Research Article:

POLYMERASE CHAIN REACTION VALUE IN DETECTION OF BACTERIAL MENINGITIS AMONG NEONATES AND CHILDREN

By

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ABSTRACT

Background*:** Meningitis is a serious communicable disease with high morbidity and mortality rates. It is an endemic disease in Egypt caused mainly by *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*. In some settings, bacterial meningitis is documented depending mainly on positive cerebrospinal fluid (CSF) culture results, missing the important role of prior antimicrobial intake which can yield negative culture. *Aim of the Work*: This study aimed to utilize molecular technology in order to diagnose bacterial meningitis in both culture-negative and culture-positive CSF samples. ***Subjects and Methods: The current study is a cross sectional descriptive study performed in the Oncology Diagnostic Unit-Research department at Faculty of Medicine-Ain Shams University in collaboration with Pediatrics Department, Faculty of medicine-Al-Azhar University during the period from 1/8/2015 to 1/8/2017**;** a total of 55 CSF samples for meningitis patients were included collected from Al Mataria teaching Hospital and Abbassia Fever Hospital. CSF samples were examined by traditional culture method and molecular analysis using real-time quantitative polymerase chain reaction (RT-qPCR). ***Result***s: Our study revealed that the overall expression **for detection** of S. pneumoniae, N. meningitidis, and H. influenzae. A total of 15 (27%) samples had a specific etiologic agent identified, with S. pneumoniae (n = 5), N. meningitidis (n = 5), and H. influenzae (n = 5); these results were obtained by culture. However, the detection efficiency of RT-qPCR for identification of bacterial meningitis causative organism was 100% in the three studied microorganisms. 13 cases showed positivity to H.Influenzeae*;* 13 for *N.meningitidis* and 29 for *S.pneumoniae* which was the commonest pathogen in this study. ***Conclusion***: Referred to these data;RT- qPCR considered a highly efficient test for detection of bacterial pathogen in meningitis patients; its efficiency exceeds traditional culture methods especially in settings with high rates of culture-negative results.

Key words: Diagnosis, Bacterial Meningitis, Real-Time Quantitative Polymerase Chain Reaction.

INTRODUCTION

Bacterial meningitis is the most common infection of the central nervous system. Bacterial meningitis is more common in developing countries where

morbidity and mortality rates are even higher in this region ***(Saravolatz et al., 2003)***. Bacterial meningitis is a grave and sometimes lethal infection affecting the central nervous system ***(Luciane et al., 2005)***.

Neisseria meningitidis is a leading etiologic agent of severe invasive disease characterized by rapid onset, of which meningitis and septicaemia are the most common and important manifestations ***(Khatami and Pollard, 2010)***.

Although meningococcal infections may appear in every age group, infants and young children are at the highest risk of invasive meningococcal disease (IMD). A second peak of IMD incidence is observed in adolescence ***(Harrison et al., 2001)***.

Rapid diagnosis and treatment of meningitis are important, because stable neurological sequelae such as hearing loss, mental retardation, seizures, and behavioral changes may occur in up to one-half of survivors. Antibiotic treatment is empirically initiated on the basis of clinical findings ***(Zaidi et al., 2009)***.

Traditional laboratory technique of culture for the detection of bacterial meningitis takes up 36 h or more. Furthermore, it was observed that antimicrobial therapy before to sample collection; decreases the sensitivity of culture assay to approximately 30% ***(Schuurman et al., 2004)***. The current standard for the identification of bacterial meningitis is microscopic examination and consequent culture of cerebrospinal fluid (CSF). However, this approach might have some disadvantages with regard to the desired rapidity and sensitivity ***(Aletayeb et al., 2010)***.

Perfect therapy guided by the results of culture, which may take 24-48 h to obtain; antibiotic susceptibility testing may need an additional 24 h ***(Marandi et al., 2010)***.

The isolation of the etiologic agent by culture is essential for epidemiological surveillance, considered "gold standard", and allows the final characterization of the agent (antigenic, genetic, and antimicrobial resistance). However, approximately 50% of suspected cases are not culture-confirmed, due to problems related to improper transportation and seeding or previous antibiotic treatment. Molecular diagnosis can be useful in situations where the antibiotic treatment was initiated, because it does not require a viable organism to confirm the diagnosis ***(Telóet al., 2007)***.

In recent years, PCR-based assays have become accessible to provide an early and accurate diagnosis of bacterial meningitis ***(Salgado et al., 2013)***. Recent evidence suggests that some of these tests are aimed at specific pathogens of bacterial meningitis, such as *Neisseria meningitidis (N. meningitidis)*, *Streptococcus pneumoniae (S. pneumoniae)* and *Haemophilus influenzae (H. influenzae)* **(Wang et al., 2011; and Salgado et al., 2012)**, whereas others use broad-range bacterial PCR **(Wang et al., 2012; and Favaro et al., 2013)**.

PATIENTS AND METHODS

The Study was performed in the Oncology Diagnostic Unit-Research Department at Faculty of Medicine, Ain Shams University, in collaborate with Pediatrics Department, Faculty of Medicine, Al-Azhar University, during the period from 1/8/2015 to 1/8/2017.

Ethical Considerations:

- Informed consent was obtained from each patient's parent after informing him or her about the steps of the procedure and the expected effects.
- The approval of Research Ethics Committee of Faculty of Medicine, Al-Azhar University was taken.
- The approval of Research Ethics Committee of Faculty of Medicine, Ain Shams University was taken.
- No conflict of interest was found from any source.

Patients:

The study was conducted on 65 cases of neonates and children in Al-Mattarya Teaching Hospital and Abbassia Fever hospital of both sexes suspecting bacterial meningitis.

Inclusion criteria:

1. Age group ranges from 0 – 4 years. Patients selected based on clinical criteria for suspected cases of bacterial meningitis (clinical suspension: fever, neck stiffness, mental status changes and signs of meningeal irritation, such as neck stiffness, Brudzinski and Kernig signs whether or not received antibacterial therapy before enrollment in the study (*Partially treated*).
2. CSF analysis for all suspected cases based on the diagnostic laboratory criteria for bacterial meningitis included the following: glucose concentration less than 40 mg/L, protein concentration more than 50 mg/dL, a white cell count more than 100 cells per mm3, and neutrophil percentage more than 50%

3. CSF culture results for all suspected cases based on clinical data and CSF analysis; whether positive or negative

Exclusion criteria:

- Patients who refused to sign a consent form.
- Patients more than 4years old.
- Patients having any of the following criteria will be excluded:
  1. Neurosurgical conditions.
  2. Known cerebral palsy.
  3. Known chronic a febrile seizure disorder.
  4. Cranial fractures with or without cerebrospinal fluid (CSF) leak.
  5. Evidence of viral infection (measles, mumps, chicken box, etc.).

Patients were divided into three groups:

- ***Group (I***): Meningitis Patients (**Culture-positive**): included 15 patients who were diagnosed as meningitis patients based on clinical symptoms and signs; CSF analysis results, and showed *positive CSF culture* results.
- ***Group (II):*** Meningitis patients (**Culture-negative**): included 40 patients who were diagnosed as meningitis patients based on clinical symptoms and signs; CSF analysis results and showed *negative CSF culture* results.
- All selected patients were further subcategorized according to antibiotics received into two subgroups:
  - ***Untreated patients*** who did not receive antibiotics before collection of samples.
  - ***Partially treated patients*** who received irregular or interrupted courses of antibiotics before collection of samples; as misdiagnosed as upper respiratory tract infections, gastroenteritis etc.

Methods:

A) Sample collection:

CSF samples were obtained from patients of all ages. According to routine clinical care, lumbar punctures were performed on patients with suspected acute meningitis (except in those with contraindications such as signs of raised intracranial pressure), defined as sudden onset of fever > 38 °C and one of the following signs: neck stiffness, altered consciousness with no other alternative diagnosis, or other meningeal sign. CSF samples were collected and processed immediately at microbiology laboratory. The following steps were performed:

- **Macroscopic Examination:** samples were examined macroscopically prior to centrifugation.
- **Biochemical analysis:**
  - Glucose level was determined by using the BS-800 chemistry analyzer (Mindray).
  - Protein level was determined by spectrophotometer (Eppendorf UV-Vis spectrophotometer).
  - The diagnostic laboratory criteria for bacterial meningitis included the following: glucose concentration less than 40 mg/dL, protein concentration more than 50 mg/dL, a white cell count more than 100 cells per mm^3, and neutrophil percentage more than 50%.
- **Microscopic Examinations:** Differential cell counts and Gram staining were performed by light microscopy.

B) CSF culture: Samples were cultured on human blood agar, chocolate blood agar, and Mac-Conkey agar, and incubated at 37 °C with 5% CO2 for 48 h. Human blood agar was used as a substitute for sheep/horse blood agar due to inconsistent availability of animal blood from local suppliers. Any resultant growth was subject to identification by standard methods.

C) Remaining CSF was held at room temperature for one week and monitored for cloudiness (indicative of bacterial growth), and all specimens and the stored at -80°C until use for RT-qPCR analysis.

D) Real-Time PCR analysis (RT-qPCR):

1. ***Nucleic acid extraction and RT-qPCR:*** were performed on all studied patients samples. 55 Samples were thawed and DNA extraction and RT-qPCR performed according to WHO guidelines10. In brief, samples were thawed and 200 µL used for DNA extraction. If less than 200 µL was available, all remaining volume was used, and for samples with no visible liquid the specimen tube was rinsed with 200 µL of TE buffer. CSF was centrifuged at 7300 × g for 10 min, and the pellet subjected to bacterial lysis performed by enzymatic digest in lysozyme (0.4 g/mL) and mutanolysin (0.075 mg/mL) in TE buffer incubated at 37 °C for 1 h followed by the addition of lysis buffer and Proteinase K and incubation for a further 30 min at 56 °C. Lysis was

completed and DNA extraction performed using the *QIAamp DNA Mini Kit (Qiagen),* according to the manufacturer's instructions, and DNA eluted in 50 µL.

2. ***Real-Time PCR amplification:*** RT-qPCR, 2 µL of template was used in 25 µL singleplex reactions containing TaqMan Universal Probes Supermix (Bio-Rad) and primers and probes specific for *ply A* (*S. pneumoniae*), *ctr A* (*N. meningitidis*), and *bex A* (*H. influenzae*). Samples were analysed using the Step One Real Time PCR Analyser (***Applied BioSystems, USA***) Thermal Cycler (Figure 1). The cycling protocol was (95 °C for 3 min followed by 40 cycles of 95 °C for 5 sec and 60 °C for 20 sec). Samples with a Ct value < 35 were considered positive, > 40 negative, and 35–40 equivocal, unless otherwise specified. Equivocal samples were diluted 1:4 and 1:10 to dilute possible inhibitors and retested**.**

Statistical Data Management and Analysis:

The collected data was revised, coded, tabulated and introduced to a PC using Statistical Package for Social Science (SPSS 22 for windows; SPSS Inc, Chicago). Data was presented and a suitable analysis was done according to the type of data obtained for each parameter.

E) Descriptive statistics:

- Mean, Standard deviation (± SD) and range for parametric numerical data, while Median and Interquartile range (IQR) for non-parametric numerical data.
- Frequency and percentage of non-numerical data.

F) Analytical statistics:

- **Student T** Test was used to assess the statistical significance of the difference between two study group means.
- **ANOVA test** was used to assess the statistical significance of the difference between more than two study group means.
- **Correlation analysis (using Pearson's method):** To assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "r" defines the strength and direction of the linear relationship between two variables.
- P- value: level of significance; $P<0.05$: significant (S).

RESULTS

Table (1): Demographic Characteristic of Studied Groups.

		Male	Female
Studied groups	55	38(69%)	17(31%)
Neonates (1-28 days**)**	12(22%)	8(67%)	4(33%)
Infant (>1 month- 24 months)	28(51%)	19(68%)	9(32%)
Childhood (>24 months**)**	15(27%)	9(60%)	6(40%)

Males are predominated in all studied groups.

Table (2): Bacteriological Results among Different Studied Groups

Variable	Subgroup	n	Percent
Meningitis patients		55	100%
Culture - negative	Total Culture - negative	40	73%
Culture – positive	Total Culture – positive	15	27%
	Culture – positive H. infuluenzae	5	9%
	Culture – positive S. pneumoniae	5	9%
	Culture – positive N. meningitidis	5	9%

Table (3): Correlation between Ages and CSF Bacteriological CSF Culture Results.

Age groups (n=55)	CSF Negative culture (n=40)	CSF Cultur -positive n=15			p-value
		Haemophilus influenzae (n=5)	Streptococcus pneumoniae (n=5)	Neisseria meningitidis (n=5)	
Neonates (1-28 days) (n=12 (22%)	**10(83%)**	**0**	**2(17%)**	**0**	**<0.001****
Infant (>1 month- 24 months) n= 28(51%)	**15(54%)**	**5(33%0**	**3(20%)**	**5(33%)**	**<0.001****
Childhood (>24 months) n=15(27%)	**15(100%)**	**0**	**0**	**0**	**<0.001****

ANOVA test F= 29.683; p-value <0.001** highly significant

Data collected revealed significant variations between age ranges in different studied groups. Neonates are mostly presented with Streptococcus pneumoniae infection (positive CSF culture). In children more than 2years old all cases found to be CSF culture negative most probably due to antibiotics administration prior to sample collection.

Table (4): Correlations between Gender Types among CSF Bacteriological CSF Culture Results.

Gender n=55	CSF Negative culture (n=40)	CSF Cultur -positive n=15			p-value
		Haemophilus influenzae (n=5)	Streptococcus pneumoniae (n=5)	Neisseria meningitidis (n=5)	
Males n=38 (69%)	27(68%)	3	4	3	0.937
Females n=17(31%)	13(32%)	2	1	2	0.937

Chi-square test x^2= 1.281; p-value 0.937 Non significant

There are no significant differences between gender type among different studied groups

Table (5): Comparison of CSF Results among CSF Bacteriological CSF Culture Results.

Variable	Mean±SD				p-value
	CSF Negative culture (n=40)	CSF positive culture for			
		Haemophilus influenzae (n=5)	Streptococcus pneumoniae (n=5)	Neisseria meningitidis (n=5)	
Glucose (mg/dl)	59.5±24.5	8.4±4.8	17.0±3.5	16.8±2.2	<0.001**
Protein (mg/dl)	107.4±110.8	310.4±26.3	296.6±95.9	17.0±3.5	<0.001**
WBC Cells(total/mm^3)	581.6±22.3	3829.0±629.5	2338±149.9	1876±666.5	<0.001**
PMNLs %	37.2±34.7	89.6±3.6	92.6±3.9	85.0±7.1	0.027*
Lymphocytes%	34.2±34.1	11.0±2.6	10.8±5.6	12.0±4.9	<0.001**

ANOVA test *p-value <0.05 S; **p-value <0.001 highly significant

There are highly significant with the results of glucose, protein, WBC count and lymphocytes especially with CSF culture positive for Haemophilus influenzae; while significant only with PMNLs% results in culture positive for Streptococcus pneumoniae.

Table (6): Correlation between Bacteriological CSF Culture Results and Patients Treatment Conditions.

Patients n=55	CSF Negative culture (n=40)	CSF positive culture for			p-value
		Haemophilus influenzae (n=5)	Streptococcus pneumoniae (n=5)	Neisseria meningitidis (n=5)	
Untreated with antibiotics (n=20) 36%	10 (50%)	2(10%)	5(25%)	3(15%)	0.011*
Partially treated with antibiotics (n=35) 64%	30 (85.7%)	0	5 (14.3%)	0	0.001**

Chi-square test x^2: 6.483 *p-value <0.05 S; **p-value <0.001

Data presented suggesting significant differences between untreated patients and their culture results; while revealed a highly significant differences between partially treated patients and culture results.

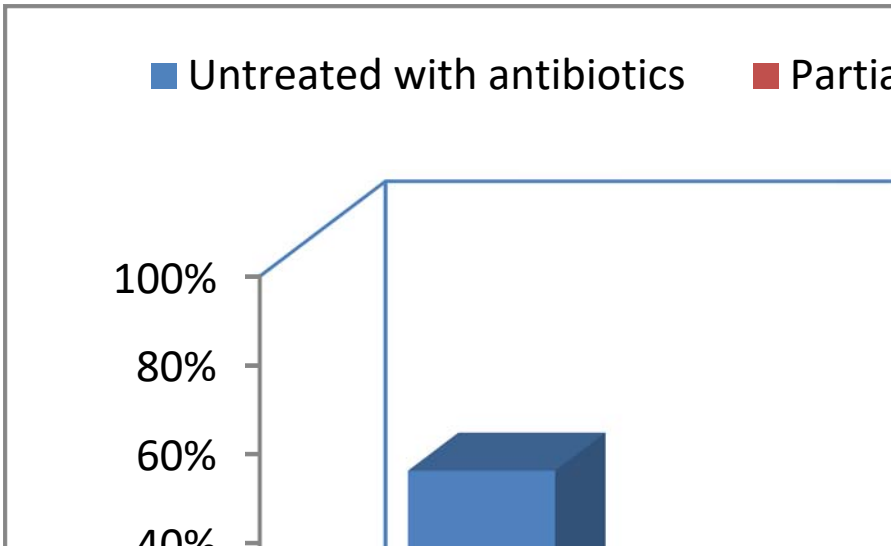


Figure (1): Correlation between Bacteriological CSF Culture results and Patients Treatment Conditions.

Table (7): Bacteriological Results of CSF RT-qPCR.

Variable	Subgroup	Frequency	Percent
CSF + ve RT-qPCR	Total CSF + ve RT-qPCR	55	100%
	CSF+ve RT-qPCR H. infuluenzae	13	14%
	CSF+ve RT-qPCR S. pneumoniae	29	52%
	CSF+ve RT-qPCR N. meningitidis	13	14%
Total		55	100%

Referred to these data; RT-qPCR considered a highly efficient test for detection of bacterial pathogens in meningitis patients; its efficiency exceeded traditional culture methods.

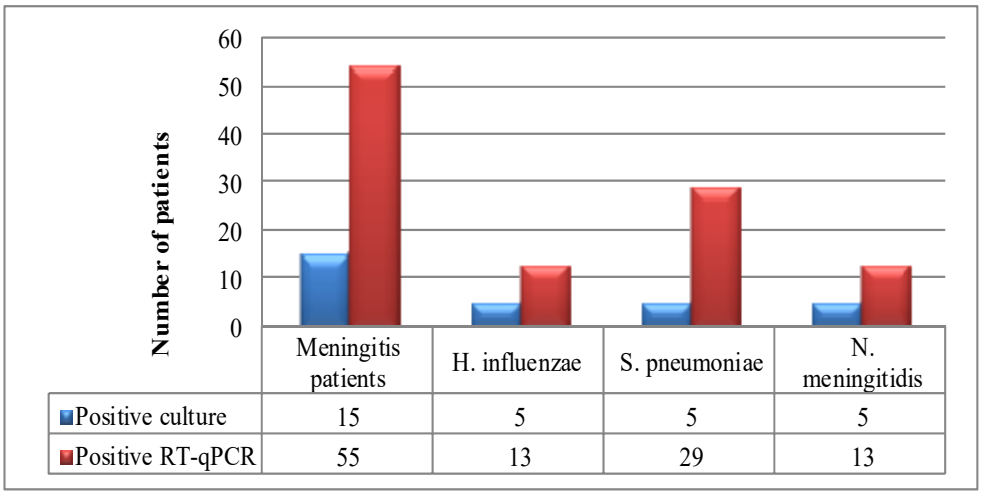


Figure (2): Comparison between RT-qPCR and traditional CSF culture efficiencies in detection of bacterial meningitis causative pathogens.

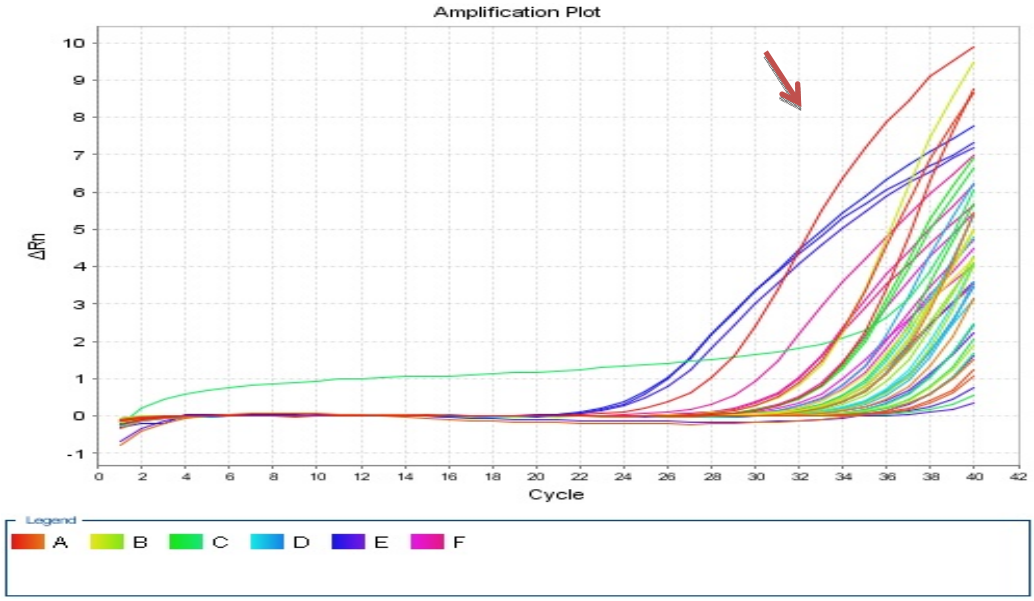


Figure (3): Amplification plots for bacterial meningitis genes of CSF samples by real-time-qPCR. Real-time. PCR amplification plot for p*ly A* gene specific for *Streptococcus pneumoniae*. Horizontal green line represents the threshold value of fluorescence. (b) Upward curves of positive samples for *Streptococcus pneumoniae*.

Table (8): Frequencies of Specific Bacterial Gene Expression (Log^{10}) in CSF; (bex A for (Haemophilus influenzae), ctr A for (Neisseria meningitidis) and ply A for Streptococcus pneumoniae) among studied subjects

Variable	Number	Bacterial meningitis gene expressions (Log^{10})		
		Haemophilus influenzae *bexA* (Log^{10})	Streptococcus pneumoniae *(plyA* (Log^{10})	Neisseria meningitidis *ctrA* (Log^{10})
Mean±SD	55	4.19±12.7	3.2±8.1	11.1±27.3
Median		0.57	0.52	0.56
Minimum		0.01	F = highest growth	l
Maximum		62.11	36.12	135.6

ANOVA test F= 11.921;
p-value <0.001** highly significant differences between all three microorganisms regarding gene expression

Table (9): Correlation between Culture Results and PCR Results.

	Culture Result			PCR Result			p-value
Culture - negative	Total Culture - negative	40	73%	CSF - ve RT- qPCR	0	0.0%	<0.001**
Culture – positive	Culture – positive	15	27%	CSF + ve RT- qPCR	55	100.0%	<0.001**
	H. infuluenzae in	5	9%	H. infuluenzae in CSF+ve RT- qPCR	13	23.6%	0.049*
	S. pneumoniae in	5	9%	S. pneumoniae in CSF+ve RT- qPCR	29	52.7%	<0.001**
	N. meningitidis in	5	9%	N. meningitidis in CSF+ve RT- qPCR	13	23.6%	0.049*

Chi-square test
*p-value <0.05 S; **p-value <0.001 high significant differences in S. pneumoniae CSF + RT-qPCR.There are highly significant differences between total culture positive results and CSF + RT- qPCR

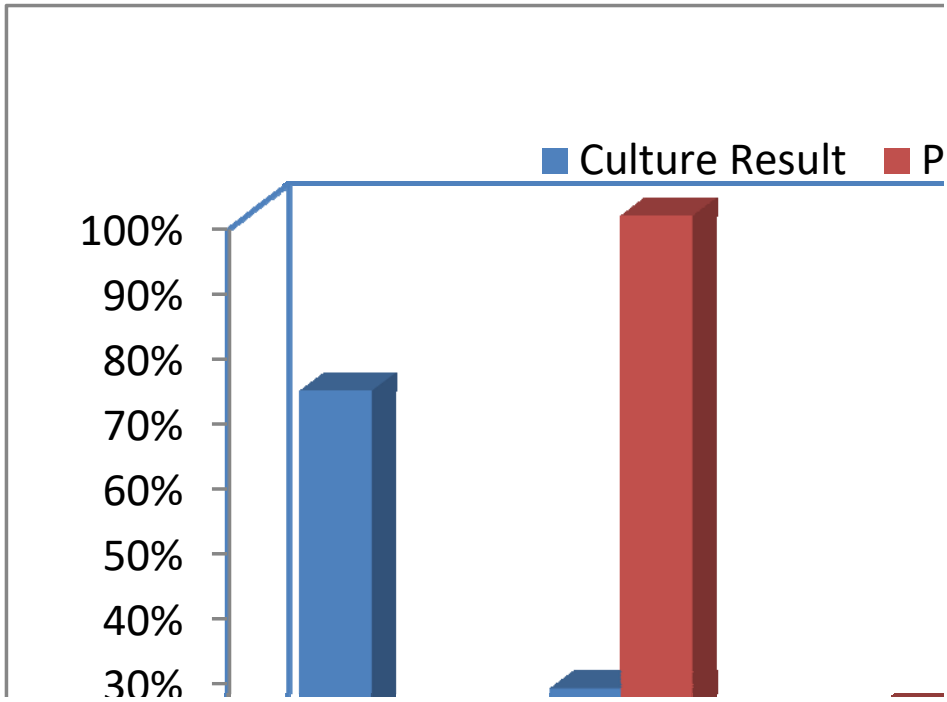


Figure (4): Correlation between culture results and PCR results.

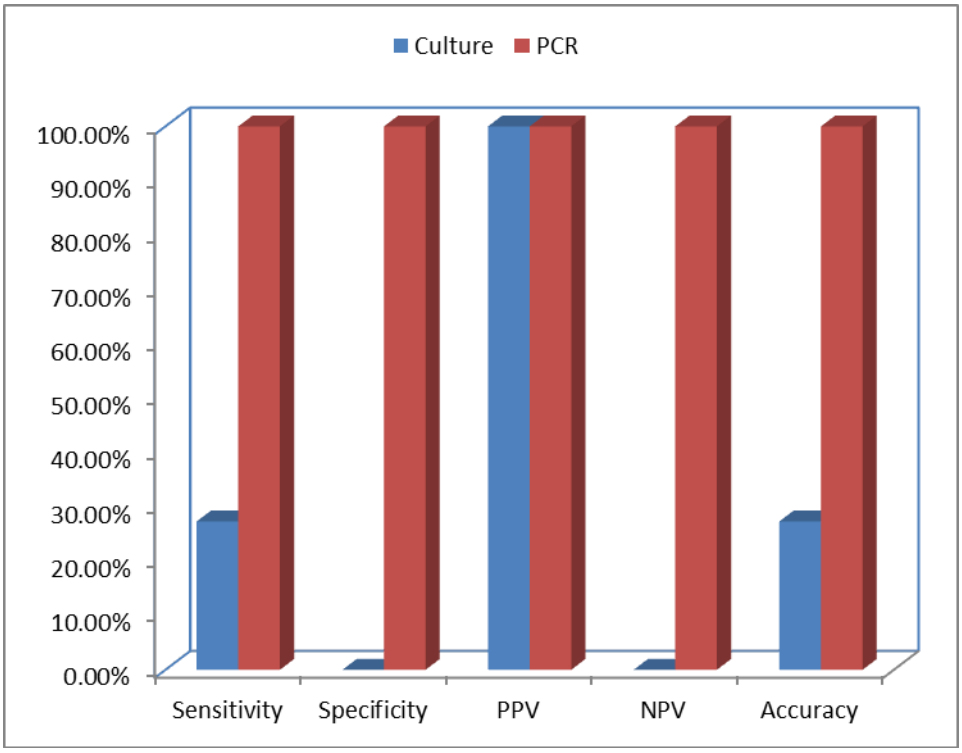
Table (10): Sensitivity and Specificity in Relation to Traditional culture results and PCR Results.

		PCR	
		Positive (N=55)	Negative (N=0)
Culture	Positive	15 (27.3%)	0 (0%)
	Negative	40 (72.7%)	0 (0%)

	Culture	PCR
Sensitivity	27.3%	100%
Specificity	0.0%	100%
PPV	100%	100%
NPV	0.0%	100%
Accuracy	27.3%	100%

Data presented suggesting that PCR is highly sensitive and specificity 100%, in comparison to traditional culture revealed sensitivity 27.3%,

specificity 0%.High positive and negative predictive values100% for PCR.



DISCUSSION

Although bacterial culture is considered to be the standard method, the negative effect of prior antimicrobial drug usage on its sensitivity makes necessary to search for non-culture techniques for diagnosis. Among non-culture diagnostic tests, PCR is the most accurate and reliable method, especially among patients with a history of antimicrobial drug use before spinal tap (**Ceyhan et al., 2008).**

Molecular diagnostic tests, including polymerase chain reaction (PCR), can improve the diagnosis of infectious diseases by rapid detection of microbial nucleic acids, including from non-viable organisms (**Yang et al., 2004**). PCR may be more sensitive than culture for the diagnosis of meningitis, especially where prior

antibiotic treatment reduces the sensitivity of culture (**Wu HM et al., 2013).**

There are a number of advantages to using real-time PCR (RT-PCR) methods as compared to conventional endpoint PCR methods, namely elimination of the need for post-amplification processing, minimization of laboratory contamination, and a more rapid turn-around time. In addition, the development of multiplex RT-PCR assays provides the opportunity to detect multiple potential pathogens simultaneously (**Boving et al., 2009**). Such multiplex RT-PCR assays can offer a very comprehensive panel of potential pathogens allowing for extensive and exhaustive investigations of patients with suspected meningitis (**Rhein et al., 2013; and Wootton et al., 2016)**.

However, in practice in specific geographic settings and in particular patient population groups, a far more limited number of pathogens constitute the vast majority of cases of meningitis. The inclusion of a large number of target pathogens into commercial or in-house multiplex-PCR assays increases the cost and complexity of such assays (**Rhein et al., 2013; and Wootton et al., 2016)** and may prevent implementation of these in routine laboratory testing in low to middle income countries.

Recently, PCR-based assays have become available to provide an early and accurate diagnosis of bacterial meningitis (**Schuurman et al., 2004**). This assay can detect as few as 10–100 cfu/mL of bacteria in CSF (**Kennedy et al., 2007**).

The usefulness of this diagnostic test would be to determine whether empirical antimicrobial therapy should or should not be administered and thus potentially eliminate unnecessary administration of antimicrobial therapy to some patients (**Saravolatz et al., 2003**).

In the present study 55 patients were selected from fever hospital diagnosed as meningitis patients based on CSF analysis results, CSF culture and clinical symptoms and signs, they were divided into two subgroups according to culture being positive or negative, 40 suspected patients discovered to have negative culture while only 15 patients found to have positive culture for Haemophilus influenzae, Strepto-coccus pneumoniae and Neisseria meningitidis. In contrast to

Mohamed et al., 2014; where a total of 110 CSF samples were collected from 110 patients only who were diagnosed with acute bacterial meningitis, based on clinical symptoms and CSF findings, while in **Khumalo et al., 2017**, samples collected based on the clinician's decision to perform a lumbar puncture, together with the presence of an abnormal CSF (suspected cases) only and the results of both viral and bacterial RT-PCR assays were compared to the final diagnosis at discharge as recorded in the patient's clinical records. Over the 12-month period in **Khumalo et al., 2017;** 3236 CSF samples from children at The Red Cross War Memorial Children's Hospital (RCWMCH) were submitted for microbiological testing. Of these 516 met the inclusion criteria for the study and sufficient residual CSF was available for testing with the multiplex RT- PCR assays in 292 patients. In which the accuracy of the bacterial RT-PCR assay compared to CSF culture and using World Health Organization definitions of laboratory confirmed bacterial meningitis. From 292 samples, bacterial DNA was detected in 12 (4.1%) and viral nucleic acids in 94 (32%). Compared to CSF culture, the sensitivity and specificity of the bacterial RT-PCR was 100% and 97.2% with complete agreement in organism identification. None of the cases positive by viral RT-PCR had a bacterial cause confirmed on CSF culture. Only 9/90 (10%) of patients diagnosed clinically as bacterial meningitis or partially treated bacterial meningitis tested positive with the bacterial RT-PCR.

In agreement with our study results, **Mohamed et al., 2014**; Multiplex PCR test was positive in 60/110 patients (54.5%). While in **Khumalo et al., 2017,** bacterial target DNA was detected in 12 samples (4.1%) and viral target nucleic acids in 94 samples (32%). In **Khater, 2016** study, thirty-six samples (90%) were positive for Streptococcus pneumoniae by real-time PCR, whereas Neisseria meningitidis and Haemophilus influenzae were not detected in any of the samples (0%) and 4 samples (10%) were negative for all three organisms.

In **Mohamed et al., 2014**, among the multiplex PCR positive cases, S. pneumoniae was detected at the highest incidence in 39 patients (65%), followed by N. meningitidis in 8 cases (13.3%), Listeria monocytogenes in 7 cases (11.7%), Group B streptococcus in 4 cases (6.7%), and Hib in 2 cases (3.3%). The sensitivity of multiplex PCR was 100%. 50

cases of acute bacterial meningitis were diagnosed by multiplex PCR, while both gram stain and bacterial culture were negative in them.

In the present study, there were few Hib meningitis cases, 13/ 55 patients (24 %). This result is similar to that found by **Mohamed et al., 2014** (3.3%). Very low numbers of bacterial meningitis cases of Hib were detected in the study, either by conventional or molecular testing. This may be partly due to the decreasing burden of disease due to *H. influenzae* type b following implementation of successful vaccination programmes targeting these pathogens in 1999.

S. pneumoniae the commonest pathogen in this study, was detected in 29/55 patients (52.7%) by RT-qPCR. This prevalence is consistence with what was detected by **Mohamed et al., 2014**, (65%). In contrast to **Khumalo et al., 2017** bacterial pathogens were isolated from CSF in 4 samples, comprising *S. pneumoniae* (3 cases) and *H. influenzae* (1 case). In this study 13/55 for ***N. meningitidis*** (23.6%) was detected by RT-qPCR looks similar to **Mohamed et al., 2014** (13.3%).

In this research, the bacterial culture was positive in a total of 15 patients (27%) had a specific etiologic agent identified, with S. pneumoniae (n=5), N. meningitidis (n=5), and H. *influenzae* (n=5) while was (34.5%) in **Mohamed et al., 2014**. The low rate of positive bacterial culture may be due to the preceding antibiotic therapy of the patients before lumbar puncture.

However, the above information denotes that the gram stain and bacterial culture cannot be considered as a method for early and exact diagnosis of meningitis. **Khumalo et al., 2017;** reported that using CSF culture as a reference standard, the sensitivity and specificity of the bacterial RT-PCR were 100% (95% CI, 51.0% - 100%) and 97.2% (95% CI, 94.6 %–98.6%) respectively. Correlation between culture results and PCR results were illustrated in the table (8).

While compared to the WHO IB-VPD Surveillance Network definition of laboratory confirmed cases, the sensitivity and specificity were 60% (95% CI, 31.3%–83.2%) and 97.9% (95% CI, 95.4%-99.0%) respectively. The use of qPCR significantly improved identification of S. pneumoniae, N. meningitidis, and H. influenzae, more than doubling the detection rate.

In conclusion, the PCR has high sensitivity and specificity for the detection of bacterial pathogens such as S. pneumoniae in the CSF. Further refinements in this technique may make it useful for the diagnosis of bacterial meningitis, especially when results of CSF gram stain and bacterial culture are negative and when patients had received antibiotics before the lumbar puncture was done. Due to the need to antimicrobial susceptibility testing, PCR should be considered as an adjunct to bacterial culture and antimicrobial susceptibility testing.

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الملخص العربى

المقدمة: الالتهاب السحائى هو التهاب الأغشية الواقية التي تغطي المخ والنخاع الشوكي. ويمثل مشكلة خطيرة للصحة العامة تتطلب التشخيص المبكر والعلاج الفعال والوقاية والمكافحة. وهو سبب رئيسي من أسباب الاعتلال والوفيات بين الرضع والأطفال.

يصيب الالتهاب السحائى البكتيرى حوالي 3 لكل 100000 شخص سنويا في الدول الغربية ويؤدى إلى 171000حالة وفاة على مستوى العالم سنويا. هناك ثلاثة أسباب بكتيرية رئيسية تمثل أكثر من 90٪ من أسباب حالات الالتهاب السحائى في الأطفال على مستوى العالم وهى مينينجوكوكل والنيموكوكل وهيموفيلس انفلونزي.

الهدف من الدراسة: تهدف هذه الدراسة إلى الاكتشاف المبكر للإصابة بالالتهاب السحائي البكتيري عن طريق سلسة تفاعل البلمرة واثبات دقته في اكتشاف هذا المرض وبالتالي كفاءة العلاج.

طريقه البحث: دراسة مقارنة مع مجموعة ضابطة.

المواضيع والأساليب:

المواضيع : تمت هذه الدراسة فى مستشفى المطرية التعليمى ومستشفى الحميات على عدد 65 من الأطفال و الرضع من كلا الجنسين منهم 10 حالات من الأصحاء للمناظرة و55حالة مرضية من المشتبه في وجود التهاب سحائي بكتيري.

معايير الدمج :

1- معايير الحالات المؤكدة من الالتهاب السحائى البكتيرى (المعملية).
2- يتراوح العمر من 0 – 4 سنوات
3- عدم وجود تاريخ مرضى لمرض عصبى.

معايير الاستبعاد :

1- المرضى الذين رفضوا التوقيع على استمارة موافقة.
2- سيتم استبعاد المرضى الذين يعانون من وجود أي من الأمراض التالية:
- أمراض عصبية جراحية
- الشلل الدماغي.
- كسور الجمجمة مع أو بدون تسرب السائل النخاعي.
- دليل على وجود عدوى فيروسية مثل الحصبة والنكاف .... الخ.

الأساليب: خضعت الحالات أثناء استقصاء التاريخ المرضي للآتي :

1- السؤال عن أعراض المرض مثل آلام بالظهر أو الرقبة، حمى، الخوف من الضوء أو التشنجات أو تغير في حالة الوعي.
2 - الفحص الإكلينيكى : تيبس الرقبة أو علامات تهيج سحائي ودرجه الوعى.
3-البزل النخاعى، وتم جمع عينة السائل النخاعي قبل بدء العلاج لفحصها في المختبر من أجل :

- عدد الخلايا الكمى و النوعى.
- تحديد مستوى الجلوكوز و البروتين.
- فحص ميكروسكوبى بصبغه الجرام.
- جمع عينة أخرى من السائل النخاعي لفحصها عن طريق البلمرة في ذات الوقت.

4- تحديد مستوى الجلوكوز بالدم.
5- مزرعة دم.
6- سلسلة تفاعل البلمرة

معايير الحالات المؤكدة : يتم تأكيد التشخيص بالالتهاب السحائى البكتيرى إذا توفرت أحد المعايير التالية:
1- وجود بكتيريا عصويه سالبه الجرام أو بكتيريا كرويه سواء إيجابية أو سلبية الجرام فى السائل النخاعي.
2- مزرعة السائل النخاعي إيجابية للمينينجوكوكل أو للنيموكوكل أو للهيموفيلس بكتيريا .
3- عدد كرات الدم الأبيض فى السائل النخاعي أكثر من 100 /مليلتر ، نسبة كرات الدم الأبيض متعددة النوايا أكثر من 50 ٪ ونسبة الجلوكوز في الدم /السائل النخاعي أقل من 0,5.
4- عدد كرات الدم الأبيض فى السائل النخاعي اكثر من 100/مليلتر ، نسبة كرات الدم الأبيض متعددة النوايا أكثر من 50 ٪ والجلوكوز السائل النخاعي أقل من 30 ملغ / دل.
5- عدد كرات الدم الأبيض فى السائل النخاعي أكثر من 1000//مليلتر ، نسبة كرات الدم الأبيض متعددة النوايا أكثر من 50 ٪.
6- التفاعل الايجابي للبلمرة.

الموافقة الأخلاقية: تم أخذ موافقة لجنة أخلاقيات البحث العلمي بكلية طب جامعة الأزهر قبل بدء الدراسة.

- تم اخذ موافقة خطية من الآباء والأمهات قبل بداية الدراسة وذلك بعد شرح طبيعة الدراسة لهم.

- تم أخذ موافقة لجنة أخلاقيات البحث العلمي بكلية طب جامعة عين شمس قبل بدء الدراسة.

التحليل الإحصائى: نتائج البحث تم تجميعها و تنظيمها فى جداول ويتم تحليلها بالطرق الإحصائية المناسبة والمتعددة لتوضيح النتائج النهائية للبحث.

نتائج البحث: أظهرت نتائج البحث الذي اقيم على عدد 65 حالة منهم 10 حالات من الأصحاء للمناظرة 55حالة مرضية من المشتبه في وجود التهاب سحائي بكتيري بينما تأكد وجود ميكروب في 15 حالة عن طريق عمل مزرعة للسائل النخاعي من بينهم 5حالات مصابة ببكتيريا الهيموفيلس أنفلونزا ، 5 حالات أخرى تبين إصابتها ببكتيريا ستربت نيموني كما تبين إصابة آخر 5 حالات بميكروب نيسيريا منتجيتيز؛ بينما أظهرت نتائج المزرعة للسائل النخاعي نتائج سلبية لعدد 40 حالة مرضية. ويرجع ذلك لتعرض هؤلاء المرضى لتناول المضادات الحيوية بشكل منتظم أو متقطع نتيجة تشخيصات مرضية أخرى تتشابه مع مرض الالتهاب السحائي البكتيري مثل التهابات الجهاز التنفسي العلوي و النزلات المعوية ؛ والتي أثرت على نتائج مزرعة السائل النخاعي وجعلتها سالبة .

بعد عمل تحليل سلسلة تفاعل البلمرة للسائل النخاعي لجميع الحالات المشتبه بإصابتهم بالتهاب سحائي بكتيري وعددهم 55 مريض وتبين ظهور نتائج ايجابية لجميع المرضى المشتبه بهم بنسبة 100% مما يؤكد فاعلية وحساسية تحليل سلسلة تفاعل البلمرة للسائل النخاعي في التشخيص وجعله اختيار فحصي مثالي لتحديد مدى الإصابة بالالتهاب السحائي البكتيري وكذا تحديد نوع الميكروب بدقة وفي أسرع وقت ممكن لا يتجاوز ال6 ساعات وان نتائجه لا تتأثر بتناول المضادات الحيوية لأنه يتمكن من اكتشاف وجود البكتيريا بنسب ضئيلة جدا في السائل النخاعي.

تمت مقارنة نتائج البحث بعدة أبحاث عالمية أجرت دراسات شبيهة بهذه الدراسة وتوصلت إلى نتائج مماثلة.

توصيات البحث: ان يضاف تحليل سلسلة تفاعل البلمرة للسائل النخاعي لتشخيص الإصابة بالالتهاب السحائي البكتيري كأسرع وأدق وسيلة للتشخيص بالإضافة إلى الفحوصات الروتينية للحصول على أفضل النتائج وأسرع وأحسن طرق العلاج وبذلك تخفض نسبة المضاعفات والتي تؤثر على الأطفال والمجتمع.

كما تدعو الدراسة إلى توفير هذه التقنية التشخيصية بأسعار أكثر تدعيما من الحكومة ووزارة الصحة حتى تتم الاستفادة المرجوة منها بشكل أفضل.