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Original article

Clinical usefulness and accuracy of polymerase chain reaction in the detection of bacterial meningitis agents in pediatric cerebrospinal fluid

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ABSTRACT

Bacterial meningitis poses enormous healthcare challenges due to a high mortality, morbidity and sequelae. *Neisseria* (*N.*) *meningitidis*, *Haemophilus* (*H.*) *influenzae*, *Streptococcus* (*S.*) *pneumoniae* and *S. agalactiae* remain among the most prevalent infectious agents that cause bacterial meningitis in children. The objective of this study was the simultaneous detection of these pathogens in suspected cerebrospinal fluid (CSF) by using multiplex polymerase chain reaction (mPCR) and compare PCR results with standard diagnostics currently used in clinical practice. CSF specimens were obtained from 515 children (< 5 years) clinically suspected of having acute bacterial meningitis. Based on bacterial culture, four isolates of *salmonella sp* and one *Citrobacter freundii* isolate were identified. The remaining 510 CSF specimens, having negative culture, were subjected to mPCR. Twenty-three (4.51%) CSF samples yielded a PCR positive signal. The pathogens identified were: *S. pneumoniae* ($n = 13$), *H. influenzae* ($n = 7$) and *N. meningitidis* ($n = 3$). *S. agalactiae* was not detected. Using sequential multiplex PCR, serogrouping of *S. pneumoniae* revealed 3 different serotypes: serotype 19A ($n = 6$), 19F ($n = 4$) and serotype 23F ($n = 3$). Only the serotype A was identified for the 3 *N. meningitidis* isolates. Despite vaccination, *S. pneumoniae* remains a leading cause of pediatric invasive disease. Detecting causative organism remains the most critical aspect for management of children with suspected meningitis. PCR method is more sensitive and rapid than culture for detecting the infectious agents. Institution of PCR diagnostics is recommended for early and appropriate therapy.

1. Introduction

Bacterial meningitis (BM) remains a major cause of morbidity and mortality worldwide. In Asia and Africa, about 20 to 25% of survivors suffers from long-term sequelae [1]. The survivors BM may have a deleterious impact on intelligence quotient and should be routinely offered screening for cognitive deficits and developmental delay in addition to hearing loss [2]. Before the introduction of vaccines, 90% of reported cases of BM in children were caused by *Haemophilus* (*H.*) *influenzae* type b (Hib), *Streptococcus* (*S.*) *pneumoniae*, and *Neisseria* (*N.*) *meningitides* [3]. In addition, *S. agalactiae* (Group B Streptococci: GBS) is in the process of being now the most common pathogen causing purulent meningitis mainly in infants less than 3 months of age [4]. The knowledge of the etiologic agents of meningitis and their antibiotic susceptibility in different age groups is essential to minimize adverse outcomes. In tertiary care setting, most the patients receive prior antibiotic therapy before being referred, mainly in low-income countries. In this case, routine and traditional microbiological testing of cerebrospinal fluid (CSF) is of limited clinical benefit since administration of antibiotics prior to lumbar puncture may decrease the yield of culture [5]. Polymerase chain reaction (PCR) can improve the diagnosis of infectious diseases by rapid detection of microbial nucleic acids, including from non-viable organisms [6]. The aim of this study was to determine the clinical usefulness of multiplex PCR for the simultaneous detection of *N. meningitidis*, *S. pneumoniae*, *H. influenzae* type b, and *S. agalactiae* in CSF samples of suspected cases of meningitis in children and comparison with standard diagnostics currently used in clinical practice.

2. Materials and methods

2.1. Patients

This study involved 515 children aged < 5 years with suspected bacterial meningitis admitted to Taif children's hospital, Saudi Arabia, from October 2016 to

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June 2017. Request of verbal consent to participate in this study, clinical examination, lumbar puncture were performed by the pediatricians attending the admitted child. Since the pathogens responsible for neonatal meningitis are different from older patients, neonates (patients < 1 month of age) were not included in the study. The diagnosis of bacterial meningitis was made based on clinical presentation that met World Health Organization clinical criteria of suspected meningitis [7].

2.2. Sample collection, bacterial isolation and antimicrobial susceptibility testing

In the laboratory of bacteriology, we collected CSF samples as per routine clinical practice and monitored them by culture. The bacterial growth identification and antimicrobial susceptibility testing were determined using Becton Dickinson BD Phoenix™ 100 automated identification and susceptibility testing system (USA) as recommended by the manufacturer.

2.3. DNA extraction, bacterial detection and serogroup determination

Two to three hundred microliters (μl) of CSF samples were used for DNA extraction using QIAamp DNA mini Kit (Qiagen) according to the manufacturer's instructions. Eluted DNA was stored at −20 °C for later analysis. Five to ten μl of the purified DNA solution was used as a template for PCR. Simultaneous detection of bacterial meningitis agents was accomplished using the primer sets and multiplex PCR conditions as previously reported [8]. *S. pneumoniae* serogrouping was performed using primers and sequential multiplex PCR (sm-PCR) as previously reported [9]. For *N. meningitides*, serogroup prediction was accomplished as previously described [10,11]. For positive controls, reference strains were used: serial dilutions of the purified DNAs of *S. pneumoniae* ATCC 6305, *H. influenzae* ATCC 10211, *N. meningitidis* ATCC 13090 and *S. agalactiae* ATCC 2759 were performed and up to 50 to 60 ng of the DNA could be detected at each reaction. Negative controls consisting of PCR grade water instead of the CSF DNA samples were used in each assay. The PCR products were visualized on 2% agarose gels stained with ethidium bromide. The size of the PCR products was determined by comparison with a molecular size standard.

3. Results

In the 515 cases of suspected meningitis children, there were 323 males (62.72%) and 192 females (37.28%). The boy-to-girl ratio was 1.68:1. In 314 cases (60.97%), it was unknown whether antibiotics had been used or not before lumbar puncture. The main clinical symptoms of these 515 suspected cases of bacterial meningitis were summarized in Table 1. The most common clinical features were fever (93.59%), followed by headaches (71.06%), vomiting (69.32%), somnolence (52.23%), restlessness/irritability (47.57%) and convulsions (39.22). The levels of white blood cells (WBCs), polymorphonuclear cells, glucose and proteins in CSF routine biochemical tests for the 515 children with suspected bacterial meningitis are shown in Table 2. The culture of the 515 CSF samples showed that the positive bacterial growth was obtained from 5 CSF specimens. The pathogens identified were 4 isolates of *Salmonella sp* and one isolate of *Citrobacter freundii*. The 4 *Salmonella sp* isolates were sensitive to almost all antibiotics used in the Taif children's hospital except amikacin, gentamicin, cephalothin, cefoxitin, and ceferoxim; whereas the *Citrobacter freundii* isolate was sensitive to all cephalosporins tested and resistant only to aminoglycosides, carbapenems and fluroquinolones. The remaining 510 CSF samples, having negative culture, were subjected to multiplex PCR (mPCR) for detecting simultaneously the 4 bacterial meningitis agents. Twenty-three (4.51%) CSF specimens yielded mPCR positive signal. Pathogens identified were: *S. pneumoniae* ($n = 13$; one fragment of 80 bp was amplified), *H. influenzae* ($n = 7$; one amplified fragment of 181 bp) and *N. meningitidis* ($n = 3$; amplification of one fragment of 110 bp). *S. agalactiae* was not detected by PCR assays. PCR had a specificity of 100% for detection of *S. pneumoniae, H. influenzae* and *N. meningitis* in CSF samples (according to ATCC strains DNA used as positive control). The remaining of CSF specimens with culture negative and PCR negative were sent to the virology laboratory for Enterovirus and Herpes simplex virus testing. These analyses showed that three types of echoviruses (E9, E11 and E30) are associated with 16 cases of meningitis and a subsequent diagnosis revealed that 11 cases were as respiratory infections (Laboratory Staff: personnel communication). Using sm-PCR, serogrouping for *S. pneumoniae* revealed 3 different serotypes: serotype 19A ($n = 6$), 19F ($n = 4$) and serotype 23F ($n = 3$). The serotype 19A is included in the PCV-13 vaccine, whereas serotype 19F and 23F are covered by PCV-7 vaccine. The 3 *N. meningitidis* isolates had the serotype A.

Table 1
Main clinical manifestations of 515 cases of suspected cases of meningitis.

Clinical manifestations	Cases	Percentage (%)
Fever	482	93.59
Headaches	366	71.06
Vomiting	357	69.32
Somnolence	269	52.23
Restlessness/irritability	245	47.57
Convulsions	202	39.22

Table 2
CSF analysis results of 515 children with suspected meningitis.

CSF analysis results	Cases	Percentage (%)
Level of WBCs		
$\leq 100 \times 10^6$/L	131	25.44
101×10^6–500×10^6/L	81	15.73
501×10^6–1000×10^6/L	246	47.76
$> 1000 \times 10^6$	57	11.07
Polymorphonuclears		
> 50%	416	80.77
Glucose ratio (CSF/blood)		
< 2/3	267	51.85
Level of proteins		
< 0.45g/L	108	20.97
> 0.45g/L	407	79.03

CSF: cerebrospinal fluid; WBC: white blood cells.

4. Discussion

The golden diagnosis criterion of BM is to find the evidence for the existence of bacteria in CSF. In this study, 0.97% of CSF specimens had positive culture. In a similar study, only 0.42% grew suspected CSF bacterial pathogens were reported [12]. These positive culture rates of CSF were too lower as compared to other previously reported data [8,13]. The small number of microorganisms present in the specimens, the low quality of CSF samples and the use of antibiotics prior to the lumbar puncture could negatively affect the CSF culture and explain these false culture negative results [14]. All patients had at least three features consistent with clinical symptoms and signs of BM (Table 1). CSF routine and biochemical examinations showed inflammatory changes in almost all patients: increase of WBCs ($> 500.10^6$/L), level of polymorphonuclear cells > 50%, significant increase of protein content (> 0.45 g/L) and significant decrease of sugar (< 2.4 mmol/L) (Table 2). However pathogens were detected, by PCR, only in 4.51% cases (23/510). Similar rate PCR detection was previously described [15]. Signs are quite important to the diagnosis and treatment of BM however these symptoms are varied and some symptoms may not be observed in neonates, in the elderly and in patients with neutropenia [16]; in addition young infants lack the ability to describe their conditions; this situation could lead easily to misdiagnose the infection [13]. On the other hand, the count of leucocytes is not always indicative because meningitis can occur in the absence of the increased number of polymorphonuclears [17]. Moreover, in aseptic meningitis, there are clinical signs of meningitis and inflammatory changes in CSF along with negative cultures prior to antibiotics

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