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

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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.

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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

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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 abnormalies, 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.

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