COMPARISON OF CULTURE AND PCR METHODS IN THE DIAGNOSIS OF BACTERIAL MENINGITIS

1Dr. Bakhtawer Mehmood, 2Dr. Maham Shakoor, 3Dr. Farhan Nasir
1,2MBBS; King Edward Medical University Lahore, Pakistan., 3MD; Grodno State Medical University, Belarus.

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Abstract:
Our aim in this study is to understand the standard culture method of positive bacterial meningitis, 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 in a hospital at clinical microbiology carried out between December 2017 and April 2018.

The diagnosis of bacterial meningitis was made based on the clinical findings, laboratory test anomalies, CSF analysis results, and the radiological images. Development was analysed in the CSF cultures of 10 out of the 57 patients included in the study (17.5%) and Streptococcus pneumonia was isolated in all of them. The CSF samples of 34 patients (59.6%) were positive according to Streptococcus pneumonia and detected in 33 of the samples (97.05%), while Neisseria meningitides was established in one sample (2.95%).

In a total of 10 patients, Streptococcus pneumonia was both isolated in the CSF culture. The culture was negative in 23 of the CSF samples. There was no sample in which the CSF culture was positive. While it seems 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.

Keywords: Bacterial meningitis; Culture; Cerebrospinal fluid.
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. The incidence and mortality rates of bacterial meningitis vary according to the geographical region, the type of pathogen and the age groups.

Since permanent neurological sequelae are observed in almost half of the survivors, a rapid diagnosis and treatment is crucial. Excluding the neonatal period, Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae are the most frequently observed agents causing bacterial meningitis.

The clinical symptoms observed in patients with bacterial meningitis are fever, headache, meningeismus, 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. 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).

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.

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. 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.

MATERIAL AND METHODS:
In this study we have retrospectively analysed the adult patients with acute bacterial meningitis. 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 .

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 (with the use of WTB Binder Device, 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.

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.

RESULTS:
Fifty-seven patients who were diagnosed and treated for acute bacterial meningitis between December 2017 and April 2018 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).

| 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%)    |

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 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%).
A total of 10 patients, S. pneumoniae was both isolated in the CSF culture and detected in the. In 24 out of the 34 patients with positive results (70.5%), the culture was negative. Among the 24 positive samples, 23 (95.8%) were positive for S. pneumonia while the remaining 1 (4.2%) was positive for N. meningitidis. The culture and they were negative in 23 of the CSF samples.

**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. 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.

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. 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.

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. 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. It can be considered as less costly compared to other molecular testing methods as it analyzes the most common bacterial meningitis agents Streptococcus pneumoniae, 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.

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 results (44.1%) had a history of antibiotic treatment before the LP.

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. 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.

CONCLUSION:
In conclusion, considering that 42.1% of the patients in our study had received antibiotics before the diagnostic tests, we can conclude that 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 in the diagnosis and treatment of bacterial meningitis in adults.

REFERENCES:
1. H. Trotman, O. Olugbuyi, M. Barton, D. McGregor, S. Thomas Pneumococcal meningitis in Jamaican children West Indian Med J, 58 (6) (2009), pp. 585-588
2. L.D. Saravolatz, O. Manzor, N. VanderVelde, J. Pawlak, B. Belian Broad-range bacterial polymerase chain reaction for early detection of bacterial meningitis Clin Infect Dis, 36 (1) (2003), pp. 40-45
3. World Health Organization Laboratory Methods for the Diagnosis of Meningitis Caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenza WHO Meningitis Manual (2011)
4. M.L. Durand, S.B. Calderwood, D.J. Weber, et al. Acute bacterial meningitis in adults. A review of 493 episodes N Engl J Med, 328 (1) (1993), pp. 21-28
5. A.R. Tunkel Acute meningitis G.B.J. Mandell, R. Dolin (Eds.), Principles and Practice of Infectious Diseases (7th ed.), Churchill-Livingstone, New York, USA (2010), pp. 1205-1206
6. M. du Plessis, A.M. Smith, K.P. Klugman Rapid detection of penicillin-resistant Streptococcus pneumoniae in cerebrospinal fluid by a seminested-PCR strategy J Clin Microbiol, 36 (2) (1998), pp. 453-457
7. M.C. Brouwer, A.R. Tunkel, D. van de Beek Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis Clin Microbiol Rev, 23 (3) (2010), pp. 467-492
8. T. Meyer, G. Franke, S.K. Polywka, et al. Improved detection of bacterial central nervous system infections by use of a broad-range PCR assay J Clin Microbiol, 52 (5) (2014), pp. 1751-1755
9. http://www.vircell.com/fileadmin/Material_Tecnico/Dossier_Speed-oligo_Ingles_v3.2.pdf Granada, Spain.
10. M.C. Brouwer, G.E. Thwaites, A.R. Tunkel, D. van de Beek Dilemmas in the diagnosis of acute community-acquired bacterial meningitis Lancet, 380 (9854) (2012), pp. 1684-1692.
11. Celal, G.M. Faruk, H. Salih, C.M. Kemal, A. Serife, K.O. Faruk Characteristics of acute bacterial meningitis in Southeast Turkey Indian J Med Sci, 58 (8) (2004), pp. 327-333.