1.2 million cases of bacterial meningitis is estimated to occur annually worldwide.[1] The incidence and mortality rates of bacterial meningitis vary

* *Corresponding author.*
E-mail: m_bekcibasi@hotmail.com (M. Bekçibaşı).
http://dx.doi.org/10.1016/j.bjm.2016.06.014

according to the geographical region, the type of pathogen and the age groups.[2] Since permanent neurological sequelae are observed in almost half of the survivors, a rapid diagnosis and treatment is crucial.[3] Excluding the neonatal period, *Streptococcus pneumoniae*, *Neisseria meningitis* and *Haemophilus influenzae* are the most frequently observed agents causing bacterial meningitis.[4]

The clinical symptoms observed in patients with bacterial meningitis are fever, headache, meningismus, cerebral dysfunction (altered consciousness ranging from confusion to delirium, lethargy and coma). Only two thirds of the adult patients with acute bacterial meningitis present the triad: involving fever, nuchal rigidity and altered mental state; however, at least one of these symptoms is observed in all the patients.[5] These classical symptoms may not be observed in neonates, the elderly and in patients with neutropenia. In these individuals, the altered mental state should not be attributed to other causes until meningitis is excluded through the analysis of the cerebrospinal fluid (CSF).[6]

The diagnosis of bacterial meningitis is based on the blood and CSF cultures and the microscopic and chemical analyses of the CSF samples. Empirical antibiotic treatment is to be initiated immediately based on the clinical findings. For an effective therapy of bacterial meningitis, the microorganisms and their antibiotic susceptibility patterns should be rapidly identified.[7]

While the CSF culture is the gold standard in the diagnosis of bacterial meningitis, the low bacterial growth rates particularly in the patients who have received antibiotic treatment before the lumbar puncture (LP) necessitated the development of new test methods.[8] Nucleic acid amplification tests such as the PCR can detect small amounts of pathogen DNA independently from the growth of the microorganism causing the disease.[9]

In this study our aim was to compare the standard culture method with the Speed-Oligo® Bacterial Meningitis Test (SO-BMT) which is a PCR-based molecular test during the CSF examination of the patients with the pre-diagnosis of acute bacterial meningitis (ABM) and to describe the optimum strategy to identify the bacterial pathogen.

Materials and methods

University of Dicle School of Medicine is the largest tertiary referral hospital in South Eastern region of Turkey with 1400 inpatient bed capacity. In this study we have retrospectively analyzed the adult patients with acute bacterial meningitis treated at University of Dicle School of Medicine, Infectious Diseases and Clinical Microbiology Clinic in Diyarbakır, Turkey, between December 2009 and April 2012.

The diagnosis of bacterial meningitis was made based on the clinical findings, laboratory test abnormalities, CSF analysis results and the radiological images. Patients with clinical and laboratory findings supporting meningitis and with specific pathogen growth in the CSF cultures were diagnosed with acute bacterial meningitis. Patients with negative CSF cultures, but with clinical symptoms consistent with bacterial meningitis were diagnosed with acute bacterial meningitis if the microscopic examination results of the CSF were as follows: >20 leukocytes/mm^3, neutrophil predominance, CSF protein concentration >45 mg/dL; simultaneous CSF glucose/blood glucose ratio <50–75%. Clinical symptoms of bacterial meningitis were fever, headache, nausea, vomiting, nuchal rigidity, Kernig and Brudzinski signs, convulsions, rash, and regional neurological symptoms. Exclusion criteria included age <16, malformations of the central nervous system; and viral, fungal or tuberculosis meningitis.

Before practicing the lumbar punctures (LP), the patients have undergone fundus examinations or cranial CT imaging when indicated in order to detect any counter indications for LP. Lumbar punctures were carried out by experienced clinicians under aseptic conditions and CSF samples were collected in 3 sterile tubes (0.5–1 mL). The first sample was used for the biochemical analysis, the second was used in the microscopic examination and culture inoculation, and the third sample was stored at −20 °C for the SO-BMT.

The CSF samples were centrifuged at 4000 rpm for 5 min and were inoculated to 5% sheep blood agar, EMB agar and chocolate agar. Samples inoculated to the media were stored in the incubator (WTB Binder, Germany) at 37 °C for 24 and 48 h. At the end of the incubation period, the plates were assessed through the conventional method. Identification and antibiotic susceptibility of the plates on which growth was observed was carried out using the PHOENIX 100 (Becton Dickinson, USA) device. The antibiotic susceptibility of the samples with growth was also verified with Disc Diffusion Tests (Oxoid, UK).

After the CSF samples were centrifuged at 3000 rpm for 10 min, the DNA extraction was performed in line with the manufacturer's instructions using the QIAamp DNA mini kit (Qiagen, USA). The extracted samples we obtained were amplified using the Speed-Oligo® Bacterial Meningitis kit (Vircell Microbiologists, Spain).[10] In this kit, the regions specific to the *lyt*A, *bex*A, and *ctr*A genes were selected for the detection of *S. pneumoniae*, *H. influenzae*, and *Neisseria meningitidis*, respectively. The separate strips containing the complementary probes for the target genes were placed on a single test strip to detect these three types of bacteria. Through this kit we used, serial dilutions of the purified DNAs of *S. pneumoniae*, *N. meningitidis* serogroup A, *N. meningitidis* serogroup B, *N. meningitidis* serogroup C, and *H. influenzae* were performed on the negative samples and up to 50 fragments of the DNA could be detected at each reaction of the kit. The test procedures and the evaluation of the results were performed according to the manufacturer's recommendations.

Statistical analysis

The statistical analyses were carried out using the SPSS for Windows software package version 18 (SPSS Inc., Chicago, IL). The comparison the sensitivity of the pathogens identified through the CSF culture and the molecular method was performed using Fisher's exact test. Variables with a p-value < 0.05 were considered as significant.

Table 1 – Demographic characteristics of the 57 ABM[a] patients.

Age (mean ± SD)	32.92 ± 16.1
Gender	
Male	28 (49.1%)
Female	29 (50.9%)
Antibiotic treatment before hospitalization	
Yes	24 (42.1%)
No	33 (57.9%)
Symptoms and signs	
Fever	46 (80.7%)
Consciousness	
Awake	12 (21.1%)
Confused	25 (43.8%)
Coma	20 (35.1%)
Nuchal rigidity	57 (100%)
Kernig	15 (26.3%)
Brudzinski	21 (36.8%)
Petechial rash	1 (1.8%)

[a] ABM, acute bacterial meningitis.

Results

Fifty-seven patients who were diagnosed and treated for acute bacterial meningitis between December 2009 and April 2012 were included in the study. The demographic characteristics of the patients are shown in Table 1. Among the patients, 29 (50.9%) were female while 28 (49.1%) were male. The mean age of the patients was 32.92 ± 16.1 years (range: 16–79 years).

The laboratory results of the patients are presented in Table 2. The growth was observed in the CSF cultures of 10 patients (17.5%) and *S. pneumoniae* was isolated in all of them. The CSF samples of 34 patients (59.6%) were positive according to the SO-BMT and *S. pneumoniae* was detected in 33 of the samples (97.05%), while *N. meningitidis* was found in 1 sample (2.95%). *S. pneumoniae* was isolated in the blood culture of 4 patients (7%).

Table 2 – Laboratory results of the patients.

Characteristics	All the patients ($n = 57$)
Blood	
Peripheral white blood cells	
<10.000/mm^{-3}	15 (26.4%)
>10.000/mm^3	42 (73.6%)
Serum C-reactive protein (mg/dL)	
0–8	7 (12.3%)
>8	50 (87.7%)
Erythrocyte sedimentation rate (mm/h)	
0–15	11 (19.3%)
>15	46 (80.7%)
CSF	
WBC count (mm^3)	
0–100	2 (3.5%)
101–500	8 (14.1%)
501–1000	6 (10.5%)
>1000	41 (71.9%)
Glucose ratio (CSF/blood)	
<2/3	57 (100%)
Protein (mg/dL)	
>45	57 (100%)
CSF culture positivity	10 (17.5%)

CSF, cerebrospinal fluid; WBC, white blood cells.

Table 3 – Comparison of the CSF culture and the SO-BMT results.

		CSF culture ($n = 57$)		Total
		+	–	
SO-BMT	+	10 (17.5%)	24 (42.1%)	34 (59.6%)
	–	0 (0%)	23 (40.4%)	23 (40.4%)
Total		10 (17.5%)	47 (82.5%)	57

$p = 0.004$.
CSF, cerebrospinal fluid; SO-BMT, Speed-Oligo® Bacterial Meningitis Test.

A total of 10 patients, *S. pneumoniae* was both isolated in the CSF culture and detected in the SO-BMT. In 24 out of the 34 patients with positive SO-BMT results (70.5%), the culture was negative. Among the 24 SO-BMT positive samples, 23 (95.8%) were positive for *S. pneumonia* while the remaining 1 (4.2%) was positive for *N. meningitidis*. The culture and the SO-BMT were negative in 23 of the CSF samples. There was no sample in which the CSF culture was positive although the SO-BMT was negative (Table 3). SO-BMT was observed to be a more efficient method than the CSF culture to determine the pathogens. Consequently, the agent was detected with the SO-BMT method in 59.6% of all the samples, while this ratio was 17.5% with the bacterial culture.

Discussion

The identification of the pathogen causing the bacterial meningitis in the CSF and the early initiation of the appropriate treatment is the most critical stage in the management of the disease. Even short delays in the diagnosis and treatment increase the rate of sequelae and mortality.[11] The CSF culture is the gold standard for the diagnosis of bacterial meningitis. The diminished sensitivity of the CSF culture in the patients who received antibiotics before the LP and the 72-h test period hinder clinicians from reaching a prompt diagnosis and starting the treatment in the ideal period.[12]

The latex agglutination test is a rapid diagnostic method that may detect the bacterial meningitis agents in less than 15 min. This test is recommended to be used in patients under the suspicion of bacterial meningitis in which no bacteria are observed in the gram staining of the CSF and no growth occurs in the CSF culture.[13] Studies have shown that the latex agglutination test has a very low sensitivity especially in the patients who have received antibiotic treatment before the lumbar puncture, which limits the use of his method.[14,15]

Delays in the diagnosis and treatment can be avoided through the routine use of PCR-based molecular methods in the patients under the suspicion of bacterial meningitis. This method, which is highly sensitive and specific, can also indicate the microorganisms in the CSF in patients who have used antibiotics before the LP.[5] Various nucleic acid amplification tests are currently in use to identify the bacterial meningitis agents. Through frequently employed methods

such as the real-time PCR or multiplex PCR, microorganisms in the CSF can be detected with high sensitivity and specificity. However, these methods are not preferred except in referral centers in developing countries since they require high cost equipment.[16] The SO-BMT can be considered as less costly compared to other molecular testing methods as it analyzes the most common bacterial meningitis agents *Streptococcus pneumonia*, *N. meningitidis* and *H. influenzae*. Also, the amplification step in SO-BMT takes between 15 and 75 min due to the thermocycler and the dipstick test takes only 5–10 min.

Since the SO-BMT kit is manufactured to test the three bacteria most frequently associated with bacterial meningitis in adults, unlike the previous studies where this test was used, we have only included adult patients to our study. The most commonly observed pathogen in our study was *S. pneumoniae* (97.05%), followed by *N. meningitidis* (2.95%). We could not isolate *H. influenzae*. Similarly to our study, *S. pneumoniae* is the most frequently isolated agent in the CSF cultures of the patient series reported from our hospital, also followed by *N. meningitidis*. *H. influenzae* could not be isolated.[12,17] This result may be associated with our exclusion of the pediatric patients and the high natural immunity to *H. influenzae* in the adult age group in Turkey.[18] Among the studies in the literature investigating the efficiency of the SO-BMT, Saglam et al.,[19] have detected *S. pneumoniae* and *H. influenzae* as the agents using the SO-BMT method. In this study, *H. influenzae* was the second most common agent, which may be explained with the inclusion of the pediatric patients. On the other hand, Gultepe et al.,[20] have observed *S. pneumoniae* followed by *N. meningitidis* as the most common agents, which was also in line with our study.

In this study, two out of the 10 patients with growth in the CSF culture (20%) and 15 out of the 34 patients with positive SO-BMT results (44.1%) had a history of antibiotic treatment before the LP. These results show that SO-BMT is much more sensitive than the culture method in the patients who have received antibiotics before the collection of the CSF sample.

Our study had certain limitations. First of all, since it is a retrospective design, we were not able to form a non-infectious control group. On the other hand, we did not include all the patients with the pre-diagnosis of bacterial meningitis and tried to minimize bias by selecting patients according to strict criteria based on clinical and laboratory results. Secondly, we have compared the SO-BMT method with the culture method which is considered as the gold standard in the diagnosis of bacterial meningitis. However, we did not include methods such as real-time PCR or multiplex PCR known to have high sensitivity and specificity in the diagnosis of bacterial meningitis. Since our study did not have a prospective design, we did not have the chance to include such high-cost tests into our comparison.

In conclusion, considering that 42.1% of the patients in our study had received antibiotics before the diagnostic tests, we can conclude that SO-BMT is a superior method than culturing to determine the pathogens most frequently causing bacterial meningitis in adults. Further studies conducted on larger populations are needed in order to assess its efficiency and use of SO-BMT in the diagnosis and treatment of bacterial meningitis in adults.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. World Health Organization. *Control of Epidemic Meningococcal Disease*. second ed. WHO Practical Guidelines; 1988.
2. Trotman H, Olugbuyi O, Barton M, McGregor D, Thomas S. Pneumococcal meningitis in Jamaican children. *West Indian Med J*. 2009;58(6):585–588.
3. Saravolatz LD, Manzor O, VanderVelde N, Pawlak J, Belian B. Broad-range bacterial polymerase chain reaction for early detection of bacterial meningitis. *Clin Infect Dis*. 2003;36(1):40–45.
4. World Health Organization. *Laboratory Methods for the Diagnosis of Meningitis Caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae*. WHO Meningitis Manual; 2011.
5. Durand ML, Calderwood SB, Weber DJ, et al. Acute bacterial meningitis in adults. A review of 493 episodes. *N Engl J Med*. 1993;328(1):21–28.
6. Tunkel AR. Acute meningitis. In: Mandell GBJ, Dolin R, eds. *Principles and Practice of Infectious Diseases*. 7th ed. New York, USA: Churchill-Livingstone; 2010:1205–1206.
7. du Plessis M, Smith AM, Klugman KP. Rapid detection of penicillin-resistant *Streptococcus pneumoniae* in cerebrospinal fluid by a seminested-PCR strategy. *J Clin Microbiol*. 1998;36(2):453–457.
8. Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. *Clin Microbiol Rev*. 2010;23(3):467–492.
9. Meyer T, Franke G, Polywka SK, et al. Improved detection of bacterial central nervous system infections by use of a broad-range PCR assay. *J Clin Microbiol*. 2014;52(5):1751–1753.
10. http://www.vircell.com/fileadmin/Material_Tecnico/Dossier_Speed-oligo_Ingles_v3.2.pdf Granada, Spain.
11. Brouwer MC, Thwaites GE, Tunkel AR, van de Beek D. Dilemmas in the diagnosis of acute community-acquired bacterial meningitis. *Lancet*. 2012;380(9854):1684–1692.
12. Celal A, Faruk GM, Salih H, Kemal CM, Serife A, Faruk KO. Characteristics of acute bacterial meningitis in Southeast Turkey. *Indian J Med Sci*. 2004;58(8):327–333.
13. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. *Clin Infect Dis*. 2004;39(9):1267–1284.
14. Nigrovic LE, Kuppermann N, McAdam AJ, Malley R. Cerebrospinal latex agglutination fails to contribute to the microbiologic diagnosis of pretreated children with meningitis. *Pediatr Infect Dis J*. 2004;23(8):786–788.
15. Tarafdar K, Rao S, Recco RA, Zaman MM. Lack of sensitivity of the latex agglutination test to detect bacterial antigen in the cerebrospinal fluid of patients with culture-negative meningitis. *Clin Infect Dis*. 2001;33(3):406–408.
16. Wang Y, Guo G, Wang H, et al. Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis. *BMC Pediatr*. 2014;14:224.

17. Hosoglu S, Ayaz C, Geyik MF, Kokoglu OF, Ozen A. Acute bacterial meningitis in adults: analysis of 218 episodes. *Ir J Med Sci*. 1997;166(4):231–234.
18. Sönmez C, Çöplü N, Kurtoğlu D, et al. Levels of *Haemophilus influenzae type B* (*Hib*) antibody in Turkey before routine immunization. *Turk J Med Sci*. 2010;40(6):959–964.
19. Sağlam M, Zer Y, Balci İ. Causative agents of bacterial meningitis. *Afr J Microbiol Res*. 2013;7(20):2221–2227.
20. Gültepe B, Bayram Y, Güdücüoğlu H, Çıkman A, Berktaş M. Investigation of bacterial and viral meningitis agents with different PCR methods at a university hospital. *Abant Med J*. 2015;4(2):125–129.