

COMPARISON OF THE UTILITY OF CONVENTIONAL CULTURE VERSUS MULTIPLEX PCR IN THE DIAGNOSIS AND MANAGEMENT OF SEPSIS AND MENINGITIS

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ABSTRACT: OBJECTIVE: Sepsis and meningitis are few of the most important causes of morbidity and mortality. Even so, establishing a microbial diagnosis for is still arduous and is often achieved in only half of cases by conventional culture techniques. This study was designed to compare the multiplex PCR method with the traditional culture method in sepsis and meningitis. The other aim was to evaluate the reliability of multiplex PCR method.

METHODS: Forty-four patients with symptoms of sepsis and meningitis were included in the study. Both culture and multiplex PCR methods were performed for the isolation of most commonly seen pathogen, from bronchoalveolar lavage (BAL) and cerebrospinal fluid (CSF) samples.

RESULTS: The conventional culture method detected at least one bacterial isolation in 19 patients. Whereas, the number for multiplex PCR was 44 (100%). The pathogens most commonly detected by PCR were Pseudomonas, Candida, S. pneumonia and CMV. In terms of detection of multiple pathogens, multiplex PCR was significantly efficient than conventional culture ($p < 0.05$).

CONCLUSION: The traditional methods, such as culture are often inadequate in detection of the pathogens in sample from patients of sepsis and meningitis. Multiplex PCR assays proved highly sensitive and rapid. Widespread use of PCR methods will not only provide the immediate and appropriate "agent specific antibiotic treatment" of sepsis and meningitis, it will also contribute to a reduction in antibiotic resistance.

KEYWORDS: Sepsis, Meningitis, Multiplex PCR, SES, Culture.

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INTRODUCTION: Prompt goal-directed haemodynamic therapy and appropriate antibiotics are a mainstay of treating patients with life threatening conditions such as sepsis^[1] or meningitis.^[2] Empirical antibiotic treatment is subsequently adjusted accordingly by results obtained from blood or cerebrospinal fluid (CSF) culture, which yield important particulars about the nature of culprit pathogens and in vitro sensitivity of antibiotics. The conventional cultural methods (CC) are still considered as the gold-standard test for diagnosis of both sepsis and meningitis. However, low sensitivity, delayed results and vulnerability to contamination are biggest limitations of traditional microbiological techniques.^[3] In order to overcome these flaws, multiplex PCR based DNA macrochip (Syndrome Evaluation System, SES) is developed.

In this study, our primary aim was to compare the clinical value of multiplex PCR (SES) with that of CSF and bronchoalveolar lavage (BAL) culture for the diagnosis of sepsis or meningitis. The reliability of multiplex PCR method was also evaluated.

The secondary aim of our study included the identification of causative microorganisms and implementation of these results as a guide for the choice of antibiotic regimen.

PATIENTS AND METHODS:

Setting: The HSG hospital, Bengaluru is a 500-bedded tertiary care teaching hospital with 25-bed critical care unit catering the morbidly ill patients.

Study Design: We conducted an observational study on 44 consecutive patients who had been admitted to the critical care unit over a period of one year. We collected 13 CSF and 31 BAL samples from patients admitted unit with sepsis or meningitis.

Inclusion and Exclusion Criteria:

- All patients aged above 18 years with informed consent from their legally eligible relative were included.
- Adult patients suspected of having meningitis and/ or encephalitis requiring CSF analysis.
- All patients having/ suspected of having pulmonary infections requiring invasive sampling like BAL.

Exclusion Criteria: Patients with a history of hospitalization and antibiotic use within the prior 90 days and with accompanying immunosuppression, respiratory failure, malignancy, and congestive heart failure were not included in the study.

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METHODOLOGY: All patients aged ≥ 18 years who had been admitted to the critical care unit with possible meningitis and an indication for CSF collection during the study period were eligible for inclusion in the study.

Table 1 describes the conditions used for the diagnosis of sepsis. The diagnosis of meningitis was based upon presence of clinical manifestations including fever, headache, stiff neck, altered mental status, nausea, vomiting, photalgia, sleepiness, confusion, irritability, delirium and coma. The study was approved by the Ethics Committee of HSG hospital, Bengaluru.

CULTURE: The BAL samples were obtained by the pulmonologist by using the fiberoptic bronchoscope (Olympus B-150) and were stored into a sterile container. After gram staining and direct microscopic examination, BAL samples were inoculated into appropriate culture media and incubated.

CSF samples (1-3 ml) were centrifuged at 1000 g for 15 min. The pellet was used for direct microscopy and culture. Direct microscopy was done by India ink wet mount and culture was done on brain heart infusion (BHI) agar (HiMedia, Mumbai, India) with gentamicin (26 $\mu\text{g/ml}$). Culture tubes were incubated at 37°C.

After 24-hour incubation of cultures, bacterial colonies with growth of 10⁵ and above were considered as pathogenic. Results were then analysed.

Multiplex PCR (SES=Syndromic Evaluation System): Multiplex polymerase chain reaction (Multiplex PCR) is a modified method of polymerase chain reaction in which multiple DNA sequences are amplified simultaneously and are analysed, which otherwise would require to be done multiple times with repeated use of reagents. Hence it is time saving and helps in detection of multiple pathogenic organisms in clinical samples in a single setting.

Statistical Analysis: SPSS version 21 software (IBM Corporation, USA) was used for statistical analysis. Independent samples t-test was used for comparison of parametric data among groups, Mann-Whitney U test was used for comparison of nonparametric data, and χ^2 test was used for multiple non-parametric group comparisons. The limit for statistical significance was accepted as $p < 0.05$.

RESULTS: A total of 44 patients (30 male) were included in the study. The patients' ages ranged from 13 to 81. The general characteristics of the patients are summarized in Table 2.

Table 2: General characteristics of the patients included in the study. ARDS, acute respiratory distress syndrome.

CULTURE RESULTS: At least one pathogen isolate was detected in 19 (43.2%) patients' culture. The most commonly grown bacteria based on the culture studies were Pseudomonas, Acetobacter and Klebsiella.

There were no significant correlations between patients' culture isolate and gender. However, there was a significant difference between age and culture growth ($p = 0.033$).

Multiplex PCR Results: The detection rate of the pathogen was significantly higher in multiplex PCR method compared to the culture method ($p < 0.005$). At least one pathogen was detected in 40 patients (90.9%). The most frequently detected species by PCR were pseudomonas and candida and CMV. The PCR-detected bacteria and their numbers according to nature of sample are given in Table 3.

When we compared the bacteria detection rate in multiplex PCR based on the sample type, we found that the pathogen detection rate was higher in BAL (100%) than CSF (69.23%). There were no significant differences between detection of pathogen by PCR and gender.

Table 3: The detection of causative pathogens by CC and SES in BAL and CSF samples.

Multiplex PCR and Culture Comparison Results: When we compared the pathogen detection rates using the multiplex PCR and culture methods, that of multiplex PCR was significantly higher ($p < 0.005$). While pathogen growth was detected in cultures from 19 patients, the PCR method was able to detect pathogens in all 44 patients. The multiplex PCR method detected bacteria in 4 out of 44 patients with positive cultures. There were only no patients who had positive cultures but negative multiplex PCR. In all patients who had Pseudomonas, Acinetobacter and Klebsiella in the culture, the same pathogens were detected in the multiplex PCR as well.

When culture is considered to be the gold standard, the sensitivity of PCR method was 0.96, and the positive predictive value was found to be 0.95. There was a significant difference between the culture and PCR methods in terms of detection rates of multiple pathogens ($P < 0.001$). Culture method was not able to detect multiple pathogens, while PCR detected multiple pathogens in 35 cases.

DISCUSSION: Prior recognition of culprit pathogens in sepsis and meningitis can dramatically decrease morbidity and prevent misuse/overuse of antibiotic agents. Traditional techniques, such as culture and serology are often inadequate to distinguish pathogenic microorganisms causing sepsis or meningitis. Thus, there is dire need of novel diagnostic methods.^[4] We, in this study, evaluated multiplex PCR (SES) and conventional culture method for their clinical efficacy and reliability. We observed that the rate of identification of pathogens in samples by multiplex PCR methods (100%) was almost double to that with conventional culture method (54.5%).

It is widely known that the traditional culture tests suffer from low sensitivity and specificity for detecting pathogenic microorganisms.^[5] Other key limitations include the need of different culture media for different organisms. The result of the test becomes hard to analyze due to diversity of microorganism. Many published articles have documented the higher identification rates with multiplex PCR

method.^[6,7,8] In a study conducted in pneumonia patients, the detection rate with multiplex PCR method was 65.2% compared to 39.1% with culture method.^[9] In yet another study of comparison, the authors have published the data showing higher pathogen identification rate in BAL samples from LRTI patients using multiplex PCR method. Interestingly, the rate increased by 20% in *S. pneumoniae*, from 20% in *H. influenzae* and increased from 2 to 20 patients in detection of dual pathogen presence.^[10] Furthermore, Honkinen et al reported that the multiplex PCR method could detect bacterial presence in sputum samples of pediatric patients with pneumonia who, initially, had negative culture results.^[11]

In our analysis, there were no cases whereby multiplex PCR did not detect any causative microorganism. Notably, we had 5 CSF and BAL samples where microorganisms detected by multiplex PCR were different from those by conventional culture. Hence, our study indicates that the multiplex method is more reliable in detection of pathogens. Previous studies have reported 94-100% sensitivity and specificity of multiplex PCR method.^[12,13] Our data analysis reflects the similar high sensitivity and predictive values.

Yet one more vital finding of our study was the ability of multiplex PCR to detect multiple pathogens. While conventional culture was not able to find more than 1 pathogen in any sample, multiplex PCR detected multiple causative pathogens in 4 CSF and all 31 BAL samples. Our findings are supported by many previously published articles. In a Chinese study, the authors have reported that the PCR identified multiple bacteria in 35% of children with LRTI.^[14] Lieberman and colleague from Israel were able to find multiple microorganisms in 35% of adult patients.^[15] Mustafa et al have documented that 17.7% of their patients showed multiple pathogens.^[8] In our analysis, more than three-fourth of our patients showed multiple pathogens.

The judicious selection of antibiotic regimen and its success are highly dependent on the understanding of distribution and treatment-resistance of pathogens in the general population. In our country, many national studies and programs, based on the results from conventional culture and serological methods, have given the firm foundation to the currently-prescribed empirical treatment. Nonetheless, there are only a few studies done using multiplex PCR. Simpler to the data of previously conducted studies, we identified the commonly detected pathogens in both conventional culture and multiplex PCR were *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Staphylococcus aureus* and *S. pneumoniae*.

There was few limitation to our study. The multiplex PCR kit detected atypical infectious bacteria like *Chlamydia*. However, we did not cross-check this detection with conventional culture. As it is generally known, there are some limitations in identifying atypical pathogen during routine practice of culture. The growth of these bacteria is slow (3-5 weeks) and difficult in regular growth media. Various studies across the world have reported to furnish high sensitivity and specificity in concurrent and rapid identification of atypical bacteria. Our study has documented

In summary, multiplex system is reliable and highly effective in identification of multiple microorganisms. It provides rapid detection of pathogens, aiding in timely management of critically ill patients, however large scale multicentre studies of such nature are essential to further strengthen the role of multiplex PCR.

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General Variables	Alteration in mental status, Alteration in body temperature (> 38.3°C or < 36°C), Tachycardia (> 90 beats/minute or greater than 2 SD above normal value for age), Tachypnea (> 20 respirations per min or PaCO2 < 32 mm Hg), Hyperglycemia (plasma glucose >120 mg/ml or 7.7 mmol/L in the absence of diabetes), significant edema
Inflammatory variables	Altered WBC count (leukocyte count > 12,000/mm ³ ; leukocytosis, < 4,000/mm ³ ; leukopenia), Normal white blood cell count with an increased number of immature neutrophils cells (> 10% immature [band] cells), Plasma C-reactive protein (>2 SD above the normal value) and Plasma procalcitonin (>2 SD above the normal value)
Hemodynamic variables	Arterial hypotension (Systolic blood pressure (SBP) <90 mm Hg, Mean arterial blood pressure <70, or an SBP decrease of >40 mm Hg in adults or <2 SD below normal for age), Mixed venous oxygen saturation (Svo ₂) >70% (should not be used in children and newborns as a sign of sepsis since their Svo ₂ is normally lower [75-80%]), Cardiac index >3.5 L. min ⁻¹ .M ⁻²
Table 1: Signs and symptoms of sepsis (adopted from^[41])	

Characteristics	n(%)
Patients	44
Male	30
Female	14
Indications	
Meningitis	2
Sepsis	16
Septic shock	5
ARDS	2
Sample	
BAL	31
CSF	13
Paramater	Mean (±SD)
Age	56 (±14) Years
Table 2	

Micro-organism	CSF		BAL	
	CC	SES	CC	SES
Human herpesvirus 6		1		1
Pseudomonas	1	4	10	27
Acinetobacter	1	3	6	3
Klebsiella			1	4
Candida		3		16
A. Baumannii				10

Mycobacterium tuberculosis				4
s.aerogenes		1		4
S.pneumoniae		2		11
CMV				16
S.aureus				14
GBS				5
E.coli				9
HSV		1		7
Enterococcus		1		6
Staphylococcus		1		
VZV				1
B.fragilis				1
Aspergillus				1
N.meningitidis				1
Dengue		1		
Table 3				